Textbook of Military Medicine

MEDICAL ASPECTS of CHEMICAL and BIOLOGICAL WARFARE

Introduction

Foreword by The Surgeon General

Preface

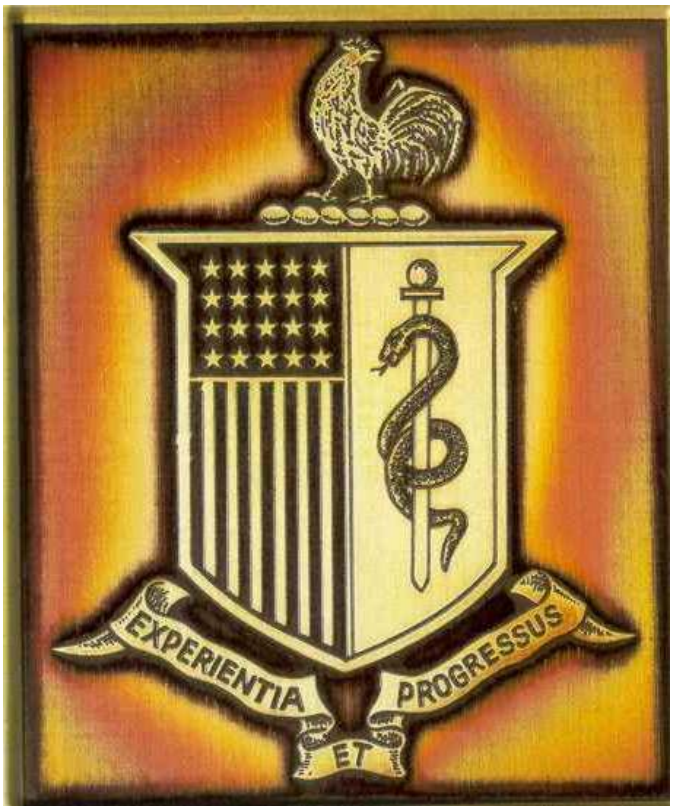
Patient Flow in a Theater of Operations

Medical Aftermath of the Persian Gulf War

1. Overview: Defense Against the Effects of Chemical and Biological Warfare Agents
2. History of Chemical and Biological Warfare: An American Perspective
3. Historical Aspects of Medical Defense Against Chemical Warfare
4. The Chemical Warfare Threat and the Military Healthcare Provider
5. Nerve Agents
6. Pretreatment for Nerve Agent Exposure
7. Vesicants
8. Long-Term Health Effects of Nerve Agents and Mustard
9. Toxic Inhalational Injury
10. Cyanide Poisoning
11. Incapacitating Agents
12. Riot Control Agents
13. Field Management of Chemical Casualties
14. Triage of Chemical Casualties

15. Decontamination
16. Chemical Defense Equipment
17. Healthcare and the Chemical Surety Mission
18. Historical Overview of Biological Warfare
19. The U.S. Biological Warfare and Biological Defense Programs
20. Use of Biological Weapons
21. The Biological Warfare Threat
22. Anthrax
23. Plague
24. Tularemia
25. Brucellosis
26. Q Fever
27. Smallpox
28. Viral Encephalitides
29. Viral Hemorrhagic Fevers
30. Defense Against Toxin Weapons
31. Staphylococcal Enterotoxin B and Related Pyrogenic Toxins
32. Ricin Toxin
33. Botulinum Toxins
34. Trichothecene Mycotoxins
35. Medical Challenges in Chemical and Biological Defense for the 21st Century

MEDICAL ASPECTS OF CHEMICAL AND BIOLOGICAL WARFARE



The Coat of Arms
1818
Medical Department of the Army

A 1976 etching by Vassil Ekimov of an original color print that appeared in The Military Surgeon,
Vol XLI, No 2, 1917

The first line of medical defense in wartime is the combat medic. Although in ancient times medics carried the caduceus into battle to signify the neutral, humanitarian nature of their tasks, they have never been immune to the perils of war. They have made the highest sacrifices to save the lives of others, and their dedication to the wounded soldier is the foundation of military medical care.

Textbook of Military Medicine

Published by the

Office of The Surgeon General
Department of the Army, United States of America

Editor in Chief
Brigadier General Russ Zajtchuk, MC, U.S. Army
Director, Borden Institute
Commanding General
U.S. Army Medical Research and Materiel Command
Professor of Surgery
F. Edward Hébert School of Medicine
Uniformed Services University of the Health Sciences
Bethesda, Maryland

Managing Editor
Ronald F. Bellamy, M.D.
Colonel, MC, U.S. Army (Retired)
Borden Institute
Associate Professor of Military Medicine
Associate Professor of Surgery
F. Edward Hébert School of Medicine
Uniformed Services University of the Health Sciences
Bethesda, Maryland

The TMM Series

Part I.	Warfare, Weaponry, and the Casualty Medical Consequences of Nuclear Warfare (1989) Conventional Warfare: Ballistic, Blast, and Burn Injuries (1991) Military Psychiatry: Preparing in Peace for War (1994) War Psychiatry (1995) Medical Aspects of Chemical and Biological Warfare (1997) Military Medical Ethics
Part II.	Principles of Medical Command and Support Military Medicine in Peace and War
Part III.	Disease and the Environment Occupational Health: The Soldier and the Industrial Base (1993) Military Dermatology (1994) Military Preventive Medicine: Mobilization and Deployment Medical Aspects of Deployment to Harsh Environments
Part IV.	Surgical Combat Casualty Care Anesthesia and Perioperative Care of the Combat Casualty (1995) Rehabilitation of the Injured Soldier Military Surgery

Foreword

The thought of chemical and biological warfare terrifies us. What is it in the human psyche that makes being attacked with conventional weapons—that kill and maim—more acceptable than being attacked with molecules that alter the body chemistry or with organisms that cause disease? For some, the wearing of chemical protective clothing seems to exemplify our fear of an unknown agent that we cannot see, do not understand, and think must be immoral.

World events have conspired to increase the threat of the use of chemical and biological weapons. The end of the Cold War brought not only the hoped-for change of swords into plowshares but also political and economic turbulence in the former Soviet Union, unemployed and disenchanted weapons specialists and scientists, the rise of religious fundamentalism in southwest Asia, state-sponsored terrorism, and blurring of the lines between terrorism and traditional warfare.

In addition, the nature of war is changing. We no longer expect a war to last years, as World War II did, but rather days, as we saw with the Persian Gulf War. Worse, the weapons of war have also changed. Many countries do or could possess chemical and biological agents—bypassing the tremendous financial outlay required to acquire conventional weapons.

Until this decade, our military forces had not faced chemical and biological weapons since World War I, and the prevailing attitude has been "out of sight, out of mind." The Persian Gulf War changed all that. Just the *threat* that such weapons would be used was itself an effective weapon, as it required us to expend tremendous logistical resources to supply our troops in the desert. Now we know that we must master all relevant aspects of defense against chemical and biological warfare. The Biological Weapons Convention, ratified in 1975, did not slow the massive Soviet program, which continued until early 1992, nor did it prevent the buildup in Iraq between 1985 and 1990. At this time, experts are severely questioning whether verification of compliance with the treaty can be certain. Similar concerns delayed ratification of the Chemical Weapons Convention by the U.S. Senate; nevertheless, the senate ratified the treaty on 24 April 1997.

A primary value of the *Textbook of Military Medicine* series is to preserve the lessons of past wars and, by so doing, demonstrate how current doctrine is built on knowledge that was

gained at so high a cost. Medical officers should read this volume, *Medical Aspects of Chemical and Biological Warfare*, and learn its lessons well. Civilians expect that we in the military will know how to manage chemical and biological casualties. Indeed, if we do not, then who will? The nation expects us to be prepared to defend against all attacks and will be unforgiving of any incapacity on our part.

Lieutenant General Ronald R. Blanck
The Surgeon General
U.S. Army

May 1997
Washington, D. C.

Preface

Until recently, we in the United States have not given much thought to the specter of chemical and biological warfare. Our fathers and grandfathers who fought in World War I are almost all gone now, and the poet's image of gassed soldiers fumbling for their helmets has been considered merely a historical footnote—if it is remembered at all. But forgetting is a luxury we can no longer afford. In 1917, the Army War College stated:

> The employment of poisonous gases as a means of offensive warfare has made it imperative that medical officers should have some knowledge of the action of the various gases that are likely to be met with and of rational lines of treatment which may be adopted in cases of gas poisoning.[1(p5)]

Nothing has changed except the increased availability of chemical and biological weapons; now more than ever we must be able to both defend against attack and manage chemical and biological casualties.

The good news is that the development of passive countermeasures for chemical and biological defense (pretreatments, therapies, timely detectors, effective protective equipment) has significantly reduced the threat to our military forces. Although the biological defense countermeasures program is not yet as advanced as its chemical counterpart, new developments in biotechnology have allowed us to take tremendous strides forward. In the meantime, we can educate our healthcare providers now, at minimal cost and with great potential benefit. One of the reasons that chemical and biological weapons are considered so dangerous is that we medical officers, in our daily clinical practice, hardly ever see patients whose conditions have any similarity to casualties of chemical and some of the more exotic biological agents.

This textbook focuses on the management of casualties. Its publication may be even more timely than we had expected, especially considering the increased threat of terrorism—both foreign and domestics. Terrorist attacks at home and abroad have heightened the interest of civilian healthcare providers and first-responders, and of other governmental agencies such as the

Federal Emergency Management Agency and the Public Health Service that would be required to respond in case of an attack on our own soil. These nonmilitary healthcare providers will also find this textbook to be extremely useful.

The scientists who organized and are responsible for this textbook are recognized worldwide as the foremost experts in the medical aspects of chemical and biological warfare. Their overriding goal is this: to produce a force that understands the threats of chemical and biological weapons and how to respond to them, and, by understanding the threats, sustains fewer casualties.

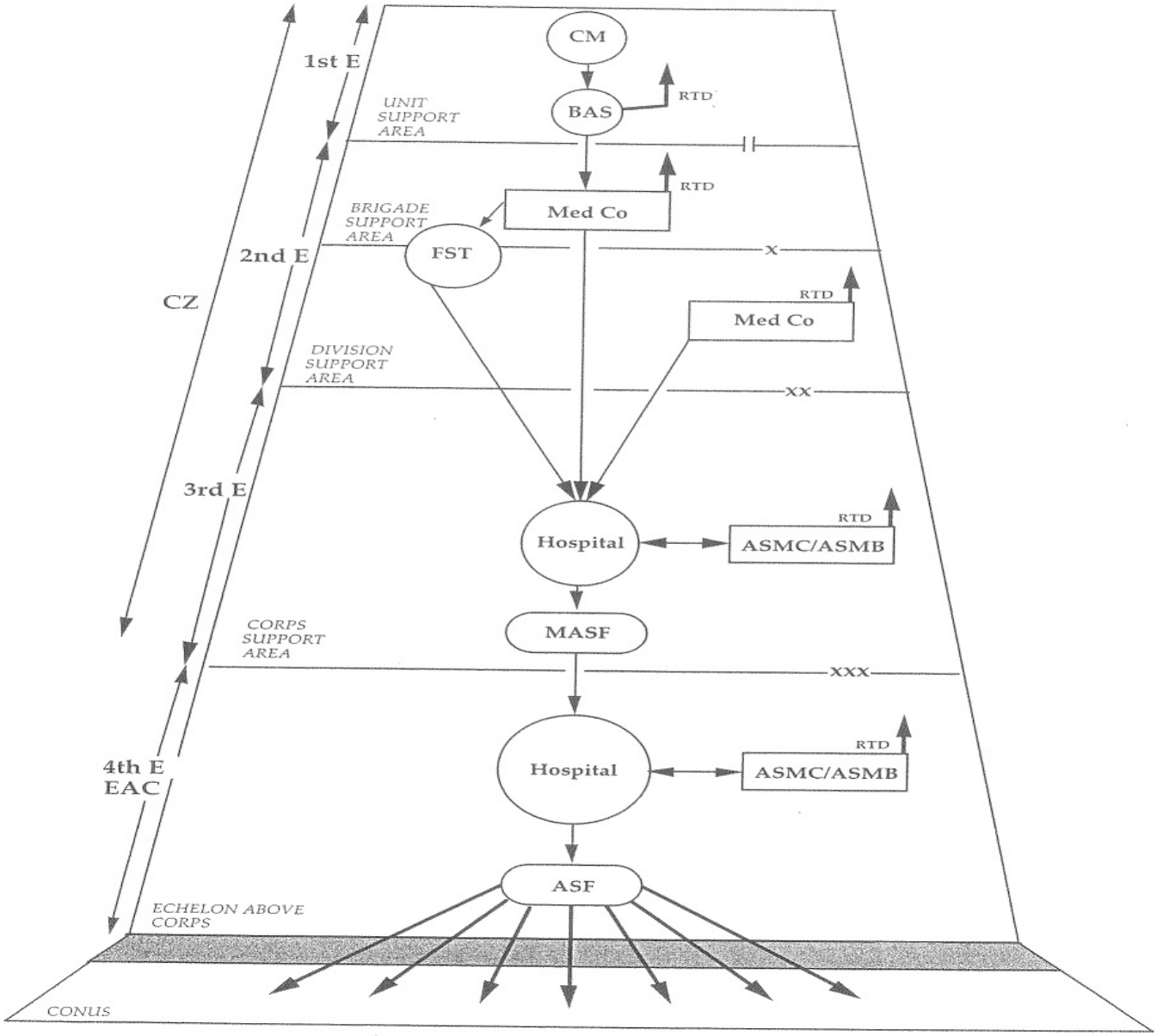
Brigadier General Russ Zajtchuk
Medical Corps, U.S. Army

May 1997
Washington, D. C.

1. Army War College. *Memorandum on Gas Poisoning in Warfare, With Notes on its Pathology and Treatment.* Washington, DC: Government Printing Office; 1917: 5.

The current medical system to support the U.S. Army at war is a continuum from the forward line of troops through the continental United States; it serves as a primary source of trained replacements during the early stages of a major conflict. The system is designed to optimize the return to duty of the maximum number of trained combat soldiers at the lowest possible echelon. Far-forward stabilization helps to maintain the physiology of injured soldiers who are unlikely to return to duty and allows for their rapid evacuation from the battlefield without needless sacrifice of life or function.

Medical Force 2000 (MF2K)
PATIENT FLOW IN A THEATER OF OPERATIONS



ASF:	Aeromedical Staging Facility, USAF	E:	Echelon
ASMB:	Area Support Medical Battalion	EAC:	Echelon Above Corps
ASMC:	Area Support Medical Company	FST:	Forward Surgical Team
BAS:	Battalion Aid Station	MASF:	Mobile Aeromedical Staging Facility, USAF
CM:	Combat Medic	Med Co:	Medical Company
CONUS:	Continental United States	RTD:	Return to Duty
CZ:	Combat Zone		

Medical Aftermath of the Persian Gulf War

The editors of the *Textbook of Military Medicine* are mindful that some veterans of the Persian Gulf War (1990-1991) face continuing health problems. Although readers might have hoped to find a discussion in this textbook devoted to the illness known as Gulf War syndrome, the medical aftermath of that war is incompletely understood. A formal academic treatment now would not only be premature, it would soon be outdated. One fact seems clear at this time (May 1997): the scientific community has not yet reached a consensus on the medical consequences of serving in the Persian Gulf. Most observers agree that some of the 697,000 U.S. soldiers who were deployed there are sick and have wide-ranging symptoms, but the cause, or causes, have not yet been established. Investigations into the etiology and epidemiology of these illnesses have reached inconclusive and contradictory conclusions. Even the popular name of the illness, Gulf War syndrome, is perhaps misleading because the array of signs and symptoms does not fit the usual medical definition of a syndrome: a set of symptoms that occur together; the sum of signs of any morbid state; the aggregate of signs and symptoms associated with any morbid process that constitute together the picture of the disease. The level of scientific inquiry into the problem, already high, has increased in recent months; we hope that these questions (particularly those pertaining to etiology and epidemiology, and from there, treatment) can be answered soon. Subsequent editions of this or other textbooks in this series will give the medical aftermath of the Persian Gulf War the attention it deserves.

Chapter 1

OVERVIEW: DEFENSE AGAINST THE EFFECTS OF CHEMICAL AND BIOLOGICAL WARFARE AGENTS

FREDERICK R. SIDELL, M.D.*; AND DAVID R. FRANZ, D.V.M., PH.D.†

INTRODUCTION

HISTORICAL PRECEDENTS

INTRODUCTION TO CHEMICAL AND BIOLOGICAL AGENTS

IMPLICATIONS FOR THE MILITARY MEDICAL DEPARTMENTS

* *Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014*

†*Colonel, Veterinary Corps, U.S. Army; Commander, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

INTRODUCTION

“Gas! Gas!” This warning cry, so common in World War I, almost became real to U.S. forces again as they prepared to liberate Kuwait in late 1990. The threat of chemical, and even biological, warfare was foremost in the minds of U.S. military personnel during Operation Desert Shield, the preparation for the Persian Gulf War. Iraq was known to have a large stockpile of chemical weapons and had demonstrated during its conflict with Iran that it would use them. It was not until after the Persian Gulf War that the U.N. Special Commission on Iraq confirmed that Saddam Hussein also had biological agents loaded in weapons. The chemical and biological threats were major concerns to those in the military medical departments who would be called on to care for poisoned or infected casualties, possibly in a chemically contaminated environment. Fortunately the ground war of the Persian Gulf War (Operation Desert Storm) was brief, and even more fortunately, our adversary did not employ these weapons.

In the desert, during the fall and winter of 1990–1991, the threat of chemical warfare became very real to our military medical personnel. The threat of biological warfare was no less feared. The military medical departments realized that medical personnel were not prepared to provide care to chemical or biological casualties or to function in a contaminated environment. This textbook should help accelerate the assimilation of medical defense information in the next war; in the past, such information has not been readily accessible. Two handbooks have also been prepared: *Medical Management of Chemical Casualties Handbook*, Chemical Casualty Care Office, Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland (September 1994); and *Medical Management of Biological Casualties Handbook,* U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland (March 1996).

Rapid and intense teaching programs helped prepare our medical healthcare providers, so that by the onset of Operation Desert Storm, they were as ready as any military medical personnel might be to go to war. Hundreds of thousands of troops were supplied with chemical pretreatment and therapeutic agents and thousands were immunized against anthrax and the botulinum toxins, the two most likely biological battlefield threats.

Two lessons were learned from this conflict, lessons that should never be forgotten by those in the military. The first was that there are countries that have chemical and biological weapons, and there are other countries that might obtain or produce them. The second was that the U.S. military medical departments must be prepared at all times to treat

both types of casualties. As long as potential adversaries exist, the U.S. military might face a chemical or biological battlefield.

Military medical personnel of the United States have not treated a chemical casualty on the battlefield for nearly 8 decades, and they have never treated a biological casualty. Chemical agents have not been used as weapons in a major war or in any military conflict in which the United States has been involved since World War I. Despite the recent dissolution of the Warsaw Pact, the breakup of the Soviet Union, and other events that have seemingly reduced the conventional military threat to the United States, a textbook for military medical personnel on the management of chemical and biological agent casualties is still urgently needed. The breakup of the Soviet Union, and the consequent glut of biowarfare experts on the world employment market, may have actually increased the threat of biological proliferation. In addition to the recent experience in the Persian Gulf, a review of other events of the past 2 decades bears out this conclusion (Exhibit 1-1).

EXHIBIT 1-1
RECENT TARGETS OF CHEMICAL OR BIOLOGICAL AGENTS

Laos (mid to late 1970s; alleged)

Kampuchea (late 1970s and early 1980s; alleged)

Afghanistan (1980s; alleged)

Iran (1980s; Iran–Iraq War; confirmed)

Iraqi Kurds (1988; confirmed)

HISTORICAL PRECEDENTS

During the Arab–Israeli War (also called the Yom Kippur War) of 1973, chemical weapons were not used. While processing captured soldiers, however, Israeli troops found that the Egyptians carried personal protective equipment, a decontamination kit containing items unfamiliar to U.S. personnel, and an antidote with which we were also unfamiliar. This evidence suggested that the Egyptians were prepared for a chemical battlefield, and the components of the antidote suggested that they were prepared for the use of the nerve agent soman. (The antidote was a mixture of three compounds: atropine, benactyzine, and the oxime, TMB4.) The U.S. military soon issued the antidote to U.S. troops, only to withdraw it about 5 years later.

In the mid to late 1970s, reports began to appear that chemicals were being used against Hmong tribesmen in Laos. The Hmong had been loyal to the United States and had served this country in many ways during the

Vietnam War; it was suggested that chemicals were being used against the Hmong in retaliation. Investigations were conducted by U.S. State Department personnel, by a medical team sent by The U.S. Army Surgeon General, and by international groups. Little definitive evidence was discovered, primarily because the alleged attacks took place deep in Laos. The victims took weeks to travel to Thailand to be examined, and outsiders could not enter Laos to examine the attack sites. The Hmong who reached Thailand provided graphic accounts of attacks by sprays and bombs from airplanes and how these “smokes,” which were of all colors, killed many in their villages. One member of the medical team brought back a sample of a yellow substance on the outer (barklike) layers of a bamboo culm (ie, stalk); the sample had been given to him by a Hmong, who claimed that the material had killed many of his fellow villagers. This yellow substance, along with samples from many other locations, later became known as “yellow rain” (see Chapter 34, Trichothecene Mycotoxins, which discusses yellow rain in greater detail).

Moreover, in the late 1970s and early 1980s, allegations were made of chemical agent use against refugees fleeing the barbaric conditions that existed in Kampuchea at that time.[1] The clinical response of the exposed humans did not fit what we understood about the effects of classic chemical agents. Tearing and itching looked like the effects of tear gas. Convulsions suggested nerve agents. But the occurrence of internal hemorrhage and skin lesions could not be explained. Analysis of a leaf sample collected in Kampuchea 24 hours after an attack implicated trichothecene mycotoxins, a family of toxins produced by fungi but having characteristics more like chemical than biological agents.

In August 1981, based on limited physical evidence, the U.S. government announced that trichothecene mycotoxins had been used but the findings were less than convincing to some in the scientific community and the issue became extremely contentious. This controversy was never totally resolved, and the question of which, if any, agents were used against civilians was not answered. If mycotoxins were, in fact, used it was the first recorded use of biological agents since before World War II, when the Japanese used them against the Chinese in the early 1940s.[2]

In the 1980s, Soviet troops battled Afghan rebels protesting the communist Afghan regime. During this lengthy conflict, frequent allegations were made of the use of chemical agents against the Afghans. One of these chemicals, known as Blue-X, was said to cause instant immobilization, the victim remaining in place for a number of hours before recovering. The use of other, more lethal agents was also alleged, but again no definitive evidence was found.

The most widespread and most open use of chemical weapons on a battlefield in recent decades was by Iraq in its conflict with Iran. This time

the evidence of chemical use was conclusive. Undetonated shells were sampled and their contents were analyzed by several laboratories in Europe. A vesicant or blister agent (mustard) and a nerve agent (tabun) were identified. About 100 Iranian soldiers with chemical wounds were sent to European hospitals for care; their wounds were consistent with vesicant (mustard) injury. A team appointed by the U.N. secretariat went to Iranian battlefields and hospitals and found chemical shells and patients with chemical injuries. The public outcry at the use of these weapons was less than overwhelming. Ignoring protests from the world community, Iraq continued to use these agents.

Evacuating wounded soldiers to Europe not only lessened the burden on the medical facilities in Iran (although the number sent was a small fraction of the total) and provided soldiers with good medical care, but it also provided the rest of the world with evidence that Iraq was using these weapons. In general, the casualties were sent privately, not through governmental connections. Physicians in Europe accepted the patients and assumed responsibility for their care, usually in private hospitals (a situation that made a retrospective analysis of the care rendered and the effectiveness of different treatment regimens difficult).

A similar situation enabled three physicians from the U.S. Army medical community to examine several casualties from Iraq's use of chemical weapons. On March 19, 1988, Iraqi airplanes bombed the village of Halabja, in Iraq. The inhabitants were Kurdish Iraqi citizens, a tribespeople who live in the region where the borders of Turkey, Iran, and Iraq meet. The casualties from this raid received worldwide media attention. The chemical weapons allegedly used were nerve agents, cyanide, and mustard. The casualties were cared for by Iran, and five of them (a man, a woman, and three young children, all unrelated) were sent to the United States for care by an Iranian physician living here. On examination by three authors of chapters in this textbook, the casualties were found to have skin lesions and pulmonary pathological changes (as determined by radiograph) consistent with mustard exposure.

Other items in the news over the past decade have suggested that the proliferation of chemical and biological agents is greater than we might hope. For example, numerous accounts claimed that Libya had built a facility capable of chemical agent production at Rabta—Libya's protestation that this facility was a pharmaceutical plant notwithstanding. One report even noted that monthly production was about 30 tons of mustard.

In 1979, an accident at a previously undetected biological weapons plant in Sverdlovsk, Russia, surprised even the intelligence community.[3] At least 66 humans living or working downwind of the plant died of pulmonary anthrax. Soviet troops quickly attempted to decontaminate the

facility and the city following airborne release of anthrax spores, and medical teams instituted preventive therapy, but the message was clear. The Soviet biological warfare program was thriving, more than 6 years after the Soviet Union had signed the Biological Weapons Convention.

In addition to their being used on the battlefield, chemical and biological agents might also be used in terrorist attacks. The nerve agent sarin was twice used in Japan. The first incident, in Matsumoto in June 1994, produced more than 200 casualties including 7 fatalities. In the second incident—in the Tokyo subway system on 20 March 1995—5,510 people were taken to medical facilities or sought medical assistance. About 20% of these were hospitalized, and 12 died. The cult that was accused of both attacks was found to have a large facility for manufacturing both chemical and biological agents.

In the face of overwhelming evidence, the Soviet Union continued to officially deny having an offensive biological weapons program until 1992, when Russian President Boris Yeltsin admitted publicly to having maintained a program until March of that year. Since then, visits by teams from the United States and the United Kingdom to former biological warfare facilities under the Joint United States/United Kingdom/Russia Trilateral Statement on Biological Weapons have clearly documented the capabilities to produce biological warfare agents in massive quantities.

Verification of compliance with agreements such as the Trilateral and with the chemical and biological weapons conventions are plagued by the “dual-use” nature of the facilities in which these agents are developed and produced. A legitimate chemical facility can be converted fairly easily for the manufacture of chemical agents. On threat of inspection by an international group, the facility can readily be converted back to a legitimate use. The dual-use nature of production facilities is even more applicable to the production of biological agents. Partly for this reason, chemical and biological weapons have been called “the poor man’s atom bomb.” It has also been said that agents can be made in a bathtub, which may be true to a limited extent for a skilled microbiologist or chemist. Production of even tactical quantities of these agents and their deployment on the battlefield, however, is not a trivial undertaking.

INTRODUCTION TO CHEMICAL AND BIOLOGICAL AGENTS

Chemical and biological agents differ in several important ways. Chemical agents are typically manmade through the use of industrial chemical processes. Biological agents are either replicating agents (bacteria or viruses) or nonreplicating materials (toxins or physiologically active proteins or peptides) that can be produced by living organisms.

Some of the nonreplicating biological agents can also be produced through either chemical synthesis, solid-phase protein synthesis, or recombinant expression methods. Almost none of the biological agents are dermally active (the mycotoxins are a rare exception) and none are volatile. On the other hand, most of the chemical agents are dermally active, volatile, or both.

Therefore, while many of the dermally active or volatile chemical agents can be disseminated as liquids or aerosols, and the biological agents must be dispersed as respirable aerosols (particles approximately 1–10 µm in diameter). Dispersing a respirable aerosol on a battlefield requires a high-energy generating system to produce the small particle size, appropriate weather conditions to assure that the aerosol cloud stays near the ground, and adequate infectivity or toxicity of the agent to produce the desired effect. Except for infectivity, these are all important practical requirements for the field use of chemical, as well as biological, warfare agents.

In World War I, the use of chemical agents began with the small-scale use of irritants (known today as riot control agents). Chlorine, the first agent used on a large scale, and phosgene caused large numbers of deaths. Cyanide was introduced in midwar, but the agent that caused the greatest number of casualties was the vesicant mustard, which was introduced late in the war. Cyanide, phosgene, and mustard are still potential chemical weapons today.

In the period before World War II, German scientists synthesized the first nerve agents; during the war, Germany had thousands of tons of nerve agents stockpiled in munitions. The United States and the Soviet Union captured the stockpiles and manufacturing facilities late in the war, and they began to manufacture and stockpile these agents. Nerve agents are 15- to 100-fold more potent than the chemical agents used in World War I. In the 1950s, the United States put the incapacitating compound BZ into munitions (which have been destroyed); late in that decade, the currently used riot control agent CS was introduced for military use.

Military chemical agents are classified as “persistent” and “nonpersistent.” Persistent agents are those with low volatility or which evaporate slowly. Since they do not readily evaporate, they stay on terrain, materiel, or equipment for days, weeks, or months, depending on the weather. Chief among the persistent agents are the vesicant mustard and the nerve agent VX. Nonpersistent agents are those that are volatile and hence evaporate quickly; they are not expected to be present for more than several hours. The nonpersistent agents are phosgene, cyanide, and the G series of nerve agents. Each type has military advantages. Advancing troops might disperse a nonpersistent agent ahead of their attack to have the advantage of its effects on the enemy and later to have

uncontaminated terrain into which to advance. A persistent agent might be used to contaminate terrain, supplies, and equipment, denying the enemy their use.

Biological weapons may contain either replicating or nonreplicating agents. Although hundreds of naturally occurring bacteria, viruses, and toxins, as well as “designer compounds,” could potentially be considered agents by an aggressor, a finite number of these are actually useful as area weapons on the battlefield. The agents’ utility is limited by ease of production, stability, and infectivity (bacteria and viruses), or toxicity/effectivity (toxins and other physiologically active materials). *Bacillus anthracis,* for example, is often touted as the best of bacterial agents. Stability of the spore form and ease of production are its greatest strengths as weapons material. Among viral agents, Venezuelan equine encephalitis virus is easily grown to extremely high titers, making it a potential incapacitating agent. The bacterial agents that cause tularemia, Q fever, and brucellosis are infective at extremely low doses (1–10 organisms per person). Finally, the extraordinary toxicity (1,000- to 10,000-fold more toxic than the classic nerve agents) of the staphylococcal enterotoxins as incapacitants and the botulinum toxins as lethal agents makes them candidates for weaponization.

Most of the chemical compounds noted above have characteristics that make them uniquely suited to warfare. Closely related chemical substances, however, and some of the threat agents, are found throughout the civilian community. Unlike the chemical warfare agents, which are not found in nature, essentially all of the biological agents described are found in nature and cause the same or very similar disease syndromes. Military medical personnel might encounter persons exposed to the organisms as endemic disease agents on remote battlefields.

Similarly, civilians as well as military personnel could be exposed during peacetime to commercial chemicals closely related to chemical warfare agents. Thousands of tons of cyanide, for example, are manufactured annually for industrial use and are shipped to users by truck and train throughout the country. Phosgene is also manufactured in large amounts and shipped cross-country. The nerve agents are not available outside the military, but they are closely related to most pesticides or insecticides that are sprayed on orchards or used by the backyard rose gardener. The effects of these agricultural compounds are nearly identical to those of nerve agents, and medical therapy is the same. The incapacitating agent BZ (3-quinuclidinyl benzilate) is used in small amounts in research pharmacology (where it is known as QNB). Also, BZ is pharmacologically related to anticholinergic drugs, which are present in many over-the-counter preparations, such as sleeping medications.

Unlike the chemical warfare agents, essentially all of the biological

agents described cause syndromes that mimic or are identical to naturally occurring diseases. Outbreaks of disease caused by bacteria or viruses or isolated intoxications caused by toxins may result in syndromes similar to those seen in biological warfare attacks. In the case of these agents, the route of exposure—universally via the airways on the battlefield—may cause slightly or significantly different clinical presentations. General principles of prophylaxis and therapy presented in this text, however, often apply. Although the reader may initially think that the information presented in this textbook is needed only in wartime, much of the contents will also be useful to the physician in a busy emergency room.

On the battlefield, knowledge of the chemical or biological agent threat and its medical and physical countermeasures can actually reduce the threat. In World War I, the death rate for chemical casualties was about 3%. Data are not available for the Iran–Iraq War, but informal reports indicate that the death rate for those chemical casualties who reached medical care was probably less than 5%, despite the use of the highly toxic nerve agents against relatively unprotected troops. With well-trained troops and well-prepared medical personnel, these figures will be lower. For the chemical agents, real-time detectors allow exploitation of the excellent individual physical protective mask, effective pretreatment, and therapy.

These countermeasures, in conjunction with training of our forces, can make an enormous difference and actually serve as a deterrent to chemical agent use. A chemical attack on a battlefield will not be the devastating event that some military medical personnel fear. Soldiers will survive and return to duty. For the biological agents, field detectors are still not responsive enough to allow timely warning of a cloud moving across the battlefield. Although the mask is protective, adequate warning may still be a problem. Knowledge of the meteorological conditions necessary for effective deployment of biological and chemical agents can at least limit the time during which a force must be on highest alert. In addition, effective medical countermeasures (vaccines, drugs, and diagnostics) are available for many of the agents of greatest concern. An integrated system of countermeasures for the chemical and biological agents can significantly reduce the threat by raising the cost/benefit ratio for the would-be aggressor. If the agents are used, appropriate medical care from well-informed medical care providers that enables soldiers to survive could be the factor determining whether a battle is won or lost.

IMPLICATIONS FOR THE MILITARY MEDICAL DEPARTMENTS

From 18 January to 28 February 1991, 39 Iraqi-modified SCUD missiles reached Israel. [4] Although many were off target or malfunctioned,

some of them landed in and around Tel Aviv. Approximately 1,000 people were treated as a result of missile attacks, but only 2 died. Anxiety was listed as the reason for admitting 544 patients and atropine overdose for hospitalization of 230 patients. Clearly, these conventionally armed SCUDs were not effective mass casualty weapons, yet they caused significant disruption to the population of Tel Aviv. Approximately 75% of the casualties resulted from inappropriate actions or reactions on the part of the victims. Had one of the warheads contained a chemical or biological agent that killed or intoxicated a few people, the “terror effect” would have been even greater.

The likelihood of such a weapon causing panic among military personnel decreases, however, when the leaders and troops become better educated regarding these agents. As General John J. Pershing wrote after World War I: “Whether or not gas will be employed in future wars is a matter of conjecture. But the effect is so deadly to the unprepared that we can never afford to neglect the question.”[5(p623)] The experience in the Persian Gulf War reinforced General Pershing’s warning. Despite the improvement in relations between the East and the West, potential adversaries still exist and potential adversaries have chemical and biological agents. These agents have been used in recent years, and probably will be used again on the battlefield or in small, regional conflicts. They might also be used in acts of terrorism within the United States, in which case, by authority of Presidential Decision Directive 39 (1995), the military will assist civilian authorities and medical personnel. Fortunately, U.S. troops and medical personnel have not been involved in these attacks; it is hoped that they never will be. We must be prepared, however. The purpose of this textbook is to assist in that preparation.

REFERENCES

1. McDermott J. *The Killing Winds*. New York, NY: Arbor House; 1987: 49–60.
2. Williams P, Wallace D. *Unit 731: Japan’s Secret Biological Warfare in World War II*. New York, NY: The Free Press (Macmillan); 1989: 65–70.
3. Meselson M, Guillemin J, Hugh-Jones M, et al. The Sverdlovsk anthrax outbreak of 1979. *Science*. 1994;266:1202–1208.
4. Karsenty E, Shemer J, Alshech I, et al. Medical aspects of the Iraqi missile attacks on Israel. *Isr J Med Sci.* 1991;27:603–607.
5. Pershing JJ. Final report of General John J. Pershing. *Annual Report.* Vol 1, Part 1; 1919. Quoted by: Brown FJ. *Chemical Warfare. A Study in Restraints.*

Princeton, NJ: Princeton University Press; 1968: 623.

Chapter 2

HISTORY OF CHEMICAL AND BIOLOGICAL WARFARE: AN AMERICAN PERSPECTIVE

JEFFERY K. SMART, M.A. *

INTRODUCTION

PRE–WORLD WAR I DEVELOPMENTS

WORLD WAR I

THE 1920s: THE LEAN YEARS

THE 1930s: THE GROWING THREAT OF CHEMICAL AND BIOLOGICAL WARFARE

THE 1940s: WORLD WAR II AND THE NUCLEAR AGE

THE 1950s: HEYDAY OF THE CHEMICAL CORPS

THE 1960s: DECADE OF TURMOIL

THE 1970s: THE NEAR END OF THE CHEMICAL CORPS

THE 1980s: THE RETURN OF THE CHEMICAL CORPS

THE 1990s: THE THREAT MATERIALIZES

SUMMARY

** Command Historian, U.S. Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Maryland 21010-5423*

INTRODUCTION

Webster's Ninth New Collegiate Dictionary defines the term "chemical warfare," first used in 1917, as "tactical warfare using incendiary mixtures, smokes, or irritant, burning, poisonous, or asphyxiating gases." A working definition of a chemical agent is "a chemical which is intended for use in military operations to kill, seriously injure, or incapacitate man because of its physiological effects. Excluded from consideration are riot control agents, chemical herbicides and smoke and flame materials." [1(p1-1)] Chemical agents were usually divided into five categories: nerve agents, vesicants, choking agents, blood agents, and incapacitants.

Webster's dictionary likewise defines "biological warfare" as "warfare involving the use of living organisms (as disease germs) or their toxic products against men, animals, or plants." A working definition of a biological agent is "a microorganism (or a toxin derived from it) which causes disease in man, plants or animals or causes deterioration of material." [2(p1-1)] Biological warfare agents were normally divided into three categories: antipersonnel, antianimal, and antiplant.

Prior to World War I, the United States had little knowledge about the potential of chemical and biological warfare. Particularly in terms of preparing soldiers for future wars, the possibility of chemical or biological warfare went virtually unnoticed by the U.S. Army. By the end of World War I, the situation had drastically changed. Chemical warfare had been used against and by American soldiers on the battlefield. Biological warfare had been used covertly on several fronts. In an effort to determine what had gone wrong with their planning and training, U.S. Army officers prepared a history of chemical and biological warfare. To their surprise, they found numerous documented cases of chemical and biological agents having been used or proposed to influence the outcome of a battle or campaign. In addition, they discovered that the technology to protect against chemical and biological agents already existed, and, in some cases, was superior to the equipment used during the war. In hindsight, these officers realized that the army had failed to recognize and prepare for these two already existing types of warfare.

[This chapter focuses primarily on the development of chemical and biological weapons and countermeasures to them, thus setting the stage for Chapter 3, Historical Aspects of Medical Defense Against Chemical Warfare, which concentrates on medical aspects of chemical warfare. To avoid excessive duplication of material, protective equipment of the modern era is illustrated in Chapter 16, Chemical Defense Equipment.—Eds.]

PRE–WORLD WAR I DEVELOPMENTS

The chemical agents first used in combat during World War I were, for the most part, not recent discoveries. Most were 18th- and 19th-century discoveries. For example, Carl Scheele, a Swedish chemist, was credited with the discovery of chlorine in 1774. He also determined the properties and composition of hydrogen cyanide in 1782. Comte Claude Louis Berthollet, a French chemist, synthesized cyanogen chloride in 1802. Sir Humphry Davy, a British chemist, synthesized phosgene in 1812. Dichloroethylsulfide (commonly known as mustard agent) was synthesized in 1822, again in 1854, and finally fully identified by Victor Meyer in 1886. John Stenhouse, a Scotch chemist and inventor, synthesized chloropicrin in 1848.[3]

Many biological agents were naturally occurring diseases thousands of years old. Others were generally discovered or recognized in the 19th and 20th centuries. For example, plague was recognized about 3,000 years ago. Smallpox was known in China as early as 1122 BC. Yellow fever was first described in the 1600s. Carlos Finlay, a Cuban biologist, identified mosquitoes as the primary carrier of yellow fever in 1881, while Walter Reed, a U.S. Army physician, proved the agent to be a virus. Casimir-Joseph Davaine

isolated the causative organism of anthrax in 1863, followed by Robert Koch, a German scientist, who obtained a pure culture of anthrax in 1876. Koch also discovered the causative agent for cholera in 1883. Rocky Mountain spotted fever was first recognized in 1873; Howard T. Ricketts, an American pathologist, discovered the causative agent in 1907. Ricketts also identified the causative organism of typhus in 1909. F. Loffler and W. Schutz identified glanders in 1882. Sir David Bruce, a British pathologist, discovered the causative organism of brucellosis (it was named after him) in 1887. Ricin toxin was identified in 1889. Tularemia was first described in Tulare County, California (after which it was named), in 1911, and the causative agent was identified the next year.[3]

Early Chemical Weaponization Proposals and Usage

There are numerous examples of chemical weapons used or proposed during the course of a campaign or battle. The Chinese used arsenical smokes as early as 1000 BC. Solon of Athens put hellebore roots in the drinking water of Kirrha in 600 BC. In 429 and 424 BC, the Spartans and their allies used noxious smoke and flame against Athenian-allied cities during the Peloponnesian War. About 200 BC, the Carthaginians used Mandrake root left in wine to sedate the enemy. The Chinese designed stink bombs of poisonous smoke and shrapnel, along with a chemical mortar that fired cast-iron stink shells. Toxic smoke projectiles were designed and used during the Thirty Years War. Leonardo da Vinci proposed a powder of sulfide of arsenic and verdigris in the 15th century.[3]

During the Crimean War, there were several proposals to initiate chemical warfare to assist the Allies, particularly to solve the stalemate during the siege of Sevastopol. In 1854, Lyon Playfair, a British chemist, proposed a cacodyl cyanide artillery shell for use primarily against enemy ships. The British Ordnance Department rejected the proposal as “bad a mode of warfare as poisoning the wells of the enemy.”[4(p22)] Playfair’s response outlined a different concept, which was used to justify chemical warfare into the next century:

> There was no sense in this objection. It is considered a legitimate mode of warfare to fill shells with molten metal which scatters among the enemy, and produced the most frightful modes of death. Why a poisonous vapor which would kill men without suffering is to be considered illegitimate warfare is incomprehensible. War is destruction, and the more destructive it can be made with the least suffering the sooner will be ended that barbarous method of protecting national rights. No doubt in time chemistry will be used to lessen the suffering of combatants, and even of criminals condemned to death.[4(pp22–23)]

There were other proposals for chemical warfare during the Crimean War, but none were approved.

During the American Civil War, John Doughty, a New York City school teacher, was one of the first to propose the use of chlorine as a chemical warfare agent. He envisioned a 10-in. artillery shell filled with 2 to 3 qt of liquid chlorine that, when released, would produce many cubic feet of chlorine gas.

> If the shell should explode over the heads of the enemy, the gas would, by its great specific gravity, rapidly fall to the ground: the men could not dodge it, and their first intimation of its presence would be by its inhalation, which would most effectually disqualify every man for service that was within the circle of its influence; rendering the disarming and capturing of them as certain as though both their legs were broken.[5(p27)]

As to the moral question of using chemical weapons, he echoed the sentiments of Lyon Playfair a decade earlier:

> As to the moral question involved in its introduction, I have, after watching the progress of events during the last eight months with reference to it, arrived at the somewhat paradoxical conclusion, that its introduction would very much lessen the sanguinary character of the battlefield, and at the same time render conflicts more decisive in their results.[5(p33)]

Doughty's plan was apparently never acted on, as it was probably presented to Brigadier General James W. Ripley, Chief of Ordnance, who was described as being congenitally immune to new ideas.[5] A less practical concept, proposed the same year by Joseph Lott, was to fill a hand-pumped fire engine with chloroform to spray on enemy troops.[6]

The1864 siege of Petersburg, Virginia, generated several chemical warfare proposals. Forrest Shepherd proposed mixing hydrochloric and sulfuric acids to create a toxic cloud to defeat the Confederates defending Petersburg.[5] Lieutenant Colonel William W. Blackford, a Confederate engineer, designed a sulfur cartridge for use as a countertunnelling device.[7] The Confederates also considered using Chinese stink bombs against the Union troops. Elsewhere, the same year, Union Army Captain E. C. Boynton proposed using a cacodyl glass grenade for ship-to-ship fighting.[5] Other than possibly Blackford's cartridge, none of the proposals were used on the battlefield.

Two wars at the turn of the century also saw limited use of chemical weapons. During the Boer War, British troops fired picric acid–filled shells, although to little effect.[8] During the Russo–Japanese War, which was closely observed by those who would plan World War I, Japanese soldiers threw arsenal rag torches into Russian trenches.[3]

In 1887, the Germans apparently considered using lacrimators (tear agents) for military purposes. The French also began a rudimentary chemical warfare program with the development of a tear gas grenade containing ethyl bromoacetate, and proposals to fill artillery shells with chloropicrin.[9]

Early Biological Warfare Proposals and Usage

There were many examples of proposed usage or actual use of biological weapons on the battlefield. Hannibal hurled venomous snakes onto the enemy ships of Pergamus at Eurymedon in 190 BC. Scythian archers used arrows dipped in blood and manure or decomposing bodies in 400 BC. The use of dead bodies as the carrier of the biological agent proved particularly effective against an enemy's water supply. Barbarossa used this tactic at the battle of Tortona in 1155. De Mussis, a Mongol, catapulted bubonic plague–infected bodies into Caffa in 1346. The Spanish tried wine infected with leprosy patients' blood against the French near Naples in 1495. One of the more unique attempts at biological warfare was initiated in 1650 by Siemenowics, a Polish artillery general, who put saliva from rabid dogs into hollow spheres for firing against his enemies. The Russians cast plague-infected bodies into Swedish-held Reval, Estonia, in 1710.

The proposed use of biological weapons was not limited to Europe and Asia. In 1763, during Pontiac's Rebellion in New England, Colonel Henry Bouquet, a British officer, proposed giving the Indians at Fort Pitt, Pennsylvania, blankets infected with smallpox. The disease, whether purposely disseminated or not, proved devastating to the Native American population. A similar plan was executed in 1785, when Tunisians threw plague-infected clothing into La Calle, held by the Christians.

The 19th-century wars continued the same trend. In 1861, Union troops advancing south into Maryland and other border states were warned not to eat or drink anything provided by

unknown civilians for fear of being poisoned. Despite the warnings, there were numerous cases where soldiers thought they had been poisoned after eating or drinking. Confederates retreating in Mississippi in 1863 left dead animals in wells and ponds to deny water sources to the Union troops.

A more carefully planned use of biological weapons was attempted by Dr. Luke Blackburn, a future governor of Kentucky, who attempted to infect clothing with smallpox and yellow fever and then sell it to unsuspecting Union troops. At least one Union officer's obituary stated that he died of smallpox attributed to Blackburn's scheme. Yellow fever, however, could not be transferred in this manner. Since more soldiers died of disease during the Civil War than were killed on the battlefield, the effectiveness of Blackburn's work was difficult to judge.

Biological agents were also considered for antianimal weapons during the 19th century. Louis Pasteur, the French chemist and biologist usually recognized for his humanitarian accomplishments, also experimented with the use of salmonella as an agent to exterminate rats. Others successfully used chicken cholera to exterminate rabbits and dysentery to kill grasshoppers.[3]

Early Protective Devices

Parallel to the development and use of chemical and biological weapons was the design of protective equipment for use against toxic chemicals and biological agents. Although conventional protective masks started appearing in the 19th century, the earliest recorded mask proposal was written by Leonardo da Vinci in the 15th century. He envisioned a fine cloth dipped in water for defense against a sulfide of arsenic and verdigris powder he was proposing for a toxic weapon.[10]

The earliest known patent for a protective mask in the United States was filed by Lewis P. Haslett in 1847. His design included a moistened woolen fabric mask with an exhaust.[11] Benjamin I. Lane's patent in 1850 included an air tank, goggles, and a rubber nose piece.[12] John Stenhouse developed a velvet-lined copper mask with a charcoal filter in 1854. The same year, George Wilson, a professor of technology at the University of Edinburgh, proposed that the British Board of Ordnance issue charcoal masks to soldiers to protect them from bombs employing suffocating or poisonous vapors during the Crimean War.[13]

Between the American Civil War and World War I, there were numerous additional patents and designs for protective devices that were used in industry, for fire fighting, and in mines. These included an improved mask by Lane, which had a rubber facepiece with an exhaust; Theodore A.Hoffman's mask, which was made of cotton with an elastic border to protect against aerosols (Figure 2-1); Samuel Barton's mask with a metal-and-rubber facepiece, hood, goggles, and a charcoal filter; and Charles A. Ash's mask, which added an air supply for use by miners.[3]

Attempts to Control Chemical and Biological

Warfare

Most of the early attempts to control chemical and biological warfare were bilateral or unilateral agreements directed at the use of poisons. These included the 1675 agreement between the French and Germans, signed in Strassburg, to ban the use of poison bullets, and U.S. Army General Order No.100, issued in 1863 during the American Civil War, which stated: “The use of poison in any manner, be it to poison wells, or food, or arms, is wholly excluded from modern warfare.”[14(p687)]

Fig. 2-1. Theodore A. Hoffman patented this respirator in 1866. It is representative of the already developing protective mask designs of the post–American Civil War era. Ironically, these masks were superior to the ad hoc emergency masks used by the Allies after the Germans began chemical warfare in World War I. Reprinted from US Patent No. 58,255; 25 Sep 1866.

The first international attempt to control chemical and biological weapons occurred in 1874, when the International Declaration Concerning the Laws and Customs of War was signed in Brussels and included a prohibition against poison or poisoned arms. The First Hague Peace Conference in 1899 also banned the use of poisons and was ratified by the United States. However, a separate proposition stated: “The contracting Powers agree to abstain from the use of projectiles the sole object of which is the diffusion of asphyxiating gasses.”[14(p685)] Although 27 nations, including Germany, France, Russia, Austria-Hungary, and Great Britain, eventually agreed to this additional statement, the United States delegation declined to approve it.

Captain Alfred T. Mahan, a U.S. Navy delegate plenipotentiary, gave three reasons for opposing the additional restrictions: (1) currently used weapons were despised as cruel and inhumane when first introduced, (2) since there were no current chemical weapons stockpiles, it was too early to ban them, and (3) chemical weapons were not any more inhumane than any other weapon. The 1907 Second Hague Peace Conference retained the ban against poisons.[15]

WORLD WAR I

When Europe was caught up in the crises of 1914 after the murder of Archduke Francis Ferdinand at Sarajevo and the declarations of war among Austria-Hungary, Serbia, Germany, France, Russia, and Great Britain that followed within a month, few observers expected the 19th-century chemical and biological paper proposals to be transformed into actual battlefield operations. The United States, remaining neutral under the policy of President Woodrow Wilson, certainly made no preparations for chemical and biological warfare.

Early Allied Chemical Warfare Plans

With the outbreak of hostilities, both the French and the British apparently considered, investigated, and tested various chemical weapons at home and on the battlefield. During the German invasion of Belgium and France, the French used their ethyl bromoacetate grenades against the Germans, but with no noticeable effect. Although the grenades were considered of no military worth, the French apparently continued to consider the further use of tear agents against the Germans.

In the early stages of the war, the British examined their own chemical technology for battlefield use. They initially investigated tear agents also but later turned to more toxic chemicals. In January 1915, several chemists at Imperial College successfully demonstrated ethyl iodoacetate as a tear gas to the War Office by gassing a representative.

Another officer suggested using sulfur dioxide as a chemical weapon. Field Marshal Lord Kitchener, Secretary of State for War, was not interested in the concept for the army but suggested trying the navy. At the Admiralty, the idea found a sympathetic ear in Winston

Churchill in March 1915. The suggestion included a plan to use a sulfur dioxide cloud against the Germans, screen the operation with smoke, and provide British troops a gas-proof helmet. Churchill declined to accept the sulfur dioxide plan but did put the officer in charge of a committee the next month to discuss the use of smoke on land and sea.[9]

German Chemical Warfare Plans

Possibly aware of the Allied interest in chemical weapons, the Germans also examined their own chemical technology for war applications. Their strong dye industry and the technical knowledge supplied by university professors in Berlin created the right combination for pursuing the concept of offensive chemical weapons. From the suggestion of Professor Walther Nernst, a physical chemist at the University of Berlin, or one of his colleagues, the Germans filled 105-mm shells with dianisidine chlorosulfate, a lung irritant, for use on the western front. To evade the 1899 international ban, the Germans also put shrapnel in the shell so the “sole” purpose was not gas dissemination.

On 27 October 1914, the Germans fired 3,000 of these projectiles at the British near Neuve-Chapelle, but with no visible effects. The explosive aspect of the shells destroyed the chemical aspect. In fact, the British were apparently unaware that they were the victims of the first large-scale chemical projectile attack.

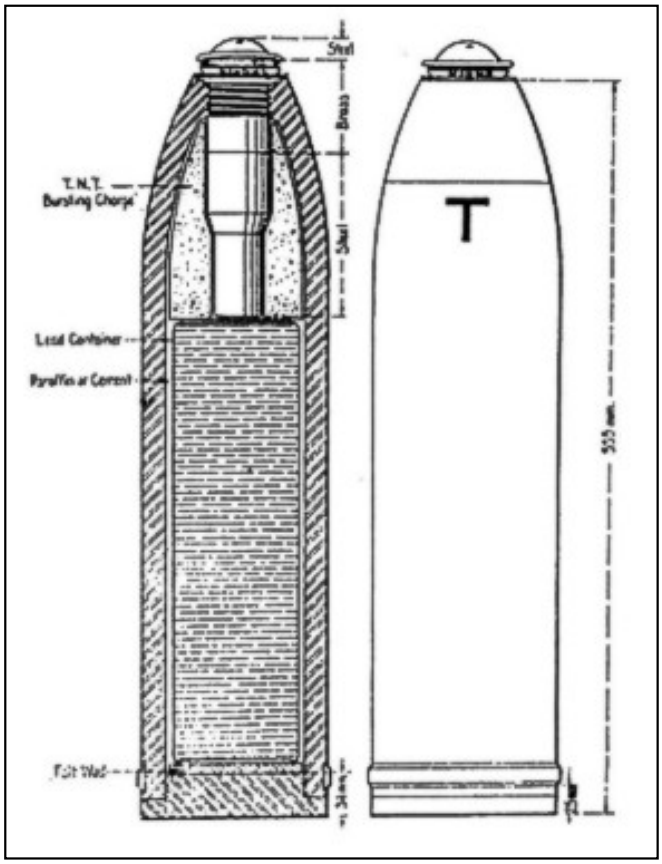


Fig. 2-2. The German 150-mm T-Shell, which mixed xylyl bromide with an explosive charge. Note that the explosive charge was in the front and the chemical agent in the rear compartment. This design is similar to the one proposed in 1862 by John Doughty during the American Civil War (see Figure 3-1). Reprinted from Army War College. *German Methods of Offense.*Vol 1. In: *Gas Warfare.* Washington, DC: War Department; 1918: 59.

The Germans continued researching chemical shells, and by November 1914, Dr. Hans von Tappen, assigned to the Heavy Artillery Department, designed a 150-mm howitzer shell containing 7 lb of xylyl bromide and a burster charge for splinter effect (Figure 2-2). The Germans moved these to the eastern front and experimented by firing more than 18,000 of the shells at Russian positions near Bolimov. In this case, the weather came to the aid of the Russians by providing cold temperatures that prevented the vaporization of the gas. The Germans tried the same shells again on the western front at Nieuport in March 1915 with equally unsuccessful results. [9,14,16]

Ypres, April 1915: The First Successful German Chemical Attack

The concept of creating a toxic gas cloud from chemical cylinders was credited to Fritz Haber of the Kaiser Wilhelm Physical Institute of Berlin in late 1914. Owing to shortages of artillery shells, Haber thought a chemical gas cloud would negate the enemy’s earthworks without the use of high explosives. In addition, gas released directly from its storage cylinder would cover a far broader area than that dispersed from artillery shells. Haber selected chlorine for the gas since it was abundant in the German dye industry and would have no

prolonged influence over the terrain.

On 10 March 1915, under the guidance of Haber, Pioneer Regiment 35 placed 1,600 large and 4,130 small cylinders containing a total of 168 tons of chlorine opposite the Allied troops defending Ypres, Belgium. Haber also supplied the entire regiment with Draeger oxygen breathing sets, used in mine work, and a portion of the surrounding German infantry with small pads coated with sodium thiosulfate. Once the cylinders were in place, the Germans then waited for the winds to shift to a westerly direction.[9,14,17]

The Germans believed this means of attack, nonprojectile, was still within the guidelines of the Hague ban and hoped the cylinders would produce a potent cloud. The comments of General von Deimling, commanding general of the German 15th Corps in front of Ypres, written sometime after the war, however, perhaps better reflect the reason for initiating chemical warfare:

> I must confess that the commission for poisoning the enemy, just as one poisons rats, struck me as it must be to any straight-forward soldier: it was repulsive to me. If, however, these poison gases would lead to the fall of Ypres, we would perhaps win a victory which might decide the entire war. In view of such a high goal, personal susceptibilities had to be silent.[18(p5)]

On 22 April 1915, the Germans released the gas with mixed success. Initially, the Allied line simply fell apart. This was despite the fact that the Allies were aware of the pending gas attack, and British airmen had actually spotted the gas cylinders in the German trenches. The success of the attack was more significant than the Germans expected, and they were not ready to make significant gains despite the breakthrough. In addition, fresh Allied troops quickly restored a new line further back. The Allies claimed that 5,000 troops were killed in the attack, but this was probably an inflated number for propaganda purposes.[18]

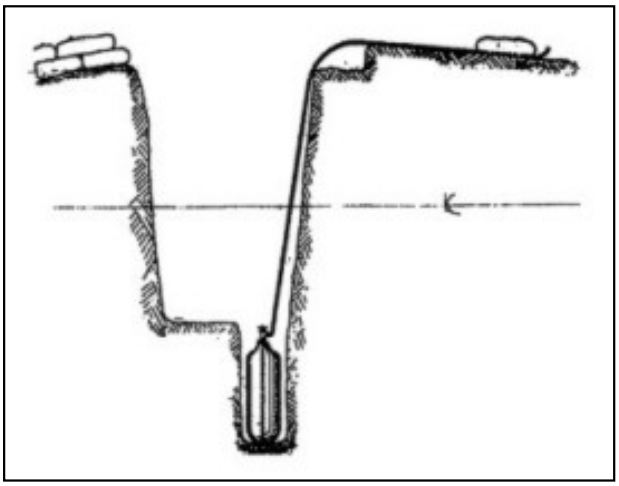


Fig. 2-3. A typical German chemical cylinder set up and ready for discharge. The discharge from thousands of cylinders created the gas cloud. Reprinted from Army War College. *German Methods of Offense*. Vol 1. In: *Gas Warfare*. Washington, DC: War Department; 1918: 14.

The Germans used chlorine again at Ypres on 24 April 1915 and four more times during May 1915 (Figure 2-3). These additional attacks gained additional ground. As one British soldier stated:

> Nobody appears to have realized the great danger that was threatening, it being considered that the enemy's attempt would certainly fail and that whatever gas reached our line could be easily fanned away. No one felt in the slightest degree uneasy, and the terrible effect of the gas came to us as a great surprise.[19(p3)]

Another observer, in reflecting about the attack at Ypres and the first major use of chemical warfare, wrote: "The most stupendous change in warfare since gunpowder was invented had come, and come to stay. Let us not forget that."[20(p3)] Yet chemical warfare

failed to be decisive and the German attack against Ypres was halted short of its objective.

Allied Chemical Warfare Retaliation

That same month, the British and the French began planning to retaliate with chemical weapons. The Allied response to the chemical attacks evolved into three general categories:

1. protective devices for the troops
2. toxic gases of their own, and
3. weapons to deliver the toxic gases to the enemy lines.

Shortly after the first chlorine attack, the Allies had primitive emergency protective masks. In September, they launched their own chlorine attack against the Germans at Loos (Figure 2-4). This initiated a deadly competition to develop better protective masks, more potent chemicals, and long-range delivery systems to more widely disperse the agents.

The Germans quickly escalated to phosgene to replace the less-effective chlorine. In May 1916, the Germans started using trichloromethyl chloroformate (diphosgene), while the French tried hydrogen cyanide 2 months later and cyanogen chloride the same year. In July 1917, the Germans introduced mustard agent to provide a persistent vesicant that could attack the body in places not protected by gas masks. To further complicate defensive actions, both sides mixed agents and experimented with camouflage materials to prevent quick identification.[3]

Fig. 2-4. A French cylinder attack on German trenches in Flanders. The critical importance of the wind is apparent. Condensation of water vapor caused the cloudlike appearance of the gas. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

German Biological Warfare Plans

While the German chemical warfare program was extensively documented after the war, the German use of biological weapons during World War I unfortunately was poorly documented and much debated. Apparently in 1915, the Germans initiated covert biological warfare attacks against the Allies' horses and cattle on both the western and the eastern fronts. In that year, they also allegedly used disease-producing bacteria to inoculate horses and cattle leaving U.S. ports for shipment to the Allies. Other attacks included a reported attempt to spread plague in St. Petersburg, Russia, in 1915.[3,21]

The activities of German agents operating in the United States in 1915 came to light after the war. Erich von Steinmetz, a captain in the German navy, entered the United States disguised as a woman. He brought with him cultures of glanders to inoculate horses intended for the western front. After trying unsuccessfully, he posed as a researcher and took the cultures to a laboratory, where it was determined the cultures were dead.

Anton Dilger was an American-educated surgeon who specialized in wound surgery at Johns Hopkins University, Baltimore, Maryland. After joining the German army in 1914, he suffered a nervous breakdown and was sent to his parents' home in Virginia since the United States was still neutral in the war. At the request of the German government, he brought along strains of anthrax and glanders to begin a horse-inoculation program. With his brother Carl, he set up a laboratory in a private house in Chevy Chase, Maryland, to produce additional quantities of the bacteria.

The bacteria from “Tony’s lab” were delivered to Captain Frederick Hinsch, who was using a house at the corner of Charles and Redwood Streets in Baltimore, Maryland. Hinsch inoculated horses in Baltimore that were awaiting shipment to Europe. Dilger also attempted to establish a second biological warfare laboratory in St. Louis, Missouri, but gave up after a cold winter killed the cultures. Although the impact of these German agents’ activities was not determined, the year 1915 is considered to be the beginning of 20th-century antianimal biological warfare. [22]

Additional biological attacks reportedly occurred throughout the war. In 1916, a German agent with intentions to spread a biological agent was arrested in Russia. German agents also tried to infect horses with glanders and cattle with anthrax in Bucharest in 1916. In 1917, Germany was accused of poisoning wells in the Somme area with human corpses, and dropping fruit, chocolate, and children’s toys infected with lethal bacteria into Romanian cities. German agents tried to infect horses with glanders and cattle with anthrax in France. A more successful attack was the infection of some 4,500 mules with glanders by a German agent in Mesopotamia. Another reported attack was with cholera in Italy. A 1929 report also accused the Germans of dropping bombs containing “plague” over British positions during the war. Many of these reports were of questionable authenticity and were vehemently denied by the Germans. As had happened during the American Civil War, the rampant spread of naturally occurring disease during World War I made the impact of planned biological warfare attacks impossible to determine.[3,21]

Pre-War Interest in the United States in Chemical Warfare

The production and use of offensive chemical weapons in the European war did not go completely unnoticed in the United States. The combination of the use of chemical warfare at Ypres in April, followed by the sinking of the *Lusitania* by a German U-boat off the Irish coast on 7 May 1915, shocked the nation. Americans began to take greater interest in the nature of warfare taking place in Europe and elsewhere. In May 1915, President Woodrow Wilson proposed that Germany halt chemical warfare in exchange for the British ending their blockade of neutral ports. Germany (and Great Britain) refused to comply.

Helpful suggestions from armchair scientists proved to be of little help to the army. *The Army and Navy Register* of 29 May 1915 contained the following report:

> Among the recommendations forwarded to the Board of Ordnance and Fortifications there may be found many suggestions in favor of the asphyxiation process, mostly by the employment of gases contained in bombs to be thrown within the lines of the foe, with varying

effects from peaceful slumber to instant death. One ingenious person suggested a bomb laden to its full capacity with snuff, which should be so evenly and thoroughly distributed that the enemy would be convulsed with sneezing, and in this period of paroxysm it would be possible to creep up on him and capture him in the throes of the convulsion.
23(p12)

By the fall of 1915, the War Department finally became interested in providing American troops with some form of a protective mask. By then, the British already had the P helmet, a flannel bag treated with sodium phenate and sodium hyposulfite that fitted over the head and was effective against chlorine and phosgene gases. The Germans were slightly ahead with a rubberized facepiece, unbreakable eyepieces, and a drum canister.[24]

In the United States, the mask project was assigned to the Army Medical Department. The Medical Department sent several medical officers to Europe as observers, but accomplished little else. Since the United States was not at war, no particular emphasis was placed on the project. Ultimately, all major participants in World War I attempted to develop protective masks (Figure 2-5).

As relations with Germany declined over its unrestricted use of submarines, the war overtones did energize several key civilians in the U.S. government. One, Van H. Manning, Director of Bureau of Mines, Department of the Interior, called together his division chiefs on 7 February 1917 to discuss how they could assist the government if the country was drawn into war. At this meeting, George S. Rice suggested that the bureau might turn its experience in mine gas and rescue apparatus toward the investigation of war gases and masks.

The next day, Manning sent a letter to Dr. C. D.Walcott, Chairman of the Military Committee of the National Research Council (NRC), which had been created the year before, offering the Bureau's services in creating a chemical warfare program for the army. On 12 February 1917, Dr. Walcott replied to Manning's letter, stating that he would bring the matter to the attention of the Military Committee. Events, however, moved quicker than the Military Committee.

On 2 April 1917, President Wilson addressed the U.S. Congress and called for a declaration of war. The next day, the Military Committee acted on Manning's proposal and established the Subcommittee on Noxious Gases under the chairmanship of the director of

the Bureau of Mines, and to include ordnance and medical officers from both the army and the navy, as well as two members of the Chemical Committee of the NRC. Their mission was to investigate noxious gases, the generation of chemical warfare agents, and the discovery of antidotes for war purposes. Three days later the United States declared war on Germany when congress approved the president's request.[17,25,26]

Fig. 2-5. A potpourri of World War I–vintage protective masks. This extraordinary photograph gives some indication of the great effort made by the warring parties to develop an effective and practical (and frequently unsuccessful) defense against the chemical warfare threat. Top row, left to right: U.S. Navy Mark I mask; U.S. Navy Mark II mask; U.S. CE mask; U.S. RFK mask; U.S. AT mask; U.S. KT mask; U.S. model 1919 mask. Middle row, left to right: British Black Veil mask; British PH helmet; British BR mask; French M2 mask; French artillery mask; French ARS mask. Bottom row, left to right: German mask; Russian mask; Italian mask; British Motor Corps mask; U.S. Rear Area mask; U.S. Connell mask. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The United States Organizes for Chemical Warfare

The new Subcommittee on Toxic Gases got off to a quick start. Within a short time, the subcommittee began organizing research into chemical agents at universities and industries across the nation, while mobilizing a large portion of the chemists in the country. This initial phase was the groundwork that later led to the establishment of the Chemical Warfare Service, the forerunner of the Chemical Corps. Thus the country's civilian scientists, engineers, and chemistry professors rescued the army from its unpreparedness for chemical warfare.

Eventually, the War Department also began to plan for chemical warfare. The Medical Department was assigned responsibility for chemical defense and the Ordnance Department responsibility for chemical munitions. The Corps of Engineers was designated to provide engineers to employ the new weapons. This diversified arrangement did not last long.

When General John J. Pershing faced the task of organizing the American Expeditionary Forces (AEF) in France in the summer of 1917, he decided to place responsibility for all phases of gas warfare in a single military service, and he recommended that the War Department at home do likewise. On 3 September 1917, the AEF established a centralized Gas Service under the command of Lieutenant Colonel Amos A. Fries.[25,26] The new organization had many hurdles to overcome. The troops had virtually no chemical warfare equipment of U.S. design and relied on the British and French to supply equipment from gas masks to munitions.

U.S. Troops Introduced to Chemical Warfare

Despite the Allied support, the U.S. Army was not ready for chemical warfare. For example, on 26 February 1918, the Germans fired 150 to 250 phosgene and chloropicrin projectiles against the Americans near Bois de Remieres, France. The first attack occurred between 1:20 AM and 1:30 AM. There was a blinding flash of light and then several seconds elapsed before the projectiles reached their target. Some exploded in the air and others on

the ground. A second and similar attack occurred about an hour later. The attack and its casualties were recorded by many observers, including the following selected accounts [27]:

- A corporal saw the projectiles burst 10 ft in the air with flash and smoke. As the shells burst, he got his mask on without smelling any gas. When he took his mask off an hour and a half later, however, he could smell gas.

- One private said the gas smelled like sour milk and had a sharp odor. It hurt his eyes and nose. Another private forgot to hold his breath while putting on his mask. The gas smelled sweet and he became sick to the stomach and his lungs hurt. Still, he kept his mask on for 4 hours.

- One man in panic stampeded and knocked down two others who were adjusting their masks. The panicked man rushed down the trench screaming and made no attempt to put on his respirator; he died shortly after reaching the dressing station.

- Another man threw himself in the bottom of the trench and began to scream. Two others, trying to adjust his respirator, had their own pulled off and were gassed. The screaming man was finally carried out of the area but died not long after.

- An officer was gassed while shouting to the men to keep their respirators on.

The Americans suffered 85 casualties with 8 deaths, approximately 33% of their battalion. The problem was a lack of discipline. Because a good American mask was not yet available, the soldiers were issued two gas masks: a French M2, which was comfortable but not extremely effective; and a British small-box respirator (SBR), which was effective but uncomfortable with its scuba-type mouthpiece and nose clip. At the first sign of gas, some of the men could not find their gas masks in time. Others were able to get their SBRs on, but then either removed their masks too quickly or decided to switch to the more comfortable French mask and were gassed in the process.[27]

An editorial later summed up the lesson learned from this first fiasco:

> A stack of standing orders a mile high will not discipline an army. Neither can you so train men at the outbreak of hostilities that they can protect themselves against the gas which will be used by the enemy. We must train our Army to the last degree during peace.[28(p2)]

Creation of the Chemical Warfare Service

In the spring of 1918, the U.S. government began centralizing gas warfare functions in the War Department under a senior Corps of Engineers officer, Major General William L. Sibert (Figure 2-6). When President Wilson transferred the research facilities that had been set up by the Bureau of Mines to the War Department, the stage was set for the inauguration of a new consolidated organization. On 28 June 1918, the War Department formally established the Chemical Warfare Service (CWS) under Sibert as part of the National Army (ie, the wartime army, as distinguished from the regular army), with full responsibility for all facilities and functions relating to toxic chemicals.

Fig. 2-6. Major General William L. Sibert was the first commanding general of the U.S. Army Chemical Warfare Service. He had previously commanded the 1st Division in France in early 1918. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The CWS was organized into seven main divisions. The Research Division was located at American University, Washington, D. C. Most of the weapons and agent research was conducted by this division during the war. The Gas Defense Division was responsible for the production of gas masks and had a large plant in Long Island City, New York. The Gas Offense Division was responsible for the production of chemical agents and weapons, with its main facility located at Edgewood Arsenal, Maryland. The Development Division was responsible for charcoal production, and also pilot-plant work on mustard agent production. The Proving Ground Division was collocated with the Training Division at Lakehurst, New Jersey. The Medical Division was responsible for the pharmacological aspects of chemical defense.

The offensive chemical unit for the AEF was the First Gas Regiment, formerly the 30th Engineers. This unit was organized at American University under the command of Colonel E. J. Atkisson in 1917, and was sent to France in early 1918.[17,25]

The U.S. Army finally had an organization that controlled offensive chemical production, defensive equipment production, training, testing, and basic research, along with a new chemical warfare unit, the First Gas Regiment, under one general. This organization helped lead the AEF to victory, although much of its work, including the construction of toxic gas–production and –filling plants and gas mask factories, was only partially completed by the end of the war.

Agent Production

Agent production and shell-filling were initially assigned to the Ordnance Department and then to the CWS. The primary facility was Edgewood Arsenal, Maryland, erected in the winter of 1917–1918. The plant was designed to have four shell-filling plants and four chemical agent production plants. The first shell-filling plant filled 75-mm, 155-mm, 4.7-in., and Livens projectiles with phosgene. A second filling plant was added to fill 155-mm shells with mustard agent or chloropicrin (Figure 2-7). Two additional shell-filling plants were started but not completed before the end of the war.

The four agent production plants produced the highest priority agents thought to be required for the western front in 1917. These were chlorine, chloropicrin, phosgene, and mustard agent (Figure 2-8). By 1918, the first two were no longer critical agents, although chlorine was used in the production of phosgene. Over 935 tons of phosgene and 711 tons of mustard agent were produced at the arsenal by the end of the war. Government contractors also produced these four agents and Lewisite, named after Captain W. Lee Lewis, a member of the CWS Research Division. The Lewisite, however, never reached the front: it was dumped somewhere in the Atlantic Ocean (ie, sea dumped) after the armistice.[3,17,26]

Chemical Weapons

During the war, the CWS used foreign technology for offensive weapons. The initial mode of offensive chemical attack was the portable chemical cylinder, designed to hold 30 to 70 lb of agent. Soldiers simply opened a valve and hoped the wind continued to blow in the right direction. The resulting cloud could drift many miles behind enemy lines, or, if the wind changed, could gas friendly troops.

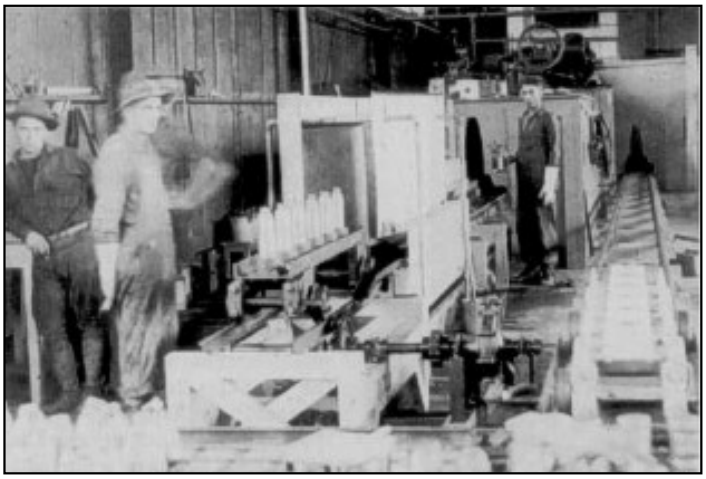


Fig. 2-7. Filling 75-mm artillery shells with mustard agent at Edgewood Arsenal, Md. Facilities designed to fill shells with chemical agents were notoriously hazardous. Anecdotal reports from mustard shell-filling plants indicated that over several months, the *entire* labor force could be expected to become ill. These workers' apparent nonchalance to the hazards of mustard would not be tolerated by the occupational medicine standards of a later era (see Figure 2-31). Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

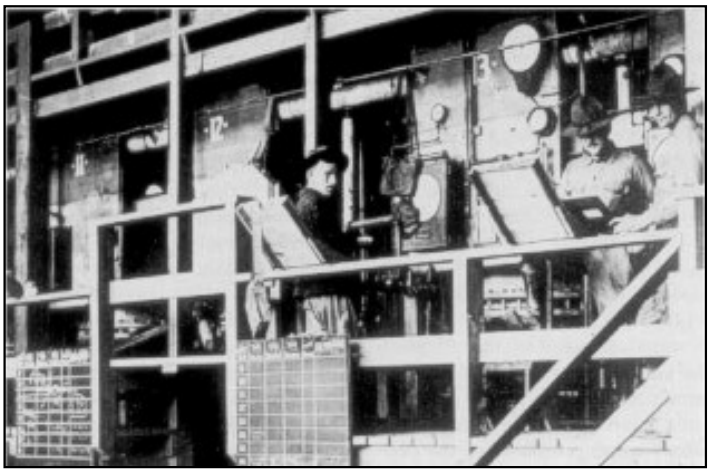


Fig. 2-8. Interior view of the Mustard Agent Production Plant at Edgewood Arsenal, Md. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The British improved on the delivery system, developing the Livens projector, an 8-in. mortarlike tube that shot or projected the cylinder into the enemy's lines (Figures 2-9 and 2-10). The range was a respectable 1,700 yd, with a flight time of 25 seconds. There were several problems with the system.

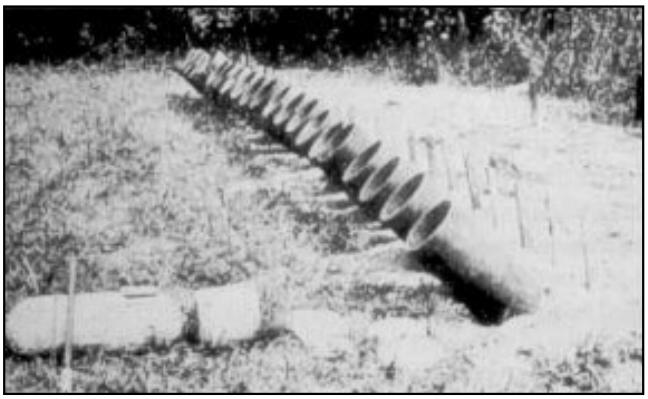


Fig. 2-9. A battery of dug-in Livens projectors, with one gas shell and its propellant charge shown in the foreground. Electrically controlled salvo firing was the usual mode of operation. Emplacement was a slow process, and it limited the surprise factor for attack. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

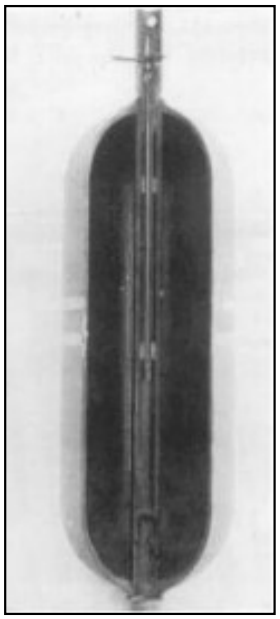


Fig. 2-10. Sectionalized view of a Livens projectile. The central tube contains a small explosive charge, which, when detonated by the contact fuze, breaks the shell and aids in the dissemination of the chemical agent. The usual weight of the chemical agent was 30 lb; the shell weighed an additional 30 lb. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Being electrically fired, a battery of Livens projectors required extensive preparation and could not be moved once set up. Normally, a battery could only be emplaced and fired once a day. This limited mobility required the element of surprise to prevent the Germans from taking counter actions.

British 4-in. trench mortars, called Stokes mortars (Figure 2-11), provided a solution to some of the problems with Livens projectors. The Stokes mortar did not require extensive preparation and could be moved as needed. Since it was not rifled, the range was only 1,200 yd, which meant about a 14-second flight time. The small size of the shell only held about 6 to 9 lb of agent, but experienced gunners could fire 25 rounds per minute. American troops used both Livens projectors and Stokes mortars during the war. Ordnance officers tried making their own Stokes mortars, but none reached the front before the end of the war.

In addition to the special chemical weapons, the CWS fired chemical rounds from 75-mm, 4.7-in., 155-mm, and larger-caliber guns. Many of these had ranges of 5 to 10 miles, with payloads of as much as 50 lb of agent. Owing to a shortage of shell parts and the late completion of U.S. shell-filling plants, U.S. troops primarily fired French phosgene and mustard agent rounds.[3,14,26]

Biological Warfare Weapons

By 1918, the United States was apparently aware of the German biological warfare program, but the only agent examined was a toxin for retaliatory purposes. Ricin, derived from castor beans, could be disseminated two ways. The first involved adhering ricin to shrapnel bullets for containment in an artillery shell. The results of this work were stated in a technical report in 1918:

> These experiments show two important points: (1) easily prepared preparations of ricin can be made to adhere to shrapnel bullets, (2) there is no loss in toxicity of firing and even with the crudest method of coating the bullets, not a very considerable loss of the material itself. ... It is not unreasonable to suppose that every wound inflicted by a shrapnel bullet coated with ricin would produce a serious casualty. ... Many wounds which would otherwise be trivial would be fatal.[29(p112)]

Fig. 2-11. A complete Stokes mortar with ammunition and accessories for firing. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The second involved the production of a ricin dust cloud, but due to limited amounts of ricin being produced and the inefficient delivery via the respiratory tract, little work seems to have been pursued in this means of dissemination. Although both approaches were laboratory tested, neither was perfected for use in Europe before the end of the war.[29]

Protective Equipment

The early unsuccessful efforts to produce a gas mask were resolved by CWS researchers at American University and other CWS research facilities. In the spring of 1918, the CWS issued the Richardson, Flory, and Kops (RFK) mask, which was an improved version of the British SBR. Over 3 million were produced for U.S. troops. Late in 1918, the CWS merged the best aspects of the RFK mask with a French design that eliminated the scuba-type mouthpiece. Designated the Kops Tissot Monro (KTM) mask, only 2,000 were produced before the end of the war.[14,30–32] Humans were not the only creatures requiring protection against chemical agents: the CWS developed protective masks for horses, dogs, and carrier pigeons.

Other efforts at individual protection were not very successful. Sag Paste derived its name from Salve Antigas and was intended as an ointment that would prevent mustard agent burns. It was made of zinc stearate and vegetable oil and, for a short period, provided some protection against large doses of mustard agent. However, once the paste absorbed the mustard, injuries occurred. In addition, there was the problem of an individual's having to apply the paste to all the parts of his body using his contaminated hands and while

remaining on the battlefield. Over 900 tons of Sag Paste was shipped to the AEF during the war.[14,26]

The early concerns with collective protection primarily concentrated on providing a group of soldiers a gas-proof place in the trenches where they could remove the uncomfortable early gas masks. To accomplish this objective, studies were conducted on blankets to hang over dugout doorways, and various coatings or impregnates were examined for agent resistance. The result was a regular cotton blanket treated with dugout-blanket oil, a special heavy oil (Figure 2-12). Over 35,000 such blankets were shipped to the AEF.[26]

For ventilation of the dugout, there was the special antigas fan known as the Canvas Trench Fan. A 1918 War College gas warfare manual dedicated seven pages to the use of the fan, although all the fan really did was disperse the gas (Figure 2-13). Still, over 25,000 trench fans were sent to the front.[26,33]

Decontamination

There was also the problem of cleaning up the chemical agents after the gas attack. Mustard agent was a significant problem when it came to decontaminating the ground. The Germans apparently used chloride of lime to decontaminate the ground after an explosion at Germany's first mustard agent factory in Adlershof. For the AEF, bleaching powder (also known as chloride of lime or calcium hypochlorite) was the primary decontaminant during the war. Obtained from the bleaching industry, this white powder proved effective in neutralizing mustard agent on the ground. Almost 2,000 tons of bleaching powder was sent to the AEF during the war. As for mustard-contaminated clothing, the recommendation was to expose it to the open air for 48 hours or longer if the weather was cold. A quicker method was to leave the clothing inside a steam disinfecting chamber for 3 hours, but steam chambers were normally not available to front line troops.[14,26,34]

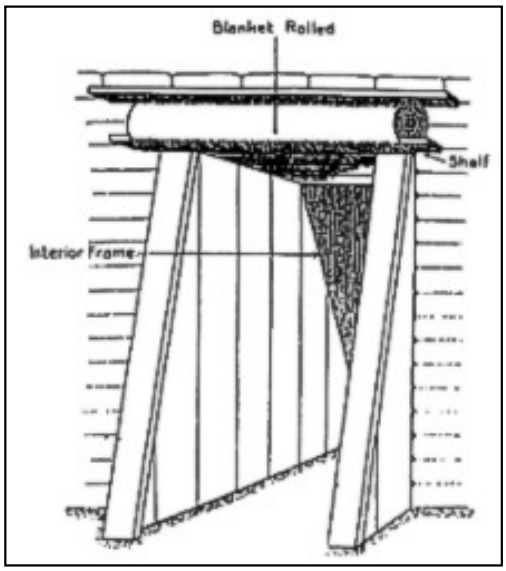


Fig. 2-12. Early attempts at collective protection during World War I included the dugout blanket, which was used to cover the doorways to dugouts. Reprinted from Army War College. *Methods of Defense Against Gas Attacks.* Vol 2. In: *Gas Warfare.* Washington, DC: War Department; 1918: Figure 18.

Detection and Alarms

The CWS also studied the critical need for chemical agent detectors and alarms. Initially, World War I soldiers relied on their own senses (smell, and throat and nose irritation) to detect chemicals. Eventually, the CWS was able to produce various dyes that changed color when contaminated with mustard agent. Most of the formulas for the detector paints, however, were British, and the CWS had trouble duplicating their work.[35]

At least one organic detector was also studied. One of the more interesting investigations was that of using snails as detectors. U.S. Army scientists reported that in the presence of mustard gas, snails waved their tentacles wildly in the air and then withdrew into their shells.

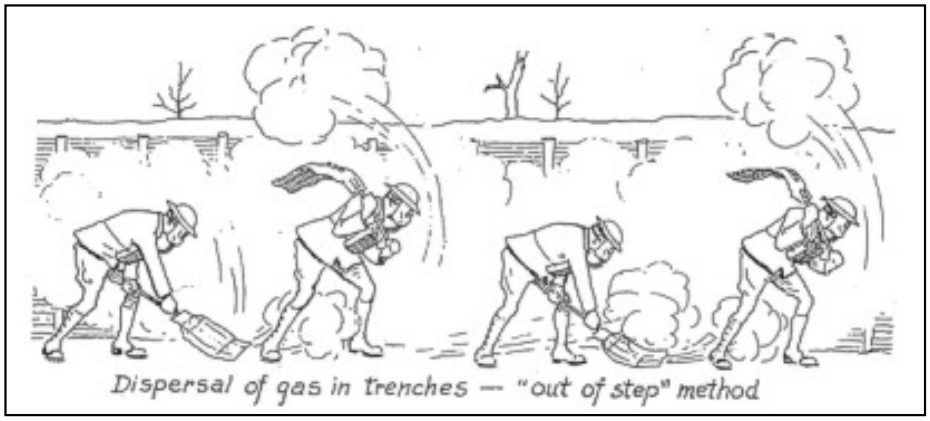


Fig. 2-13. Procedures for using the trench fan to remove chemical agents from trenches. The fan was a failure. Reprinted from Army War College. *Methods of Defense Against Gas Attacks*. Vol 2. In: *Gas Warfare*. Washington, DC: War Department; 1918: Figure 25.

When a prominent French physiologist was asked about this, he burst out laughing and said that French soldiers would eat the snails first. A test was conducted using French snails, but the conclusion was that the foreign snails were more conservative in their impulse to wave their tentacles.[36]

Once chemical agents were detected, the alarm was sounded by horns, rattles, bells, or whatever loud noise was available. These alarms created problems of their own, as the rattles often sounded like machine-gun fire, and it was difficult to distinguish from other nonchemical alarms. By the end of the war, the ability to detect chemical agents and alert the troops was still in a very primitive state.

Gas Casualty Treatments

A month after the United States entered the war, the U.S. Army War College issued *Memorandum on Gas Poisoning in Warfare with Notes on its Pathology and Treatment,*[37] a short manual for medical officers written by a committee of consultant physicians and physiologists. The memorandum directed that "Rest is the most important point of all in the general treatment of gas casualties"[37(p18)] and recommended using morphia to calm gassed soldiers who were too restless. Next in importance to rest were oxygen; protection from cold; special stimulants or drugs (particularly ampules of ammonia for inhalation, but also brandy in small sips, and pituitrin, administered hypodermically every 3 h); venesection (to relieve headaches); and removing "serous exudate" from the lungs (by drinking water and tickling the back of throat to produce vomiting; later treatments included potassium iodide, atropine, and steam tents with tincture benzoin compound). The manual concluded by admitting: "Knowledge on the various points discussed in this pamphlet is still far from being stable."[37(p32)]

The final version of the manual, issued in November 1918, made many changes to the original and reflected battlefield experience. For example, morphia was recognized as a "dangerous drug to use when the respiration is seriously affected. Its use should therefore be restricted to severe cases."[38(p22)] The most significant addition was information on mustard agent, which included sections for the treatment for the various organs exposed to the agent. For the skin, after cleaning the mustard agent off a soldier with soap and water,

> [a] dusting powder of zinc oxide mixed with boracic acid, chalk, and starch, or a calamine lotion with lime water may be used after the bath to allay skin irritation. The blisters may be evacuated by pricking.[38(p34)]

The delayed action of mustard agent required quick personnel decontamination actions. One solution was to bathe the soldiers thoroughly with soap and water within half an hour of mustard agent exposure. This was thought to prevent or greatly reduce the severity of the mustard burns. The army established degassing units that used a 5-ton truck with a 1,200-gal water tank, fitted with heaters and piping to connect it to portable showers. A second truck held extra uniforms. Two degassing units were assigned to each division. After the showers, the troops were give a drink of bicarbonate of soda water and then had their eyes, ears, mouths, and noses washed with the soda water.[38]

Mustard agent was a significant problem for untrained soldiers. In September 1918, one Field Artillery general instructed his troops:

> In view of the many casualties recently resulting in other commands from German mustard gas, each organization commander will take the following precautions: (a) Each soldier will place a small piece of soap in his gas mask container, (b) Each Chief of Section will keep constantly on hand in each gun-pit or gun position, two large bottles of soapy water—empty bottles may be purchased at wine shops from Battery Fund, (c) In case of a gas attack, and if opportunity permits, soapy water will be rubbed under the arms and between the legs around the scrotum, of soldiers affected, this serving to neutralize the pernicious effect of the gas. This effect will be explained to the soldiers of each organization, who can only hope to prevent becoming casualties through the strictest gas discipline.[39]

Despite the many warnings, mustard agent earned its designation of King of the Battlefield by killing approximately 600 U.S. soldiers and injuring over 27,000.[40]

Lessons Learned

The armistice of November 1918 ended the world's first chemical and biological war. Of the approximately 26 million casualties suffered by the British, French, Russians, Italians, Germans, Austro-Hungarians, and the Americans, some 1 million were gas casualties. Of the total 272,000 U.S. casualties, over 72,000 were gas casualties, or about one fourth. Of the total U.S. gas casualties, approximately 1,200 either died in the hospital or were killed in action by gas exposure. There were no casualties or deaths attributed to biological warfare.[40]

Thus the U.S. Army completed its introduction to 20th-century chemical warfare. With the help of the CWS, the army successfully recovered from its early poor performance and survived repeated toxic chemical attacks against its troops. Likewise, by the end of the war, the First Gas Regiment and numerous U.S. artillery units successfully used toxic chemical agents in retaliation and during offensive operations.

At the end of the war, the United States could proudly point to the best protective mask, abundant munitions, and trained troops. The CWS had 1,680 officers and 20,518 enlisted personnel controlling the army's chemical warfare program.[25]

The only negative aspect was the dire prediction of future chemical wars, as expressed by one U.S. Army officer:

> Gas was new and in an experimental stage throughout the war and hence the man who plans for future defense must consider the use of gas to have been in its infancy. He must draw very few lessons for the future use of gas based on past performances. He must only use those lessons as pointing the way and not as approaching a final result. The firing of steel as shell passed its zenith with the passing of the Argonne fight. Never again will the world see such a hail of steel on battlefields, but in its place will be concentrations of gas and high explosives as much greater than the World War as that was greater than the Civil War.[41(p4)]

In contrast, Fritz Haber, the Nobel laureate chemist who, more than anyone else, was responsible for the development and fielding of chemical weapons for use by Kaiser Wilhelm II's army, downplayed the importance of chemical warfare as a weapon of mass destruction after the surprise was gone. In an interview published in New York in 1921, he concluded: "Poison gas caused fewer deaths than bullets."[42(p10)]

General John J. Pershing summed up his opinion of the new chemical warfare shortly after the conclusion of World War I:

> Whether or not gas will be employed in future wars is a matter of conjecture, but the effect is so deadly to the unprepared that we can never afford to neglect the question.[43(p77)]

THE 1920s: THE LEAN YEARS

The Chemical Warfare Service Made Permanent

Following the successful conclusion of World War I, the U.S. Army almost immediately tried to forget everything it had learned during the war about being prepared for future chemical warfare. The first major concern of the new CWS was to ensure that it survived demobilization. The army had organized the CWS as a temporary war measure, a part of the National Army only, and that temporary existence was due to expire within 6 months after the end of the war. This 6 months was later extended to 30 June 1920. During hearings before the U.S. Congress, Secretary of War Newton D. Baker testified, "We ought to defend our army against a gas attack if somebody else uses it, but we ought not to initiate gas."[44(p3)] He and Chief of Staff General Peyton C. March both used this philosophy to recommend both abolishing the CWS and outlawing chemical warfare by a treaty.[45]

Even General Sibert, when asked about the need for a permanent CWS and the possibility of chemical warfare in future wars, replied:

> Based on its effectiveness and humaneness, [chemical warfare] certainly will be an important element in any future war unless the use of it should be prohibited by international agreement. As to the probability of such action, I cannot venture an opinion.[46(p87)]

To persuade congress to keep the CWS, several prominent civilian and military leaders lobbied to include a permanent chemical warfare organization. Lieutenant Colonel Amos A. Fries, a CWS officer and one of the strongest proponents of a permanent organization, stressed the need for a central organization, one that covered all aspects of chemical warfare (Figure 2-14). He drew on the lessons learned from the previous war:

> Had there been a Chemical Warfare Service in 1915 when the first gas attack was made, we would have been fully prepared with gases and masks, and the army would have been trained in its use. This would have saved thousands of gas cases, the war might easily have been shortened six months or even a year, and untold misery and wasted wealth might have been saved.[47(p4)]

He also stressed that both offensive and defensive research must be conducted:

> Just as developments in masks have gone on in the past just so will they go in the future. Just as from time to time gases were found that broke down or penetrated existing masks, just so in the future will gases be found that will more or less break down or penetrate the best existing masks. Accordingly, for thorough preparation, mask development must be kept absolutely parallel with development in poisonous and irritating gases. Mask development cannot, however, be kept parallel unless those working on masks know exactly what is going on in the development of poisonous gases. Thus a nation that stops all investigation into poisonous gases cannot hope to be prepared on the defensive side should the time ever come when defense against gas is needed.[20(pp7–8)]

Fig. 2-14. Amos A. Fries, shown here as a major general, was chief of the Chemical Warfare Service between 1921 and 1929. “With his dynamic personality and extensive contacts in Congress and the chemical industry, he quite literally kept the CWS alive.” Quotation: Brown FJ. *Chemical Warfare—A Study in Restraints.* Princeton, NJ: Princeton University Press; 1968: 130. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fries also disagreed with the premise that treaties could prevent chemical warfare:

> Researches into poisonous gases cannot be suppressed. Why? Because they can be carried on in out-of-the-way cellar rooms, where complete plans may be worked out to change existing industrial chemical plants into full capacity poisonous gas plants on a fortnight’s notice, and who will be the wiser?[20(p3)]

Although Fries was very persuasive and eloquent in his comments, a young lieutenant, who published the following poem in 1919, more graphically expressed the opinion of those who understood the nature of chemical warfare:

> There is nothing in war more important than gas
> The man who neglects it himself is an ass
> The unit Commander whose training is slack
> Might just as well stab all his men in the back.[48(cover iv)]

The chemical warfare specialists won the argument. On 1 July 1920, the CWS became a permanent part of the Regular Army. Its mission included development, procurement, and supply of all offensive and defensive chemical warfare material, together with similar

functions in the fields of smoke and incendiary weapons. In addition, the CWS was made responsible for training the army in chemical warfare and for organizing, equipping, training, and employing special chemical troops (Figure 2-15).[25,49]

Despite the encouragement of permanent status and surviving demobilization, the years after 1920 were lean (ie, austere) ones for the CWS, as indeed they were for the army as a whole. The CWS was authorized only 100 Regular Army officers but never actually achieved that number. The low point was 64 in 1923. Enlisted strength dropped to a low of 261 in 1919 and averaged about 400 the rest of the decade. Civilian employees numbered fewer than 1,000. The low point in funding was in 1923, when the amount was $600,000.[25]

Fig. 2-15. The first temporary Chemical Warfare School building at Edgewood Arsenal, Md., shortly after the end of World War I. The school was later moved to a permanent structure. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

After 1919, almost all the work of the CWS moved to Edgewood Arsenal, Maryland, with only the headquarters remaining in Washington, D.C. Edgewood became the center of training, stockpiling, and research and development.

Initially, the CWS was authorized to train only its own troops in all aspects of chemical warfare, while the General Staff permitted only defensive training for other army elements (Figure 2-16). The CWS protested this limitation and finally in May 1930, the Judge Advocate General ruled that both offensive and defensive training were allowed for all troops.[50]

Leftover stocks of chemicals from World War I were deemed sufficient for the army's stockpile. In 1922, to comply with the Limitation of Arms Conference, the War Department ordered that "[t]he filling of all projectiles and containers with poisonous gas will be discontinued, except for the limited number needed in perfecting gas-defense appliances."[51] The CWS was only allowed to continue limited research and development based on perceptions of future wars.[51,52]

To improve its standing with the taxpayers and the growing pacifist movement, the CWS also expanded its research capabilities into nonmilitary projects. These special projects included such activities as preserving wooden dock structures (1923) and fighting boll weevils (1925–1927.[53–55]

Fig. 2-16. Soldiers wearing protective clothing are firing 75-mm mustard agent shells at Edgewood Arsenal, Md., in 1928. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground.

New Chemical Weapons

In 1928, the CWS formalized the standardization of chemical agents. Seven chemical agents and

smokes were selected as the most important. The seven, with their symbols, were mustard agent (HS), methyldifluorarsine (MD), diphenylaminechlorarsine (DM), chloroacetophenone (CN), titanium tetrachloride (FM), white phosphorus (WP), and hexachlorethane (HC). Phosgene (CG) and Lewisite (L) were considered of lesser importance. Chloropicrin (PS) and chlorine (Cl) were rated the least important.[3]

Delivery systems were also improved. As early as 1920, Captain Lewis M. McBride experimented with rifling the barrel of the Stokes mortar. In 1924, a Stokes mortar barrel was rifled and tested. In truing the inside diameter of the 4-in. barrel preparatory to rifling, the bore was enlarged to 4.2 in. in diameter. This work increased the range of the mortar from 1,100 yd to 2,400 yd. In 1928, the improved mortar was standardized as the M1 4.2-in. chemical mortar and became the CWS's prized ground weapon for the delivery of toxic chemical agents as well as smoke and high explosives (Figures 2-17 and 2-18).[26]

Fig.2-17. An experimental 4.2-in. chemical mortar, showing (1) the standard, (2) the barrel with the shock-absorbing mechanism, and (3) the tie rods connecting the standard to the baseplate. This weapon differed from the Stokes mortar, its predecessor, in that it was easier to set up and it was rifled; the spiral grooves can be seen on the inside of the barrel at its muzzle. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

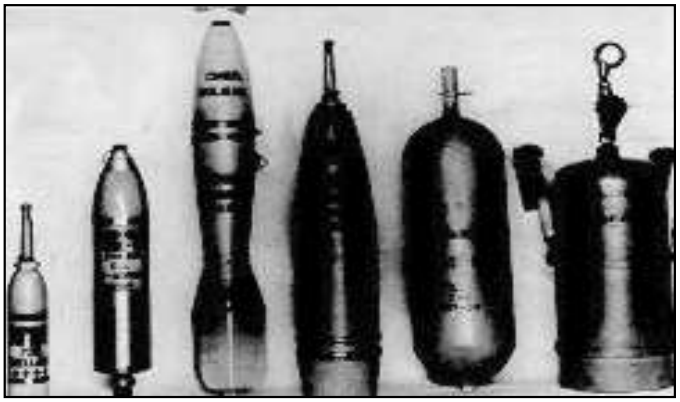


Fig. 2-18. The chemical weapons of the 1920s and 1930s. From left to right: the 75-mm mustard shell; the 4.2-in. white phosphorus shell; the M1 30-lb mustard bomb; the Mk II 155-mm mustard shell; the Livens phosgene projectile; and the Mk I portable chemical cylinder. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md

One much-discussed topic was the role that airplanes would take in the next chemical war. Fries predicted:

> The dropping of gas bombs of all kinds upon assembly points, concentration camps, rest areas and the like, will be so fruitful a field for casualties and for wearing down the morale of armies in the future that it will certainly be done and done on the very first stroke of war.[56(pp4–5)]

To meet this need, the CWS standardized the M1 30-lb chemical bomb. It held only about 10 lb of agent owing to its thick shell. As a test of the use of airplanes in a chemical war, the CWS first demonstrated simulated chemical attacks against battleships in 1921.[3,57]

New Protective Equipment

The CWS concentrated, however, on defensive work. After the war, the CWS continued working on the KTM mask, which became known as the Model 1919. In 1921, the mask officially became the M1 Service Gas Mask (Figure 2-19); it had a rubber facepiece and was available in five sizes.[30,58] The hope was to issue a protective mask to every soldier in the

army. One proponent described the reason why:

> To put the matter briefly, a modern army which enters on a campaign without respirators is doomed from the outset. It is asking to be attacked by gas, most certainly will be, and equally certainly will be destroyed. A soldier without a respirator is an anachronism.[59(p129)]

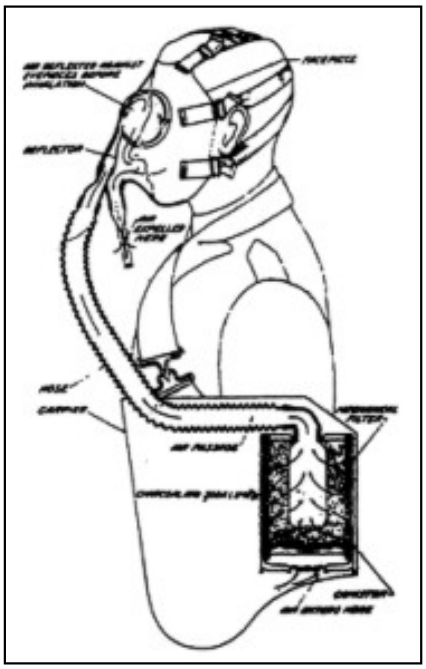


Fig. 2-19. A schematic diagram showing the M1 Service Gas Mask. The M1 eliminated the nose clip and mouthpiece of the box respirators of World War I vintage. By directing the incoming air over the eyepieces, it also helped eliminate lens fogging. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Biological Warfare Program

During the early 1920s, there were several suggestions from within the CWS that it undertake more research into biological agents. Fries, who had been promoted to major general and had replaced Sibert as the Chief Chemical Officer in 1920, however, decided it was not profitable to do so. In 1926, he wrote in the annual report of the CWS:

> The subject of bacteriological warfare is one which has received considerable notice recently. It should be pointed out in the first place that no method for the effective use of germs in warfare is known. It has never been tried to any extent so far as is known.[60(p8)]

The new League of Nations, which had been quoted in the annual report, concluded the same:

> [Biological] warfare would have little effect on the actual issue of a war because of protective methods available; that filtering and chlorinating drinking water, vaccination, inoculation, and other methods known to preventive medicine, would so circumscribe its effect as to make it practically ineffective.[60(p8)]

Chemical–Biological Warfare Use and Plans

Throughout the 1920s, rumors of chemical warfare attacks plagued the world. Besides the United States and the major World War I powers, several other countries began to develop a chemical warfare capability. Some of the countries with chemical weapons used them in their military operations. During the Russian Civil War and Allied intervention in the early 1920s, both sides had chemical weapons, and there were reports of isolated chemical attacks. Later accounts[3,21] accused the British, French, and Spanish of using chemical warfare at various times during the 1920s. One country in particular attracted the attention of the United States. As early as 1924, the CWS began to take note of the growing Italian chemical warfare capability. That was the year the Italians established the Centro Chemico Militaire, a unified chemical warfare service and began production of chemical agents.[61–63]

Two events related to biological warfare probably went unnoticed by the Americans. In 1928, a Japanese officer by the name of Shiro Ishii began promoting biological warfare research and took a 2-year tour of foreign research establishments, including the United States. After his tour, he concluded that all the major powers were secretly researching biological warfare. Although his conclusion was erroneous for the United States, it was

probably accurate for the Soviet Union. In 1929, the Soviets reportedly established a biological warfare facility north of the Caspian Sea. [3,21,64,65]

While the CWS struggled to survive and keep the army ready for a chemical war, international attempts were made to prohibit chemical warfare. The Treaty of Versailles, completed in 1919, prohibited Germany from producing, storing, importing, or using poisons, chemicals, and other chemical weapons. The treaty was not ratified by the United States. A separate treaty with Germany did not mention chemical warfare, but the United States agreed to comply with the provisions of the Treaty of Versailles in relation to poisonous gases.

Although the new League of Nations concluded in 1920 that chemical warfare was no more cruel than any other method of warfare used by combatants, the Limitation of Arms Conference, held in Washington, D. C., in 1922, banned the use of poisonous gases except in retaliation. The United States ratified the limitation, but France declined to ratify the treaty and therefore it was never implemented.

This unsuccessful attempt was followed by the 1925 Geneva Protocol, which was signed by 28 countries, including the United States. This agreement condemned the use of gas and bacteriological warfare. The U.S. Senate, however, refused to ratify the Protocol and remained uncommitted by it. The senate had apparently decided that chemical warfare was no more cruel than any other weapon and therefore should not be banned. The general policy of the U.S. government, however, still tended toward the discouragement of all aspects of chemical warfare, but was tempered by a policy of preparedness should chemical warfare occur again.[66–69]

THE 1930s: THE GROWING THREAT OF CHEMICAL AND BIOLOGICAL WARFARE

Further international attempts to ban not only the use of chemical weapons but also all research, production, and training caused a response that developed into a new U.S. policy on chemical warfare. The U.S. Army Chief of Staff, General Douglas MacArthur, stated the policy in a letter to Secretary of State Henry L. Stimson in 1932:

> In the matter of chemical warfare, the War Department opposes any restrictions whereby the United States would refrain from all peacetime preparation or manufacture of gases, means of launching gases, or defensive gas material. No provision that would require the disposal or destruction of any existing installation of our Chemical Warfare Service or of any stocks of chemical warfare material should be incorporated in an agreement. Furthermore, the existence of a War Department agency engaged in experimentation and manufacture of chemical warfare materials, and in training for unforeseen contingencies is deemed essential to our national defense.[45(p118)]

There were no other major attempts to ban chemical and biological warfare during the 1930s.

New Chemical Agents and Weapons

The CWS continued to maintain stockpiles of the key World War I–chemical agents during the 1930s. Captain Alden H. Waitt, then Secretary of the Chemical Warfare School at Edgewood Arsenal and later Chief Chemical Officer, summed up the CWS's planning for the next war in 1935:

> Foreign writers agree that at least for the first few months of any war, should one occur within a few years, the gases that were known at the end of the

> World War would be used. Of these, the opinion is unanimous that mustard gas would be the principal agent and the most valuable. Opinion in the United States coincides with this.[70(p285)]

> In 1937, Edgewood Arsenal rehabilitated their mustard agent plant and produced 154 tons of mustard agent to increase their stockpile (Figure 2-20). The same year, the phosgene plant was renovated for additional production, and the CWS changed phosgene from substitute standard to standard (Figure 2-21).[71]

Fig. 2-20. The Mustard Manufacturing Plant at Edgewood Arsenal, Md. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The result of the CWS's confidence in these selected agents was that the CWS missed the development of several key new agents. Waitt wrote:

> Occasionally a statement appears in the newspapers that a new gas has been discovered superior to any previously known. Such statements make good copy, but not one of them has ever been verified. Today no gases are known that are superior to those known during the World War. It is unlikely that information about a new gas will be obtained until it is used in war. The chemical agent is too well adapted to secrecy. The only insurance against surprise by a new gas is painstaking research to find for ourselves every chemical agent that offers promise for offensive or defensive uses. It seems fairly safe to say that today mustard gas is still the king of warfare chemicals and to base our tactical schemes on that agent as a type.[70(p285)]

Yet already the reign of mustard agent was ending. In 1931, Kyle Ward, Jr., published an article describing nitrogen mustard, a vesicant agent with no odor. The CWS investigated the new substance and found it to be less vesicant than sulfur mustard. The U.S. Army eventually standardized nitrogen mustard as HN-1, although it was the Germans who took a great interest in the new vesicant.[3]

In 1936, German chemist Dr. Gerhart Schrader of I. G. Farbon Company discovered an organophosphorus insecticide, which was reported to the Chemical Weapons Section of the German military prior to patenting. The military was impressed with the effects of the compound on the nervous system and classified the project for further research. The military assigned various names to the new substance, including Trilon-83 and Le 100, but tabun was the name that stuck. After World War II, the CWS designated it GA, for "German" agent "A."

About 2 years later, Schrader developed a similar agent, designated T-144 or Trilon-46 and eventually called sarin, which was reportedly 5 times as toxic as tabun. The United States later designated this agent GB. The Germans assigned a large number of chemists to work on these new nerve agents and began building a pilot plant for production in 1939, the year World War II started.[3,72,73]

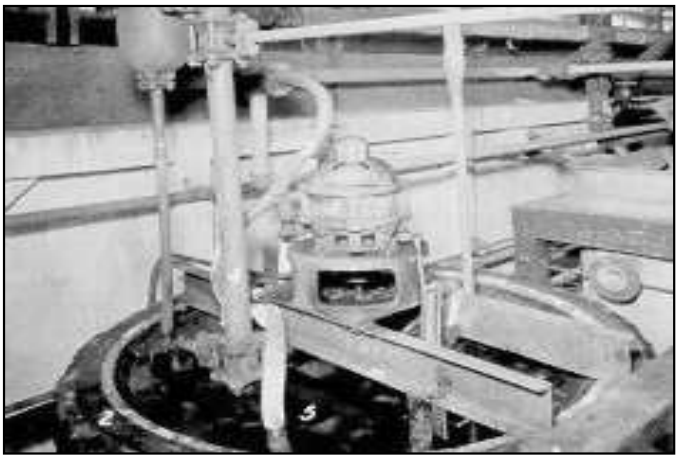


Fig. 2-21. Interior view of the Phosgene Production Plant at Edgewood Arsenal, Md. The low level of chemical engineering technology apparent in this World War II-era photograph is relevant to the problem of chemical agent proliferation today. Elaborate, expensive equipment is not required for mass-producing the less-sophisticated chemical agents. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

During the 1930s, the CWS stockpiled the chemical weapons used by World War I ground forces in preparation for a future war. These were primarily Livens projectors, Stokes mortars, and portable cylinders. In addition, there were chemical shells for 75-mm, 105-mm, and 155-mm artillery pieces (Figures 2-22 and 2-23).

Fig. 2-22. A Field Artillery unit prepared for chemical war. Both the men and the horses required protection against the agents. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fig. 2-23. Battery D, 6th Field Artillery, firing a 75-mm gun while in protective clothing at Edgewood Arsenal, Md., in 1936. The overgarments of the 1920s were made of rubberized cloth or cloth impregnated with substances such as linseed oil. These overgarments were heavier and hotter than today's protective clothing. Note the lack of overboots. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The production of the new 4.2-in. chemical mortar eventually made that weapon the key ground delivery system for the CWS. Between 1928 and 1935, the army attempted to make the 4.2-in. mortar a mechanized weapon by mounting it on various vehicles (Figure 2-24). The CWS also began experiments in 1934 to make the mortar a more versatile weapon by testing high-explosive shells as an alternative to chemical rounds.

In 1935, the improved M1A1 mortar was standardized. The M1A1 had an improved barrel and baseplate, and a new standard connected to the baseplate by two tie-rods for support. The M1A1 had a maximum range of 3,200 yd (1.8 miles). Each shell held 5 to 7 lb of either phosgene, mustard agent, cyanogen chloride, white phosphorus, or smoke agent.[3,26]

Additional new delivery systems also included the first standardized chemical land mine for mustard agent in 1939. Designated the M1, this 1-gal gasoline-type can held 9.9 lb of mustard agent and required detonating cord to burst the can and disseminate the agent.[74]

For air delivery, the CWS standardized the first good airplane smoke tank, the M10, in 1933. This tank held 30 gal of mustard (320 lb), Lewisite (470 lb), or smoke material. The system was rather simple. Electrically fired blasting caps shattered frangible seals in the air inlet and the discharge line, which allowed air and gravity to force the liquid out. The slipstream of the plane then broke up the liquid into a spray.[74]

Biological Warfare Developments

While chemical warfare received some attention during the 1930s, biological warfare received very little. In 1933, Major Leon A. Fox, Medical Corps, U.S. Army, wrote an article on bacterial warfare for *The Military Surgeon* that began:

> Bacterial warfare is one of the recent scare-heads that we are being served by the pseudo-scientists who contribute to the flaming pages of the Sunday annexes syndicated over the Nation's press.[75(p189)]

Fig. 2-24. A 4.2-in. chemical mortar mounted on a light cargo carrier in 1928. The carrier had a speed of 20 mph. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

He then proceeded to point out the difficulties of trying to weaponize biological agents. For example, bubonic plague would create significant problems for friendly troops as well as the enemy:

> The use of bubonic plague today against a field force, when the forces are actually in contact, is unthinkable for the simple reason that the epidemic could not be controlled. Infected personnel captured would provide the spark to set off possible outbreaks of pneumonic plague in the ranks of the captors. Infected rats would also visit and spread the condition. An advance over terrain infected with plague-bearing rats would be dangerous. Therefore, except as a last desperate, despairing hope of a rapidly retreating army, the use of plague by forces in the field is not to be considered.[75(p202)]

After dismissing the causative organisms of malaria, yellow fever, anthrax, and other such agents, he concluded:

> I consider that it is highly questionable if biologic agents are suited for warfare. Certainly at the present time practically insurmountable technical difficulties prevent the use of biologic agents as effective weapons of warfare.[75(p207)]

The same year that Fox wrote his article, Germany began military training in offensive biological warfare and reportedly covertly tested *Serratia marcescens,* considered a biological simulant, in the Paris Metro ventilation shafts and near several French forts. Three years later they conducted antianimal experiments with foot and mouth disease at Luneburger Heide. The next year the German Military Bacteriological Institute in Berlin began developing anthrax as a biological weapon, while the Agricultural Hochschule in Bonn examined the spraying of crops with bacteria.[3,65]

Even the future allies of the United States in World War II were working on biological warfare programs. By 1936, France had a large-scale biological warfare research program working on bacterial and viral viability during storage and explosive dispersal. The same year, Britain established a committee to examine offensive and defensive biological warfare issues. By 1940, the British chemical laboratory at Porton Down had a biological warfare laboratory. Canada initiated biological warfare research under Sir Frederick Banting at Connaught Laboratories, Ile Grosse, and at Suffield in 1939. The Canadians started work on anthrax, botulinum toxin, plague, and psittacosis.[3,65]

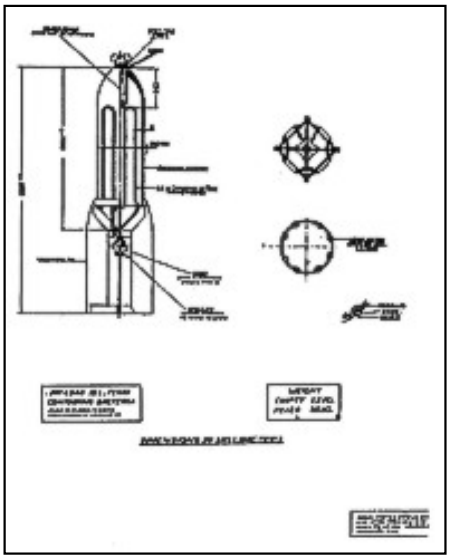


Fig. 2-25. The Japanese UJI bacterial bomb, drawn from sketches given to Lieutenant Colonel Murray Sanders, Chemical Warfare Service, in 1945. Porcelain rather than metal was used to form the “shell” because it could be shattered by a much smaller explosive charge. This protected the biological agent, assuring that it would be subjected to less heat and pressure. Reprinted from Scientific and Technical Advisory Section, US Army Forces, Pacific. *Biological Warfare.* Vol 5. In: *Report on Scientific Intelligence Survey in Japan.* HQ, US Army Forces, Pacific; 1945: appended chart.

One man who definitely thought differently from Fox was Japan’s Ishii. In 1933, he set up an offensive biological warfare laboratory in occupied Manchuria, later designated Detachment 731, which developed and tested a biological bomb within 3 years and also tested biological agents on Chinese prisoners. Additional biological warfare facilities were established in 1939, the same year that Japanese troops allegedly entered Russia to poison animals with anthrax and other diseases.

By 1940, Ishii had developed and tested in the field nine different kinds of biological bombs and had produced over 1,600 bombs, although some had been expended in research. The 40-kg Ha bomb, filled with a mixture of shrapnel and anthrax spores, and the 25-kg Type 50 UJI bomb, also filled with anthrax spores, were considered the most effective (Figure 2-25). His early discoveries that conventional bombs filled with biological agents failed to disseminate the agent properly did, however, confirm some of Fox’s beliefs. The Japanese were able to disseminate typhus rickettsia, cholera bacteria, and plague-infested fleas through Ning Bo in China, where 500 villagers died from plague epidemics. By the beginning of World War II, Ishii was concentrating on the use of vectors such as the common flea to carry the biological agents.[3,64,76]

New Defensive Equipment

The M1 gas mask design proved to be a reliable choice for over a decade. In 1934, minor modifications to the head-harness straps and the mounting of the eyepieces resulted in the M1A1 mask.

In 1935, the first major modification to the original design was introduced as the M1A2 mask. The M1A2 was constructed from a flat rubber faceblank with a seam at the chin. This design allowed the mask to be issued in one universal size, although the small and large sizes of the M1A1 continued in production. This mask became the standard mask for the army up to the beginning of World War II. By 1937, Edgewood Arsenal was producing over 50,000 masks per year (Figure 2-26).[30]

Collective protection during the 1930s began the advancement from the passive dugout blanket of World War I to the modern mechanical systems. Although most major powers initiated work on collective protection for troops in the field during the 1920s, the CWS did not standardize its first unit until 1932. That year, the M1 Collective Protector, a huge, 1,210-lb, fixed installation unit providing 200 cu ft of air per minute, was typed classified for use primarily in coastal forts. The level of protection was the same as that provided by the standard gas mask canister.[77]

For decontamination, the CWS concentrated on mustard agent decontaminants. Ordinary bleach, used during World War I, was considered the most effective but was corrosive to metals and had only a 3-week storage life in the tropics (Figure 2-27). Early work, starting in 1930, used simple tanks filled with DR1 emulsion, a soap prepared with magnesium carbonate, animal fat, and kerosene, and designed primarily for ship decontamination. The next development involved commercial items such as insecticide sprayers, fire extinguishers, agricultural spreaders, and road sprinklers. The best sprayer was the 3-gal Demustardizing Apparatus, Commercial Type, standardized in 1938. The 1 1/2-qt Demustardizing Apparatus was used for lighter work. This fire extinguisher–type sprayer was recommended for standardization in 1937.

Agricultural spreaders and road sprinklers proved less successful at disseminating the proper amount of decontaminant. Just before the beginning of World War II, the CWS also investigated the power-driven demustardizing apparatus, which was based on a commercial orchard sprayer with a 300-gal tank and an 8-hp engine.

In 1938, the CWS made the important discovery of the decontaminating capability of the compound RH-195, developed by the Du Pont Company, when mixed with acetylene tetrachloride. This combination was later designated Decontaminating Agent, Non-Corrosive (DANC). DANC was a whitish powder that liberated chlorine more slowly than ordinary bleaching material and therefore was more stable in storage. One gallon of DANC could decontaminate 15 sq yd of

Fig. 2-26. In addition to the standard Service Gas Mask, the Chemical Warfare Service also designed diaphragm masks for speaking capability. Note the hood, which covered the skin of the head, face, and neck. The soldier also wears chemically protective gloves. Since the uniform was impregnated with a substance that hindered the penetration of mustard, in theory, no portion of his skin was subject to mustard injury. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

heavily contaminated soil.[34]

Fig. 2-27. Cleaning up mustard agent in the field with bleaching powder and soil. The labor-intensive nature of mustard decontamination is readily apparent. Note that the exercise is being conducted in the winter; no doubt the chemical protective garments shown here would have constituted a considerable thermal load. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Italian–Ethiopian War

The first major use of chemical weapons after World War I came in 1935 during the Italian–Ethiopian War. On 3 October 1935, Benito Mussolini launched an invasion of Ethiopia from its neighbors Eritrea, an Italian colony, and Italian Somaliland. Ethiopia protested the invasion to the League of Nations, which in turn imposed limited economic sanctions against Italy. These sanctions, although not crippling, put a deadline pressure on Italy to either win the war or withdraw.

The initial Italian offensive from Eritrea was not pursued with the proper vigor in Mussolini's opinion, and the Italian commander was replaced. The new commander, Marshal Pietro Badoglio, was ordered to finish the war quickly. He resorted to chemical weapons to defeat the Ethiopian troops led by Emperor Haile Selassie. Despite the Geneva Protocol of 1925, which Italy had ratified in 1928 (and Ethiopia in 1935), the Italians dropped mustard bombs and occasionally sprayed it from airplane tanks. They also used mustard agent in powder form as a "dusty agent" to burn the unprotected feet of the Ethiopians. There were also rumors of phosgene and chloropicrin attacks, but these were never verified.

The Italians attempted to justify their use of chemical weapons by citing the exception to the Geneva Protocol restrictions that referenced acceptable use for reprisal against illegal acts of war. They stated that the Ethiopians had tortured or killed their prisoners and wounded soldiers.[78–90]

Chemical weapons were devastating against the unprepared and unprotected Ethiopians. With few antiaircraft guns and no air force, the Italian aircraft ruled the skies. Selassie emotionally described the nightmare to the League of Nations:

> Special sprayers were installed on board aircraft so they could vaporize over vast areas of territory a fine, death-dealing rain. Groups of 9, 15, or 18 aircraft followed one another so that the fog issuing from them formed a continuous sheet. It was thus that, as from the end of January 1936, soldiers, women, children, cattle, rivers, lakes, and pastures were drenched continually with this deadly rain. In order more surely to poison the waters and pastures, the Italian command made its aircraft pass over and over again. These fearful tactics succeeded. Men and animals succumbed. The deadly rain that fell from the aircraft made all those whom it touched fly shrieking with pain. All those who drank poisoned water or ate infected food also succumbed in dreadful suffering. In tens of thousands the victims of Italian mustard gas fell.[83(pp151–152)]

By May 1936, Italy's army completely routed the Ethiopian army. Italy controlled most of Ethiopia until 1941 when British and other allied troops reconquered the country.

The U.S. Army closely followed the war and sent Major Norman E. Fiske as an observer with the Italian army and Captain John Meade as an observer with the Ethiopian army. Their different conclusions as to the role of chemical warfare in the war reflected the sides they observed. Major Fiske thought the Italians were clearly superior and that victory for them was assured no matter what. The use of chemical agents in the war was nothing more than an experiment. He concluded:

> From my own observations and from talking with [Italian] junior officers and soldiers I have concluded that gas was not used extensively in the African campaign and that its use had little if any effect on the outcome.[88(p20)]

His opinion was supported by others who felt that the Ethiopians had made a serious mistake in abandoning guerrilla operations for a conventional war.

Captain Meade, on the other hand, thought that chemical weapons were a significant factor in winning the war. They had been used to destroy the morale of the Ethiopian troops, who had little or no protection, and to break up any attempts at concentration of forces. Captain Meade concluded:

> It is my opinion that of all the superior weapons possessed by the Italians, mustard gas was the most effective. It caused few deaths that I observed, but it temporarily incapacitated very large numbers and so frightened the rest that the Ethiopian resistance broke completely.[88(p20)]

Major General J. F. C. Fuller, assigned to the Italian army, highlighted the Italian use of mustard agent to protect the flanks of columns by denying ridge lines and other key areas to the Ethiopians. He concluded:

> In place of the laborious process of picketing the heights, the heights sprayed with gas were rendered unoccupiable by the enemy, save at the gravest risk. It was an exceedingly cunning use of this chemical.[85(p143)]

Still another observer stated:

> I think [where mustard] had [the] most effect was on animals; the majority of

> the Ethiopian armies consisted of a number of individual soldiers, each with his donkey or mule on which he carried rations. These donkeys and mules ate the grass and it killed them, and it was that which really broke down morale more than anything.[86(p81)]

B.H. Liddell Hart, another military expert, compromised between the two schools of thought and concluded:

> The facts of the campaign point unmistakably to the conclusion that mechanization in the broad sense was the foundation on which the Italians' military superiority was built, while aircraft, the machine gun, and mustard gas proved the decisive agents.[87(p330)]

All observers, however, seemed to agree that the Italians would eventually have won whether chemical agents were used or not.

In general, the U.S. Army learned little new from this war. The annual report for 1937 stated that "situations involving the employment of chemical agents have been introduced into a greater number of problems." [89] The CWS Chemical Warfare School concluded that "the use of gas in Ethiopia did not disclose any new chemical warfare tactics,"[90] but only reconfirmed existing tactical use expectations. The school also initiated a class for Army Air Corps personnel (Figure 2-28).[90] One senior air corps officer, perhaps noting the successful Italian use of spray tanks, commented, "We want that course repeated again and again until all of our people are thoroughly awake to the necessity for training and preparation."[91(p153)]

Japanese Invasion of China

The next war that drew the interest of chemical warfare experts was the Japanese invasion of China in 1937. The Japanese, in addition to their biological work, had an extensive chemical weapons program and were producing agent and munitions in large numbers by the late 1930s. During the resulting war with China, Japanese forces reportedly began using chemical shells, tear gas grenades, and lacrimatory candles, often mixed with smoke screens.

By 1939, the Japanese reportedly escalated to mustard agent and Lewisite. Against the untrained and unequipped Chinese troops, the weapons proved effective. The Chinese reported that their troops retreated whenever the Japanese used just smoke, thinking it was a chemical attack.[21,92]

Fig. 2-28. Aerial spraying of a Chemical Warfare School class with tear gas during a training event. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Preparing for the Next War

After the Italian–Ethiopian War, the possibility of war in Europe became the primary concern of the U.S. Army. The CWS closely studied the chemical warfare capabilities of Germany and Italy, although it missed the German development of nerve agents.

The United States, although largely isolationist in policy, followed the declining political situation in Europe and decided to begin a gradual improvement in its military posture. Official policy, however, was against the employment of chemical warfare, and initially the CWS met with much resistance. President Franklin D. Roosevelt detested chemical warfare and in 1937 refused to permit the redesignation of the CWS as a corps. There was no ongoing chemical warfare in Europe to learn from, and public opinion continued to be solidly against any use of chemical weapons. In addition, the issue of whether the CWS should field ground combat units, particularly chemical mortar battalions, distracted policy makers and was only resolved by the U.S. Army Chief of Staff, who finally approved two battalions just before the beginning of World War II.[45]

THE 1940s: WORLD WAR II AND THE NUCLEAR AGE

The start of World War II in 1939 and the rapid collapse of France in the spring of 1940 stimulated a major increase in the rate of American rearmament. Although no major use of chemical and biological agents occurred, rumors and reports of incidents of chemical and biological warfare attracted the attention of intelligence officers. Although much of Germany's and Japan's chemical and biological weapons programs did not become known until after the war, the actual threat was impressive.

During the war, Germany produced approximately 78,000 tons of chemical warfare agents. This included about 12,000 tons of the nerve agent tabun, produced between 1942 and 1945. Germany also produced about 1,000 lb of sarin by 1945. The key nerve agent weapons were the 105-mm and 150-mm shells, the 250-kg bomb, and the 150-mm rocket. The latter held 7 lb of agent and had a range of about 5 miles when fired from the six-barrel Nebelwerfer launcher (Figure 2-29). Mustard agent, however, was still the most important agent in terms of production, and the Germans filled artillery shells, bombs, rockets, and spray tanks with the agent. Phosgene, of somewhat lesser importance, was loaded in 250- and 500-kg bombs. The Germans were the greatest producers of nitrogen mustards and produced about 2,000 tons of HN-3. This was filled in artillery shells and rockets.

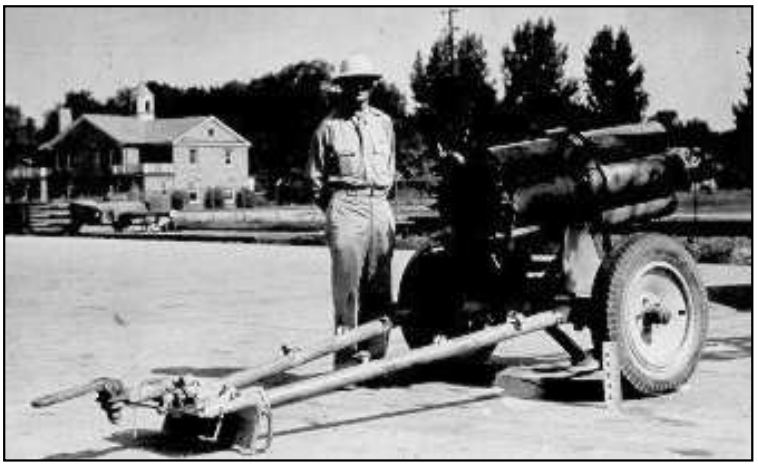


Fig. 2-29. The 150-mm German Nebelwerfer rocket projector was developed in the 1930s; one of its intended uses was to disseminate chemical agents. This fact was supposed to be disguised by naming it the Nebelwerfer (literally "smoke-screen layer"). As events transpired during World War II, Nebelwerfers were used exclusively as rocket artillery, firing high-explosive projectiles. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

They also had a large number of captured chemical munitions from France, Poland, USSR,

Hungary, and other occupied countries.[3,26]

Germany's biological warfare program was much less extensive than its chemical program. Most of the Germans' work was apparently with antipersonnel agents such as the causative organisms of plague, cholera, typhus, and yellow fever. They also investigated the use of vectors to attack animals and crops.[21,65]

Japan produced about 8,000 tons of chemical agents during the war. The Japanese loaded mustard agent, a mustard–Lewisite mixture, and phosgene in shells and bombs and gained experience in their use during their attacks on China. They also filled hydrogen cyanide in mortar and artillery shells, and in glass grenades. Japan's biological warfare program was also in full swing by World War II, and many weapons had been laboratory and field-tested on humans.[26]

The possibility that massive chemical or biological attacks could happen any day kept CWS officers pushing for preparedness. A newspaper article reflected the common prediction circulating in the press:

> European military authorities have predicted that gas would be used in the present war, if at any time the user could be sure of an immediate and all-out success from which there could be no retaliation.[93(p37)]

Major General William N. Porter (Figure 2-30), the new chief of the CWS, warned that Hitler was likely to use chemical weapons "at any moment." He also felt that "No weapon would be too bad to stop or defeat Hitler"[94(p31)] and wanted to "fight fire with fire in the event an enemy chooses to use poison gas." [95(p36)]

Fig. 2-30. Major General William N. Porter commanded the Chemical Warfare Service during World War II. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Preparing for Chemical Warfare

During the massive 1941 training maneuvers, the U.S. Army used a scenario that called for no first use of chemical weapons by either side. Troops carried gas masks, but were to wear them only in areas designated as being under gas attack. Simulated chemical agent attacks were made by placing signs stating "Mustard Gas" in various areas and, in some cases, using molasses residuum, a popular mustard simulant. However, in the latter case, the army ran into a serious problem of getting the stains out of their uniforms. Despite this hitch, at least one participant concluded:

> There was clear evidence that distinct progress was made during the maneuvers in arousing interest in the use of chemical warfare means and methods under battlefield conditions. As a result, a greater portion of the command, staff, and rank and file are undoubtedly more cognizant of how chemicals might be used against them, and what countermeasures to take.[96(p17)]

While planning for a more traditional, European-style war, the CWS also monitored

Japan's use of chemical weapons in China. U.S. Army interest in chemical warfare preparation rose significantly, since Japan was already employing chemical weapons.[97]

The CWS, however, found itself hardly prepared to fight a major chemical war on the level of World War I. Increased budgets and personnel helped with war planning, but to actually field chemical weapons and build chemical stockpiles first required industrial mobilization and massive production.

The national emergency declared prior to the war increased the size of the CWS to over 800 officers and over 5,000 enlisted men, with civilian strength keeping pace. Appropriations, which had already passed $2 million per year, jumped to $60 million as successive military supplements increased the fiscal 1941 budget. The CWS rapidly increased its productive capacity and improved nationwide procurement district offices to expand its mobilization basis.[25]

The Growth of the Chemical Warfare Service

When World War II finally engulfed the United States on 7 December 1941, the transition to wartime conditions was much less sudden than in 1917, primarily owing to the extensive mobilization activity of the preceding 2 years. Porter, who served as the chief throughout the war, found under his command not the skeletonized CWS of the 1930s but a large and rapidly growing organization, whose personnel numbered in the thousands, physical facilities were scattered throughout the eastern half of the country, and products were in urgent demand by an army rapidly growing to multimillion-man strength.

More than 400 chemical battalions and companies of varying types were activated during the course of the war, and a large proportion of them saw service overseas. Chemical mortar battalions and companies, using high-explosive and smoke shells in the 4.2-in. chemical mortars, gave close artillery-type support to infantry units in every theater. Smoke generator battalions and companies screened troop movements as well as fixed installations. Depot companies stored, maintained, and issued material; processing companies kept up theater stocks of protective clothing; decontamination companies backed up chemical defense postures; and laboratory companies provided technical intelligence assessments of captured chemical material. Chemical maintenance companies repaired and reworked equipment, performing especially critical tasks in keeping the mortar units firing. Chemical service units, organized to provide a broad spectrum of capabilities, performed most or all of the service and logistical functions already mentioned on a smaller scale where full-sized specialized companies were not authorized, or not available. Finally, a full complement of chemical service units supported the operations of the Army Air Force, especially in the storage and handling of incendiary bombs. In addition to the field organizations, each theater, army group, and army headquarters had a chemical staff in their headquarters elements.

The production and storage needs of a rapidly growing military establishment could not be met by Edgewood Arsenal alone. The CWS quickly constructed new installations: arsenals at Huntsville, Alabama, Denver, Colorado, and Pine Bluff, Arkansas; a chemical/biological proving ground in Utah; protective clothing plants at Columbus, Ohio, Kansas City, Missouri, and New Cumberland, Pennsylvania; charcoal-filter plants at Zanesville and Fostoria, Ohio; and impregnate factories at Niagara Falls, New York, East St. Louis, Illinois, and Midland, Michigan.[25,26]

Chemical Agents

The CWS agent production initially concentrated on the World War I agents.

Approximately 146,000 tons of chemical agents was produced by the United States between 1940 and 1945. Phosgene (CG) was produced at Edgewood Arsenal; the new Huntsville Arsenal; and the Duck River Plant owned by Monsanto Chemical Company in Columbia, Tennessee. These plants produced about 20,000 tons of the agent during the war. Mustard agent (HS) was produced at Edgewood Arsenal; Rocky Mountain Arsenal, Denver, Colorado; Pine Bluff Arsenal; and Huntsville Arsenal (Figures 2-31 and 2-32). By the end of the war, these plants produced over 87,000 tons of the agent. Lewisite (L) was produced at a small pilot plant at Edgewood Arsenal and later at Huntsville Arsenal, Pine Bluff Arsenal, and Rocky Mountain Arsenal. Approximately 20,000 tons of the agent was produced before the plants were shut down in 1943. Cyanogen chloride (CK) was produced at the American Cyanamid Company plant in Warners, New York, and at the Owl Plant in Azusa, California. About 12,500 tons of the agent was procured during the war. Hydrogen cyanide (AC) was produced by Du Pont and the American Cyanamid Company. Only about 560 tons of the agent was procured by the CWS.

The leadership of the CWS took interest in the nitrogen mustards after they learned that the Germans were producing it. HN-1 was produced at Edgewood Arsenal in a small pilot plant and later at Pine Bluff Arsenal, which produced about 100 tons of the agent. The British also investigated HN-2 and HN-3, but the United States did not produce the latter two agents.

Investigation of ways to improve the purity of mustard agent resulted in the discovery that washing the agent with water and then distilling it produced a much more pure product. The new agent was called distilled mustard agent (HD). Edgewood Arsenal used a pilot plant to produce some of the agent in 1944 and then a full-scale plant was completed at Rocky Mountain Arsenal the next year. By the end of the war, over 4,600 tons of the agent was produced.[26]

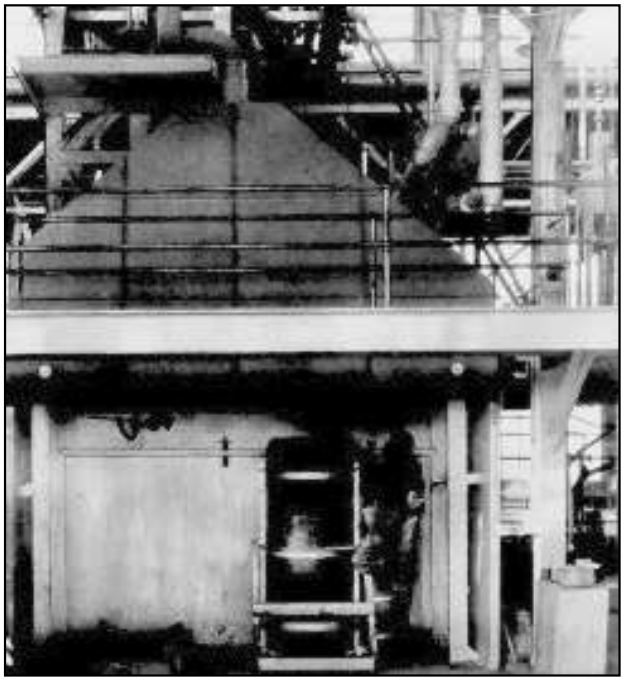


Fig.2-31. Interior view of the Mustard Agent Plant at Edgewood Arsenal, Md., showing a soldier filling a 1-ton container with the agent. The operator is wearing a protective mask. Concerns regarding occupational hazards evidently dictated a higher standard of personal protection than was apparent during World War I (see Figure 2-7). Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Chemical Weapons

The heart of the CWS offensive capability was the chemical mortar. In December 1941, there were only 44 chemical mortars on hand. This was quickly corrected, as the demand for the versatile weapon increased after each major usage. The continued need for greater range, accuracy, durability, and ease in manufacturing resulted in the improved M2 4.2-in. mortar in 1943. The M2 had a maximum range of 4,400 yd, which was later increased to 5,600 yd by modifying the propellant in test firings at Edgewood Arsenal in 1945. Despite a slow start, the M2 series 4.2-in. chemical mortar rapidly became the central weapon of the CWS, not only for chemical agent delivery if needed, but also for high-explosive, smoke, and white phosphorus rounds. Over 8,000 chemical mortars were procured by the CWS for chemical mortar battalions during the war.[3,26,98]

The other offensive weapons for chemical agent attack were to be delivered by either the artillery or the air force. The artillery had available 75-mm, 105-mm, and 155-mm chemical rounds that were filled primarily with mustard agent but that also contained Lewisite (Figure 2-33). In 1945, the CWS standardized the first chemical rockets: a 7.2-in. version used phosgene and cyanogen chloride, fired from a 24-barrel, multiple–rocket launcher platform; and a smaller 2.36-in. cyanogen chloride–filled bazooka round.

Fig. 2-32. Unloading mustard agent from 1-ton containers on flat cars at Pine Bluff Arsenal, Arkansas, in 1943. Apparently unloading and loading mustard agent were considered to constitute different hazards (see Figure 2-31). Note that the operator is wearing a face shield, apron, and gloves, but not a protective mask. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

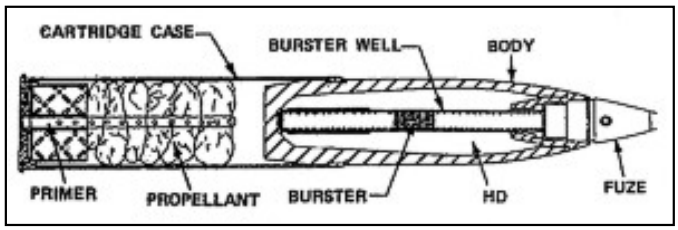


Fig. 2-33. Diagram of the M60 105-mm mustard shell, with the cartridge case attached. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The U.S. Army Air Force had 100-lb mustard agent bombs (Figure 2-34); 500-lb phosgene or cyanogen chloride bombs; and 1,000-lb phosgene, cyanogen chloride, or hydrocyanic acid bombs. In addition, the new M33 spray tank could hold 750 to 1,120 lb of mustard agent or Lewisite. None of these chemical weapons were used on the battlefield during the war.[3,99,100]

The prepositioning of chemical weapons in forward areas in case of need resulted in one major disaster and several near disasters. The one major disaster occurred 2 December 1943, when the SS *John Harvey*, loaded with 2,000 M47A1 mustard agent bombs, was destroyed after a German air raid at Bari Harbor, Italy. The only members of the crew who were aware of the chemical munitions were killed in the raid. As a result of the destruction of the ship, mustard agent contaminated the oily water in the harbor and caused more than 600 casualties, in addition to those killed or injured in the actual attack. The harbor cleanup

took 3 weeks and used large quantities of lime as a decontaminant.[101]

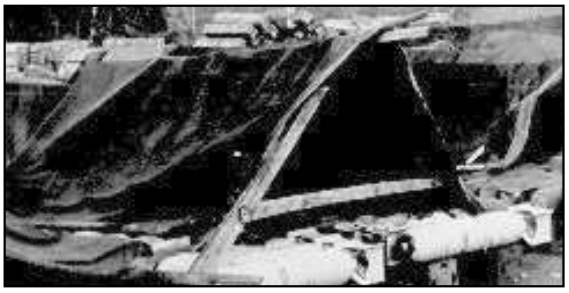


Fig. 2-34. Open storage of M47 100-lb chemical bombs on Guadalcanal Island in 1944. This important lesson is frequently forgotten: it was necessary to take along the full spectrum of chemical weaponry wherever U.S. troops were deployed. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Defensive Equipment

At the beginning of the war, the CWS designed and issued the M1 Training Mask, which used a small, lightweight filter connected directly to the facepiece. The facepiece was the first to use a fully molded rubber faceblank. The original concept of a training mask was that complete protection from all chemical agents was not required; therefore, there was no need for the state-of-the-art canisters. However, soldiers liked the new facepieces enough that the CWS standardized the M1 Training Mask as the M2 Service Mask in 1941. The mask utilized the original M1A2 mask's M9A1 canister, which was a bulky steel canister that, when combined with the facepiece, weighed 5 lb (Figure 2-35). Over 8.4 million of the M2 series masks were procured during the war, but they were used only for training.

The existing M1 and M2 series protective masks, with their molded rubber faceblanks and heavy canisters, proved a significant problem for the military. First, there was a shortage of rubber during World War II. Second, the weight of the mask with canister needed to be reduced, particularly for amphibious assaults. The continued need for a lightweight combat mask resulted in the M3 series mask. First standardized in August 1942, the M3 made several changes to the M2 design. In the facepiece, a nosecup covering the nose and mouth was added to prevent lens fogging. The canister was modified to be carried on the chest instead of the side; was much lighter (the overall weight decreased to just 3.5 lb); and had a more efficient absorbent (Figure 2-36). Eventually, over 13 million M3 series masks were procured during the war.

Fig. 2-35. The M2 series Service Mask. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fig. 2-36. The M3 series lightweight gas mask. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Production problems with the new molds, however, caused the CWS to issue the M4 series lightweight mask. This mask used a modified M2 series facepiece with a nosecup to prevent lens fogging. Only about 250,000 of the masks were produced.

By 1944, with a major invasion of Europe by U.S.forces pending, the army requested a better assault mask that was even lighter and less bulky than the M3 series. To meet this requirement, the CWS returned to the original German World War I design, which put the canister directly on the facepiece.

The result was the M5 Combat Mask, which was standardized in May 1944 (Figure 2-37). Due to the shortage of rubber, the M5 mask was the first to use synthetic rubber (neoprene) for the facepiece. This mask eliminated the hose from canister to facepiece by mounting the new M11 canister directly on the cheek. The M11 canister used ASC Whetlerite charcoal, which proved better protection against hydrocyanic acid, a chemical agent discovered in a Japanese grenade shortly after the attack on Pearl Harbor (Figure 2-38). Although the M5 weighed a mere half-pound less than the M3, more than 500,000 were procured during the war. The U.S. soldiers who landed at Normandy carried this mask with them.

Fig. 2-37. The M5 Combat Service Mask, the first U.S. mask with the canister placed directly on the cheek. The M5 mask was part of the personal equipment of the troops who landed at Normandy on 6 June 1944. Post-war tests indicated that it might have protected against respiratory exposure to the nerve agent tabun if the Germans had chosen to use it against the invasion armada. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fig. 2-38. A Japanese frangible hydrocyanic acid grenade, copper stabilized type. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

During the war, the CWS also initiated a major civil defense program to protect civilians against both chemical and biological weapons. Of particular concern were protective devices for children. With the help of Walt Disney, a Mickey Mouse gas mask was designed for children, in the hope that they would not be frightened if they had to wear it, and a tentlike protector was designed for infants. [26,30,102–104]

For collective protection, the CWS concentrated on improving the bulky M1 Collective Protector for field use. A somewhat lighter version, the M2, was standardized in 1942. It provided the same amount of air but weighed just over 600 lb. A still-lighter version, the M3, was also standardized the same year. It weighed only 225 lb and provided 50 cu ft of air per minute.[77]

The CWS also tried to improve the detection capability for toxic chemical agents, particularly blister agents. The early war efforts included the M4 Vapor Detector Kit, which could detect even faint concentrations of mustard agent; M5 liquid detector paint; M6 liquid detector paper; and the M7 detector crayon. These all proved relatively good for detecting mustard and Lewisite. The development of the M9 Chemical Agent Detector Kit in 1943 proved to be one of the most significant developments of the CWS during the war. Described in news releases as being as “effective as a modern burglar alarm,”[105] the kit consisted of a sampling pump, four bottles of reagents, and six clips of detector tubes. The kit could detect small amounts of mustard agent, phosgene, and arsenicals by color changes. It was simple to use and did not require a chemist to make the tests.

An improved version of the World War I orchard sprayer decontamination apparatus was fielded to provide ground and equipment decontamination. It could also be used for plain water showers for soldiers (Figure 2-39). For treatment of gas casualties, the CWS standardized the M5 Protective Ointment Kit. This kit came in a small, waterproof container and held four tubes of M5 Protective Ointment wrapped in cheesecloth and a tube of BAL (British anti-Lewisite) Eye Ointment. The protective ointment was used to liberate chlorine to neutralize vesicant agents on the skin. The BAL ointment neutralized Lewisite in and around the eye by changing it to a nontoxic compound. Over 25 million of the kits were procured for the army.[26,35,105]

Fig. 2-39. The 400-gal decontaminating apparatus was also used to provide water showers for the troops on Iwo Jima. Like the actual weapons, all the associated paraphernalia of chemical warfare had to go with the deployed combat forces. Useful alternative work was found for decontamination apparatuses, however, in contrast to the bombs shown in Figure 2-34. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Biological Warfare Program

The apparent use of cholera, dysentery, typhoid, plague, anthrax, and paratyphoid by the Japanese against Chinese troops finally led to an American decision to conduct research

and establish a retaliatory biological warfare capability. In response to the potential threat, in 1941 (prior to the attack on Pearl Harbor), Secretary of War Harry L. Stimson asked the National Academy of Sciences to appoint a committee to study biological warfare, appropriately named the Biological Warfare Committee. This committee did not have time to prepare before the war came. This left the army unprepared for the threat of biological warfare by Japan.

Immediately following the attack on Pearl Harbor, the army's Hawaiian Department took special precautions against biological attack by both external enemies and local residents. Guards were placed on the water supplies in Hawaii to protect against sabotage by biological warfare, and daily checks for chlorine content were made. Food production plants were also guarded, and drinking fresh (but not canned) milk, in particular, was banned. A general order was issued prohibiting the sale of poisons to the general public except under special circumstances.

In February 1942, the Biological Warfare Committee recommended that the United States should take steps to reduce its vulnerability to biological warfare. In response, Secretary Stimson recommended to President Roosevelt that a civilian organization should be established to accomplish the mission. After the president approved the plan, the War Research Service (WRS) was formed in August 1942 under the leadership of George W. Merck, president of Merck Company, a pharmaceutical company. The WRS was only a coordinating committee attached to the Federal Security Agency; it used existing government and private institutions for the actual work. It drew its scientific information from a committee of scientists from the National Academy of Sciences and the National Research Council.

In December 1943, U.S. intelligence reports predicted that Japan might use biological warfare. At the same time, tests indicated that masks made in the United States gave poor protection against simulated biological agents. In response to these threats, the CWS (1) developed a special outlet-valve filter for the masks and (2) rushed delivery of some 425,000 under special security conditions to the island of Saipan in case biological warfare actually started.

In January 1944, the complete biological warfare program was transferred from the WRS to the War Department, and the WRS was abolished. The War Department divided the biological warfare program between the CWS and The U.S. Army Surgeon General. The CWS took responsibility for agent research and production, foreign intelligence, and defensive means. The Surgeon General was to cooperate with the CWS on the defensive means. Merck, the former leader of the WRS, became a special consultant to the program.

This arrangement was modified in October 1944, when the secretary of war established the U.S. Biological Warfare Committee with Merck as the chairman. The CWS assigned the biological warfare program to its Special Projects Division. At its peak, this division had 3,900 army, navy, and civilian personnel working on various programs.[26,106]

Initially, the army's biological warfare program was centered at Edgewood Arsenal. In April 1943, Detrick Air Field near Frederick, Maryland, was acquired by the CWS and was activated as Camp Detrick. Four biological agent production plants were started at Camp Detrick to meet the army's needs (Figure 2-40). Pilot Plant No. 1, activated in October 1943 for the production of botulinum toxin, was located in the Detrick Field hangar. Pilot Plant No. 2, completed in March 1944, produced the anthrax

simulant *Bacillus globigii* and actual anthrax spores. Pilot Plant No. 3, completed in February 1945, produced plant pathogens. Pilot Plant No. 4 was completed in January 1945 and produced, in embryonated eggs, the bacteria that cause brucellosis and psittacosis (Figure 2-41). Additional smaller pilot plants were set up to explore the many other antipersonnel, antianimal, and antiplant agents examined in Camp Detrick's laboratories.

Fig. 2-40. The first biological warfare agent laboratory at Camp Detrick, Md. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The existing Vigo Ordnance Plant near Terre Haute, Indiana, was also acquired by the CWS in 1944 for conversion into a biological agent–and weapon–production plant. This plant was divided into four main subplants: agent production, munitions assembly, munition packaging and storage, and the animal farm. Although the plant was considered ready to produce biological agents by the summer of 1945, none were actually produced.

Fig. 2-41. Camp Detrick, Md., 16 July 1945. These technicians at the Egg Plant are disinfecting and drilling eggs prior to inoculating them with *Brucella suis* or *Chlamydia psittaci*, the bacteria that cause brucellosis and psittacosis. Viral agents such as Venezuelan equine encephalitis virus were also produced in eggs. This pilot facility had incubator capacity for approximately 2,000 chicken eggs. Depending on the agent being produced, eggs were incubated for approximately 1 to 10 days between inoculation and harvest. The work was done by hand, in assembly-line fashion, with little mechanical assistance. Preparing biological warfare agents in this manner is a labor-intensive process. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Weaponization of biological agents made tremendous progress, considering that the CWS started from nothing. Anthrax was considered the most important agent. Although no dissemination of anthrax in a weapon was accomplished in the United States before the end of the war, anthrax simulant was tested in large 100-lb and 115-lb bombs, and small 10- lb bombs, shotgun shell (SS) bombs, and the 4-lb SPD Mk I bomb. The smaller bombs, suitable for use in larger cluster bombs, proved the most successful in static tests. Only the SPD Mk I bomb was considered ready for production, and the first and apparently only large-scale munition order was placed at Vigo in June 1944 for production of 1 million of the bombs. The order was canceled with the end of the war.

The U.S. Biological Weapons Program also targeted German and Japanese vegetable crops. Tests of anticrop bombs included using spores of brown spot of rice fungus and 2,4-dichlorophenoxyacetic acid (known as VKA, for vegetable killer acid) in the SPD Mk II bomb and a liquid (VKL, for vegetable killer liquid) in the M-10 spray tank. Scientists also worked on defoliants in the program.[3,106]

In 1944 and 1945, there was a sudden interest in the possibility that Japan was attempting to attack the United States by placing biological agents on balloons that then floated across the ocean. In fact, some 8,000 to 9,000 balloons were launched by Japan against the United States, however, those recovered in the United States contained only high-explosive and incendiary bombs meant to start forest fires. These balloons continued to turn up several years after the war.[26]

U.S. Chemical Warfare Policy

President Roosevelt established a no-first-use policy for chemical weapons early in the war. In 1943, this was reiterated in an official statement: “We shall under no circumstances resort to the use of such [chemical] weapons unless they are first used by our enemies.”[107(p6)] The policy was backed up by a statement of warning:

> Any use of gas by any axis power, therefore, will immediately be followed by the fullest possible retaliation upon munition centers, seaports and other military objectives throughout the whole extent of the territory of such axis country.[107(pp6–7)]

Neither Germany nor Japan chose to initiate chemical warfare with the United States. The CWS spent the war training troops; designing chemical, incendiary, smoke, high explosive, and flame weapons, and protective equipment; and planning for a chemical war that never occurred. It was a tremendous “just-in-case” effort.

Toward the end of the war with Japan, the combination of President Roosevelt’s death, the extremely costly battles of Iwo Jima and Okinawa, and the planned invasion of the Japanese homeland led the army to look at the possibility of initiating chemical warfare to save American lives. One such proposal began:

> Our plan of campaign against the Japanese is one which we think will bring the war against Japan to the quickest conclusion and cut our cost in men and resources to the minimum. Japan’s complete defeat is assured providing we persevere in this plan, the only question remaining being how long the war will last and what the cost will be of achieving final victory. These questions will be answered not alone by the tactics employed in the execution of the plan but also by the weapons used. Gas is the one single weapon hitherto unused which we can have readily available and which assuredly can greatly decrease the cost in American lives and should materially shorten the war.[108]

The proposal concluded by recommending that the president change the policy on no first use of chemical weapons and coordinate the plan with the British and Russians.[108]

The senior staff, however, concluded that chemical warfare would only complicate the invasion of Japan and would not be a decisive weapon. In addition, coordinating and preparing America’s allies for chemical warfare were also perceived as major problems. The use of the atom bomb in 1945 effectively ended the discussion.[45,109]

Lessons Learned

The U.S. Army learned several lessons from this nongas war that the CWS followed. Although perhaps more a finger-pointing exercise, the phrase “had the United States been prepared for war in 1939, there would not have been a war”[110(p24)]was taken as a self-evident truth. The CWS needed to be a permanent organization that concentrated on training, research and development, and chemical warfare preparedness. This same lesson, from a slightly different angle, was reflected in the words of Under Secretary of War Kenneth C. Royall to the chemical warfare specialists, “The better job you do the less likely it is that you will have to put to actual use the products of your work.”[111(p41)]

Demobilization and the Creation of the Chemical Corps

The army began demobilization activities almost immediately on the president’s proclamation of the end of hostilities. By early 1946, the CWS was effectively demobilized, and its military strength approached prewar levels. One observer commented: “Gas warfare

is obsolete! Yes, like the cavalry and horsedrawn artillery, it is outmoded, archaic, and of historical interest only. This is the atomic age!”[112(p3)]

To preserve the CWS from total disintegration, Major General Porter, the chief of the CWS, made a vigorous advocacy of the distinctive character and important role of the CWS before an army board considering postwar organization. The result was the permanency long sought by the chemical program, a corps designation. The army finally agreed that the CWS, along with the other Technical Services, should continue its existence as a distinct entity in the peacetime army. On 2 August 1946, Public Law 607 changed the name of the CWS to the Chemical Corps.[113]

After World War II, as western defense became increasingly based on the threatened use of nuclear weapons, the Chemical Corps’s mission expanded to include radiological protection as well as chemical and biological research and development. At the same time, the Corps concentrated on producing and fielding nerve agent weapons and the assorted detection and decontamination equipment required.

Major General Alden H. Waitt, who replaced Porter in November 1945, assessed the future of chemical warfare in 1946:

> The fact that toxic gas was not used in the late war does not justify a conclusion that it will not be used in the future. Gas has not been outmoded as a weapon. The Germans developed new gases during World War II. The magnitude of their preparedness for gas warfare is indicated by the fact that they had amassed more than a quarter of a million tons of toxic gas; their failure to use this gas against us is attributable largely to their fear of our retaliatory power. We cannot count upon other nations refraining from the use of gas when it would serve their purpose. There were numerous instances in the late war in which the use of gas might have had far-reaching results. Thus, there is no good reason for assuming that the considerations which prevented the employment of gas in World War II will prevail in the future.[114]

On the topic of biological warfare, he acknowledged it as a new field that still required much work:

> The tremendous potentialities of biological warfare in the future demand that the necessary tactics and employment in the field be worked out well in advance so that such means may be used immediately and effectively once a decision to do so is made. It is essential that Chemical Officers on the staffs of divisions and higher units, including equivalent Army Air Force elements, be in a position to advise their Commanders relative to the capability, limitations and means of protection against this new method of attack. Further, they must be able to prepare suitable offensive and defensive plans and to supervise such training of troops in these methods as may be required.[114]

Demilitarization of Captured Weapons

Following the occupation of Germany and Japan, the Allies initiated a sea-dumping and weapons disposal program to eliminate the large stockpiles of captured chemical agents (Figure 2-42). Operation Davy Jones Locker involved sinking ships that contained German weapons in the North Sea. However, not all the German weapons were destroyed. Between 1945 and 1947, over 40,000 of the 250-kg tabun bombs, over 21,000 mustard bombs of various sizes, over 2,700 nitrogen mustard rockets, and about 750 tabun artillery shells of various sizes were shipped to the United States. In addition to disposing of the enemy stockpiles, the United States also dumped the U.S. Lewisite stockpile into the sea during Operation Geranium in 1948.[3,115]

Fig. 2-42. Dumping weapons into the sea was not the Allies' only method of disposing of them. These 150-mm German nitrogen mustard (HN-3) rockets are wired with prima cord for destruction. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Post–World War II Developments

Although the late 1940s was not a time for many dramatic developments, the Chemical Corps was able to issue a new gas mask in 1947. Designated the M9 series, it was an improved version of the M5 mask (Figure 2-43). This mask utilized a superior synthetic rubber composition that worked better in cold weather than the neoprene of the earlier mask.[30]

In 1948, the army partially standardized sarin and the year after, tabun. In 1948, the army also issued a new circular[116] on G-series nerve agents and a technical bulletin[117] on the treatment of nerve agent poisoning.[3] The circular provided current information on detection, protection, and decontamination of nerve agents. For detection, the M9 and the improved M9A1 detection kits, standardized in 1947, could detect vapor after a complicated procedure:

> To make test, tear off lead wrapper and heating pad. Insert blue dot end of the glass tube into pump. Slowly take 25 full pump strokes. Remove from pump, and heat tube with matches or cigarette lighter for about 5 seconds. (Avoid excessive heating of tube, since this will char contents of the tube and invalidate the test results.) After tube is cool, add liquid from blue bottle to unmarked end of the tube. If gas is present, a blue ring will form in the upper end of the tube.[116]

For droplets, the M5 detector paint and the M6 detector paper both turned from olive green to red. None of the detectors provided any advance warning, and all merely confirmed the presence of the agents after the fact.

For protection, the circular simply stated that the current mask “gives protection for the eyes and respiratory tract and for the skin covered by the facepiece.”[116] Additional required items were impermeable clothing worn over a layer of ordinary clothing, rubber boots, and rubber gloves.

Decontamination of nerve agents was still a problem. DANC was not suitable. Bleach slurry and dilute water solutions of alkalies were reported as effective decontaminants. Hot soapy water was also recommended, while cold water only partially decontaminated the agents.[116]

The technical bulletin [117] pointed out that contamination by nerve agents could come via breathing the vapors or body contact with the liquid, and that death could occur in a few minutes. The bulletin then outlined the suggested treatment for exposures:

Fig. 2-43. The M9 series gas mask. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

> The treatment of poisoning is based essentially upon the blocking of excessive nervous activity, due to the direct effects of the poison and to apprehension, fear, physical activity, and external stimuli. Quiet, reassurance, and gentle handling of the casualty are therefore essential. Atropine sulfate (1.0 mg) should be given by intravenous or intramuscular injection very promptly. This effectively blocks the excessive activity of smooth muscle and glands, and also controls convulsions.[117]

However, should this not work:

> In the event of impending respiratory failure, all drug therapy may be ineffective. Under such circumstances, artificial respiration may prove to be the only life-saving procedure.[117]

How artificial respiration could be conducted in a contaminated environment was not addressed.

Beginning of the Cold War

The declining relations with the Soviet Union caused that country to become the number one intelligence target for chemical warfare preparations. Intelligence reports noted with alarm that toward the end of World War II, the Soviets had captured a German nerve agent production facility and had moved it back to their country.[15,118]

Other studies described the Soviets as ready to conduct chemical attacks should open warfare break out. In 1949, Waitt reported:

> Intelligence reports indicate extensive preparation for gas warfare by the USSR with current Soviet superiority over the U.S. in this field as to stockpiles of gas munitions, currently operating war-gas plant capacity, and Soviet ability to maintain this superiority for at least 12 months after the start of hostilities, assuming the U.S. gas warfare position is not improved prior to M-Day.[118]

His recommendations were to increase chemical training, replace the aging World War II–era equipment and munitions, and then achieve a much higher state of readiness.[118]

One of the first Cold War actions that involved the Chemical Corps was the Berlin Blockade in 1949. Cold weather caused frost to build up on the airplanes flying to Berlin with supplies. The accepted method of ice removal was to use brooms to sweep the ice off. This slow and dangerous work was replaced by the corps's using decontamination trucks to spray isopropyl alcohol, which was used as a deicer since glycerin was not readily available. A large plane could be deiced in about 5 minutes, and the corps was credited with keeping the airplanes from being delayed by frost.[119]

THE 1950s: HEYDAY OF THE CHEMICAL CORPS

Korean War

In June 1950, with the onset of the Korean War, the Chemical Corps participated in its first military action. The corps quickly implemented an increased procurement program to supply the army with a retaliatory chemical capability and defensive equipment. Major General Anthony C. McAuliffe, the new Chief of the Chemical Corps, concluded that this ability was the number one lesson learned from World War II:

> It required the experiences of World War II to demonstrate that the most important basic factor in a nation's military strength is its war production potential and ability to convert smoothly and quickly its industry, manpower, and other economic resources.[120(p284)]

Within a short time, however, the army's policy on chemical warfare and the lessons learned from the past were hotly disputed, particularly as the military situation in Korea changed. First, the Chemical Corps lost its high-visibility ground weapon, the 4.2-in. chemical mortar. Responsibility for research, development, procurement, storage, issue, and maintenance of all 4.2-in. mortars and ammunition was transferred to the Ordnance Department on 31 December 1947 by order of the Chief of Staff, Department of the Army. The exception was the responsibility for chemical fillings for mortar shells, which remained with the Chemical Corps. This event represented the end of the Chemical Corps's role in the development of the 4.2-in. chemical mortar. In 1951, the Ordnance Department completed the development of a new 4.2-in. (later designated the 107-mm) mortar, the M30, to replace the M2. The loss of the 4.2-in. mortar moved the Chemical Corps away from being a combat arm and left it a combat support arm. [121]

The action in Korea also brought up the subject of whether to initiate chemical warfare to save lives. Many of the Chemical Corps's supporters favored the use of chemical weapons as humane weapons of war, particularly to offset the enemy's superior numbers. One writer, upset with negative public opinion toward chemical weapons and the army's policy of retaliation only, wrote:

> Has this concept and this attitude been reflected in our military planning and our military preparations? If, in an effort to "make the most" of our military expenditures we have failed to stock up to the fullest requirements in the matter of toxic weapons on the premise that such weapons "might not be used again, as they were not used in World War II," we may have made a major military decision on the basis of a fatally unsound assumption.[122(p3)]

Another officer stated it much more bluntly:

> The use of mustard, Lewisite and phosgene in the vast quantities which we are capable of making and distributing offers the only sure way of holding Korea at the present time. We are not playing marbles. We are fighting for our lives. Let's use the best means we have to overwhelm the enemy scientifically and intelligently.[123(p3)]

Again, however, neither side chose to initiate chemical and biological warfare and the corps supported the war through its many other programs, particularly smoke and flame. Much as it had done during World War II, the United States did not change its policy about no first use of chemical weapons.

Although there were allegations by the North Koreans and the Chinese that U.S. forces employed chemical and biological weapons on the battlefield, the Chemical Corps apparently did not use such weapons. The corps did, however, use riot control agents to quell riots of prisoners of war. In 1968, a Czech general defected to the United States and reported that U.S. prisoners of war were used for biological tests by the Russians in North Korea. These allegations have yet to be confirmed by the Russians and were vigorously denied by the North Koreans. [124]

The Chemical Corps ended the Korean War in a much stronger position than it faced after the end of World War II. The corps reduced its units and manpower somewhat, and terminated many of its procurement contracts in the months following the 1953 armistice. Still, Major General Egbert F. Bullene, the new Chief Chemical Officer summed up the feeling of the corps about the Korean War and the Cold War in general: "Today, thanks to Joe Stalin, we are back in business." [125(p8)]

Changes in the Chemical Corps

During the 1950s, the concept of warfare, and chemical and biological warfare continued to change radically. The phrase that one could "push a button" to start a war became exceedingly popular. The lesson learned from the Korean War—the concept of a limited war, fought without nuclear weapons and possibly against satellite states, not the "real enemy"—determined much of the army's planning. The fact, however, that two wars had come and gone without the employment of chemical and biological weapons made it necessary for successive Chief Chemical Officers to work continually to remind the army and the country that this might not be the case again, and that the capabilities of the Chemical Corps constituted insurance against the possibility of chemical or biological attack in the future.

Throughout the 1950s, the corps conducted several extensive studies to change its organization and improve its training capabilities. One significant improvement was the activation of a new training center at Fort McClellan, Alabama, in 1951, which offered more space and training options. The Chemical School, after more than 30 years in Maryland, moved there early in 1952.[3]

The emphasis on individual training for chemical and biological warfare resulted in the elimination of the unit gas officers in 1954. Originally, an officer or noncommissioned officer

had been responsible for chemical and biological training and readiness. With this change, the troop commanders assumed the responsibility and were expected to include chemical and biological training in all their field exercises and maneuvers.[126]

Nerve Agent Production and Development

In 1950, the Chemical Corps began construction of the first full-scale sarin production complex based on pilot plant work accomplished at the Army Chemical Center, which had formerly been called Edgewood Arsenal (Exhibit 2-1). The production of sarin was a five-step process that was divided between two sites. For the first two steps of the process, the corps constructed a plant at Muscle Shoals, Alabama, later designated Site A or the Muscle Shoals Phosphate Development Works, which was completed in 1953. The last three steps of the process were conducted at a new plant at Rocky Mountain Arsenal, Colorado. In 1951, the corps fully standardized sarin and by 1953 was producing the agent. After only 4 years of production, the plants stopped manufacturing since the stockpile requirements for the agent had been met. The plants then went into inactive status with layaway planned. The related munitions filling plants also went into standby status a year later.[3,127]

EXHIBIT 2-1
RECENT NAME CHANGES OF EDGEWOOD ARSENAL

25 Oct 1917	Construction begun on a shell-filling plant called Gunpowder Neck Reservation
2 Apr 1918	Gunpowder Neck Reservation designated Gunpowder Reservation
4 May 1918	Name changed from Gunpowder Reservation to Edgewood Arsenal
10 May 1942	Name changed from Edgewood Arsenal to Chemical Warfare Center
2 Aug 1946	Name changed from Chemical Warfare Center to Army Chemical Center
1 Jan 1963	Name changed from Army Chemical Center to Edgewood Arsenal
1 July 1971	Edgewood Arsenal discontinued as a separate installation and designated Edgewood Area, Aberdeen Proving Ground

Part of the reason for the shut down of the sarin plant was the development of a new nerve agent. Chemists at Imperial Chemicals, Ltd., in the United Kingdom, while searching for new insecticides, came across compounds that were extremely toxic to humans. The British shared the discovery with the United States in 1953. The Chemical Corps examined the new compounds and determined that a new series of nerve agents had been discovered that were more persistent and much more toxic than the G-series agents. This new series was designated the V-series agents in 1955, because they were “venomous” in nature. These agents would enter the body through the skin, thereby bypassing the protective mask. They were 1,000-fold more toxic than sarin when applied to the skin, and 2- to 3-fold more toxic when inhaled. A drop the size of a pinhead on bare skin could cause death within 15 minutes.[3,128]

The Chemical Corps gave top priority to the investigation of these compounds. Of the compounds investigated, VX was selected in 1957 for pilot plant development and dissemination studies. It was standardized in December 1957. The annual report for that year concluded: “The reign of mustard gas, which has been called the King of Battle gases since it was first used in July 1917, will probably come to an end.”[129(p100)]

The initial plan was to contract with private industry for a 10-ton per day production plant. A later decision put the plant at the inactivated Dana Heavy Water Plant of the Atomic Energy Commission at Newport, Indiana, within the Wabash River Ordnance Works. A patent dispute that resulted in a restraining order by the Chief Justice of the United States

and problems with contractors visiting the new site delayed construction. Finally in 1959, Food Machinery and Chemical Company, the low bidder, got the contract and construction was planned for 1960. Shortly after the approval, the Chemical Corps supplemented the contract to provide for a VX weapon-filling plant.[129,130]

Chemical Weapons

During the 1950s, the Chemical Corps concentrated on the weaponization of sarin. For air delivery, the first items standardized in 1954 were the 1,000-lb M34 and M34A1 cluster bombs (Figure 2-44). These clusters held 76 M125 or M125A1 10-lb bombs, each containing 2.6 lb of sarin (Figure 2-45).

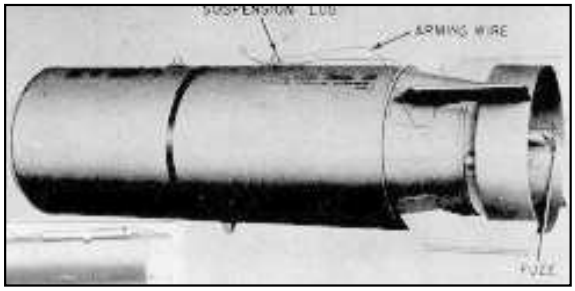


Fig. 2-44. The M34 series sarin cluster bomb was the first major nerve agent bomb standardized by the U.S. military after World War II. Photographs: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

In 1959, the Chemical Corps standardized the first nonclustered bomb, designated the MC-1 750-lb sarin bomb. This was a modified general purpose demolition bomb, suitable for high-speed aircraft, and held about 215 lb of sarin filling. For ground delivery, the Chemical Corps standardized the M360 105-mm and the M121 155-mm shells in 1954. The smaller shell held about 1.6 lb of agent and the larger about 6.5 lb.[3,130]

Although delivery systems for VX nerve agent were initiated during the 1950s, no system was standardized. In addition, many of the sarin delivery systems took longer to develop than planned and some were never standardized.

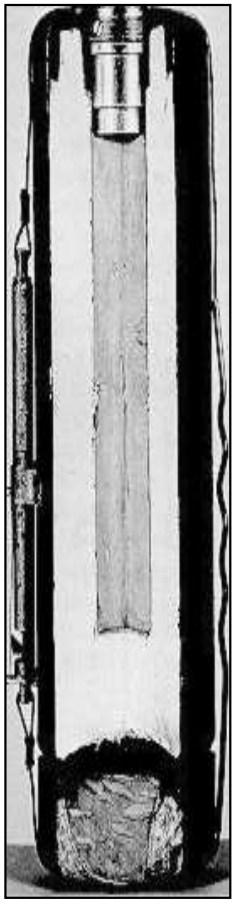


Fig. 2-45. The M125 series sarin bomblet, which was contained in the M34 cluster bomb. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Biological Agents

During the 1950s, the biological warfare program was one of the most highly classified programs, owing to its nature and the ongoing Cold War, and many of the details of the program have never been declassified. The corps concentrated on standardizing the agents investigated during World War II and weaponizing them at Fort Detrick, the Chemical Corps biological warfare center. The highest priority was placed on the antipersonnel agents, as the antianimal and antiplant programs both experienced major disruptions during the decade.

A number of antipersonnel agents were standardized during the early 1950s, but in 1953, Major General Bullene, Chief Chemical Officer, gave an overriding priority to the development of anthrax, which had also been the highest-priority agent during World War II.

One of the more interesting stories was the standardization in 1959 of the yellow fever virus for use, with a mosquito as vector. The virus came from an individual in Trinidad who had been infected with the disease during an epidemic in 1954. Scientists inoculated rhesus monkeys with the serum to propagate the virus. In tests conducted in Savannah, Georgia,

and at the Avon Park Bombing Range, Florida, uninfected mosquitoes were released by airplane or helicopter. Within a day, the mosquitoes had spread over several square miles and had bitten many people, demonstrating the feasibility of such an attack. Fort Detrick's laboratory was capable of producing half a million mosquitoes per month and had plans for a plant that could produce 130 million per month.[130]

Fort Detrick, however, was limited in its production capability and required an expanded facility. Since the World War II–era Vigo Plant, inactivated in the postwar years, was not reopened (and was eventually sold in 1958), Pine Bluff Arsenal was selected to be the site of the new biological agent production plant. The plant was designated the X-201 Plant, later renamed the Production Development Laboratories, and was completed in 1954. This plant could produce most of the agents standardized by the Chemical Corps, and could fill bombs within 4 days after receipt of an order.

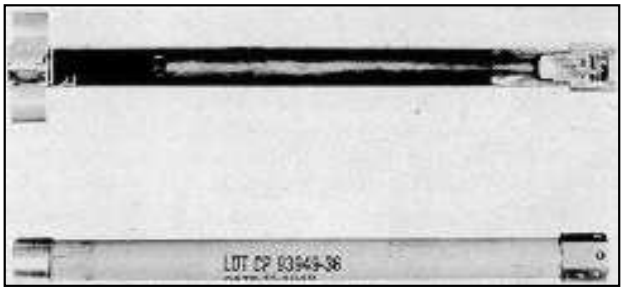
The antianimal program started off strong in 1952 when the Chemical Corps activated Fort Terry, on Plum Island, New York, to study animal diseases. In 1954, however, the army terminated all antianimal agent work with exception of rinderpest and the completion of the foot-and-mouth disease research facility. The Department of Agriculture then took over the defensive aspects of the antianimal program, including Fort Terry, the same year.

The antiplant program made some progress when, in 1955, wheat stem rust became the first antiplant pathogen standardized by the Chemical Corps for use primarily against cereal crops. Additional antiplant agents were standardized shortly thereafter. In 1957, however, the army ordered the corps to stop all antiplant research and development since the air force, primarily, would be delivering the agent. This was accomplished by 1958 with the termination of the program. Then the decision was reversed the next year after additional funding was found. Fort Detrick had to restart the program, which delayed any significant accomplishments for some time. Fort Detrick also began to concentrate more on the chemical defoliants, conducting the first large-scale military defoliation effort at Fort Drum, New York, using the butyl esters of 2,4-D and 2,4,5-T, later designated Agent Purple. [3,131]

Biological Weapons

Although many biological agents were standardized and many delivery systems developed, only a few biological weapons were standardized. The first was the M114 4-lb antipersonnel bomb, which held about 320 mL of *Brucella suis* (Figure 2-46). This was a small, 21-in.-long tube with a 1 5 /8-in. diameter, similar to a pipe bomb. One hundred eight of the M114s were clustered in the M33 500-lb cluster bomb (Figure 2-47). The bombs were also tested at Dugway Proving Ground, Utah, throughout the 1950s with various other fillings.

The M115 500-lb antiplant bomb was standardized in 1953 for the dissemination of wheat stem rust. This filling consisted of dry particulate material adhered to a lightweight, dry carrier (ie, feathers). Thus, the bomb was normally referred to as the feather bomb.



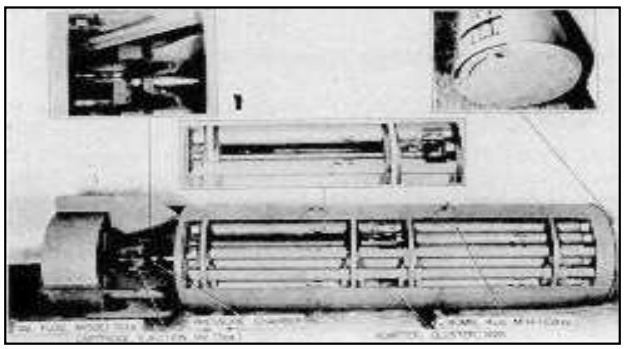


Fig. 2-46. The M114 4-lb biological bomb was the first biological weapon standardized by the U.S. military. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fig. 2-47. The M33 500-lb biological cluster bomb, which held 108 of the M114 bombs. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

There were numerous other experimental delivery systems. The E61R4 half-pound antipersonnel bomb held only about 35 mL of agent, but four of the little bomblets produced twice the area coverage of one M114. The E133R3 750-lb cluster bomb held 544 bomblets.

Copying the method the Japanese developed during World War II, the Chemical Corps developed the 80-lb antiplant balloon bomb. The bomb itself was a cylinder 32 in. in diameter and 24 in. high that served as the gondola of the balloon. Inside the insulated gondola were five agent containers, each holding feathers and an antiplant agent. The agent containers were grouped around a chemical-type heater. A barometric and mechanical time mechanism opened the gondola at a preselected altitude, releasing the agent.

Other delivery systems included spray tanks, missiles, aerosol generators, drones, and marine mines. Of these, the submarine mine was one of the more covert forms of delivery. It was designed to be fired from a torpedo tube, to sink to the bottom for a specified period up to 2 hours, and then rise to the surface and expel about 42 L of agent. After dissemination of the agent, it scuttled itself. [3,132]

Although both simulants and small amounts of live agents were used in open-air testing during the 1950s, for sheer size, Operation Large Area Coverage (LAC) covered the largest geographical area. To test the feasibility of contaminating a large area of the continent with biological organisms, in 1957 the Chemical Corps dropped a myriad of microscopic fluorescent particles of zinc cadmium sulfide along a path from South Dakota to Minnesota. In the first test, the air stream turned north and took the bulk of the material into Canada. Still, a test station in New York was able to detect the particles. In the second test in 1958, the particles were carried into the Gulf of Mexico. Special collectors were located at 63 Civil Aeronautics Authority sites and 112 Weather Bureau stations. Over 2,200 samples were mailed back to the corps from these sites.

Two additional tests covered from Ohio to Texas, and from Illinois to Kansas. All demonstrated that the particles were widely disseminated. Although it had been only theoretical prior to this test, Operation LAC provided the first proof that biological agents were indeed potential weapons of mass destruction.[129]

Medical Research on Human Volunteers

The Chemical Corps's concern with the effects of nerve and other chemical agents on soldiers led to extensive studies to determine the dangers of exposure and the proper kinds of treatment. This program exposed soldiers to low levels of agents to demonstrate the effects of treatment and to answer questions about how agents affect humans.

Prior to the 1950s, the use of humans in testing had been conducted on a somewhat ad hoc basis, with little documentation surviving. A more-formal volunteer program was established at the Army Chemical Center during the 1950s. This program drew on local military installations and utilized a specific consent procedure that ensured that each volunteer was prebriefed and was truly a volunteer in the experiment. Between 1955 and 1975, over 6,000 soldiers participated in this program and were exposed to approximately 250 different chemicals. [133]

Although biological agents had been tested on animals, the question arose as to whether the same agents would be effective on humans. In 1954, the Chemical Corps received permission to use human volunteers in the evaluation of biological agents. The plan to assess the agents and vaccines, which was approved by both the U.S. Army Surgeon

General and the Secretary of the Army, was produced at Fort Detrick. A medical school under contract conducted most of the investigation. By 1955, the corps had tested many of the known agents on the volunteers in laboratory situations.

The army, however, also wanted to know the effects of biological agents in natural settings. After receiving approval from the secretary of the army, the first open-air test was conducted at Dugway Proving Ground, Utah, where 30 volunteers were exposed to an aerosol containing *Coxiella burnetii*, the rickettsia that causes Q fever. These open-air tests gave valuable data on the infectivity of biological warfare agents. [134,135]

The Incapacitant Program

During the 1950s, the Chemical Corps became interested in developing chemical weapons that incapacitated rather than killed its targets. In 1951, the corps awarded a contract with the New York State Psychiatric Institute to investigate the clinical effects of mescaline and its derivatives. The contractor tested 6 derivatives, while the corps tested 35 derivatives. The results of the investigation indicated that mescaline and its derivatives would not be practical as agents, because the doses needed to bring about the mental confusion were too large. [131]

In 1955, the Chemical Corps formally established a new project called Psychochemical Agents. The next year, the program was redesignated K-agents. The objective was to develop a nonlethal but potent incapacitant that could be disseminated from airplanes in all environments. The program was conducted at the Army Chemical Center and examined nonmilitary drugs like lysergic acid (LSD) and tetrahydrocannabinol (related to marijuana). None of these drugs, however, were found to be of military worth.[129,131,134,135]

New Protective Equipment

The changing need for protective equipment created by the new threats of chemical, biological, and radiological warfare was reflected in 1951, when the Chemical Corps officially changed the name of all its gas masks to “protective masks.” The M9A1 mask, standardized the same year, was the first to be so designated.

Starting in 1952, the Chemical Corps began work on a new mask to replace the M9 series. The corps wanted a mask that was more reliable, suitable for any face size and skin texture, and more comfortable in any climate. Utilizing previous work on canisterless civilian masks and an earlier military prototype, Dr. Frank Shanty, a young engineer assigned to the Army Chemical Center, thought of the concept for a new mask on a late-night train to Cincinnati, Ohio. The final result was the M17 Protective Mask, the first canisterless military mask, which was standardized in 1959. The new mask eliminated the problem of having left- and right-handed masks, weighed less, and had reduced breathing resistance.[30]

Other mask work included the first tank mask, the M14, standardized in 1954 as part of the M8 3-Man Tank Collective Protector. In 1959, the corps standardized an improved head-wound mask, designated the M18, that allowed soldiers with head wounds to wear protective masks in contaminated environments.[3]

Chemical Agent Detection

The inability to instantly detect nerve agents and sound an alarm to alert surrounding troops was the primary concern of the Chemical Corps during the 1950s. Many detector kits from World War II were updated to improve detection of nerve agents, but these only provided confirmation, without providing advance warning.

The M5 Automatic G-Agent Fixed Installation Alarm, standardized in 1958, was the first detector and alarm for G-series agents. The unit could detect a G-series agent and sound

an alarm in about 10 seconds. Unfortunately, the unit was 7 ft high and 2 ft square. It was not suitable for the field and was primarily used at Rocky Mountain Arsenal in sarin production and filling plants.[130]

The M6 Automatic G-Agent Field Alarm, standardized also in 1958, was the first automatic electronic alarm for the detection of G-series agents for field use. Owing to various problems, the alarm was primarily used by the navy for dock monitoring. The alarm was contained in a 24-lb aluminum case approximately 7 in. wide by 15 in. high. The operation of the alarm was based on the color formed when any G-series agent came into contact with a combined solution of o-dianisidine and sodium pyrophosphate peroxide. Design of the alarm provided that a drop of this combined solution was placed on a paper tape, which was moved (every 5 min) under two sampling spots, one of which sampled ambient air while the other acted as a monitor to minimize the effects of variations in light reflected from the paper and fluctuations in electronics. The two spots on the paper were viewed by two balanced photo cells. If color developed on the sample side, unbalance occurred between the cells and the buzzer alarm triggered. As designed, it would function continuously unattended for a 12-hour period, at which time it required fresh solutions and new tape. One problem with the alarm was that it did not function at temperatures below 32°F and therefore was not what the army needed. [134,136]

A secondary approach to detection and alarm was the beginning of the remote sensing capability. In 1954, the Chemical Corps began development of a small, simple alarm commonly called LOPAIR (**lo**ng-**pa**th **i**nfra**r**ed) (Figure 2-48). The principle behind the operation of this device was that the G-series agents absorb certain portions of the infrared spectrum. Such a device would scan the atmosphere continuously in advance of troops and sound a warning alarm when G-series agents were spotted. The prototype performed satisfactorily up to about 300 yd, but it weighed over 250 lb and used too much electrical power. An improved version reduced both the weight (to 34 lb) and the power consumption. Its response time was 3 to 10 seconds. A third version combined the best of each unit, with a slight increase in weight but less power consumption, and a range of a quarter mile. Although the corps worked continuously on this approach, it would not come to completion for another 40 years.[135]

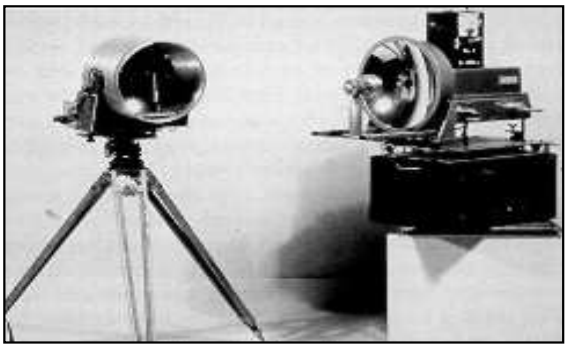


Fig. 2-48. A prototype ***l***ong-***pa***th ***i***nfra***r***ed (LOPAIR) alarm, the E33 Area Scanning Alarm consisted of an infrared source, optical reflector, optical collecting system, grating monochromator, and associated electronics. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Decontamination

Although the Chemical Corps concentrated on nerve agent programs during the 1950s, there was one significant improvement for mustard agent decontamination. In 1950, the corps standardized super tropical bleach (STB) as the best decontaminant for persistent agents. The new bleach was more stable in long-term storage, particularly in temperature extremes, and was easier to spread from a decontaminating apparatus, owing to its more uniform consistency.[137]

Treatment for Nerve Agents

As a result of the introduction of nerve agents, the Chemical Corps added atropine, an antidote to G-series agent poisoning, to the World War II M5 Protective Ointment Set by replacing one of the four ointment tubes. The modified kit was designated the M5A1 Protective Ointment Set in 1950.

Since atropine had to be circulated by the blood stream to overcome the effects of the G-series agent, it was packaged in syrettes, small collapsible metal tubes filled with a solution of atropine and fitted with a hypodermic needle at one end. A soldier was required to jab the needle into his thigh muscle and force the atropine out by squeezing the tube. Later in the decade, the M5 ointment was also found to be effective against V-series agents.[74]

Many soldiers, however, reportedly were afraid to stick a needle into themselves. Therefore, a new injector was developed: an aluminum tube, about the size of a small cigar, containing a spring-driven needle and cartridge containing atropine solution. The soldier had simply to push the tube against his thigh and pull a safety pin, and the spring drove the needle into his leg. The new kit was standardized in 1959 as the M5A2 Protection and Detection Set. [130]

The recognized need for respiratory support for the apneic victim of nerve agent exposure resulted in the development of a resuscitation device that could be attached to the M9 series of protective masks (Figure 2-49).

Fig. 2-49. With the advent of nerve agents and the recognition that they cause respiratory paralysis, the army saw the need to develop first-aid methods capable of providing artificial ventilation on the battlefield. The M28 mask-to-mouth resuscitator was one such development. It consisted of three parts: (1) a hose, which connected the casualty and the rescuer; (2) a modified M9A1 protective mask; and (3) an anesthetist-type oronasal mask. The expiratory valve of the M9A1 mask was removed and replaced by the hose. The rescuer inhaled through a standard canister (out of sight in the photograph) and exhaled into the hose. Positive pressure in the hose connecting the casualty and the rescuer opened a double-acting, demand-type, inlet-and-expiration valve in the oronasal mask. This allowed the rescuer's exhaled breath to enter the casualty's lungs. The second canister (seen on the casualty's chest) protected the casualty from inhaling contaminated air when he began to breathe sponaneously. Note that none of the soldiers in this staged photograph are protected against skin absorption. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The Growing Soviet Threat

The growing Soviet threat concerned the Chemical Corps and the U.S. Army throughout the decade. Soviet Defense Minister Georgi Zhukov, while addressing the Communist Party Congress in Moscow in 1956, warned: "[A]ny new war will be characterized by mass use of air power, various types of rocket, atomic, thermo-nuclear, chemical and biological weapons." [138(p26)]

In 1959, Major General Marshall Stubbs, the new Chief Chemical Officer, assessed the growing Soviet chemical threat:

> Soviet chemical weapons are modern and effective and probably include all types of chemical munitions known to the West, in addition to several dissemination devices peculiar to the Russians. Their ground forces are equipped with a variety of protective chemical equipment and they are prepared to participate in large scale gas warfare. They have a complete line of protective clothing which will provide protection in any gas situation and a large variety of decontaminating equipment.[139(pp8–9)]

As for the biological threat, he added:

> We can assume from available knowledge that they are equally capable in biological warfare. The mass of medical and technical reports published recently by their scientists indicates increased activity in this area. Soviet microbiologists and military authorities have conducted BW tests at an isolated location over a long period of time. It is also known that the Communists have conducted research and development leading to large scale production and storage of disease producing and toxic agents.[139(p9)]

He concluded: “I believe that I have given you enough to make you aware that they pose a threat to the free nations of the world.” [139(p9)]

THE 1960s: DECADE OF TURMOIL

In 1960, Major General Stubbs talked to various groups around the country on the need for a greater sense of urgency in attaining chemical, biological, and radiological preparedness. Contending that—to both military and civilian populations—the threat of chemical and biological warfare was as great as the threat of nuclear warfare, he quoted a Soviet source who, in 1958, had described the next war as being distinguished from all past wars in the mass employment of military air force devices, rockets, weapons; and various means of destruction such as nuclear, chemical, and bacteriological weapons. Stubbs also reported that the Soviets had about one sixth of their total munitions in chemical weapons. [140]

In January 1961, Secretary of Defense Robert S. McNamara initiated about 150 projects aimed at giving him an appraisal of military capabilities. Two of these—project 112 and project 80—had significant impact on the chemical and biological weapons program.

Project 112 had as its objective the evaluation of chemical and biological weapons both for use as strategic weapons and for limited war applications. The result of this study was a recommendation to highlight chemical and biological weapons and particularly to increase long-term funding. In 1961, these recommendations were basically approved for immediate action by the deputy secretary of defense. One of the responses was the creation of Deseret Test Center, Utah, which was intended for extracontinental chemical and biological agent testing, including trials at sea, and arctic and tropical environmental testing. The new center was jointly staffed by the army, navy, and air force, with testing scheduled to begin in 1962.

Project 80 resulted in a committee to review the organization of the army. The conclusion of this committee was to eliminate the technical services and distribute their functions to various elements of the new army organization. Secretary of Defense McNamara felt that the Chemical Corps’s knowledge, experience, and training was not being “infused” into the rest of the army. The problem appeared to be that the combat troops were “structurally separated” from the Chemical Corps, particularly in the areas of research and development, and training. [141]

The chemical training of combat troops was a major concern. Colonel John M. Palmer, commanding the Chemical Corps Training Command, reflected on the problem in 1960:

> The quickest way to reduce the effectiveness of a military training program is to train without purpose or sense of urgency. Unfortunately, for 40 years an aimless approach has largely characterized unit chemical warfare training in the U.S. Army.… Much of the Army still appears to visualize chemical warfare, and related biological warfare training, as an annoying distraction from normal combat training.[142(p28)]

Based on these problems, the Defense Department ordered a far-reaching realignment of functions in 1962. Most of the Technical Service headquarters establishments, including that of the Chemical Corps, were discontinued, and their functions merged into three field commands. Thus, the training mission of the Chief Chemical Officer was assigned to the Continental Army Command; the development of doctrine to the new Combat Development Command; and the logistical function, including all arsenals, laboratories, and proving grounds, to the equally new Army Materiel Command (AMC).

The effects of the reorganization were quickly felt. Within 2 years, the chemical warfare training program had been improved significantly. One junior officer described the changes:

> We have set up special 40-hour or 80-hour schools so that we can have a trained CBR [chemical-biological-radiological] officer and noncommissioned officer in every company-sized unit. We have assigned a chemical officer down to brigade, and a chemical operations sergeant down to battalion. We set aside a certain number of hours annually for classroom instruction for the troops. We set up special blocks of instruction for surveying and monitoring teams. We list CBR defense as a subject integrated into our training schedules, and … we may even throw tear gas grenades or other agents at troops in the field.[143(p16)]

The same officer, however, concluded that even more realistic field training was still required to prepare soldiers for the modern battlefield with nuclear weapons, nerve agents, and biological weapons. [143]

Beginning of the Vietnam War

A growing guerrilla war in South Vietnam soon made the army again reexamine its training program, chemical warfare readiness, and its no-first-use policy. One observer stated in 1963: “After years of almost total lack of interest, the United States has taken up guerrilla warfare training as though it were something new under the sun.”[144(p12)] As part of that sudden interest, the role of chemical weapons again came under intense scrutiny and debate. In 1963, one author stated: “The best way for the U.S. to achieve its military aims in Southeast Asia would be to rely on chemical warfare.”[144(p12)] He described how soldiers could “sanitize” a particular large area with gases and sprays that killed everything from vegetation to humans. [144]

In 1966, a retired U.S. Army general suggested that mustard gas be used as an invaluable weapon for clearing Vietnamese tunnels. He thought the use of low-lethality chemicals would save both American and Vietnamese lives by rendering the tunnels useless. [145]

Other observers and authors also recommended revising the no-first-use policy. Public opinion and national policy opposing the use of toxic chemicals apparently was the deciding factor against their employment. The army did, however, utilize defoliants and nonlethal riot control agents in large quantities. This caused a worldwide response that required the army

to quickly explain the differences between lethal and nonlethal chemicals.

The expansion of hostilities in Vietnam caused a gradual rise in the level of development and procurement of chemical warfare–related items. By virtue of their training and their specialized equipment, Chemical Corps personnel were able to make a number of contributions, primarily in the areas of riot control and flame weapons.

Yemen Civil War

While the United States was still involved in the Vietnam War, another small war in the Middle East brought the subject of chemical warfare back from being only hypothetical. In 1962, Yemeni dissidents overthrew the monarchy and declared a republic. Royalist forces then retreated into the mountains of northern Yemen and initiated a counterrevolt against the republican forces. Egypt (which probably had had a hand in the revolt) recognized the new republic and sent military forces to help defeat the royalist troops, who were supported by the kingdoms of Saudi Arabia and later Jordan. [146]

Egyptian efforts to defeat the royalist forces and destroy their civilian support bases proved particularly difficult in the mountainous terrain. Apparently growing impatient with the successful royalist guerrilla tactics, the Egyptian air force allegedly dropped chemical-filled bombs on pro-royalist villages to terrorize or kill not only the local inhabitants but also, possibly, the royalists who were hiding in caves and tunnels. The Egyptians denied ever using chemical warfare during their support of republican forces.

Most of the early accounts of chemical warfare came from journalists in the area. The first reported incident occurred in July 1963. This alleged attack took place against the village of Al Kawma and killed seven civilians. The United Nations investigated the allegation by sending an observation team to Yemen, but their report concluded there was no evidence of a chemical attack.[147]

Newspaper articles described additional chemical attacks taking place from 1963 to 1967, although most disagreed on the dates, locations, and effects of the attacks. The United States, involved in its own controversy concerning the use of riot control agents in Vietnam, took little notice of the reports.

Much like the progression of chemicals used during World War I, the Egyptians allegedly started with tear gases, which were meant to terrorize more than kill; then progressed to mustard agents, which caused more-serious casualties; and finally to nerve agents, which were meant to kill large numbers quickly. Prior to this, no country had ever used nerve agents in combat. The combination of the use of nerve agents by the Egyptians in early 1967 and the outbreak of war between Egypt and Israel during the Six-Day War in June, finally attracted world attention to the events in Yemen.

In January 1967, an attack occurred on the Yemeni village of Kitaf. During this air raid, bombs were dropped upwind of the town and produced a gray-green cloud that drifted over the village. According to newspaper accounts,[148–152] 95% of the population up to 2 km downwind of the impact site died within 10 to 50 minutes of the attack. All the animals in the area also died. The estimated total human casualties numbered more than 200. Still another attack was reported to have taken place on the town of Gahar in May 1967 that killed 75 inhabitants. Additional attacks occurred that same month on the villages of Gabas, Hofal, Gadr, and Gadafa, killing over 243 occupants.

Shortly after these attacks, the International Red Cross examined victims, soil samples, and bomb fragments, and officially declared that chemical weapons, identified as mustard agent and possibly nerve agents, had been used in Yemen. The Saudi government protested the Egyptian use of chemical weapons to the United Nations. U Thant, Secretary-General of the United Nations, sought to confirm the use of chemical weapons with the Egyptians, but they denied it. The United Nations apparently took little further notice of the situation. The civil war officially ended in 1970 with a political agreement between the

republican and royalist factions.

Egypt had been a signatory of the 1925 Geneva Convention, which outlawed the use of chemical weapons. Some accounts attributed the chemical weapons to German scientists, usually described as being former Nazis, who had been brought to Egypt by President Nasser. Several sources reported that the Soviet Union, through its friendship with Egypt, used Yemen as a testing ground for its chemical research program. Other reports mentioned Communist China as being the supplier, while still other accounts had Egypt using old chemical munitions left behind from World War II stockpiles. [147–154]

Much of what the U.S. Army learned from the Yemen Civil War was negative. Reports of possible chemical use in certain areas of the world, particularly those inaccessible to official and technical observers, were difficult to confirm or even to condemn without accurate and verifiable information. News reports alone proved informative but unreliable. Even samples from the alleged attacks apparently did not lead to further political or military action. Most importantly, with the world distracted by the Arab–Israeli Six-Day War and events in Vietnam, politics discouraged a universal condemnation and follow-up response. In effect, the world powers let the event pass much as they had when Italy used chemical warfare against Ethiopia in the 1930s.

1967 Arab–Israeli Six-Day War

The 1967 Arab–Israeli Six-Day War was described as having come very close to being the first major war where both combatants openly used nerve agents and biological warfare. Fearing a pending attack from its Arab neighbors, on 5 June 1967, the Israelis launched a preemptive strike against Jordan, Egypt, and Syria. This action included an invasion of the Sinai Peninsula, Jerusalem's Old City, Jordan's West Bank, the Gaza Strip, and the Golan Heights.

Reports soon appeared that the Egyptians allegedly had stored artillery rounds filled with nerve agents in the Sinai Peninsula for use during a war. The Israelis, reflecting on Egypt's possible testing of the weapons in Yemen earlier in the year, suddenly realized that their troops and cities were vulnerable to attack. The fact that chemical weapons were not used during the war was possibly due to the Israelis' preemptive action or possibly to the newspaper reports of the Yemen Civil War. The Israelis felt threatened enough to place frantic orders for gas masks with Western countries. However, this last-minute plea for gas masks and nerve agent antidote came too late to have prevented enormous casualties if nerve agents had been employed. The Egyptians, on the other hand, claimed that Israel was preparing for biological warfare. A United Nations—sponsored cease-fire ended the fighting on 10 June 1967, and the potential chemical–biological war did not occur. [84,148,149,155]

Chemical Agents

While concern over the potential and actual use of chemical agents grew during the 1960s, the United States also continued its chemical agent production program. Construction of the United States's VX agent production plant at Newport, Indiana, was completed in 1961, when the first agent was produced (Figure 2-50). The production plant was only operated for 7 years, and it was placed in standby in 1968. [3]

The first and only incapacitating agent (excluding riot control agents) standardized by the army completed development in 1962. Designated BZ, 3-quinuclidinyl benzilate was a solid but was disseminated as an aerosol. The major problem with the agent for military purposes was its prolonged time of onset of symptoms. The estimate was 2 to 3 hours before the enemy would become confused and therefore vulnerable. This was a disappointment to those hoping for a quick-use, nonlethal agent as an alternative to lethal agents. A second

problem was its visible cloud of smoke during dissemination, which limited the element of surprise.[141]

Fig. 2-50. The first three steps of VX nerve agent production were completed in these structures at Newport, Indiana. The technological level of chemical engineering needed to make this agent is vastly more complicated than that required to make mustard and phosgene during the World War I era. Photograph: Chemical and Bioogical Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

New Chemical and Biological Weapons

Having concentrated on nerve agent bombs during the 1950s, the Chemical Corps turned its attention to artillery, rocket, and other delivery systems during the 1960s. In 1960, the corps standardized the first nerve agent land mine, designated the M23 2-gal VX mine (Figure 2-51). This mine resembled the conventional high-explosive land mine, but held about 11.5 lb of agent. It was designed to be activated either by a vehicle's running over it or by an antipersonnel antitampering fuze.

In 1961, the Chemical Corps standardized two new VX projectiles for artillery. The M121A1 was an improved version of the earlier sarin round. Each round held about 6.5 lb of agent. The M426 8-in. sarin or VX projectile held more than 15.5 lb of agent.[3]

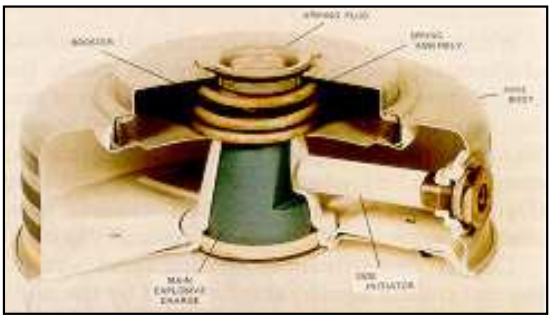


Fig. 2-51. The M23 VX land mine. Most of the interior was to be filled with the nerve agent VX. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

The early 1960s was the peak of the nerve agent rocket program. The program was first started at the end of World War II to duplicate the German V-2 missiles used against England. The United States eventually developed both short-range and long-range rockets.

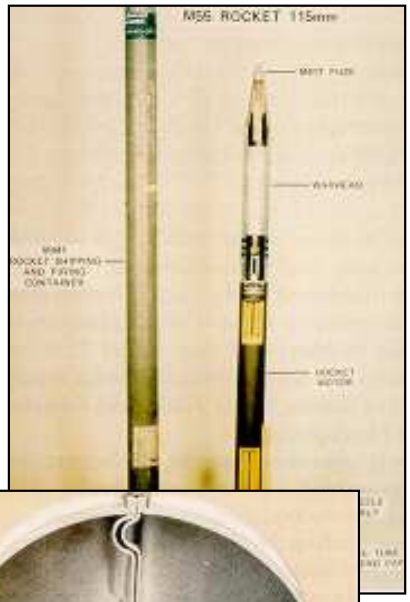
For short-range tactical support, the Chemical Corps standardized the M55 115-mm rocket in 1960 (Figure 2-52). Described as the first significant ground capability for the delivery of chemical agents since the 4.2-in. chemical mortar, the M55 was loaded with 11 lb of VX or sarin nerve agent. The range when fired from the M91 multiple rocket launcher was over 6 miles. Each launcher held 45 rockets that could be fired simultaneously. The army initially approved 40,000 sarin-filled and 20,000 VX-filled rockets, but many more were actually filled.[3,156]

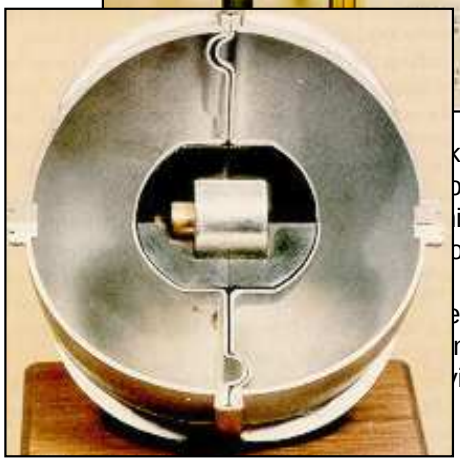
For middle-range tactical support, the Chemical Corps standardized the M79 sarin warhead for the 762-mm Honest John rocket in 1960 (Figure 2-53). The rocket had a range of 16 miles, and the warhead held 356 M134 4.5-in. spherical bomblets, each containing about 1 lb of sarin. A smaller warhead was standardized in 1964 for the 318-mm Little John rocket, which held 52 of the improved M139 4.5-in. spherical bomblets, each holding 1.3 lb of sarin (Figure 2-54).

The first long-range rocket warhead was standardized the same year for the Sergeant

missile system. The missile had a range of 75 miles and the warhead held 330 M139 sarin bomblets. More developmental projects added chemical warheads to other long-range missiles, such as the Pershing missile, which had a range of over 300 miles.

Development of rockets as delivery systems for biological agents also reached its peak during the 1960s. The M210 warhead for the Sergeant missile held 720 M143 bomblets. The M143 1-lb spherical bomblet was smaller than the sarin version, being only 3.4 in. in diameter. Each bomblet held about 212 mL of agent. If released at about 50,000 ft, the dispersion of the bomblets would cover about 60 square miles.





ket
or
inum
oon
e
nd
ving

Fig. 2-53. A chemical warhead for the Honest John rocket. It was designed to break apart and disperse the spherical bomblets of nerve agent. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fig. 2-54. The M139 4.5-in. spherical sarin bomblet used in the Little John rocket. The vanes on the outside of the bomblet created a spin that then armed the impact fuze. The explosive burster is in the center and sarin fills the two outer compartments. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

In addition to the rocket program, the Chemical Corps examined several drones for delivery of chemical and biological agents. The SD-2 Drone was a slow (300 knots), remote-controlled, recoverable drone that could hold over 200 lb of either nerve agent or biological agents. It had a range of about 100 nautical miles and could disperse agent over about 5 to 10 nautical miles. The SD-5 was an improvement that used a jet engine that gave it speeds of over Mach 0.75 and a range of over 650 nautical miles. The added horsepower allowed it to hold about 1,260 lb of chemical or biological agent, which was discharged through a tail nozzle.

The BZ program also reached weaponization status in the 1960s. In 1962, the Chemical Corps standardized the M43 750-lb BZ Bomb Cluster and the M44 175-lb BZ Generator Cluster. The M43 held 57 M138 BZ bomblets. The M44 held three 50-lb thermal generators, each holding 42 BZ canisters.[3]

Biological Agents and Weapons

By the 1960s, the U.S. Biological Warfare Program was in decline. Funding for the

program gradually decreased throughout much of the 1960s, from $38 million in 1966 to $31 million in 1969. In 1961, the army announced that new biological agents would be standardized in conjunction with munitions. This proved a further limiting factor, as the demand for biological munitions decreased.[157]

Despite budget and development constraints, the army continued to work on the antipersonnel agents. The standardization of dry *Pasteurella tularensis* was considered a significant improvement over the liquid suspension used by most agents. Dry agents were more adaptable to storage, shipping, and logistical considerations. The research into drying methods for living agents was begun during the Korean War. The method adopted for *P tularensis* was to freeze droplets of a concentrated liquid culture with liquid Freon, drying the resultant pellets, and reducing the product to a particle diameter of about 5.5 µm by means of a milling operation. The stabilizer used as a protective suspension contained skim milk and sucrose. A gram of the packaged product contained about 14.7×10^{9} viable cells and had a 3-year storage stability when stored in a dry nitrogen atmosphere at –18°C.[156]

The antiplant program was resumed for the U.S. Air Force in 1962. Agent production was conducted at Pine Bluff Arsenal. Field tests of wheat stem rust and rice blast disease were conducted at several sites in the midwestern and southern United States and on Okinawa. The same year, the Defense Department requested additional work on defoliation and antiplant activities owing to the ongoing events in southeast Asia. In 1962, the Chemical Corps initiated a crash project for the production of wheat stem rust under Rocky Mountain Arsenal supervision. Other work included trying to find pathogens suitable for use against the opium poppy crop. [141]

During the 1960s, the army conducted large-scale tests using the biological simulant *Bacillus globigii* (code name BG) at various places in the public domain to access the dangers of covert biological attacks. For example, in 1965, BG was tested at National Airport and the Greyhound Terminal in Washington, D. C. In 1966, BG was disseminated in New York City within the subway tubes and from the street into the subway stations in mid Manhattan. The results confirmed that a similar real covert attack would have infected a large number of people during peak traffic periods.

The army also conducted antianimal testing using BG at several stockyards in Texas, Missouri, Minnesota, South Dakota, Iowa, and Nebraska between 1964 and 1965. Antiplant testing using the wheat stem rust fungus was also conducted at Langdon, North Dakota, in 1960 and Yeehaw Junction, Florida, in 1968.[157]

By the 1960s, the army was in the process of developing vaccines for most of the biological agents standardized or in development. A 1965 volunteer consent form provides insight into the pertinent agents (Exhibit 2-2). Attached to the consent form for vaccines was an order to also administer selected live agents to the participants.[158,159]

New Defensive Equipment

The most significant advancement in individual protection was a new version of the M17 Protective Mask, designated the M17A1, which introduced two new concepts in 1966 that were long overdue. The first was a resuscitation device for the mask, which was required to allow soldiers to provide artificial respiration without unmasking. Although atropine injections were an effective antidote for the anticholinesterase effects of nerve agents, artificial respiration was required to counteract the effects of the agent on the respiratory system.

The second new concept was a drinking tube. The drinking capability allowed a soldier to

drink from his canteen in a contaminated battlefield without unmasking. This was considered critical because of the longer times required to wear protective gear around persistent nerve agents and the possible use of the mask in desert and tropical climates.[30]

The need to provide air conditioning and protection against chemical and biological agents to workers in the army's NIKE missile-control vans resulted in the development of a trailer-mounted unit adopted for limited production in 1961. After some improvements, the unit was standardized in 1963 as the M1 Collective Protection Equipment. Initially, 288 of the units were ordered, but additional similar needs for collective protection quickly became apparent. [160]

For the U.S. Army, one requirement that was further supported by lessons learned from the 1967 Six-Day War was the need for an automatic field-alarm system. In 1968, the army solved the 2-decades-old problem by standardizing the M8 Portable Automatic Chemical Agent Alarm. The 4-year development effort covered the gap that had left U.S. soldiers vulnerable to a surprise nerve agent attack. The unit consisted of the M43 detector unit and the M42 alarm unit. Additional alarms could be connected. [3]

EXHIBIT 2-2
CONSENT TO INOCULATION WITH EXPERIMENTAL BIOLOGICAL PRODUCTS

It has been explained to me that it is necessary for my safety and protection to be inoculated with certain biological products approved by the Army Investigational Drug Review Board but not yet approved by the Commissioner of Food and Drugs, Department of Health, Education and Welfare. I understand that the administration of these products will provide future additional evidence of their safety and usefulness.

I hereby consent * to inoculation with any or all of the following biological products to include the initial series and booster immunizations as required:

1) Venezuelan Equine Encephalomyelitis Vaccine, Live, Attenuated.
2) Live Tularemia Vaccine.
3) Anthrax Vaccine (non-viable), aluminum hydroxide adsorbed.
4) Botulinum Toxoid, Types A B C D E, aluminum phosphate adsorbed.
5) Tularemia Skin Test Antigen.
6) Rift Valley Fever Virus Vaccine.
7) Q Fever Vaccine.
8) Eastern Equine Encephalomyelitis Vaccine.
9) Western Equine Encephalomyelitis Vaccine.

WITNESSES:

_____ _____

(Date) (Signature) (Date) (Signature)

(Date) (Signature) (Date) (Signature)

SMUFD FORM 8 (Rev)
May 65

* It is unclear how the volunteer signified consent. Note that this form does not contain a blank for the volunteer's signature.

The alarm used an electrochemical point-sampling system that continuously monitored the atmosphere and sounded an audible or visible warning of even very low concentrations of nerve agents. Actual detection occurred when air was passed through an oxime solution surrounding a silver analytical electrode and a platinum reference electrode. Presence of an agent caused a reaction in the solution, which increased the potential between the electrodes. The change in potential, when amplified, triggered the alarm signal. The unit could detect almost all chemical agents, including the nerve agents.[161]

In 1960, the Chemical Corps made a significant improvement in the area of decontamination. DANC had proven to be particularly corrosive to the brass parts of the M2 Decontaminating Apparatus, so the Chemical Corps spent almost 2 decades developing Decontaminating Solution 2 (DS2, manufactured then by Pioneer Chemical Co., Long Island City, New York). DS2 was a clear solution of 70% diethylenetriamine, 28% methyl cellosolve (ethylene glycol monomethyl ether), and 2% sodium hydroxide. This decontaminating agent did not solve all problems, either. It was known to remove and soften new paint, and to discolor old paint. It was also irritating to the skin. Its good points were that DS2 was less corrosive to metals and less destructive to plastics, rubber, and fabrics. [161] In conjunction with the standardization of DS2, the Chemical Corps also developed the M11 1.5-qt Portable Decontaminating Apparatus, a fire extinguisher–type unit compatible with DS2, which was used to decontaminate vehicles and weapons. [3]

Public Hostility Toward Chemical and Biological Weapons

The growing protests over the U.S. Army's role in Vietnam, the use of defoliants, the use of riot control agents both in Southeast Asia and on the home front, and heightened concern for the environment all gradually increased the public hostility toward chemical and biological weapons. Three events particularly galvanized public attention: the sheep-kill incident at Dugway Proving Ground, Operation CHASE, and an accident with sarin at Okinawa.

Dugway Sheep-Kill Incident

The first event, according to Dugway Proving Ground's incident log, started with a telephone call on Sunday, 17 March 1968:

> At approximately 1230 hours, Dr. Bode, University of Utah, Director of Ecological and Epidemiological contract with Dugway Proving Ground (DPG), called Dr. Keith Smart, Chief, Ecology and Epidemiology Branch, DPG at his home in Salt Lake City and informed him that Mr. Alvin Hatch, general manager for the Anschute Land and Livestock Company had called to report that they had 3,000 sheep dead in the Skull Valley area.[162(pA-1)]

Skull Valley was adjacent to Dugway, one of the army's open-air testing sites for chemical weapons. Although the findings were not definite, the general opinion seemed to be that nerve agents had somehow drifted out of the test area during aerial spraying and had killed the sheep. Whether the army was guilty or not, the end result was bad publicity and, even more damaging, congressional outrage.

Operation CHASE

The second event was actually a series of sea dumps of surplus chemical warfare agents and a problem weapon system (Figure 2-55). These sea dumps created significant environmental concerns throughout the country. The surplus agents were mustard agent (primarily) and some nerve agent. The problem weapon system was the relatively new M55 rocket system. Although the M55 had been standardized only 7 years before, the thin aluminum head design proved faulty for long-term storage. The problem of leaking rockets started in 1966,

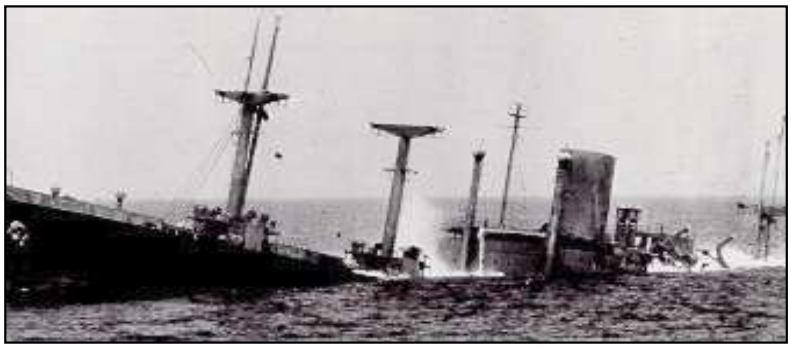


Fig. 2-55. The disposal at sea of surplus and leaking chemical munitions and radiological wastes generated environmental concerns that eventually brought sea dumping to a halt. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

and a year later, the army began disposing of the rockets, sealed in concrete vaults in the hulls of ships that were then sunk in ocean-disposal sites.

Operation Cut Holes and Sink 'Em (CHASE), an ongoing program for disposing of conventional ammunition, began accepting chemical weapons in 1967. That year, CHASE 8 disposed of mustard agent in ton containers, and M55 sarin rockets. In June 1968, CHASE 11 disposed of sarin and VX in ton containers, along with additional M55 sarin and VX rockets. In August 1968, CHASE 12 disposed of mustard agent in ton containers.[3]

The sea dumps created two major concerns. The first was that the weapons were being shipped from their storage depots by train to the loading docks. Fear of an accident along the way was paramount. Second, sea dumping and its effects on marine life were sources of environmental and commercial concern and protest.

Accident at Okinawa

The third event was a serious accident. On 8 July 1969, the army announced that 23 U.S. soldiers and 1 U.S. civilian had been exposed to sarin on Okinawa. The soldiers were cleaning sarin-filled bombs preparatory to repainting them when the accident occurred.[3]

Although none of the individuals died, the public announcement created two controversies. First, up until that time, the army had kept secret the forward positioning of chemical weapons on Okinawa. The acknowledgment created international concerns. Second, the accident pointed out the dangers of storing chemical weapons. With chemical weapons known to be stored at sites in the continental United States near cities and residential areas, the fear of an accident escalated. In response to these concerns, the Defense Department announced on 22 July 1969 that they would accelerate the previously planned removal of the chemical agents from Okinawa.[163]

Changes to the Chemical and Biological Warfare Programs

In April 1969, the secretary of defense tried to explain the U.S. chemical warfare policy to both the general public and to congress. In part, he stated:

> It is the policy of the United States to develop and maintain a defensive chemical–biological (CB) capability so that U.S. military forces could operate for some period of time in a toxic environment if necessary; to develop and maintain a limited offensive capability in order to deter all use of CB weapons by the threat of retaliation in kind; and to continue a program of research and development in this area to minimize the possibility of technological surprise.[164(p193)]

The explanation did not help. In July, the United Nations released a report on chemical and biological weapons that condemned production and stock-piling of weapons of mass destruction. Six days later, the United States acknowledged the Okinawa accident.[3]

Congress stepped in and on 11 July 1969 revealed that the army was conducting open-air testing with nerve agents at Edgewood Arsenal (the name of the Army Chemical Center had reverted back in 1963) and at Fort McClellan during training events. Shortly after the disclosure, more than 100 protesters were at the gates of Edgewood Arsenal. Three days later, buckling to the pressure, the army announced suspension of open-air testing at the two sites. Quickly rushing an independent committee together, the army promised to conduct a safety review of all such testing. The positive publicity of creating the new committee was soon forgotten when the army revealed that they had also conducted nerve agent testing in Hawaii between 1966 and 1967, something the army had previously denied.[3]

In October, the secretary of the army announced that the committee had completed its study. The committee reached the following conclusion:

> The lethal testing program at Edgewood Arsenal during the past two decades has compiled an enviable record for safety. The testing procedures that have been evolved are clearly effective in minimizing danger to base personnel and civilians in adjacent areas.[165(p16)]

The committee's only major concern was the movement of chemical agents by truck on public roads; the committee recommended resumption of lethal agent, open-air testing at Edgewood.[165]

Before testing resumed, however, the U.S. Congress passed Public Law 91-121 in November. This law imposed controls on the testing and transportation of chemical agents within the United States; and the storage, testing, and disposal of agents outside the United States. Further open-air testing of lethal chemical agents was effectively banned.[3]

In November 1969, President Richard M. Nixon took action against chemical and biological warfare. First, he reaffirmed the no-first-use policy for chemical weapons:

> I hereby reaffirm that the United States will never be the first country to use chemical weapons to kill. And I have also extended this renunciation to chemical weapons that incapacitate.[166(p5)]

Second, he decided to resubmit the 1925 Geneva Protocol to the U.S. Senate for ratification. The senate had refused to ratify the treaty when it was first signed, and President Harry S Truman had withdrawn the treaty from the senate in 1947.

Third, President Nixon renounced the use of biological weapons and limited research to defensive measures only:

> I have decided that the United States of America will renounce the use of any form of deadly biological weapons that either kill or incapacitate. Our bacteriological programs in the future will be confined to research in biological defense on techniques of immunization and on measures of controlling and preventing the spread of disease. I have ordered the Defense Department to make recommendations about the disposal of the existing stocks of bacteriological weapons.[166(p5)]

He concluded by explaining his future hopes:

> Mankind already carries in its own hands too many of the seeds of its own destruction. By the examples that we set today, we hope to contribute to an atmosphere of peace and understanding between all nations.[166(p4)]

These actions effectively stopped the production of chemical and biological weapons in the United States.[166]

THE 1970s: THE NEAR END OF THE CHEMICAL CORPS

Throughout the 1970s, the chemical and biological warfare programs experienced further restrictions and tightened controls. In February 1970, President Nixon added toxins to the banned weapons and ordered all existing stocks of toxin agents destroyed. About a month later, the army revealed that it had conducted both chemical and biological testing in Alaska but reported that the testing had stopped. The army also announced that the chemical weapons on Okinawa would be moved to Umatilla Army Depot in Oregon. This triggered a series of lawsuits that attracted the concern of congress. The next year, Public Law 91-672 was enacted, which prohibited the army from moving the weapons from Okinawa to anywhere on the U.S. mainland. Finally, Operation Red Hat moved the stockpile on Okinawa to Johnston Atoll, a small U.S. island in the South Pacific, for long-term storage and eventual demilitarization.

Demilitarization was not an easy project; heightened environmental concerns characterized the 1970s. One last sea dump took place in 1970, when, despite much negative press, CHASE 10 disposed of more M55 sarin rockets. (CHASE 10 had originally been scheduled earlier; although now out of numerical order, the designation was unchanged.) Two years later, Public Law 92-532 was enacted, which prohibited the sea dumping of chemical munitions.

Between 1971 and 1973, all remaining biological weapons were destroyed at Pine Bluff Arsenal, Rocky Mountain Arsenal, and Fort Detrick. In 1972, the United States signed the Convention on the Prohibition of the Deployment, Production, and Stockpiling of Bacteriological and Toxin Weapons. This convention banned development, production, stockpiling, acquisition, and retention of biological agents, toxins, and the weapons to deliver them. The senate ratified the Biological Warfare Convention in 1974 and President Gerald R. Ford signed it in 1975.

Although President Nixon had called in 1969 for the ratification of the Geneva Protocol, it took a few more years. In 1974, the U.S. Senate ratified the Protocol, and President Ford officially signed it on 22 January 1975. He did, however, exempt riot control agents and herbicides from inclusion in the agreement.[3] The events of 1969 had a severe impact on the future of the U.S. Army Chemical Warfare Program. Two senior department of defense personnel reflected on the impact the restrictions had during the 1970s:

> During most of the 1970s, the United States allowed its chemical retaliatory capability to decline, did little to improve chemical protection, and neglected relevant training and doctrine. The United States has not produced lethal or incapacitating chemical agents, or filled munitions since 1969.[167(p3)]

The army actually made plans to abolish the Chemical Corps entirely. In 1973, with the signing in Paris, France, of the peace pacts to end the Vietnam War, and with the end of the draft, the army recommended reducing the Chemical Corps in size and eventually merging it with the Ordnance Corps. As the first step, the army disestablished the Chemical School at Fort McClellan, Alabama, and combined it with the Ordnance School at Aberdeen Proving Ground, Maryland. Congress, however, blocked the complete disestablishment of the corps.[168–171] Still, one observer noted: “As an additional ordnance career field, the chemical specialty almost withered and died at Aberdeen.” [171(p15)]

1973 Arab–Israeli Yom Kippur War

Then another war quickly brought chemical warfare preparedness back to the forefront. The Arab-Israeli Yom Kippur War lasted only from 6 October to 24 October 1973, but the ramifications for the U.S. chemical program lasted much longer. The Egyptian and Syrian attack against Israel on Yom Kippur and the successful Israeli counterattacks ended with a cease fire. Both sides took enormous losses in personnel and equipment.

Following the Yom Kippur War, the Israelis analyzed the Soviet-made equipment they captured from the Egyptians and Syrians. They discovered (*a*) portable chemical-proof shelters, (*b*) decontamination equipment for planes and tanks, and (*c*) that most Soviet vehicles had air-filtration systems on them to remove toxic chemicals.

Another item of note was a Soviet PKhR-MV Chemical Agent Detector Kit for Medical and Veterinary Services. The set consisted of a hand pump, detector tubes, reagents in ampules, dry reagents, test tubes, and accessories. It was designed to detect nerve, blister, and blood agents. Exploitation by the U.S. specialists determined that it could detect low concentrations of nerve agents, mustard agent, cyanide, Lewisite, and heavy metals in aqueous solutions. It could also detect the same agents in addition to cyanogen chloride and phosgene in the atmosphere. One noted problem with the kit was that the procedures for using it were extremely difficult to carry out while wearing a protective suit. In addition, the glass ampules were fragile and broke easily. [172]

Overall, the experts reported finding sophisticated chemical defense materiel and a “superior quantitative capability for waging a chemical war.” [173(p3-4)] The indications were that the Soviets were ready for extensive chemical warfare and might actually be planning to initiate chemical warfare in a future war. Soviet division commanders were thought to already have authority to initiate chemical warfare.[173–176]

Restoring the Chemical Corps

The combination of (*a*) the findings of sophisticated Soviet chemical defense materiel and their capability for waging chemical war and (*b*) the decline of the U.S. Army Chemical Corps called for corrective action. The army concluded the following:

> To offset this, U.S. chemical/biological (CB) defense materiel must not only provide a protective system equivalent to or better than that of any potential enemy but the physiological and logistics burdens must be such as to permit long-term use. To cope with the hazards of any potential CB-threat environment requires the development of an integrated CB defense system. This system must contain items for individual protection, collective protection, decontamination, warning and detection, and safe devices and concepts to achieve realistic training. An effective technological base is needed from which such materiel, responsive to user needs, can be quickly developed.[173(p3-4)]

In 1976, the secretary of the army reversed the decision to abolish the Chemical Corps. He cited the heightened awareness of the Soviet Union's capability to wage chemical warfare as the primary reason. In 1977, the United States started a new effort to reach an agreement with the Soviets on a verifiable ban on chemical weapons. This effort was unsuccessful. Partly as a result, the Chemical School was reestablished at Fort McClellan in 1979. [167,177–181]

Binary Weapons Program

The end of the chemical weapons production program had stopped production but left one type of chemical retaliatory weapon still in development. Back in the 1950s, the army had begun looking at binary weapons. Until that time, chemical weapons were unitary chemical munitions, meaning that the agent was produced at a plant, filled into the munitions, and then stored ready to be used. Since most agent was extremely corrosive, unitary munitions were logistical nightmares for long-term storage. The binary concept was to mix two less-toxic materials and thereby create the nerve agent *within* the weapon *after* it was fired or dropped. Because the two precursors could be stored separately, the problems of long-term storage and safe handling of chemical weapons were therefore solved. The navy took the greater interest in the binary program during the 1960s and requested a 500-lb bomb designated the BIGEYE. Only after the production of unitary chemical munitions was halted did the binary program receive high priority in the army, however. In fact, the last open-air test with lethal agents had taken place at Dugway Proving Ground on 16 September 1969, when a 155-mm projectile filled with sarin binary reactants was test fired. Throughout the early 1970s, additional test firings took place using simulants. In 1976, the army standardized the M687 Binary GB2 155-mm Projectile.

The binary projectile used a standard M483A1 155-mm projectile as the carrier of the chemical payload (Figure 2-56). Binary chemical reactants were contained in two separate, plastic-lined, hermetically sealed containers. These leakproof canisters were loaded through the rear of the shell and fitted one behind the other in the body of the projectile. The forward canister contained methylphosphonic difluoride (DF) and the rear canister contained isopropyl alcohol and isopropylamine solution (OPA).

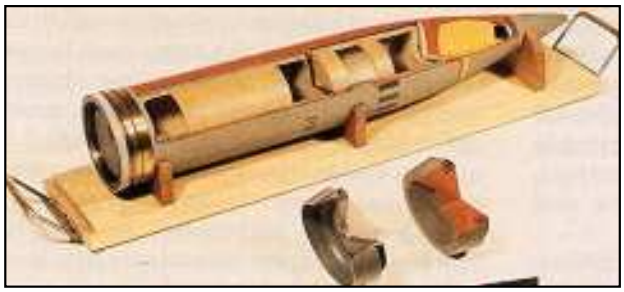
To ensure safe handling, M687 projectiles were shipped and stored with only the forward DF-filled canister in place. A fiberboard spacer occupied the cavity provided for the OPA canister. Projectiles were secured horizontally on a pallet, as opposed to the conventional vertical position for other 155-mm projectiles. This orientation permitted rapid removal of the projectile's base using a special wrench. The fiberboard spacers were removed and replaced with the OPA canisters. The fuze was then installed just prior to firing. Upon firing, setback and spin forces caused the facing disks on the canisters to rupture, allowing the reactants to combine to form sarin en route to the target. [182,183] In addition to the M687 projectile, the army also worked on the BLU-80/B (BIGEYE) VX2 bomb and projectiles of other size, including an 8-in. projectile. None of these, however, were ever standardized.

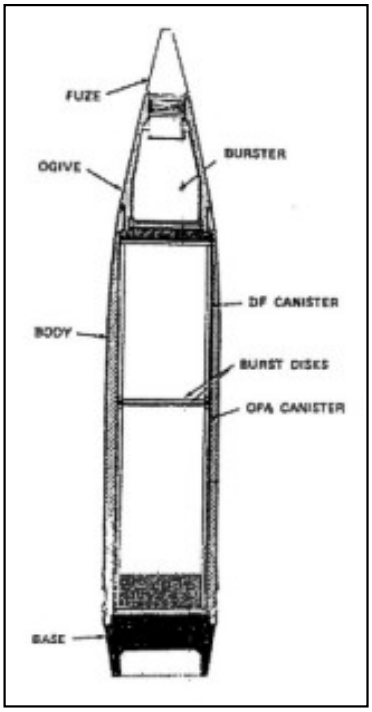
Standardization of the M687 did not lead immediately to production. The same year the M687 was standardized, the U.S. Congress passed the Department of Defense Appropriation Authorization Act, which restricted the development and production of binary chemical weapons unless the president certified to congress that such production was essential to the national interest. Thus, the army would take another decade to locate the production plants, pass environmental inspection, receive presidential approval, and begin production. [3]

Detection Improvements

Although the M8 detector/alarm solved the advance warning problem, soldiers still needed a quick test to confirm the presence of chemical agents. The problem was solved with the standardization of M8 detector paper in 1973. The paper was a Canadian development. It was packaged in booklets of 25 sheets (perforated for easy removal) sized 4 x 2 1/2 in. M8 detector paper turned dark blue for V agents, yellow for G-series agents, and red for mustard agent. [161]

a





b

Fig. 2-56. (a) The M687 GB2 binary 155-mm projectile, which was standardized in 1976 but not produced until a decade later. **(b)** A diagram of the M687 GB2 binary 155-mm projectile. To appreciate the sophistication of this weapon, the reader should compare this drawing with Figure 2-2. Photograph (a): Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md. Photograph (b): Reprinted from Department of the Army. *Binary Chemical Munitions Program.* Aberdeen Proving Ground, Md: Chemical Systems Laboratory; 1981: 5. Programmatic Environmental Impact Statement ARCSL-EIS-8101.

There were some problems with the paper, the most important of which was that some less-dangerous agents gave responses similar to mustard agent. Benzene, DANC, and defoliating agents produced a red response; sulfuric acid produced a black response; and organophosphate insecticides produced a yellow response.[184]

Collective Protection Improvements

One particular area of development that gained significantly from the resurgent interest in chemical and biological defense was collective protection. Increasing numbers of combat and combat-support vehicles with integrated chemical and biological collective protection systems were reported to be appearing throughout the Warsaw Pact area. These reports resulted in a closer look at the U.S. situation.[185]

The army was already examining collective protection for some military vehicles and, in

particular, the missile-control vans. The need for collective protection for vans and vehicles used for command posts, fire direction, rest and relief shelters, and medical aid stations resulted in the standardization of the M14 Collective Protection Equipment in 1970. This equipment was designed to protect occupants of the M291A2 and M292 series of vans against airborne toxic agents.[161]

Work during the 1960s on the CB Pressurized Pod resulted in the standardization of the M51 Shelter System in 1971. The unit was an easily transportable, pressurized enclosure. It could be air-dropped or towed to provide protection from chemical and biological agents in the field for combat, combat support, and combat service support troops. The M51 was a double-walled, air-inflated, self-supporting shelter and airlock structure. When erected, the shelter was semicircular in cross-section with a maximum inside height of 7.5 ft, with inside dimensions of 15 x 14 ft. An airlock entrance (with a set of double doors at each end) on the front was 11 (l) x 4.2 (w) x 6.7 (h) ft in dimension. The filter and support equipment were mounted on a two-wheeled trailer.[186]

Despite these developments, congress got involved in 1977 and included in the Department of Defense Appropriation Authorization Act a section that stated:

> The Secretary of the Army shall submit to the Committees on Armed Services of the Senate and House of Representatives, no later than February 1, 1978, a plan for the funding and scheduling necessary to incorporate by October 1, 1980, collective system protection against chemical and radiological agents for all main battle tanks, mechanized infantry combat vehicles, armored personnel carriers, armored self-propelled artillery vehicles, armored self-propelled air defense artillery vehicles, and other such types of equipment associated with the above in combat operations which will be in development or procurement in fiscal year 1981.[187]

This law launched an intensive effort to determine the chemical vulnerability of all army vehicles. The initial concentration was on ventilated facepieces and a mixture of positive-pressure and hybrid systems for selected rear-area vehicles.

Growing Danger of Chemical and Biological Warfare

Starting in about 1975, reports of the use of chemical and biological agents in various small wars in Southeast Asia and Afghanistan began to attract attention in the United States. Interviews with Hmong villagers in Laos suggested that Vietnamese and Russian forces might have used chemical and possibly toxin weapons against these people. Starting in 1978, similar reports from Kampuchea claimed that the Vietnamese and their allies had killed over 980 villagers using chemical weapons. Prior to the Soviet invasion of Afghanistan in December 1979, reports were already circulating that Soviet troops were using chemical weapons against the mujahidin soldiers.

The Soviets legitimized their use of chemical and biological weapons because, although they had signed the Geneva Protocol in 1928, Laos, Kampuchea, and Afghanistan were not signatories. The Soviet Union, Laos, and Afghanistan signed the Biological Weapons Convention, but the allegations of toxin use were never acknowledged by the Soviets or their allies. In fact, when the Soviets signed the Biological Weapons Convention in 1975, they added the statement: “the Soviet Union does not possess any bacteriological agents and toxins, weapons, equipment or means of delivery.” [188(p6)] Other intelligence sources thought that the Soviets considered most toxins to be chemical agents, and therefore not subject to the Biological Weapons Convention. If toxins were considered to be chemical agents, then the Soviets would be permitted under the Geneva Protocol to use them in retaliation or against nonsignatories.[189]

The use of chemical and biological weapons by the Soviets was taken as an indication that the Soviets were continuing an active chemical and biological program. This program, however, did not continue without costs. In April 1979, a sudden outbreak of anthrax occurred in Sverdlovsk, in the Ural Mountains. At the time, the etiology of the outbreak was explained by the Soviets as human ingestion of beef from cattle that had been contaminated by naturally occurring anthrax spores in the soil. U.S. intelligence officers doubted the story and used the incident to push for better chemical and biological preparedness in the army. In 1992, Russian President Boris Yeltsin confirmed that the epidemic had been caused by military researchers working with the agent. *Izvestiya*, the Russian newspaper, later reported that it took 5 years to clean up the plant after the accident. [190–192]

THE 1980s: THE RETURN OF THE CHEMICAL CORPS

The Haig Report

Despite denials by the governments involved, the United States went public with charges that chemical warfare had been used in Southeast Asia and Afghanistan in 1980. Problems with the collection of samples and the remoteness of the sites, however, prevented definitive evidence from being obtained. Furthermore, the later identification, discussion, and media debate over the origin of possible trichothecene mycotoxins in Southeast Asia also took away a significant portion of the public interest in the alleged use of conventional chemical munitions.

In 1982, Secretary of State Alexander M. Haig, Jr., presented a report titled *Chemical Warfare in Southeast Asia and Afghanistan* to the U.S. Congress. After describing the evidence, he concluded:

> Taken together, this evidence has led the U.S. Government to conclude that Laos and Vietnamese forces, operating under Soviet supervision, have, since 1975, employed lethal chemical and toxin weapons in Laos; that Vietnamese forces have, since 1978, used lethal chemical and toxin agents in Kampuchea; and that Soviet forces have used a variety of lethal chemical warfare agents, including nerve gases, in Afghanistan since the Soviet invasion of that country in 1979.[189(p6)]

Based on this evidence, senior defense department personnel concluded that the Soviet Union “possesses a decisive military advantage because of its chemical capabilities.”[167(p3)]

The Haig report, however, was not able to galvanize world opinion. Much like the situation during the Yemen Civil War, the United States was unable to prove beyond a shadow of the doubt that chemical agents and toxins had been used in Southeast Asia and Afghanistan. Instead, the accusation became a political debate between the United States and the Soviet Union during President Ronald Reagan’s administration.

Chemical Warfare in the Afghanistan and Iran–Iraq Wars

Afghanistan War

The U.S. Army monitored the war in Afghanistan throughout the 1980s. Often thinking of it as the Soviet’s “Vietnam,” the lessons learned from this war about chemical warfare provided extensive support to the U.S. chemical defense program.

The Soviets tended to use chemical weapons much like the Italians did in Ethiopia and like the U.S. Army had used nonlethal agents in Vietnam. One military writer summed up the general lesson learned:

> The use of chemical weapons by Soviet forces in Afghanistan is also significant. The use of these weapons in Afghanistan confirms, not surprisingly, that the Soviets find them put to their best use against unprotected subjects incapable of retaliation. Afghanistan is proof positive that the Soviets do not consider these devices as special weapons. Considerations of utility and not morality will govern Soviet use of them in a future conflict.[193(p27)]

Despite the use of chemical weapons, the Soviets were unable to “win” the war and, in December 1988, met with rebel forces to discuss a withdrawal of Soviet troops from Afghanistan. In January 1989 the Soviets announced the final withdrawal, which was completed a month later.[194]

Iran–Iraq War

Although the Soviet Union continued to be the number one potential chemical warfare opponent and, therefore, the United States concentrated on proposed chemical treaties with that country, the beginning of a war in the Middle East gradually began to erode that status. On 22 September 1980, the armed forces of Iraq launched an invasion against its neighbor Iran. The Iraqi army, trained and influenced by Soviet advisers, had organic chemical warfare units and possessed a wide variety of delivery systems. When neither side achieved dominance, the war quickly became a stalemate.

To stop the human wave–attack tactics of the Iranians, the Iraqis employed their home-produced chemical agents as a defensive measure against the much-less-prepared Iranian infantry. The first reported use of chemical weapons occurred in November 1980. Throughout the next several years, additional reports circulated of new chemical attacks. The result was that by November 1983, Iran complained to the United Nations that Iraq was using chemical weapons against its troops.[195–198]

After Iran repeated the claims and even sent chemical casualties to several western nations for treatment, the United Nations dispatched a team of specialists to the area in 1984, and again in 1986 and 1987, to verify the claims. The conclusion from all three trips was the same: Iraq was using chemical weapons against Iranian troops. In addition, the second mission also stressed that the use of chemical weapons by Iraq appeared to be increasing despite the publicity of their use. The reports indicated that mustard agent and the nerve agent tabun were the primary agents used, and that they were generally delivered in airplane bombs. The third mission also reported the use of artillery shells and chemical rockets and the use of chemical weapons against civilian personnel. The third mission was the only one allowed to visit Iraq.[199–201]

In the letter of transmittal to the United Nations after the conclusion of the third mission, the investigators pointed out the dangers of this chemical warfare:

> It is vital to realize that the continued use of chemical weapons in the present conflict increases the risk of their use in future conflicts. In view of this, and as individuals who witnessed first hand the terrible effects of chemical weapons, we again make a special plea to you to try to do everything in your power to stop the use of such weapons in the Iran–Iraq conflict and thus ensure that they are not used in future conflicts.
>
> . . . .
>
> In our view, only concerted efforts at the political level can be effective in ensuring that all the signatories of the Geneva Protocol of 1925 abide by their obligations. Otherwise, if the Protocol is irreparably weakened after 60 years of general international respect, this may lead, in the future, to the world facing

> the specter of the threat of biological weapons.[199]

This last comment was mirrored by another analyst:

> In a sense, a taboo has been broken, thus making it easier for future combatants to find justification for chemical warfare, this aspect of the Iran–Iraq war should cause Western military planners the gravest concern.[202(pp51–52)]

The Iran–Iraq War failed to reach a military conclusion despite Iraq's use of chemical weapons. Roughly 5% of the Iranian casualties were caused by chemical weapons. Although there were rumors of Iranian use of chemical weapons also, less attention was devoted to verifying those reports. In August 1988, Iraq finally accepted a United Nations cease-fire plan, and the war ended politically with little gained from the original objectives.[194]

Additional Reports of Chemical Warfare

The end of the Iran–Iraq War, however, did not end chemical warfare reports from circulating. Within a month of the end of the war, Iraq was again accused of using chemical weapons against the Kurds, a minority group in Iraq seeking autonomy. Shortly before, there were rumors that Libya had used chemical weapons obtained from Iran during an invasion of Chad. The United States rushed 2,000 gas masks to Chad in response. There were also reports of the Cuban-backed government of Angola using nerve agents against rebel forces.[203–206]

New Defensive Equipment

In response to the continued use of chemical agents in the Middle East and elsewhere, the army instituted a three-pronged chemical program for the 1980s, intended both to drive the Soviets to the bargaining table and to restore the United States chemical defense and retaliatory capability. First, the army improved its defensive equipment. Second, the army began production of chemical weapons for the first time since the 1969 ban. And third, the army improved its chemical warfare training and updated its training manuals.

A number of physical protection, collective protection, detection, and decontamination developments reflected the improved defensive equipment. Perhaps the most significant development was the type classification in 1987 of a new protective mask for the infantry to replace the M17 series masks. The new mask, designated the M40, returned to a canister design that provided increased protection against everything from chemical agents to toxins, smokes, and radioactive fallout particles. The canister used North Atlantic Treaty Organization (NATO) standard threads and could be worn on either side of the mask. The mask also had improved fit and comfort, voice communications, and drinking capability. It came in three sizes—small, medium, and large—which helped eliminate the logistical burden of four sizes for the M17A2 and six different stock numbers for the M9A1. In conjunction with M40, the army also standardized the M42 Combat Vehicle Mask to replace earlier tank masks from the 1960s.[207–209]

For collective protection, the army standardized the M20 Simplified Collective Protection Equipment in 1986. This system turned one room of a building into a protected area by (1) lining the walls with a chemical/biological vapor–resistant polyethylene liner and (2) providing 200 cu ft of filtered air per minute. In addition, the army concentrated on modular collective protection equipment for chemical threats to vehicles, vans, and shelters. The Department of the Army identified 43 systems in 1980 that required chemical protection. New systems that were developed each year created a major, longterm project to correct the deficiency that had been discovered after the 1973 Yom Kippur War. [207,210]

For detection, the army developed two new detectors, one using low technology and one high technology. M9 Detector Paper was an adhesive-backed paper containing B-1 dye, which turned red when contaminated with any known liquid agent. Type classified in 1980, the paper was attached to a soldier's arms or legs, or to the outside of his vehicle, and provided an indication of a chemical attack. [210]

The M1 Chemical Agent Monitor (CAM), standardized in 1988, was used to detect chemical agent contamination on personnel and equipment. It detected vapors by sensing ions of specific mobilities and used timing and microprocessor techniques to reject interferences. Its developmental history was particularly interesting, in that it was based on a United Kingdom (U.K.) design originally standardized by the U.K. in 1984. [3,211]

There were several developments in the area of decontamination. The M13 Portable Decontaminating Apparatus, designed to decontaminate large military vehicles, was standardized in 1983; and the M280 Individual Equipment Decontamination Kit, designed to partially decontaminate a soldier's personal equipment, including gloves, hood, mask, and weapon, was standardized in 1985. Both items replaced older equipment. [3]

Not all research and development utilizing current technology or foreign intelligence resulted in the standardization of a new item. One example was the truck-mounted, jet-exhaust decontaminating apparatus, designated the XM16. Based on intelligence collected on the Soviet TMS-65 decontamination system, the army started work on a similar project. The U.S. project consisted of a J60-P-6 jet engine with a control cab mounted on a 5-ton military truck. The idea was to direct high-velocity streams of hot exhaust gases onto the outer surfaces of vehicles for decontamination. In addition, the jet engine could be used as a smoke generator by adding smoke liquids to the exhaust. Because of several deficiencies in the system, the project was canceled in 1986, but the principle was continued in related developmental projects. [212]

Production of Binary Weapons

The subject of chemical weapon production was a very sensitive one. In 1984, congress created the Chemical Warfare Review Commission to look at several issues related to the military's chemical warfare preparedness. This committee visited numerous sites, interviewed experts, reviewed policy, and examined intelligence reports. Among their findings, the commission concluded

> that in spite of the approximately $4 billion that the Congress has appropriated since 1978 for defense against chemical warfare, that defense, measured either for purposes of deterrence or for war-fighting utility, is not adequate today and is not likely to become so. Chemical combat as it would exist in the late twentieth century is an arena in which—because defense must be nearly perfect to be effective at all, detection is so difficult, and surprise offers such temptation—the offense enjoys a decisive advantage if it need not anticipate chemical counterattack. Defense continues to be important to pursue, because it can save some lives and preserve some military capabilities. But for this country to put its faith in defense against chemical weapons as an adequate

> response to the Soviet chemical threat would be a dangerous illusion.[213(p50)]

The answer to the problem was simply stated by President Reagan:

> The United States must maintain a limited retaliatory capability until we achieve an effective ban. We must be able to deter a chemical attack against us or our allies. And without a modern and credible deterrent, the prospects for achieving a comprehensive ban would be nil.[214(p23)]

In 1981, the secretary of defense issued a memorandum to proceed to acquire binary chemical bombs. The appropriation restrictions of 1976, however, blocked procurement of binary munitions for several more years. The next step came in 1985 when the U.S. Congress passed Public Law 99-145 authorizing production of chemical weapons. The final step came in 1987, when President Reagan certified to congress that all their conditions had been met to start production of binary chemical weapons. [3]

The production of the M687 binary projectile began on 16 December 1987 at Pine Bluff Arsenal. This was no small feat considering modern environmental and general public concerns. To resolve political concerns, the M20 canisters were filled and stored at Pine Bluff Arsenal, while the M21 canisters were produced and filled at Louisiana Army Ammunition Plant. The filled M21 canisters and shell bodies were then stored at Tooele Army Depot, Utah. In time of need, the parts could be combined and would provide the army with a chemical retaliatory capability. [215]

In addition to the M687 round, development work continued on the BLU-80/B (BIGEYE) bomb and the XM135 Multiple Launch Rocket System (MLRS) Binary Chemical Warhead. Both utilized the binary concept. The BIGEYE was in the 500-lb bomb class and was compatible with fixed-wing aircraft belonging to the air force, navy, and marine corps. The bomb dispersed the persistent nerve agent VX after mixing two nonlethal chemical agents (designated NE and QL). The XM135 binary chemical warhead was designed as a free-flight, semipersistent, nerve agent–dispersing system. The XM135 was fired from the MLRS, a 12-round rocket launcher mounted on a tracked vehicle.[215,216]

Chemical Training

In addition to establishing a retaliatory capability, the army significantly improved its chemical training capability by *(a)* constructing a new facility at the Chemical School and *(b)* conducting more-realistic field training. In 1987, the Chemical Decontamination Training Facility (CDTF) started live–chemical agent training in a controlled environment. Major General Gerald G. Watson, the school's commandant, was "the first American to wear the battledress overgarment in a toxic chemical environment" [217(p15)] when he entered the CDTF on 19 February 1987.

For realistic field training, the army conducted such training as Operation Solid Shield 87 to test how the U.S. troops perform on a chemically contaminated battlefield. Over 40,000 personnel from the U.S. Army, Navy, Marine Corps, Air Force, and Coast Guard participated in the simulated chemical attacks. Many conclusions were drawn from the training. Of particular concern was the impact on the medical personnel trying to help both conventional and chemical casualties:

> Use of chemical weapons in an otherwise conventional warfare scenario will result in significant impact on the medical capability to treat and handle casualties. Many medical facilities might be located near chemical target areas and may be subject to contamination.

> These facilities include battalion aid stations, hospital and medical companies, casualty receiving and treatment ships, fleet hospitals, and hospital ships. Provision of medical care in a contaminated environment is extremely difficult due to the encapsulation of medical personnel in their individual protective ensembles.
>
> Medical care is best provided in an environment free of toxic agents. This environment might be provided by a collectively protected facility, or be in an uncontaminated area. Medical units ashore and afloat can expect to receive contaminated casualties and must be prepared to provide contaminated casualties with a comprehensive and thorough decontamination. This procedure is similar whether processing patients into a collectively protected facility or processing from a contaminated area to an area free of contamination.[218(p30)]

The conclusions of the training impacted all aspects of the military forces:

> All organizations must be trained in NBC detection and identification procedures, particularly units with an inherent reconnaissance mission. First aid, and casualty handling, including mass casualty handling, must also be an integrated part of training. NBC contamination, medical, operational, and logistical problems should be evaluated and responded to realistically at all command and staff levels.
>
> Particular emphasis must be placed on the ability of personnel to remove contaminated clothing and equipment while minimizing the transfer of contamination to unprotected skin or to nonprotective underclothing.[218(p31)]

One officer summed up this new way of thinking about chemical training as demonstrated by Solid Shield 87:

> NBC warfare is not a separate, special form of war, but is instead a battlefield condition just like rain, snow, darkness, electronic warfare, heat, and so on. Units must train to accomplish their wartime missions under all battlefield conditions. Whenever NBC is separated from other training events, we condition our soldiers to regard operations under NBC conditions as a separate form of warfare.[218(p31)]

To reflect the changes in concept and equipment, the army's field manuals were also rewritten and updated to incorporate chemical warfare readiness into the army's air–land battle doctrine. The five parts of the new doctrine called for contamination avoidance, individual and collection protection, decontamination, chemical weapons employment, and the deliberate use of smoke.

Soviet–United States Agreement

The increase in the United States's retaliatory and defensive capability for chemical and biological warfare, along with internal changes in the Soviet Union, helped convince the Soviets to look closely at a new chemical weapons treaty. In 1987, after admitting for the first time that they possess chemical agents, they announced the halting of chemical weapons production. In September 1989, the *Memorandum of Understanding (MOU)*

Between the Government of the United States and the Government of the USSR Regarding a Bilateral Verification Experiment and Data Exchange Related to Prohibition of Chemical Weapons, otherwise known as the Wyoming MOU, started the talks between the two countries. [3]

U.S. Demilitarization Program

While the army was producing the new binary agent weapons, it was also discovering that the destruction of the existing chemical weapons stockpile was proving a far greater challenge than originally expected. In 1982, the army announced that incineration was the best option for disposing of the chemical agents. The construction of the first such disposal system was started on Johnston Atoll in 1985. The following year, congress passed Public Law 99-145, which mandated the destruction of the U.S. stockpile of chemical weapons by 1994. This also required that the army plan for maximum protection for the environment and human health during the destruction. In 1988, congress extended the destruction date to 1997; later, this date was further extended to 2004. In 1989, construction on a second disposal system was started at Tooele, Utah.[3,219]

THE 1990s: THE THREAT MATERIALIZES

The success of the “carrot and stick” strategy with the Soviet Union led to another change in course for the chemical program. On 1 June 1990, with the fall of many of the communist governments in Eastern Europe and improved relations with the Soviet Union, the United States and the Soviet Union signed a bilateral chemical weapons destruction agreement. In support of this agreement, the secretary of defense canceled most of the new chemical retaliatory program, and the army decided to mothball its new binary chemical production facilities in 1990. [220]

Shortly after the signing of the bilateral chemical weapons destruction agreement, the army began Operation Steel Box to remove all U.S. chemical weapons from Germany. The project started in July and finished in November 1990, with all the munitions safely moved to Johnston Atoll. The same year, the Johnston Atoll Chemical Agent Destruction System (JACADS) incinerator on the island became operational. The Tooele demilitarization plant was not operational until 1996.

In 1992, however, Public Law 102-484 instructed the army to restudy incineration as the best process for demilitarization due to continuing opposition by the general public. The army then began researching both neutralization and neutralization followed by biodegradation as alternate disposal options.[3,219]

Persian Gulf War

Despite the ongoing political efforts to abolish chemical warfare, world events dictated that chemical and biological weapons would again be the subject of daily news reports. On 2 August 1990, Saddam Hussein sent Iraqi troops into Kuwait—allegedly in support of Kuwaiti revolutionaries who had overthrown the emirate. By 8 August, however, the pretense was dropped and Iraq announced that Kuwait had simply been annexed and was now a part of their country. In response, President George Bush ordered U.S. forces sent to Saudi Arabia at the request of the Saudi government as part of what became Operation Desert Shield, the buildup phase of the Persian Gulf War.

The United States’s response to Iraq’s invasion put the army’s chemical and biological warfare experience, training, production program, and lessons learned in the limelight. Not since World War I had U.S. troops been sent to face an enemy that had not only used chemical weapons extensively within the last few years, but also had publicly announced their intentions to use chemical weapons against the United States. William H. Webster,

Director of Central Intelligence, estimated that Iraq had 1,000 tons of chemical weapons loaded in bombs, artillery rounds, rockets, and missiles. Much of Iraq's biological weapon program remained unknown until after the war. [221–223]

Iraq had a large biological agent production facility at al-Hakam that produced the agents that cause botulism, anthrax, and others. Started in 1988, the plant had produced about 125,000 gal of agent by 1991. After stating for years that the plant was used to produce animal feed, the Iraqis admitted in 1995 that the plant was a biological warfare production facility. In addition to producing biological warfare agents, they also conducted live-agent tests on animals. The Iraqis also later admitted they had prepared about 200 biological missiles and bombs. [224–227]

The United States's preparation for the military phase of the Persian Gulf War had to consider all these chemical and biological threats. Vaccines for anthrax and botulinum toxin were given to U.S. troops moving into the area. [228] For nerve agent poisoning, troops had the MARK I nerve agent antidote kit, consisting of an atropine autoinjector and a pralidoxime chloride (2-PAM Cl) autoinjector. The atropine blocked the effects of nerve agent poisoning on the muscles, while the 2-PAM Cl reactivated the acetylcholinesterase. Pyridostigmine bromide tablets also were provided as a nerve agent pretreatment. [229]All military units were fully equipped with the latest chemical and biological defensive equipment, and training was continuous.

The actual attack on Iraq on 16 January 1991 as part of the United Nations's mandated effort to free Kuwait, designated Operation Desert Storm by the United States, escalated fears of a new chemical war to levels not seen since World War I. The initial air attack concentrated on Iraqi chemical-production facilities, bunkers, and lines of supply. While the air attacks were ongoing, daily news accounts addressed the potential for chemical and biological warfare. On 28 January, Saddam Hussein told Peter Arnett of CNN News that his Scud missiles, which were already hitting Israel and Saudi Arabia, could be armed with chemical, biological, or nuclear munitions. [230] Vice President Dan Quayle, while visiting the United Kingdom, was reported to have told the prime minister that the United States had not ruled out the use of chemical or nuclear weapons.[230]Likewise, the United States was reported to have threatened to target Hussein personally if he used chemical weapons against Allied troops.[230,231] Iraq, in turn, reportedly threatened to use chemical weapons against Allied troops if they continued the high-level bombings against Iraqi troops. [230]

Thus the stage was set for what many thought was going to be the second major chemical war in this century. When the Allies began the ground war on 23 February 1991, the worst was expected and planned for by chemical and biological defense specialists. Chemical alarms frequently went off across the battlefield, but all were dismissed as false alarms. On 27 February, Allied troops liberated Kuwait City and finished destroying the Iraqi divisions originally in Kuwait. No known chemical and biological attacks were made by the Iraqis.

A number of reasons surfaced after war as to why the Iraqis had not initiated large-scale chemical and biological warfare. Vice Admiral Stanley Arthur, commander of U.S. naval forces, thought that because the wind suddenly changed from blowing south at the start of the land battle, the Iraqis had probably realized that chemical weapons could harm their own troops. Some thought the speed of the campaign was the critical reason. Others reported that the combination of Allied bombing and resulting Iraqi logistical nightmares prevented the chemical weapons from ever reaching the front lines. General H. Norman Schwarzkopf, commander of Allied forces, mentioned that Iraq might have feared nuclear retaliation. [223,230,232]

After the war, however, allegations of chemical and biological exposures began to surface. Initially, the department of defense denied that any chemical and biological exposures had taken place. Veterans of the war claimed the opposite, and their ailments

collectively became known as Gulf War Syndrome. By 1996, newspapers reported that almost 60,000 veterans of the Persian Gulf War claimed some sort of medical problems directly related to their war activities. Extensive research by the department of defense failed to find any single cause for the problems. [233,234]

One controversial example of possible exposure occurred on 4 March 1991 at the Kamisiyah arsenal, northwest of Basra, involving the U.S. Army 37th Engineer Battalion. After capturing the site, the engineers blew up the Iraqi storage bunkers. According to newspaper accounts, the engineers claimed that their chemical agent detectors went off during the explosions. Later the same year, a United Nations inspection team reportedly found the remains of chemical rockets and shells in one of the bunkers and found traces of sarin and mustard agent. In 1996, the department of defense acknowledged that one of the bunkers probably contained sarin- and mustard agent–filled munitions, and that as many as 20,000 U.S. soldiers may have been exposed to chemical agents as a result. [235] A Pentagon spokesman summed up the continuing research into the possible exposure: “Our understanding of this episode is still partial.” [234(pA-10)]

Additional Allegations of Chemical Warfare

Shortly after the fighting was over between Iraq and Allied forces, reports circulated that Hussein was using chemical agents against rebellious Kurds and Shiite Moslems. The United States intercepted a message ordering the use of chemical weapons against the cities of Najaf and Karbala. President Bush’s response was that such use of chemical weapons would result in Allied air strikes against the Iraqi military organization using the chemicals. Thus, despite the end of fighting, Iraqi chemical weapons continued to be a problem for the world. [236,237]

Likewise, U.S. intelligence sources detected increased chemical-development activity in Libya. Libya constructed a chemical weapons plant at Rabta that produced about 100 tons of chemical agents. In 1990, Libya claimed that the plant was destroyed by a fire. New disclosures surfaced in 1996 that Libya was constructing a second chemical production plant at Tarhunah. U.S. intelligence sources claimed that this would be the largest underground chemical weapons plant in the world, covering roughly 6 square miles and situated in a hollowed-out mountain. With Scud missiles having a range of 180 to 300 miles, this created a significant threat to Libya’s neighbors. Libya strongly denied the accusation. [238,239]

New Defensive Equipment

During the 1990s, the army standardized several new protective masks. In 1990, the M43 CB AH-64 Aircraft Mask was standardized for use in Apache helicopters. The unique aspect of the mask was its compatibility with AH-64 display sighting system. Within 6 years, the army improved the mask and standardized the new version as the M48 CB Aviator’s Mask. [3]

Although the new M40 series was an improvement over the M17 series mask, complaints from soldiers about the M40 masks resulted in the standardization of the M40A1 Field Mask and the M42A1 Combat Vehicle Mask in 1993. The M40A1 Mask added the Quick Doff Hood/Second Skin (QDH/SS), which allowed the hood to be doffed without removing the mask. This feature resulted in faster, more efficient decontamination operations. The M42A1 Mask added the QDH/SS and a canister-interoperability system that allowed the use of NATO canisters in the system. The new masks also included an improved nosecup that provided more comfort than the previous one. These changes increased the likelihood that soldiers would survive on a chemical and biological battlefield. [3,240]

The detection of chemical and biological agents was made much easier in the 1990s. In 1990, the army issued the first XM93 series NBC Reconnaissance Systems (the FOX), a dedicated system of NBC detection, warning, and sampling equipment integrated into a high-speed, high-mobility, armored carrier. A later version, the M93A1, was standardized in 1996. The FOX was capable of performing NBC reconnaissance on primary, secondary, or cross-country routes throughout the battlefield and had the capability to find and mark chemical and biological contamination. While conducting the reconnaissance, the crew was protected by an onboard overpressure system. [3,240,241]

The remote sensing research that started in the 1950s finally resulted in a detector in 1995, when the M21 Remote Sensing Chemical Agent Alarm was standardized. The M21 was an automatic scanning, passive infrared sensor that detected vapor clouds of nerve and blister agents, based on changes in the background's infrared spectra caused by the presence of agent vapor. The detector could "see" agent clouds to 5 km. [242]

After the Persian Gulf War, General Colin Powell testified to congress that the United States was vulnerable to biological warfare. One reason was that the United States had been unable to standardize a good biological agent detector. In 1995, the army standardized the first biological alarm. The M31 Biological Integrated Detection System (BIDS) was a small truck packed with sampling and detection equipment. Each vehicle could provide 24-hour monitoring, with identification of the agent following an alarm in about 30 minutes. [3,243]

The Chemical Weapons Convention

In 1993, the long-sought Chemical Weapons Convention was signed by the United States, Russia, and other countries. This treaty prohibited development, production, stockpiling, and use of chemical weapons. Ratification by the U.S. Senate, however, was delayed for various reasons. [3] One reason was that reports of a Russian chemical-development program surfaced in U.S. newspapers. A Russian scientist claimed that in 1991 Russia had developed a new, highly toxic, binary nerve agent called Novichok. According to the scientist, the nerve agent was undetectable by U.S. chemical detectors and may have been used in the Persian Gulf War by Iraq to produce some of the Gulf War syndrome symptoms. Despite these claims, the negotiations continued and additional agreements were signed with Russia. The United States even agreed to help fund the Russian demilitarization program. [244,245]

Terrorism and Counterterrorism

The use of chemical and biological weapons for terrorism became a key concern of the U.S. Army in the 1990s. In 1994, a Japanese religious cult, Aum Shinrikyo, reportedly released nerve agent in a residential area of Matsumoto, Japan, that killed 7 and injured 500. A second attack on 20 March 1995 spread sarin through a crowded Tokyo subway. This act of terrorism killed 12 and caused more than 5,500 civilians to seek medical attention. After the attacks, news accounts reported that the cult had developed a helicopter to spray toxins, a drone for unmanned chemical and biological attacks, and their own strains of botulism. They had also allegedly attempted to obtain the Ebola virus from Zaire. [246–248]

Chemical and biological terrorism was not limited to foreign countries. The first conviction under the Biological Weapons Anti-Terrorism Act of 1989 occurred in 1995, when a U.S. citizen was sentenced to 33 months in prison for possession of 0.7 g of ricin. The same year, a nonprofit organization shipped plague bacteria, *Yersinia pestis*, to an alleged white supremacist. [249,250]

Some of the items developed by the Chemical Corps were also used as counterterrorism measures, but sometimes with unintended consequences. For example, in 1993 the Federal

Bureau of Investigation decided to use a nonlethal riot control agent while attacking the Branch Davidian compound in Waco, Texas. Fires, however, destroyed the complex and killed the 80 occupants. [3,251]

These examples, both good and bad, led many state and local officials to notify congress that they did not have the training or equipment to combat an act of chemical or biological terrorism. Senator Sam Nunn expressed similar concerns:

> I, like many of my colleagues, believe there is a high likelihood that a chemical or biological incident will take place on American soil in the next several years.[252(pA-10)]

In reference to the Tokyo subway incident, he added:

> The activities of the Aum should serve as a warning to us all. This is a lesson that we will ignore at our peril.[251(pA-10)]

A military expert described the dangers of covert biological warfare:

> A terrorist attack using an aerosolized biological agent could occur without warning, and the first sign of the attack might be hundreds or thousands of ill or dying patients, since biological clouds are not visible.[251(pA-10)]

In 1996, the U.S. Congress responded by passing a new antiterrorism training bill to prepare the United States for future chemical and biological terrorism incidents. In addition to using military experts to equip and train local chemical and biological response teams, the bill also provided funding for former Soviet republics to destroy their own chemical and biological weapons to keep them out of the hands of terrorists. [251,253]

SUMMARY

Many lessons can be learned from the past concerning chemical and biological warfare and the U.S. experience combating it. So far, the United States has been extremely lucky and has not experienced a chemical and biological “Pearl Harbor” like some other countries have. To prevent that, the U.S. military forces will have to continue to learn about chemical and biological warfare and how to accomplish their mission—on both a chemical and biological battlefield and at a chemical and biological terrorist site anywhere in the world. In the words of General Pershing, “we can never afford to neglect the question” [43(p77)]of chemical and biological preparedness again.

REFERENCES

1. Department of the Army. *NATO Handbook on the Medical Aspects of NBC Defensive Operations*. Washington, DC:HQ, DA; AMedP-6, Part 3; 1996: 1-1. Field Manual 8-9.
2. Department of the Army. *NATO Handbook on the Medical Aspects of NBC Defensive Operations.* Washington, DC:HQ, DA; AMedP-6(B), Part 2; 1996: 1-1. Field Manual 8-9.
3. Smart JK. *History of Chemical and Biological Warfare Fact Sheets*. Aberdeen Proving Ground, Md: US Army Chemical and Biological Defense Command; 1996. Special Study 50. Not cleared for public release.
4. Browne CA. Early references pertaining to chemical warfare. *Chemical Warfare*. 1922;8(9):22–23.

5. Miles WD. Chemical warfare in the Civil War. *Armed Forces Chemical Journal*. 1958;12(2):27, 33.

6. Haydon F. A proposed gas shell in 1862. *Military Affairs*. 1938;2(2):54.

7. Miles WD. Suffocating smoke at Petersburg. *Armed Forces Chemical Journal*. 1959;13(4):34–35.

8. Pringle L. *Chemical and Biological Warfare*. Hillside, NJ: Enslow Publishers; 1993: 17.

9. Haber LF. *The Poisonous Cloud: Chemical Warfare in the First World War*. Oxford, England: Clarendon Press; 1986: 15–40.

10. MacCurdy E, ed. *The Notebooks of Leonardo da Vinci*. Vol 2. London, England: Jonathan Cape; 1977: 206.

11. US Patent 6529, 12 Jun 1849.

12. US Patent 7476, 2 Jul 1850.

13. Miles WD. The velvet-lined gas mask of John Stenhouse. *Armed Forces Chemical Journal*. 1958;12(3):24–25.

14. Prentiss AM. *Chemicals in War: A Treatise on Chemical Warfare*. New York, NY: McGraw-Hill; 1937: 343–477, 533- 566, 574, 685–689.

15. US Senate Committee on Foreign Relations, Subcommittee on Disarmament. *Chemical Biological Radiological (CBR) Warfare and Its Disarmament Aspects*. Washington, DC: Government Printing Office; 29 Aug 1960: 6, 19.

16. Hogg I. Bolimow and the first gas attack. In: Fitzsimons B, ed. *Tanks and Weapons of World War I*. New York, NY: Beckman House; 1973: 17–19.

17. Fries AA, West CJ. *Chemical Warfare*. New York, NY: McGraw-Hill; 1921: 10–11, 14, 31–71, 151.

18. Hanslian R. The gas attack at Ypres: A study in military history, I. *Chemical Warfare Bulletin*. 1936;22(1):5.

19. An account of German cloud gas attacks on British front in France. Unsigned manuscript. Attachment to a letter dated 11 Feb 1922 from Major ON Solbert, Military Attaché, American Embassy, London, England, to Chief, Chemical Warfare Service, Edgewood, Md; Subject: History of gas and development of British respirators from beginning of war to armistice. Original held at US Army Chemical and Biological Defense Command Historical Office in file named Gas Warfare History (British) 1915.

20. Fries AA. Gas in attack. *Chemical Warfare*. 1919;2(2):3, 8.

21. Stockholm International Peace Research Institute. *The Rise of CB Weapons*. Vol 1. In: New York, NY: Humanities Press; 1971: 111–124, 141–142, 147, 214–230.

22. Witcover J. *Sabotage at Black Tom*. Chapel Hill, NC: Algonquin Books of Chapel Hill; 1989: 83–101, 134–151.

23. Bancroft WD. *Bancroft's History of the Chemical Warfare Service in the United States*. Washington, DC: Research Division, Chemical Warfare Service, American University Experiment Station; May 1919: 12, 16.

24. Barker ME. Gas mask development. *Chemical Warfare*. 1926;12(7):14–15.

25. Brophy LP, Fisher GJB. *The Chemical Warfare Service: Organizing for War.* Washington, DC: Office of the Chief of Military History; 1959: 3–18, 25–27, 424–471.

26. Brophy LP, Miles WD, Cochrane RC. *The Chemical Warfare Service: From Laboratory to Field*. Washington, DC: Office of the Chief of Military History; 1959: 2–27, 49–76, 85–86, 101–138, 268, 336, 436–453.

27. Spencer EW. An historical lesson. *Chemical Warfare*. 1923;9(3):2–10.

28. Discipline through training or annihilation. *Chemical Warfare*. 1920;5(11):2. Editorial.

29. Hunt R. *Ricin*. Washington, DC: American University Experiment Station; 1918: 107–117. Chemical Warfare Monograph 37.

30. Smart JK. *Preparing for Chemical Warfare: The History of the Infantry Protective Mask*. Edgewood Arsenal, Md: US Army Chemical and Biological Defense Command; 1991. Not cleared for public release.

31. Gilman ED. The evolution of the gas mask. *Chemical Warfare*. 1922;8(8):5–9.

32. Evolution of the gas mask. *Chemical Warfare*. 1920;3(1):3–11.

33. Army War College. *Methods of Defense Against Gas Attacks*. Vol 2. In: *Gas Warfare*. Washington, DC: War Department; 1918: 32–35.

34. Mankowich J, Butcosk AF, Robbins R, Roberts WB, West AL, Love S. *Decontamination of Chemical Agents*. Aberdeen Proving Ground, Md: Technical Support Directorate; 1970: 20, 88–91. Edgewood Arsenal Special Publication 300–5.

35. Falkof MM, Gehauf B. *Detectors and Alarms*. Vol 11. In: *History of Research and Development of the Chemical Warfare Service in World War II*. Army Chemical Center, Md: Chemical and Radiological Laboratories; Oct 1951: 1–3, 102.

36. Nothing equal to the trained nose. *The Catalyst*. 1996;2(2):11.

37. Army War College. *Memorandum on Gas Poisoning in Warfare with Notes on its Pathology and Treatment.* Washington, DC: Government Printing Office; 1917: 18–21, 32.

38. US Army. *Memorandum on Gas Poisoning in Warfare with Notes on its Pathology and Treatment.* Washington, DC: Government Printing Office; 1918: 22, 26–34. Army Expeditionary Force Publication 48.

39. 157th Field Artillery, 82nd Division History Box 23 (282-32.15 157 FA Brig Memos). National Archives Building, Washington, DC.

40. Clark DK. *Effectiveness of Chemical Weapons in WWI*. Bethesda, Md: Johns Hopkins University Operations Research Office. 1959: 99–123. ORO-SP-88 Staff Paper.

41. Fries AA. Chemical warfare inspires peace. *Chemical Warfare*. 1921;6(5):4.

42. Poison gas is indispensable, says German inventor. *Chemical Warfare*. 1921;17(7):10.

43. Pershing JJ. *Final Report of General John J. Pershing*. Washington, DC: US Government Printing Office; 1920: 77.

44. Knappen TM. Chemical warfare and disarmament. *Chemical Warfare*. 1921;7(11):3.

45. Brown FJ. *Chemical Warfare, A Study in Restraints*. Princeton, NJ: Princeton University Press; 1968: 74–75, 98– 110, 118, 145, 193–194, 267–269, 279–281.

46. Clark EB. As chemical warfare chieftain. *Chemical Warfare Bulletin*. 1941;27(3):87. Editorial.

47. Fries AA. Sixteen reasons why the Chemical Warfare Service must be a separate department of the Army. *Chemical Warfare*. 1920;2(7):4.

48. Clapp D. Gas. *Chemical Warfare*. 1919;2(1):cover iv. Poem.

49. Barker ME. The work of the Chemical Warfare Service. *Chemical Warfare*. 1933;19(4):1330–1339.

50. Brophy LP. Chemical corps troops and training in the years between the two wars. *Chemical Corps Journal*. 1948;2(3):25–28.

51. War Department. *General Orders No. 26*. Washington, DC: War Department; 17 Jun 1922.

52. Status of chemical warfare preparedness in the US. *Chemical Warfare*. 1924;10(8):13.

53. Edgewood seeking boll weevil poison. *Chemical Warfare*. 1924;10(9):14.

54. Fries AA. Summary of marine piling investigation. *Chemical Warfare*. 1925;11(6):11.

55. Boll weevil investigations. *CWS News Letter*. 1928;1:3.

56. Fries AA. Chemical warfare—Past and future. *Chemical Warfare*. 1920;5(1):4–5.

57. Chemical warfare bombing tests on warship. *Chemical Warfare*. 1921;6(10):6.

58. Fries AA. *Annual Report of Chief, Chemical Warfare Service*. Washington, DC: CWS; 1921: 22–24.

59. Hanslian R. The gas attack at Ypres: A study in military history, II. *Chemical Warfare Bulletin*. 1936;22(3):129.

60. Fries AA. *Annual Report of the Chief of Chemical Warfare Service*. Washington, DC: CWS; 1926: 8.

61. Fair SD. Mussolini's chemical war. *Army*. 1985;35(1):47.

62. Foreign developments in chemical warfare. *Chemical Warfare*. 1923;9(9):19.

63. Smalley VE. *Report of the CW Status of Italy (1926–1948)*. Army Chemical Center, Md: Chemical Corps Technical Command; 1948: 14, 52–53.

64. Williams P, Wallace D. *Unit 731*. New York, NY: The Free Press; 1989: 13–30.

65. Robertson AG, Robertson LJ. From asps to allegations: Biological warfare in history. *Milit Med*. 1995;160(8):369–372.

66. Thomas AVW, Thomas AJ Jr. *Basic Report*. Vol 2. In: *Development of International Legal Limitations on the Use of Chemical and Biological Weapons*. Dallas, Tex: Southern Methodist University School of Law and US Arms Control and Disarmament Agency; 1968: 53–54, 73–102.

67. The present international situation as regards chemical warfare and the impossibility of securing the assured abolition of the use of gas in future wars. *Chemical Warfare*. 1925;11(3):7.

68. Gas warfare not forbidden by any international agreement. *Chemical Warfare*. 1925;11(3):6.

69. The international legal status of chemical warfare. *Chemical Warfare*. 1926;12(10):9–14.

70. Waitt AH. Europe looks at chemical warfare. *Army Ordnance*. 1935;15(89):285.

71. Brigham CE. *Final Report on Status of Chemical Readiness by the Retiring Chief*. Record Group 114; 1937. National Archives, Suitland, Md.

72. Wiseman DJC. *Special Weapons and Types of Warfare*. London, England: The War Office; 1951: 150.

73. Intelligence Division, Chemical Warfare Service. *German Chemical Warfare, World War II*. Washington, DC: CWS; 1945: 28–30, 115–117.

74. Department of the Army. *Chemical Corps Equipment Data Sheets*. Washington, DC: HQ, DA; 1961: 3, 60, 103.Training Manual 3-500.

75. Fox LA. Bacterial warfare: The use of biologic agents in warfare. *Military Surgeon*. 1933;72(3):189–207.

76. Scientific and Technical Advisory Section, US Army Forces, Pacific. *Biological Warfare*. Vol 5. In: *Report on Scientific Intelligence Survey in Japan*. HQ, US Army Forces, Pacific; 1945.

77. West AL, Goldfield J, Mitchell J. Antigas Collective Protection Equipment. Edgewood Arsenal, Md:

1969: 20, Edgewood Arsenal Special Publication 300-4.

78. Duffield M. Ethiopia: The unconquered lion of Africa. *Command Magazine*. 1990;4:10–22.

79. Neil EJ. *Use of Gas in Ethiopia*. Washington, DC: War Department; 2 Sep 1936. War Department Memorandum.

80. Thuillier HF. The use of mustard gas by the Italians in the Abyssinian War. Porton, United Kingdom; 25 Apr 1941. Memorandum CDR5/1793.

81. Volkart W. The Gas Weapon in the Italian–Abyssinian War 1935–1936. Translated from *Allgemeine Schweizerische Militär Zeitschrift*. Typescript prepared for Army Chemical Center, Md; 1951.

82. Murphy P. Gas in the Italo–Abyssinian campaign. *Chemical Warfare Bulletin*. 1937;23(1):1–8.

83. *Time*. Quoted in: Brett HH. Chemicals and aircraft. *Chemical Warfare Bulletin*. 1936;22(4):151–152.

84. Kohn GC. *Dictionary of Wars*. New York, NY: Facts on File Publications; 1986: 226, 433–434, 524.

85. Chemicals in Ethiopia. *Chemical Warfare Bulletin*. 1936;22(3):143.

86. Use of gas in Italo–Abyssinian campaign. *Chemical Warfare Bulletin*. 1937;23(2):81.

87. Hart L. The Abyssinian War. *Ordnance*. 1937;17(102):330.

88. Clark DK. *Effectiveness of Toxic Chemicals in the Italo–Ethiopian War*. Bethesda, Md: Operations Research Office; 1959: 1–21.

89. Baker WC. *Annual Report of the Chief of the Chemical Warfare Service for the Fiscal Year Ending June 30, 1937*. Washington, DC: War Department; 1937.

90. Chemical Warfare School. *Use of Gas in Ethiopia—1936*. Edgewood Arsenal, Md: Chemical Warfare School; 22 Oct 36. Mimeo 1.

91. Brett GH. Chemicals and aircraft. *Chemical Warfare Bulletin*. 1936;22(4):153.

92. 5250th Technical Intelligence Company. *The Use of Poison Gas by Imperial Japanese Army in China, 1937–1945*. Tokyo, Japan: 5250th Technical Intelligence Company; 1946.

93. Watson M. Army is speeding chemical defense. *Baltimore Sun*. 12 Nov 1941. Quoted in: *CWS News Letter*. 1941;5(6):37.

94. Nazis may use gas, General Porter warns. *CWS News Letter*. 1941;5(5):31.

95. General Porter urges America to “Get Tough.” *CWS News Letter*. 1941;5(6):36.

96. Chemical warfare goes on maneuvers. *Chemical Warfare Bulletin*. 1942;28(1):4–24.

97. Japs reported using gas. *CWS News Letter*. 1941;5(6):33–34.

98. Miller GA. The development of the 4.2 chemical mortar. *Armed Forces Chemical Journal*. 1948;3(2):33–42; continued in 1949;3(3):35–42.

99. US War Department. *Characteristics and Employment of Ground Chemical Munitions*. Washington, DC: War Department; 1946: 108–119. Field Manual 3-5.

100. US War Department. *Employment and Characteristics of Air Chemical Munitions*. Washington, DC: War Department; 1946: 4–5. Field Manual 3-6.

101. Infield G. *Disaster at Bari*. New York, NY: Bantam Books; 1988: 209, 230–231.

102. Cochrane RC. *Research and Development*. Vol 2. In: *History of the Chemical Warfare Service in World War II*. Aberdeen Proving Ground, Md: Chemical Corps Historical Office; 1947: 245–246, 248–249. Draft.

103. Office of the Chief, Chemical Warfare Service. *Report of Activities of the Technical Division During World War II*. Washington, DC: CWS; 1946: 172–175.

104. Katz SH. *Standard US Army Gas Masks and Components*. Edgewood Arsenal, Md: Chemical Warfare Service Technical Command; 1944. Technical Division Memorandum Report 878-2.

105. Office of the Chief, Chemical Warfare Service. *New Gas Detector Kit*. Washington, DC: CWS; 12 Sep 44. Information.

106. Cochrane RC. *Biological Warfare Research in the United States*. Office of Chief, Chemical Corps: Historical Section, Plans, Training and Intelligence Division; 1947. Draft.

107. Ditto RC. Will Hitler's goose be cooked with gas? *Chemical Warfare Bulletin*. 1943;29(3):6–7.

108. Chemical Warfare Service. *US Chemical Warfare Policy*. Washington, DC: Operations Division, War Department General Staff, Strategy and Policy Group; 14 Jun 1945. Draft.

109. Wing HG. *Gas Warfare Planning: History of the Chemical Warfare Service in World War II*. 1953: 43–58.

110. Finlay WW. Why prepare? *Chemical Corps Journal*. 1946;1(1):24.

111. Royall KC. A tribute to the corps. *Chemical Corps Journal*. 1947;2(1):41.

112. King L. Exit gas warfare? *Chemical Corps Journal*. 1948;2(3):3.

113. *The Army Almanac*. Washington, DC: Government Printing Office; 1950: 89.

114. Waitt AH. *Chemical Warfare Organization and Policy in the Post-War Army*. Washington, DC: Chemical Warfare Service. 9 May 46. Memorandum.

115. Office of the Chief of Chemical Corps. *The History of Captured Enemy Toxic Munitions in the American Zone, European Theater, May 1945 to June 1947*. Headquarters: Chemical Corps, European Command; 1947.

116. Department of the Army. *Chemical Agents of the G-Series*. Washington, DC: HQ, DA; 19 Mar 1948. Circular 74.

117. Department of the Army. *Treatment of Poisoning Caused by Chemical Agents of the G-Series*. 19 Mar 1948. Washington, DC: HQ, DA; Technical Bulletin Chemical Warfare 34.

118. Waitt AH. Chief, Chemical Corps, to Director of Plans and Operations, General Staff, US Army. *Gas Warfare Policy*. Washington, DC: Chemical Corps. 2 May 1949. Memorandum.

119. Loucks CS. The "decons" and the air lift. *Armed Forces Chemical Journal*. 1949;3(6):16, 38.

120. McAuliffe AC. Korea and the Chemical Corps. *Ordnance*. 1951;35(184):284.

121. Department of the Army. *Transfer of the Responsibilities for the 4.2-inch Chemical Mortar and Related Equipment to the Ordnance Department*. Washington, DC: HQ, DA; 9 Feb 1948. Memorandum.

122. Rodier HB. Editorial. *Armed Forces Chemical Journal*. 1951;4(3):3.

123. Baker ER. Chemical warfare in Korea. *Armed Forces Chemical Journal.* 1951;4(4):3. Letter.

124. Probe into biological experiments on Korean War POWs reported. *Washington Times*. 15 Aug 1992.

125. Bullene EF. The needs of the army. *Armed Forces Chemical Journal*. 1952;6(1):8.

126. Abolish unit gas officer positions. *Armed Forces Chemical Journal*. 1954;8(5):4.

127. Hylton AR. *The History of Chemical Warfare Plants and Facilities in the United States*. US Arms Control and Disarmament Agency: Midwest Research Institute; 1972;4:59–75. ACDA/ST-197.

128. Fielding GH. *V Agent Information Summary*. Washington, DC: US Naval Research Lab; 1960: 1–2. NRL Report 5421.

129. US Army Chemical Corps. *Summary of Major Events and Problems*, FY58. Army Chemical Center, Md: US Army Chemical Center Historical Center; Mar 1959: 97–101, 108–111, 153–158.

130. US Army Chemical Corps. *Summary of Major Events and Problems*, FY59. Army Chemical Center, Md: US Army Chemical Center Historical Center; Jan 1960: 101–105, 112–114, 117–118, 160–162.

131. US Army Chemical Corps. *Summary of Major Events and Problems*, FY57. Army Chemical Center, Md: US Army Chemical Center Historical Center; Oct 1957: 97–98, 103.

132. Smart JK. Biological Weapons. Aberdeen Proving Ground, Md: US Army Chemical and Biological Defense Command; 1996. Special Study 55. Not cleared for public release.

133. The Inspector General, Department of the Army. *Use of Volunteers in Chemical Agent Research*. Washington, DC: DA, IG; 1976. DAIG-IN 21-75.

134. US Army Chemical Corps. *Summary of Major Events and Problems*, FY55. Army Chemical Center, Md: US Army Chemical Center Historical Center; Dec 1955: 48–49, 61–62, 133.

135. US Army Chemical Corps. *Summary of Major Events and Problems*, FY56. Army Chemical Center, Md: US Army Chemical Center Historical Center; Nov 1956: 128–130, 133–134, 140–141.

136. US Army Chemical Corps. *Summary of Major Events and Problems*, FY54. Army Chemical Center, Md: US Army Chemical Center Historical Center; n.d: 35–36.

137. Chemical Corps Technical Committee. *Standardization of Agent, Decontaminating, STB and Antiset, M1; Reclassification of Bleaching Material, Grade 3*. Army Chemical Center, Md: CCTC; 1950. Memorandum.

138. Creasy WM. The forward look in the Army Chemical Corps. *Armed Forces Chemical Journal*. 1957;11(4):26.

139. Stubbs M. CBR—A power for peace. *Armed Forces Chemical Journal*. 1959;13(3):8–9.

140. Stubbs M. Presentation to Directors, New York State Civil Defense Commission; 9 Feb 1960; Hotel Thayer, West Point, NY. Speech.

141. US Army Chemical Corps. *Summary of Major Events and Problems, FY1961–62*. Army Chemical Center, Md: US Army Chemical Center Historical Center; n.d: 9–20, 124–126, 131–132.

142. Palmer JM. Chemical warfare training. *Armed Forces Chemical Journal*. 1930;14(6):28.

143. Anckaitis WH. Realistic CBR training. *Armed Forces Chemical Journal*. 1964;18(3):16.

144. Harrigan A. The case for gas warfare. *Armed Forces Chemical Journal*. 1963;17(2):12.

145. United Press International. Mustard gas use suggested. *Washington Post*. 18 Oct 1966;A-2.

146. Badeeb SM. *The Saudi–Egyptian Conflict Over North Yemen*, 1962–1970. Boulder, Colo: Westview Press; 1986: 2–41.

147. Meselson M. The Yemen. In: Rose S, ed. *CBW: Chemical and Biological Warfare*. Boston, Mass: Beacon Press; 1968: 99.

148. Pearson D, Anderson J. Egypt's use of gas in Yemen verified. *Washington Post*. 6 Jun 1967.

149. Childs M. Chemical warfare and a death wish. *Washington Post*. 21 Jun 1 967;A-20.

150. How Nasser used poison gas. *US News & World Report*. 3 Jul 1967;60.

151. Cromley R. Russians use Yemen as lab for "poison gas." *Washington News*. 24 Jul 1967.

152. Nasser stoops lower. *Chicago Daily News*. 31 Jul 1967.

153. Cromley R. Why Israel stocks up on gas masks. *Detroit News*. 8 Aug 1967.

154. Dupuy TN. *The Encyclopedia of Military History From 3500 BC to the Present*. New York, NY: Harper & Row; 1986: 1279–1280.

155. Hersh SM. *Chemical and Biological Warfare: America's Hidden Arsenal*. New York, NY: Bobbs-Merrill; 1968: 286–287.

156. US Army Chemical Corps. *Summary of Major Events and Problems*, FY60. Army Chemical Center, Md: US Army Chemical Center Historical Center; 1961: 108, 115–117.

157. Department of the Army. *US Army Activity in the US Biological Warfare Programs*. Washington, DC: HQ, DA; 1977. Draft.

158. Edgewood Arsenal. *Consent to Inoculation With Experimental Biological Products*. Edgewood Arsenal, Md: Edgewood Arsenal; 1965.

159. Medical Investigation Division. *Nominees for Special Procedures Program*. Edgewood Arsenal, Md: Medical Investigation Division; 9 Nov 1965. Memorandum.

160. US Army Materiel Command. *Classification of Collective Protection Equipment, Guided Missile Van, NIKE-HERCULES, Trailer-Mounted, M1 (E1R1) as a Standard–B Type & Component of the Related Air Conditioner–Collective Protector (AC–CP)*. Washington, DC: AMC; 23 Dec 1963. Memorandum.

161. Department of the Army. *Chemical Weapons and Defense Equipment*. Washington, DC: HQ, DA; 1972: 147–148, 163, 185–186, 245. Training Manual 750-5-15.

162. Stone WW. *Report of Investigation Concerning Sheep Deaths in Skull Valley, Utah.* Washington, DC: US Army Materiel Command; n.d. A-1.

163. Associated Press. Army speeds removal of Okinawa gas. *Washington Post*; 3 Dec 1969;A-3.

164. Stockholm International Peace Research Institute. *CB Weapons Today*. Vol 2. In: *The Problem of Chemical and Biological Warfare*. New York, NY: Humanities Press; 1973: 193.

165. Ward PF. *A Summary of Ecological Investigations at Edgewood Arsenal, Maryland*: Fiscal Year 1970. Edgewood, Md: Edgewood Arsenal Research Labs; 1971: 15–23. Edgewood Arsenal Special Publication 100-101.

166. Reducing the terror of war. *Commanders Digest*. 20 Dec 1969;4–5.

167. Wagner RL, Gold TS. Why we can't avoid developing chemical weapons. *Defense*. 1982;3.

168. Fair SD. The chemical corps: Alive, well and visible. *Army*. 1972;29–32.

169. "Preferred alternative" would move chemical training to Ft. McClellan. *APG News*. 4 Apr 1979;A-1.

170. Chemical Corps School. *Chemical School*. Fort McClellan, Ala: Chemical Corps School; n.d.

171. Guiler DC Jr. Chemical Corps: A branch in search of an identity. *Army*. 1977;14, 15.

172. Foreign Science and Technology Center. *Foreign Materiel Exploitation Report, Detection Kit, Chemical Agent, Model PKhR-MV (Soviet) (U)*. Washington, DC: Foreign Science and Technology Center; 1975. AST-1640X-174-75.

173. US Army Armament Research and Development Command. *Laboratory Posture Report*. Dover, NJ: ARRADCOM; Fiscal Year 1978: 3–4.

174. Gas antidote. *Washington Star-News*. 18 Jul 1974.

175. Eifried G. Russian CW: Our Achilles' heel, Europe. *Army*. 1979;29(12):24–28.

176. Kastenmayer WW. A rebirth of chemical R&D. *Army Research, Development & Acquisition Magazine*. 1981;22 (4):13–15.

177. Siebert GW, Choi YH. Chemical weapons: Dull swords in the US armory. *Military Review*. 1985;65(3):23–29.

178. Famiglietti G. Army may switch decision, reestablish Chemical School. *Army Times*. 22 Jan 1979.

179. Chemical Corps School. *The Chemical Corps Regimental Activation Ceremony*. Fort McClellan, Ala: Chemical Corps School; 1986. Pamphlet 27.

180. US Army Chemical Corps. *Wizard of Battle*. Fort McClellan, Ala: Army Chemical Corps; n.d. Pamphlet.

181. *Fort McClellan*. Fort McClellan, Ala: National Military Publications; 1983: 3. Pamphlet.

182. Department of the Army. *Binary Chemical Munitions Program*. Aberdeen Proving Ground, Md: Chemical Systems Laboratory; 1981: 1–7. Programmatic Environmental Impact Statement ARCSL-EIS-8101.

183. Ward FP. *Construction and Operation of a 155mm M687 GB2 Binary Production Facility at Pine Buff Arsenal, Jefferson County, Arkansas*. Aberdeen Proving Ground, Md: Chemical Systems Laboratory; 1981: 3–7. Environmental Assessment ARCSL-EA-8101.

184. US Army Materiel Command. *Classification of Paper, Chemical Agent Detector, VGH, ABC-M8 as a Standard-A Type & Reclassification of the M6 as Obsolete & M6A1 as Standard-B Papers; & Completion of Task 1C643606D02202, Nonvolatile Agent Contamination Detector.* Washington, DC: AMC; 1 Nov 1963. Memorandum.

185. NBC nuggets. *NBC Readiness Bulletin*. 1978;4:1, 5.

186. US Army Materiel Command. *Approval of Minutes of Development Acceptance (DEVA) Review and the Reclassification to Standard-A of Shelter System, Collective Protection, Chemical-Biological: Inflatable, 10-man, Trailer-Transported, M51 (XM51)*. Washington, DC: AMC; 3 Aug 1971. Memorandum.

187. Department of Defense Appropriation Authorization Act. Public Law 95-79, 30 Jul 1977.

188. Keegan RJ. Definition of policy of the USA and USSR on chemical and biological warfare. *Commander's NU-CH Flash.* 1982;9:6. Bulletin of the US Army Nuclear and Chemical Agency.

189. Haig AM Jr. *Chemical Warfare in Southeast Asia and Afghanistan.* Report to the Congress from Secretary of State Alexander M. Haig, Jr. Washington, DC: US Department of State; 1982. Special Report 98.

190. Hilts PJ. '79 anthrax traced to Soviet military. *New York Times*. 18 Nov 1994.

191. Smith RJ. Yeltsin blames '79 anthrax on germ warfare efforts. *Washington Post*. 16 Jun 1992.

192. DeWeese K. Outbreak tied to microbiology site. *APG News*. 23 Mar 1994.

193. Collins JJ. The Soviet military experience in Afghanistan. *Military Review*. 1985;65(5):27.

194. Hoffman MS, ed. *The World Almanac and Book of Facts, 1990.* New York, NY: Pharos Books; 1990: 44, 46, 49, 721.

195. Spiers EM. *Chemical Weaponry: A Continuing Challenge.* New York, NY: St. Martin's Press; 1989: 121.

196. Dunn P. The chemical war: Journey to Iran. *Nuclear, Biological, and Chemical Defense and Technology International.* 1986;28–35.

197. Associated Press. Iraqi gas attack on Kurds disputed. *Washington Times*. 18 Dec 1990.

198. Rohrbaugh DK, Ward JR, Yang Y. *Comments on the Origin of Mustard in the Gulf War.* Aberdeen Proving Ground, Md: US Army Chemical Research Development and Engineering Center; 1990: 8. Technical Report.

199. Dunn P. *Chemical Aspects of the Gulf War, 1984–1987, Investigations by the United Nations*. Ascot Vale, Australia: Materials Research Laboratories; 1987.

200. United Nations. *Report of the Mission Dispatched by the Secretary-General to Investigate Allegations of the Use of Chemical Weapons in the Conflict Between the Islamic Republic of Iran and Iraq*. New York, NY: United Nations Security Council; 12 Mar 1986: 19.

201. Special to The New York Times. UN Panel says Iraq used gas on civilians. *New York Times*. 24 Aug 1988.

202. Dingeman J, Jupa R. Chemical warfare in the Iran–Iraq conflict. *Strategy & Tactics*. 1987;113:51–52.

203. Morrison J. Angola again tied to use of nerve gas. *Washington Times*. 11 Mar 1988.

204. Lancaster P. Fighting guerrillas with gas. *The Middle East*. 1 Jun 1987;17.

205. Wayne EA. Libya seeks chemical weapons in war against Chad, US charges. *Christian Science Monitor*. 5 Jan 1988;A-1.

206. Satchell M, Blaug E. A plague of “hellish poison.” *US News & World Report*. 26 Oct 1987;30.

207. Historical Office, US Army Armament, Munitions and Chemical Command. *US Army Chemical Research, Development and Engineering Center (CRDEC) Annual Historical Review (AHR).* Aberdeen Proving Ground, Md: AMCCOM; Fiscal Year 1987: 195.

208. US Army Materiel Command. *Materiel Status Record Submission 11876009*. Washington, DC: AMC; 23 Sep 1987. Memorandum.

209. New mask replaces M17 and M9A1. *Army Chemical Review*. 1988;44–45.

210. Chemical Systems Laboratory. *Summary History*. Aberdeen Proving Ground, Md: Chemical Systems Laboratory; Fiscal Year 1980: 142–143, 158.

211. Department of the Army. *NBC Protection*. Washington, DC: HQ, DA; 1992. Field Manual 3-4.

212. Historical Office, US Army Armament, Munitions and Chemical Command. *US Army Chemical Research and Development Center (CRDC) Annual Historical Review (AHR)*. Aberdeen Proving Ground, Md: AMCCOM; 1 Jul 1983–30 Sep 1984: 73–76.

213. Chemical Warfare Review Commission. *Report of the Chemical Warfare Review Commission*. Washington DC: Chemical Warfare Review Commission. Washington, DC: Government Printing Office; 1985: 50, 73.

214. The chemical strategy. *Army Chemical Review*. Jan 1988;23.

215. Historical Office, US Army Armament, Munitions and Chemical Command. *US Army Chemical Research, Development and Engineering Center (CRDEC) Annual Historical Review (AHR)*. Aberdeen Proving Ground, Md: AMCCOM; Fiscal Year 1988: 8–9, 106–108.

216. Army weaponry and equipment. *Army*. 1990;40(10):326.

217. Parker C. Toxic chemical training. *Army Chemical Review*. Sep 1987;15.

218. Polley W, Dlugopolski M, Hartzell W. 40,000 train in chemical environment. *Army Chemical Review*. Jan 1988;26–31.

219. US Congress, Office of Technology Assessment. *Disposal of Chemical Weapons: Alternative Technologies—Background Paper*. Washington, DC: Government Printing Office; 1992. OTA-BP-O-95.

220. Yockey DJ, Deputy Under Secretary of Defense (Acquisition). *United States Chemical Programs*. Washington, DC: Department of Defense; 12 Jul 1990. Memorandum For Secretaries of the Military Departments, et al.

221. Reid B. Experts debating Saddam's threat to use chemicals. *Baltimore Evening Sun*. 22 Jan 1991.

222. Baker C. Tenacity may be Iraqi ace. *Army Times*. 4 Feb 1991.

223. Atkinson R. No chemical arms found on battlefields. *Washington Post.* 7 Mar 1991.

224. Associated Press. UN arms monitors destroy Iraqi germ-weapons plant. *Baltimore Sun.* 21 Jun 1996;A-22.

225. Iraqi weapons plant destroyed. *Military Newswire.* 1996.

226. Smith RJ. Iraq had program for germ warfare. *Washington Post*. 6 Jul 1995;A-1.

227. Crossette B. Iraq gives UN full details on its germ warfare program. *New York Times*. 23 Aug 1995.

228. Barker RB. The future of the DOD chemical/biological defense program. *Defense*. 1992;May–Jun:27.

229. Herbig AT. Nerve agents—their physiological effects. *Army Chemical Review*. 1990;July:9–13. PB 3-90-2.

230. Smart JK. *Desert Storm Diary*. Aberdeen Proving Ground, Md: US Army Chemical and Biological Defense Command; 1991. Not cleared for public release.

231. Johnson SS. Cheney fears chemical attack possible. *USA Today*. 28 Jan 1991.

232. Gertz B. Nerve gas detected by Czechs during Gulf War. *Washington Times*. 7 Sep 1993.

233. Gulf War veterans link ailments to destruction of Iraqi bunker. *Baltimore Sun*. 11 Aug 1996;A-3.

234. American soldiers may have been exposed to Iraqi chemical weapons, Pentagon says. *Baltimore Sun*. 22 Jun 1996;A-10.

235. New York Times News Service. 20,000 troops may have faced gas. *The Sun*. 23 Oct 1996:A-1.

236. Matthews M. US warns Iraq against using chemical arms. *Baltimore Sun*. 10 Mar 1991.

237. Tyler PE. Baghdad reportedly told commanders to use chemicals. *Baltimore Sun*. 10 Mar 1991.

238. Wines M. US hints at chemical arms bunker in Libya. *New York Times*. 7 Mar 1991.

239. New York Times News Service. Libya close to finishing huge chemical weapons plant. *Baltimore Sun*. 25 Feb 1996;A-20.

240. US Army Chemical and Biological Defense Command. *AMC Accomplishments During General Ross' Period of Command*. Aberdeen Proving Ground, Md: CBDCOM; n.d.

241. NBCRS headed for production. *The Catalyst*. 1995;1(2):4.

242. Alarming news: M21 alarm approved for production. *The Catalyst*. 1995;1(1):1, 4.

243. Moore A. Putting the "B" back in NBC. *Soldiers*. 1996;51(4):36.

244. Adams JR. Russia's toxic threat. *Wall Street Journal*. 30 Apr 1996.

245. Englund W. Ex-Soviet scientist says Gorbachev's regime created new nerve gas in '91. *Baltimore Sun*. 16 Sep 1992.

246. Lewthwaite GA. Terrorist attacks in US expected. *Baltimore Sun*. 1 Nov 1995;A-1.

247. Associated Press. Japanese guru will hear litany of nerve-gas victims. *Baltimore Sun*. 21 Apr 1996;A-22.

248. Congress probes Japanese cult. *Military Newswire*. 1996.

249. Birch D. Bubonic plague sample delivered to white racist. *Baltimore Sun*. 18 May 1995;A-4.

250. Wire Reports. Bio-terrorism sentence. *Baltimore Sun*. 19 May 1995;A-3.

251. Associated Press. ATF officials say they've instituted reforms since Waco. *Baltimore Sun*. 1 Nov 1995;A-10.

252. Wire Reports. Anti-terrorism training bill clears senate on 96–0 vote. *Baltimore Sun*. 28 Jun 1996;A-10.

253. *Defense Against Weapons of Mass Destruction*. House Bill HR 3730; 27 Jun 1996.

Chapter 3

HISTORICAL ASPECTS OF MEDICAL DEFENSE AGAINST CHEMICAL WARFARE

ROBERT J. T. JOY, M.D., FACP*

INTRODUCTION

EARLY HISTORY

NINETEENTH CENTURY

WORLD WAR I

MEDICAL PROBLEMS CAUSED BY MUSTARD

THE INTERWAR YEARS

WORLD WAR II

THE POSTWAR YEARS: 1945 TO THE PRESENT

SUMMARY

Colonel, Medical Corps, U.S. Army (Ret); Professor Emeritus, Department of Medical History, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799

INTRODUCTION

In discussing the history of the use of any new weapon and the medical response to it, one must also describe the context of the weapon: its scientific, social, and political aspects. For chemical warfare, there is the particular idea that chemical weapons are inhumane and immoral. Medical people, who treat the wounded, may well believe that *all* weapons are inhumane. Nevertheless, even the terms are relative—consider Pope Innocent II, who, in 1139, forbade the use of the relatively new cross-bow as "Hateful to God and unfit for Christian Use." [1(pp35–36)] His prohibition was cheerfully ignored; the crossbow was used for over 300 years. In this essay, I will return to the issue of the moral use of the chemical weapon, but let us begin with the early history of chemical warfare itself.

EARLY HISTORY

In Thucydides's *History of the Peloponnesian War*, the 4th-century BC war between Athens and Sparta, we find the earliest description of chemical warfare. Thucydides describes how the Athenians were defending a fort at Delium in 423 BC, when the allies of Sparta attacked:

> The Boethians took the fort by an engine of the following description. They sawed in two and scooped out a great beam from end to end and fitted [it] together again like a pipe. They hung by chains a cauldron at one extremity, with which communicated an iron tube projecting from the beam, and this they brought up on carts to the part of the wall composed of vines and timber and inserted huge bellows into their end of the beam and blew with them. The blast passing closely confined into the cauldron, filled with lighted coals, sulfur and pitch made a great blaze and set fire to the wall. The smoke made it untenable for the defenders who left and fled, and the fort was taken.[2(p262)]

In AD 660, some thousand years later, a man named Kalinkos, who was either a Greek architect or a Syrian alchemist, invented Greek fire. The actual formula is lost, but it probably consisted of resin, pitch, sulfur, naphtha, lime, and saltpeter. Greek fire was an excellent naval weapon because it would float on water and set fire to the wooden ships of the era. [3] In the 9th century, Leo IX of Byzantium, writing on warfare, described "vases filled with quick-lime which were thrown by hand. When broken, the vase would let loose an overpowering odor which suffocates those who are near." [4(pp45–46)] Historically, then, the chemical weapons were fire and gas.

NINETEENTH CENTURY

In 1812, Admiral Thomas Cochrane of the Royal Navy of Great Britain proposed packing ships with sulfur, setting them afire, and having them sail into the French ports during the Napoleonic wars. Cochrane argued that the resultant sulfur dioxide would be carried by prevailing winds into the forts and thus incapacitate the enemy. [5–7] The Admiralty turned down his idea as impractical and further stated, "It is against the rules of warfare." [7(p22–23)]

Some 30 years later, during the Crimean War of 1854, Sir Lyon Playfair, a noted British chemist, proposed the use of cyanide-filled shells against the Russian fort at Sebastapol. The War Office rejected the idea, stating that it was "as bad as poisoning the enemy's water supply." [8(p23)] Playfair was appalled by that decision and made an interesting prophecy:

> There is no sense to this objection. It is considered a legitimate mode of warfare to fill shells with molten metal, which, scattering among the enemy, produced the most frightful modes of death. Why is a poisonous vapor which would kill men

> without suffering to be considered illegitimate? This is incomprehensible to me. But no doubt in time chemistry will be used to lessen the suffering of combatants.[8(p23)]

When the American Civil War started in 1861, the use of Greek fire was threatened but, in fact, never used. Edwin Stanton, President Lincoln's secretary of war, received an interesting letter from Mr. John Doughty of New York in 1862. Enclosing a sketch of an artillery shell (Figure 3-1), Mr. Doughty wrote:

> Above is the projectile I have devised for routing an entrenched enemy. Chlorine is so irritating in its effects upon the respiratory organs that a small quantity produces incessant and uncontrollable violent coughing. A shell holding two or three quarts of liquid chlorine contains many cubic feet of the gas.[9(p9)]

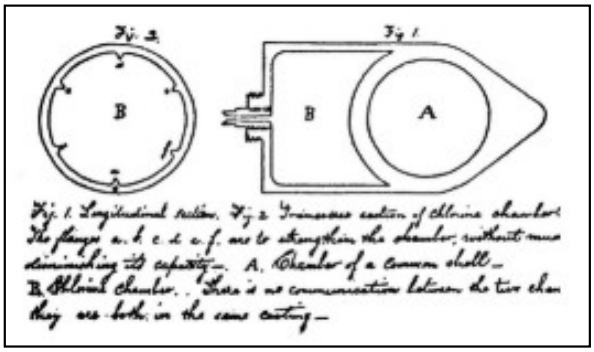


Fig. 3-1. John W. Doughty's original drawing of the artillery chlorine shell he proposed in a letter to Edwin M. Stanton, Secretary of War, in 1862. Original drawing held at Record Group 94, Records of the Adjutant General's office, entry 286, special file 62B (TR3), National Archives Building, Washington, DC.

He went on at great length in his letter to describe the potential of this shell against ships, trenches, "casemates, and bomb-proofs." He concluded by stating:

> As to the moral question involved, I have arrived at the somewhat paradoxical conclusion that its introduction would very much lessen the sanguine character of the battlefield and render conflicts more decisive in their results.[9(p9)]

Historians have been unable to find a written response to that letter. Of course, the gas shell was not used [10]. After the American Civil War, chemistry advanced rapidly as a science. As early as the 1830s, Frederick Woehler had synthesized urea, and organic chemistry began. In Germany in the 1840s, Justus von Liebig had introduced isomer chemistry and chemical fertilizers. In Sweden in the 1860s, Adolph Nobel produced trinitrotoluene (TNT) and dynamite. In 1912, a German chemist, Fritz Haber (Figure 3-2), developed the ammonia process for making nitrates. By the turn of the century, Germany had become the center of world chemistry. The six largest German firms held 950 chemical patents, whereas the six largest British firms held only 86 patents. Ninety percent of the dyes used around the world were produced in Germany. [11,12]

Fig. 3-2 is not shown due to copyright restrictions, please refer to the textbook.

Fig. 3-2. Fritz Haber was responsible for the German chemical warfare program and directed the initial German attack on Ypres. He was a strong supporter of chemical warfare.

As is usual with human advances, consideration was given to the use of chemicals (or, in the vernacular of the time, poison gases) in war. The moral question that Mr. Doughty had raised in 1862 during the American Civil War became an issue at the Hague Convention of 1899, an international meeting aimed at limiting the horrors of war. Among the issues raised was that of poison gas. The American military representative at that meeting was Rear Admiral Alfred Thayer Mahan of the U.S. Navy, who stated the official military position very well:

> It seems to me that it cannot be proved that shells with asphyxiating gases are inhumane or unnecessarily cruel or that they could not produce decisive results. I represent a people, animated by a lively desire to make warfare more humane, but which nevertheless may find itself forced to wage war, and therefore it is a question of not depriving ourselves through hastily adopted resolutions of means which we could later use with good results.[13(p46)]

The Hague Convention did outlaw chemical warfare, but the agreement had so many loopholes that it made no real difference when it came to the testing ground of World War I.

WORLD WAR I

During World War I, chemical warfare began with the German introduction of portable flamethrowers, which were not terribly effective after the initial shock wore off. There were a number of problems with flamethrowers: the flames lasted only a minute or two; the devices had a tendency to blow up and kill the operator; and they were easy to counter by shooting the operator.

Chemical warfare began in a tentative way with the French use of tear gas grenades in 1914 and early 1915. They were not particularly useful. The Germans began experimental work on chemical agents in late 1914 and produced a tear gas artillery shell. These were used against the Russians in January 1915 but were not particularly effective, owing to the cold weather. Fritz Haber, Director of the Kaiser Wilhelm Institute of Physical Chemistry in Berlin, proposed the use of chlorine gas, to be released from cylinders. [14,15]

By 1915, the trench line between the French and British forces and the Germans was established from the English Channel to the Swiss border, and a stalemate set in. At the junction of the British Expeditionary Force and a French territorial division near the old Belgian city of Ypres, an event occurred on 22 April 1915 that marked a new kind of warfare (Figure 3-3):

> Suddenly at about 4 p.m., there rose from the German trenches opposite the lines occupied by the French Colonial troops, a strange opaque cloud of greenish-yellow fumes. A light breeze from the northwest wafted this cloud toward the French who fell gasping for breath in terrible agony. Terror spread through the ranks, and a panic followed which quickly spread from front to rear lines. We saw figures running wildly in confusion over the fields. Greenish-gray clouds swept down upon them, turning yellow as they traveled over the country blasting everything they touched and shriveling up the vegetation. No human courage could face such a peril. Then there staggered into our midst French soldiers, blinded, coughing, chests heaving, faces an ugly purple color, lips speechless with agony, and behind them in the gas-soaked trenches, we learned that they had left hundreds of dead and dying comrades. It was the most fiendish, wicked thing I have ever seen.[16(p13)]

Intelligence warnings had been available for some 2 weeks about the Germans putting gas cylinders in the trenches, but the British and the French failed to heed them. The Germans released 150 tons of chlorine from 6,000 cylinders (50 lb of liquid per cylinder), and their tactical success was immediate. They punched a hole through 15,000 troops, leaving perhaps 800 dead and maybe another 2,500 to 3,000 incapacitated.

However, the German High Command was not ready for follow-up, in part because they did not trust the weapon. In part, they saw it as a civilian idea that had been pushed on them by professors Walther Nernst of the University of Berlin and Fritz Haber of the Kaiser Wilhelm

Institute. (Haber had developed the ammonia process and Nernst formulated the third law of thermodynamics. After the war, both men won Nobel prizes for their work in chemistry.) More importantly, reserve troops had been diverted to the Russian front while the Germans had been waiting for the right weather for their gas attack. [17–20]

Fig. 3-3. This photograph is reputed to show the historical German chlorine gas cloud attack at Ypres, Belgium, on 22 April 1915. Although there is little evidence to support this claim, the photograph does show a visible cloud, probably created by a cylinder attack. Photograph: Courtesy of Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Now began the race between weapon protection and weapon development. Medical involvement in chemical warfare began with the development of protective systems as well as with the treatment of patients. The Germans were the first to develop a mask. It had pads soaked in bicarbonate and sodium thiosulfate, [21(p538)] with some charcoal between the layers. The British began using “veil” respirators: the soldier put a soaked gauze pad over his nose and mouth and then wrapped millinery veiling around his head to hold the gauze in place. The British rapidly developed a flannel hood, in which a flannel bag with eyepieces was soaked glycerin and sodium thiosulfate and then pulled over the head (Figure 3-4). The French M2 mask was similar to the British mask, in which air was breathed through multiple layers of cloth impregnated with neutralizing chemicals (Figure 3-5).

a

b

Fig. 3-4. Warfare in the chemical age. **(a)** British soldiers at the Battle of the Somme appear to be wearing PH helmets in this photograph dated July 1916. **(b)** The PH helmet was an improved version of the earlier hypo and P helmets in which air was inhaled and exhaled through the fabric. The PH helmet incorporated an expiratory valve, and the cloth was impregnated with chemicals designed to destroy phosgene (the active agent was hexamethylenetetramine). This protective mask was stiflingly hot, and prolonged wear resulted in carbon dioxide retention. Source for figure legend: Prentiss AM. *Chemicals in War: A Treatise on Chemical Warfare*. New York, NY: McGraw-Hill; 1937: 536. Photograph a: Reprinted with permission from Imperial War Museum, London, England. Photograph b: Courtesy of Pictorial History, Gas Defense Division, Chemical Warfare Service, Vol 5, Edgewood Historical Files. Held at Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fig. 3-5 is not shown due to copyright restrictions, please refer to the textbook.

Fig. 3-5. The M2 protective, although ineffective was used throughout World War I.

In early 1916, the Germans introduced a far more sophisticated mask, which featured a canister containing the neutralizing chemicals attached to the front of the mask. Air was breathed through the canister (Figure 3-6). Horses were the prime movers in World War I and had to be protected from chemicals by gas masks that looked like nose bags. Artillerymen, quartermasters, and transport personnel were directed to mask their horses before masking themselves (you can't teach a horse to hold its breath). [17,19,22–26] But until a gas-warning system was implemented and soldiers routinely carried gas masks, casualty rates approached 5%, with a 25% death rate (Table 3-1). [18(App D)]

Fig. 3-6 is not shown due to copyright restrictions, please refer to the textbook.

Fig. 3-6. This mask was widely used by the German during World War I.

TABLE 3-1
SIX CHLORINE-PHOSGENE CLOUD ATTACKS:BRITISH CASUALTIES DECEMBER 1915-AUGUST 1916

Casualties	Percentages
Gas casualties as a percentage of exposed troops	4.1
Deaths due to gas as a percentage of troops	0.7
Deaths due to gas as a percentage of total gas casualties	23.6

Adapted with permission from W. *Gas Attack*. London, England: Leo Cooper; 1987: Appendix D.

By September 1915, the British were moving chlorine cylinders to the front. Major Liven of the British army developed the Livens projector, a mortar that could throw shells holding 1.5 gal of either chlorine or phosgene. The Germans continued to use gas cloud attacks; by December 1915, the standard mixture consisted of chlorine and phosgene [21(pp154–155)] (Figure 3-7). In 1916, the British developed a "box respirator" (Figure 3-8), in which the mask was connected by a hose to a canister filled with protective chemicals and filters and carried in a canvas pouch. This was later copied by the Americans. Like the British protective mask, the early American mask had a nose clip and an internal mouthpiece. Dennis Winter quoted a British officer's view:

> We gaze at one another like goggle-eyed, imbecile frogs. The mask makes you feel only half a man. The air you breathe has been filtered of all save a few chemical substances. A man doesn't live on what passes through the filter—he merely exists. He gets the mentality of a wide-awake vegetable. [27(p124)]

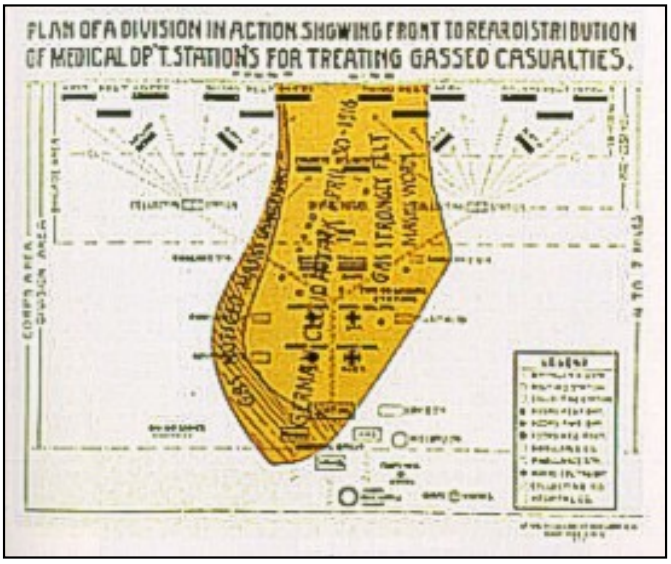


Fig. 3-7. The Germans continued to use gas cloud attacks throughout 1916, usually mixing chlorine with phosgene. Colonel H. L. Gilchrist, medical director of the American Expeditionary Force for gas warfare, prepared this illustration for chemical warfare training purposes. The drawing is based on an actual German gas cloud in 1916 but an American division is substituted for the British division that was actually attacked. The gas cloud is seen as totally interrupting the division's medical evacuation system, as well as making inoperative its two "degassing stations" (see Figure 3-20). Reprinted from Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons*. Edgewood Arsenal, Md: Chemical Warfare School; 1928: illustration 1.

Fig. 3-8. The British small-box respirator, introduced in 1916 and seen in 1918 in this photograph, was vastly more satisfactory than the earlier British helmets. The wearer breathed through a mouthpiece (like that worn by a scuba diver). Since a spring clip was applied to the nose, only air that had passed through the mouthpiece could enter the lungs. An absolute seal between the face and mask was unnecessary. The mouthpiece was connected by a tube to the canister containing neutralizing chemicals, which was worn around the trunk. Although the small-box respirator was much more protective than its predecessors, it was probably even less user-friendly. Photograph: Courtesy of Pictorial History, Gas Defense Division, Chemical Warfare Service, Vol 5, Edgewood Historical Files. Held at Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

A new weapon had come to the battlefield. It was not decisive in a strategic sense, and it did not break the stalemate of trench warfare. At the tactical level and to the soldier, however, it had a significant and frightening impact (Figure 3-9). Frederic Brown summarized it well:

> Gas is insidious. It often causes casualties without any warning. It exerts a tremendous effect on morale, especially in untrained troops. Uncertainty as to when and where gas is present and how it will act is demoralizing even to troops with high discipline. Nothing breaks a soldier's will to fight so quickly as being gassed, even slightly. His imagination magnifies his real injury 100-fold.[20(p153)]

Fig. 3-9. In this posed instructional picture of a gas attack, the soldier on the right has removed his small-box respirator and is inhaling poison gas. What message is this training photograph illustrating? That the mask is defective and is letting in the chemical agent? That the soldier thought he smelled gas, and, fearing that the mask was defective, ripped it off? Or perhaps that the soldier could not see (the lenses of the small-box respirator were notoriously subject to fogging), removed his mask, and is now suffocating? Whatever its intended purpose, this photograph reminds us that removal of the mask in the presence of chemical agents was a major cause of chemical injury in World War I. Gilchrist pointed this out in 1928:

> Investigation showed that these casualties were caused by general lack of gas discipline. It was found that the standing order that "Men will not remove the mask until ordered to do so by an officer" was absolutely disregarded by practically all units affected, and that fully 75 per cent of the casualties were due to the disobedience of this order, casualties which efficient training and discipline would have prevented.

Gas mask discipline was the key to low chemical casualty rates in the face of this insidious weapon. Quotation: Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons*. Edgewood Arsenal, Md: Chemical Warfare School; 1928: 16. Photograph: Reprinted from Moore WE, Crussell J. *US Official Pictures of the World War*. Washington, DC: Pictorial Bureau; 1920.

In April 1917, the United States entered the war, unprepared for chemical warfare. We had no organization, no equipment, and no personnel trained for chemical warfare. The U.S. Bureau of Mines was given the task of researching and developing chemical agents, primarily through contracts with universities. The Signal Corps was tasked with making the gas alarms, the Ordnance Corps with making the weapons and ammunition, and the Engineers with providing troops with chemical weapons and training them in their use. The Army Medical Department was directed to manufacture protective equipment and provide troops with training in its use. The Medical Department performed physiological studies on the energy costs and pulmonary function of individuals wearing masks. It also conducted controlled gas-exposure studies by exposing volunteers to low doses of gas to test the efficacy of various protective masks (Figure 3-10).

Fig. 3-10. The men are testing experimental canisters, probably performing a primitive form of quality assurance for equipment to protect against chemical warfare agents. Photograph: Courtesy of Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

In October 1917, at Edgewood Arsenal, Maryland, the United States began to build a huge industrial complex for making chemical warfare agents; this facility poured out chemical munitions by the ton for shipment overseas. [28–30] (Chemical warfare research done at The American University, Washington, DC, during World War I had a long-delayed fallout. In 1993, during construction of new homes in Spring Valley, a neighborhood located near the university, chemical warfare munitions from World War I were uncovered. It seems that the then-vacant wooded area was used as a testing range. The material has been removed by the U.S. Army Chemical Corps, with assistance from other agencies. [31])

Unfortunately, the first masks sent overseas with the AEF were defective (Figure 3-11), and the new AEF arrivals were fitted with French masks. General Pershing, the commanding general of the AEF, was very familiar with the divided responsibilities for chemical warfare in the United States.

Fig. 3-11. This poster from World War I was designed to encourage enthusiasm for quality assurance among women who worked manufacturing protective masks. Photograph: Courtesy of Pictorial History, Gas Defense Division, Chemical Warfare Service, Vol 5, Edgewood Historical Files. Held at Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

To prevent this from occurring in the AEF in France, he put an infantry colonel, Amos Fries, in charge of a unified Gas Warfare Service, which later became the Chemical Warfare Service (CWS, the forerunner of today's Chemical Corps). In turn, Colonel Fries chose an army physician, Colonel Harry Gilchrist (Figure 3-12), to head the medical section of his service. Gilchrist was very well known for his work in infectious diseases and was highly regarded as a researcher. (In 1929, Gilchrist gave up his medical commission, transferred to the Chemical Corps, and became a major general and the head of the corps.)

Treatment regimens were directed toward the lung irritants that produced pulmonary edema, alveolar disruption, vascular stasis, and thrombosis. Therapy consisted of good nursing, rest, oxygen, and venesection. Death from exposure to chlorine or phosgene usually occurred within 48 hours after cardiopulmonary collapse.

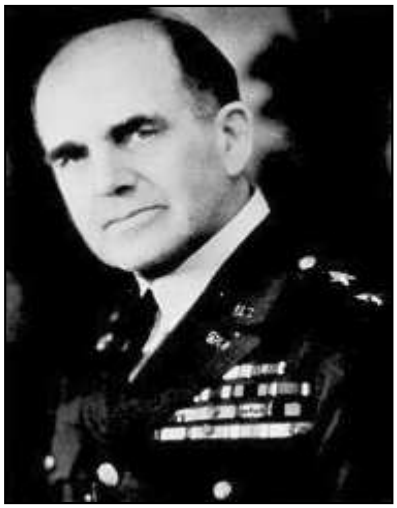


Fig. 3-12. Harry L. Gilchrist (1870–1943), shown here as a major general and head of the Chemical Corps, was the preeminent figure in the history of the U.S. Army's medical defense against chemical agents. As a Medical Corps colonel, he was medical director of the Gas Service, American Expeditionary Force 1917–1918, and was responsible for all important aspects of chemical casualty care. He was chief of the medical division of the Chemical Warfare Service at Edgewood Arsenal from 1922 to 1929 and head of the Chemical Corps from 1929 to 1934. Following his retirement from the army in 1934 and until 1940, he was editor of *The Military Surgeon*, the predecessor journal of today's *Military Medicine*. Photograph: Courtesy of Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

With the effects of the respiratory agents largely defeated by masks, the Germans changed the rules. In July 1917, they introduced dichlorethyl sulfide (mustard) against British troops at Ypres, Belgium. Delivered by artillery shells, mustard caused 20,000 casualties (Figure 3-13). To quote Gilchrist:

Fig. 3-13. This photograph from Gilchrist's study of World War I gas casualties has the following figure legend: "War photograph—Showing a small proportion of many mustard gas casualties in the United States forces resulting from a severe gas attack." Note that none of the healthcare providers are wearing protective equipment. Casualties are being unloaded from an ambulance in preparation to being triaged. Effective triage of chemical casualties was very difficult, as is apparent from this excerpt from an operational report:

> Gas cases were the most difficult of all to handle. It is impossible for the surgeon to properly diagnose his cases. One has no means of knowing whether he is dealing with delayed gas poisoning or with a simple case of Gas Fright … (but) all palpable cases of poisoning were immediately evacuated, taking precedence over other cases.

Quotation: Cochrane RC. The 3rd Division at Chateau Thierry July 1918. In: *US Army Chemical Corps Historical Studies: Gas Warfare in World War I*. Washington, DC: Office of the Chief Chemical Officer, US Army Chemical Corps Historical Office; 1959: 90. Study 14. Photograph: Reprinted from Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons*. Edgewood Arsenal, Md: Chemical Warfare School; 1928: facing page 20.

> At first the troops didn't notice the gas and were not uncomfortable, but in the course of an hour or so, there was marked inflammation of their eyes. They vomited, and there was erythema of the skin. Actually the first cases were diagnosed as scarlet fever. Later there was severe blistering of the skin, especially where the uniform had been contaminated, and by the time the gassed cases reached the casualty clearing stations, the men were virtually blind and had to be led about, each man holding on to the man in front with an orderly in the lead [Figure 3-14].[32(p44)]

Fig. 3-14. Although this photograph is frequently held to show the inhumanity of chemical warfare, the unequivocal fact is that very few mustard casualties developed permanent eye injuries—let alone blindness. Reprinted from Marshall SLA. *American Heritage History of World War I*. New York: NY: Simon and Schuster; 1964: 167.

Fig. 3-15. This photograph from Gilchrist's study of World War I gas casualties has the following figure legend: "War photograph—An old ruin heavily contaminated with mustard. Warning sign on ruin; place guarded by troops to prevent entrance." More often than not, contaminated sites were not so clearly identified. Photograph reprinted from Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons.* Edgewood Arsenal, Md: Chemical Warfare School; 1928: facing page 27.

Armies were now faced with a persistent agent. In fact, mustard has remained active (in concrete) for up to 25 to 30 years. It has a low-dose effect, does not have a strong odor, and, in addition to being a lung agent, is also a skin agent. Brown put it well:

> To the soldier, grave problems were presented by the requirements for individual and collective protection. The very air the soldier breathed and the objects he touched became potential weapons. How would the soldier eat, drink, sleep, perform bodily functions, use his weapon, or give and receive commands? How would he know his area was contaminated?[20(pp34–35)]

The presence of mustard gas meant that everyday living became a real problem. Areas previously safe from the lung gases were no longer safe from mustard (Figure 3-15). It is heavier than air and thus settles. Because of its persistence, huge areas of ground remained dangerous for days and weeks, just as if they had been mined. Effective as mustard was, chemists continued to produce new agents and combinations of agents. By the end of the war, 11 single agents and at least 7 combinations had been developed. Thousands of tons of these new weapons were produced by both sides (Figure 3-16). By 1918, approximately 25% of all artillery fire was chemical rounds. Whether for good or ill, this new weapon had come to stay.[17,33–39]

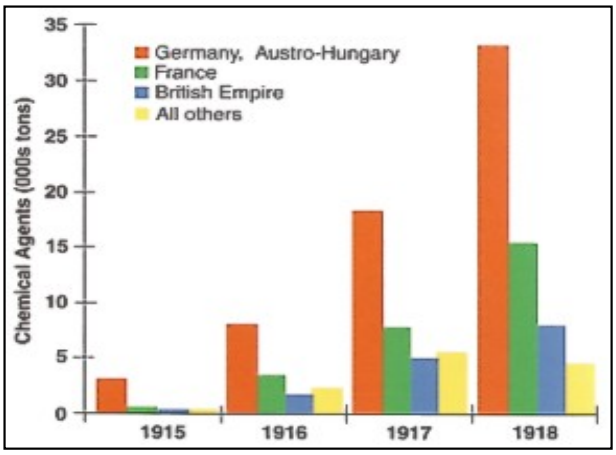


Fig. 3-16. Chemical agents used per year by major belligerents in World War I, in thousands of tons. Data source: Stockholm International Peace Research Institute (SIPRI). *The Rise of CB Weapons.* Vol 1. In: *The Problem of Chemical and Biological Warfare.* New York, NY: SIPRI; 1971: 128. Cited by: King CR. *A Review of Chemical and Biological Warfare During World War I.* Aberdeen Proving Ground, Md: US Army Materiel Systems Analysis Activity; 1979: Table 17, page 45. AMSAA-Tactical Operations Analysis Office Interim Note T-18.

MEDICAL PROBLEMS CAUSED BY MUSTARD

I will discuss in detail the medical problems with mustard gas during World War I. I have chosen mustard because the issues of diagnosis, evacuation, treatment, and contamination are similar to those with nerve agents, and because mustard is still used as a weapon today. During World War I, patients and stretcher-bearers alike had to don masks, limiting their vision and activity and making head-wounded patients difficult to mask and treat. In the U.S. forces, gassed patients were identified by a crayon cross on their foreheads because patients could appear well when evacuated but suffer from symptoms hours after exposure to mustard. In addition to the problem of triage of patients by type of exposure, there were the problems of hysteria and malingering. New troops often confused the smell of high explosives with that of gas and, as a result, made honest errors of self-diagnosis or suffered from "gas mania." [A graphic example of the problem of triage and diagnosis is apparent in the following U.S. Army afteraction report, describing an event that took place in 1918:

> One form of psychoneurosis, "Gas Fright," was very common but most cases could be restored to the lines after a few hours' rest. One instance occurred where an entire platoon of machine gunners developed this form of psychosis. These men were eating their meal just before dark when a shell fell and burst at a distance of about 100 meters. They continued eating and many of them had finished when someone yelled Gas! and said their food had been gassed. All the men were seized with gas fright and a few minutes later made their way to the Aid Station. To an inexperienced eye they could have easily been diagnosed as gassed patients. They came in in a stooping posture, holding their abdomens and complaining of pains in the stomach, while their faces bore anxious, frightened expressions and some had even vomited. After reassurance, treatment with tablets of sodium bicarbonate, and a night's rest, they were quite well again.[40(p91)] —RFB, ed.]

Gilchrist studied 281 cases consecutively admitted to a field hospital and found that only 90 of them were true gas casualties. Some were malingerers, some were misdiagnosed by battalion surgeons, and some had made honest errors of self-reporting. [13]

The mass casualties that were generated by mustard gas demanded a medical capability for quick mass decontamination of those attacked (Figure 3-17). Colonel Gilchrist organized a mobile degassing unit, a medical unit that provided showers and uniform changes for 5% of division strength. The unit (12 men and 1 officer) had the capability to decontaminate 24 men every 3 minutes. This unit was not for treating patients but for decontaminating troops who had been exposed to mustard but were not yet casualties. A water tank truck carried enough water for 700 showers of 2 minutes' duration; the water was heated by a gasoline heater at the rear of the truck. A long tent was erected, with the showers at the back of the tent. At the front of the tent, the men discarded their contaminated clothing and then stepped under the showers. The men in the medical unit who handled the contaminated clothing were protected by rubber-and-oilcloth uniforms and gas masks. [29,32]

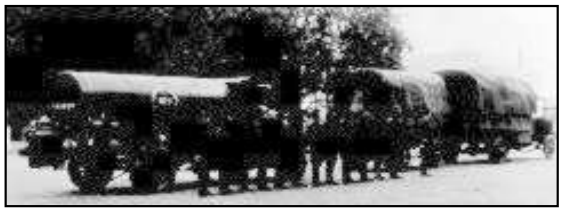


Fig. 3-17. The mobile decontamination facility was the essential part of the degassing station, two of which were to be added to each division. As events transpired, only one experimental mobile decontamination facility was actually constructed, but it was never used in combat. Its objective was "to give hot baths and clean clothing to those subjected to the fumes of mustard gas at the nearest possible points to where gas bombardments take place." Given what is now known about the speed with which mustard injury develops, attempting to slow the progression of mustard injury by this regimen was most likely an exercise in futility.

The low volatility of mustard and its ability to cause injuries at very low doses required medics to segregate the patients and to establish specialized evacuation systems and equipment, because mustard contaminated everything it came in contact with. Indeed, a single man with mustard on his uniform could easily contaminate an entire ambulance or dugout (Figures 3-18 and 3-19).

Nevertheless, by providing a shower and clean clothing, the degassing station would have played an important role in improving the general sanitation and morale of combat troops. Quotation: Gilchrist HL. Field arrangements for gas defense and the care of gas casualties. In: Weed FM, ed. *Medical Aspects of Gas Warfare.* Vol 14. In: Ireland MW, ed. *The Medical Department of the United States Army in the World War.* Washington, DC: Government Printing Office; 1926: Chap 4: 61. Photograph: Courtesy of Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Fig. 3-18. This photograph from Gilchrist's study of World War I gas casualties has the following figure legend: "War photograph—Special ambulances used for transporting mustard gas casualties rendered necessary due to insidiousness of mustard." Note that the ambulance crews appear not to be protected against mustard. Reprinted from Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons.* Edgewood Arsenal, Md: Chemical Warfare School; 1928: facing page 30.

Fig. 3-19. This photograph from Gilchrist's study of World War I gas casualties has the following figure legend: "War photograph—Special gas aid station for administering to gas casualties. Here cases suffering from different gases were, when possible, segregated." Note the lack of protective equipment; the casualty being loaded into the ambulance was apparently not deemed a threat, possibly because he was a victim of a respiratory agent. Reprinted from Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons*. Edgewood Arsenal, Md: Chemical Warfare School; 1928: facing page 31.

The acute conjunctivitis induced by mustard (Figures 3-20 and 3-21) required immediate eye irrigation. Most of the eye cleared up in several weeks. Nevertheless, during the resolution stage of mustard-related acute conjunctivitis, patients were photophobic for a considerable period. Skin burns were treated in a variety of ways (Figure 3-22). First, the patients were washed down by corpsmen who wore protective clothing. Early in the conflict, burns were initially treated with grease, which only enhanced infection. Later, sodium hypochlorite was used as a constantly running solution, soaking the skin. [25]

Fig. 3-20. A nurse is irrigating the eyes of soldier who has a probable mustard injury. Given the rapidity with which mustard damages tissue, however, we know now that eye irrigation would have provided only symptomatic relief. Reprinted from Moore WE, Crussell J. *US Official Pictures of the World War*. Washington, DC: Pictorial Bureau; 1920.

Fig. 3-21. In 1918, the British prepared for the American Expeditionary Force a series of color drawings and descriptions of injuries caused by chemical warfare agents. This drawing depicts a severely burned eye in the acute stage after exposure to mustard vapor. A portion of the original description follows:

[Severely burned eyes] may be recognized by certain characteristic features that are depicted in the drawing [right]. Whenever a dead white band crosses the exposed area of the conjunctiva, while the parts of this membrane covered by the upper and lower lids are red and oedematous, serious injury from the burning is likely to have occurred.

In the case illustrated, the caustic effect of the vapour is seen chiefly in the interpalpebral aperture. On each side of the cornea there is a dead white band due to coagulative oedema, which compresses the vessels, impairs the circulation, and thus acts as a menace to the nutrition of the cornea. The swelling in the region of this white band is slight, while the protected conjunctiva above and below it is greatly swollen and injected and may even bulge between the lids.

The exposed portion of the cornea is grey and hazy; it has lost its lustre, and when viewed with a bright light and a magnifying glass it shows a blurred “window reflex” and a typical “orange-skinned” surface. The haze gradually fades off above in the region of the protected part of the cornea where the surface is usually bright and smooth. The pupil is at first contracted as the result of irritation and congestion. In this drawing it is shown as artificially dilated by atropine ointment, which should always be used early in severe cases or where there is much pain and blepharospasm.

Reprinted from *An Atlas of Gas Poisoning.* 1918: Plate 11A. Handout provided by the American Red Cross to the American Expeditionary Force.

Fig. 3-22. An extensive mustard burn of the buttocks.
This degree of mustard injury, analogous to a second- or third-degree thermal burn, was unusual. The original description that accompanied this drawing, provided to the American Expeditionary Force by the British (also see Figure 3-21), follows:

The man sat down on ground that was contaminated by the poison and the vapour passed through his clothing, causing inflammation of the buttocks and of the scrotum. A diffuse reddening appeared twenty-four hours after exposure, and this was followed by an outcrop of superficial blisters. On the eighth day the erythema began to be replaced by a brown

staining, and the drawing was made on the eleventh day during this change of tints. Infection of the raw surface was avoided, and the healing was complete in three weeks. The blisters in this case were probably aggravated by pressure, for the inflamed skin becomes very fragile, so that the surface layer is readily loosened by pressure or careless rubbing. The blisters may be very tiny bullae, as on the eyelids, or they may coalesce into areas many inches across, covering a collection of serous fluid which perhaps itself contains enough of the irritant substance to injure other skin if it is allowed to flow over it. The blisters are usually quite superficial and almost painless in their development. But the raw surface that is left after the blister has burst becomes most acutely sensitive to all forms of mechanical irritation. Deeper destruction of the dermis may be caused by spreading necrosis where the substance attacks the skin locally in high concentration, or when secondary infections are implanted on the raw surface. Chronic and painful sores then result, and in this event the skin does not regenerate completely, so that thinly covered scars for a long time will mark the site of the burn.

Reprinted from *An Atlas of Gas Poisoning.* 1918: Plate 6. Handout provided by the American Red Cross to the American Expeditionary Force.

Patients who died from mustard inhalation had gross destruction of the tracheobronchial tree (Figure 3-23). In contrast to the pulmonary agents, mustard produced hemorrhage and alveolar edema. Mustard-induced lesions were more difficult to treat than those induced by phosgene or chlorine. How dangerous were these chemical weapons as killers? Gas was a major cause of casualties: it accounted for up to 30% of hospitalized patients (Figure 3-24). Although gas was a significant factor in casualty production, it was not especially lethal. [The AEF incurred 52,842 fatal battle injuries, but only about 1,500 were due to gas [21(p652)] RFB, ed.] (Table 3-2).

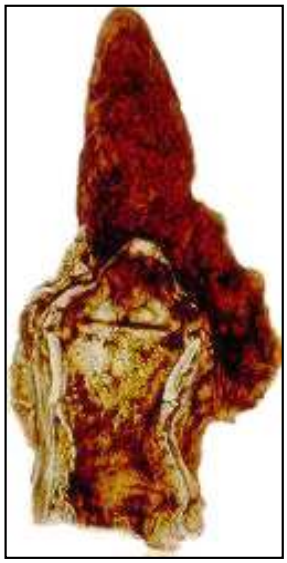


Fig. 3-23. The figure legend that was published with this photograph in the official history of the U.S. Army Medical Department in World War I reads: “Gross changes in larynx and trachea of a soldier who died four days after inhalation of mustard gas.” Purulent secretions in the smaller bronchi rather than at the glottis caused the respiratory failure that lead to the death of this soldier. The efficacy of tracheal suction in clearing the airway appears not to have been widely known during World War I. Reprinted from Weed FM, ed. *Medical Aspects of Gas Warfare.* Vol 14. In: Ireland MW, ed. *The Medical Department of the United States Army in the World War.* Washington, DC: Government Printing Office; 1926: Plate 10.

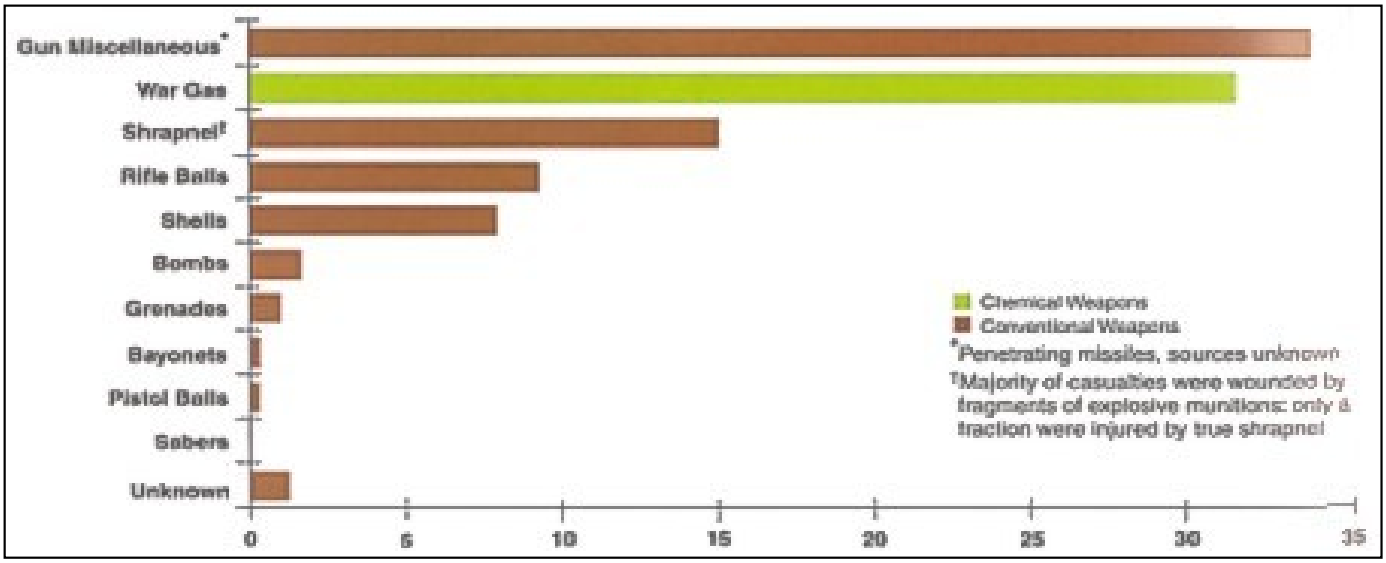


Fig. 3-24. Hospitalized casualties in World War I, in percentages by causative weapon (database: 224,089 casualties). Adapted from Gilchrist HL. A *Comparative Study of World War Casualties From Gas and Other Weapons.* Edgewood Arsenal, Md: Chemical Warfare School; 1928: Chart 7, page 19.

TABLE 3-2
CHEMICAL CASUALTIES IN WORLD WAR I

Country	Nonfatal Chemical Casualties	Chemical Fatalities	Percentage Fatal
Germany	191,000	9,000	4.5
France	182,000	8,000	4.2
British Empire	180,597	8,109	4.3
United States	71,345	1,462	2.0
Russia*	419,340	56,000	11.8

* [The data from which Prentiss (and before him, Gilchrist) derived these figures have apparently been lost to history. However, the Russians themselves analyzed their casualty statistics from World War I. The Narkomzdrav Commission found the figures for nonfatal and fatal gas casualties to be only about one tenth as great as Prentiss's values, which are the ones commonly accepted in the West (total gassed casualties: 40,000–65,000; died of gas: 6,340). Source for these data: Kohn S. *The Cost of the War to Russia*. New York, NY: Howard Fertig: 1973: Table 75; page 136; Table 76. Originally published in 1932.—RFB, ed.] Adapted from Prentiss AM. *Chemicals in War: A Treatise on Chemical Warfare.* New York, NY: McGraw-Hill; 1937: Table 11, page 653.

The Russians suffered out of proportion to the rest of the belligerents because they were late in deploying an effective mask. For the United States, the chemical agents were minor contributors to the number of soldiers killed in action: only about 200 of the total of more than 70,000 wounded by gas. [13] The real problem was the imposition of a major medical and logistical burden on the army. In the AEF, for example, gas patients had significant hospitalization periods (Table 3-3), although the great majority returned to duty. The generally low lethality and high morbidity rate led a great many people to see the chemical weapon as holding much promise for the future of war.

TABLE 3-3
AMERICAN EXPEDITIONARY FORCE: HOSPITAL DAYS DUE TO CHEMICAL WARFARE

Agent	Chemical Casualties	Average Days Hospitalized
Unknown	33,587	37.3
Chlorine	1,843	60.0
Phosgene	6,834	45.5
Mustard	27,711	46.0

Adapted from Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons.* Edgewood Arsenal, Md: Chemical Warfare School; 1928: Table 7, page 21.

THE INTERWAR YEARS

After World War I ended, work at the Edgewood medical research laboratories continued. New gas masks were developed, such as those with high-eyepoint lenses for use with binoculars, and masks with speaker diaphragms. As those who have worn mission-oriented protective posture (MOPP) gear know, one cannot really be heard through a mask. Initially, scientists at Edgewood worked on oilcloth- and-rubber uniforms for mustard protection and then developed the resin-and-chloramide uniform. Smoke and gas delivery systems were added to weapons such as tanks and airplanes. The U.S. military paid attention to gas; troops were trained, in the interwar years, in both simulated and real chemical environments. [21,41,42] In short, we took the threat of chemical warfare very seriously: research and training received considerable attention during the interwar years. [5,9,26,30]

The Army Medical Department made a big investment in research. In fact, it put more money into research on the chemical weapon than into anything else in the interwar period. Colonel Edward Vedder, Medical Corps, U.S. Army (Figure 3-25), was in charge of the medical laboratory at Edgewood that produced new mask canisters that could filter smoke in addition to the standard respiratory agents.

This development made possible the protection of American soldiers against respiratory tract effects of arsenic-based compounds—the most potent chemical agents of that period. Clinical cases were studied and animal research was performed with the agents, as well as experimentation in humans and attempts at new treatment.

In 1925, Vedder published *Medical Aspects of Chemical Warfare*, a superb book that contains excellent data on the pathology and physiology of various chemical agents (particularly mustard). Much of the text is still germane. On the inside front cover of the book is a picture of a soldier horribly wounded by shrapnel, yet alive. Vedder argued that if this is the result of a humane weapon, then the chemical weapon, by comparison, must be much more humane. [26] Vedder was not alone in this view of the relative humanity of chemical warfare. It was a predominant view of many writers who analyzed the subject. [5,9,17,21,30,33,41–45] The development of the lethal nerve gases by the Ger-mans in World War II, however, has vitiated these arguments.

Fig. 3-25. Edward B. Vedder (1878–1952) was director of pathology at the Army Medical School (now Walter Reed Army Institute of Research) from 1904 to 1913. It was during this period that he wrote his seminal book on beriberi. After serving in the Philippines during World War I, Colonel Vedder returned to the Army Medical School in 1919. It was there that he wrote this still-useful book on chemical casualties. From 1925 to 1929 he was chief of medical research for the Chemical Warfare Service. He had an illustrious civilian academic career following his retirement from the army. Photograph: Courtesy of National Library of Medicine. Bethesda, Md.

In the interwar years, a number of medically important spin-offs came from the chemical warfare program. The Americans developed Lewisite, an arsenical, at the end of World War I. It did not turn out to be a particularly effective agent, although it did lead to the development of British anti-Lewisite (BAL), which is useful as a chelating agent in metal poisoning. It was noticed in soldiers who had been exposed to mustard during the war that the white blood count fell. This was verified in 1919. Dougherty, Goodman, and Gilman showed in 1942 that the nitrogen mustards could be useful in the treatment of leukemia and lymphoma. This was the beginning of specific chemotherapy for cancer. [46–48]

Between World War I and World War II, disarmament conferences included discussions of the prohibition of gas warfare. [49] Nevertheless, the chemical weapon continued to be used but only against colonial native peoples. For example, in 1920, the British dropped mustard gas bombs on Afghan tribesmen north of the Khyber Pass. In 1925, the Spaniards used mustard bombs and mustard artillery shells against Riff tribes in Morocco. In 1935, when Mussolini moved from the Italian colony in Libya to conquer Ethiopia, the Italian troops were ambushed. Although equipped with modern arms they were heavily outnumbered, so Marshall Badoglio, the Italian commander, used aerial delivery of mustard bombs against Egyptian troop concentrations and saturated the ground on his road flanks to interdict the movement of barefoot Ethiopian troops. [50,51] (A complete list of proven or alleged use of chemical weapons between 1919 and 1970 can be found in *A Review of Chemical/Biological Warfare During World War I.* [25(pp13–14)]

WORLD WAR II

When World War II broke out, there was a general expectation and apprehension that the chemical weapon would be used. The Japanese practiced civilian operations while wearing masks. British troops trained with masks in the North African desert. In London, during the height of the blitz, schoolchildren were issued masks. German mothers and children had special capes and masks available. Americans came out with a whole series of tactical and training masks. Walt Disney designed a mask with a Mickey Mouse face for American children, so they would not be frightened by wearing the mask (Figure 3-26). Fortunately, American children never had to use these masks. By 1942, after the United States had entered the war, all U.S. troops trained in masks. Full discussions of the United States efforts in World War II are found in the U.S. Army in World War II series published by the Center of Military History. [52–54]

The United States developed a new generation of protective uniforms, which soldiers carried, along with their gas masks, on every D day in Europe. Chemical weapons were indeed used in World War II, in the form of smoke and flame. Smoke was used for screening troops and movement, especially in Europe. Americans in the Pacific used the flame weapon against Japanese caves and bunkers.

For reasons that historians are still debating, gas itself was not used. One reason the Germans did not use it was that they thought the Americans had developed new, secret nerve gases—comparable to tabun, sarin, and soman—which the Germans had developed between 1936 and 1944. The Germans may have been led to believe this because of the alleged paucity of reports on insecticide research published in the open literature in the United States, and they wrongly deduced that the Americans were now manufacturing nerve gas. In reality, however, there was no industrial base in place ready to produce nerve agents in large quantities [20] —because neither the British nor the Americans had discovered nerve agents.

Other historians have argued that because Adolph Hitler had been a gas casualty in World War I, he was personally opposed to the use of gas weapons in World War II. Similarly, many senior officers on the Allied side

Fig. 3-26. Walt Disney helped design this Mickey Mouse gas mask for American children. The intention was that children would not be frightened of the cartoon character and would therefore be more willing to wear the mask. Photograph: Courtesy of Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

in World War II had faced gas as junior officers in World War I and were highly resistant to its use in World War II. It was official U.S. policy that the United States would not use chemical warfare first but would retaliate if it were used against us or our allies. Thus, the United States was prepared to retaliate. It was in part because of this preparation that American and British troops had the only military gas casualties in World War II.

In 1943, Bari, a city on the Achilles tendon of Italy, was a major supply port for the British Eighth Army fighting in Italy. The SS *John Harvey*, an American ship in harbor, carried a highly classified load of 2,000 100-lb mustard bombs. When the Germans hit Bari harbor in a surprise raid they got 17 ships (Figure 3-27); among them was the *John Harvey*. Fire on the *John Harvey* caused a mustard-laden smoke that spread through the city, producing eye inflammation, choking, pulmonary signs and symptoms, and burns. No one really knows the extent of the civilian casualties; however, by the 9th day after the bombing, 59 military deaths had been recorded.

Shortly after the bombing, Lieutenant Colonel Stewart Alexander of the U.S. Army Medical Corps, the chemical warfare consultant on General Eisenhower's staff, was sent to Bari, where he made the diagnosis of mustard poisoning. He reported 6l7 cases in troops and merchant marine seamen, with a 14% fatality rate. This fatality rate, 3-fold higher that of World War I, was largely because the merchant marine seamen had been thrown into the sea, where they either got badly burned or swallowed mustard in the water. [55,56]

Fig. 3-27. The Bari mustard disaster, caused by a German air attack the night of 2 December 1943, resulted from the need to have chemical munitions deployed in the combat zone. The presence of those weapons was necessary to make possible an immediate retaliation should the enemy choose to initiate a chemical attack. However, the deployment of those munitions was kept secret so as not to give the enemy any justification for launching a preemptive chemical attack. Although the merchant ship carrying the mustard bombs, the SS *John Harvey,* had been docked at Bari for several days, the ship was not unloaded because the appropriate authorities did not know of the highly dangerous nature of its cargo. No photograph exists showing the *John Harvey* after the German attack; the ship was completely destroyed by the explosion of the conventional munitions that it was also carrying. Instead, this photograph shows the Bari harbor some hours after the attack. Reprinted with permission from Popperfoto. Northampton, England.

Lethal gases—pesticides, prussic acid, and cyanide, as well as carbon monoxide—were used as killing agents in gas chambers in the Nazi death camps. [57]This is obviously not a military use of the chemical weapon.

THE POSTWAR YEARS: 1945 TO THE PRESENT

Chemical agents have been used in warfare since World War II. There is a suggestion that they were considered for employment in Korea in 1950. [58] In 1963, the Egyptians used mustard bombs against the Yemen royalists in the Arabian peninsula. The United States used chemical defoliants in Vietnam for canopy clearing and crop destruction, and used tear gas for clearing tunnels and bunkers (Figure 3-28).[59] The Soviets used chemical warfare agents in Afghanistan, probably mustard and a nerve agent.[25] However, the discovery that Iraq had used chemical agents (mustard and perhaps nerve gas) in its war with Iran shocked the public in the western democracies in the 1980s.[60] Iraqi use of hydrogen cyanide and possibly a nerve gas against its own Kurdish population in 1988 was universally condemned. [61,62]

Fig. 3-28. Tear gas was used extensively by U.S. forces in the Vietnam War, especially in clearing enemy tunnel complexes. The U.S. government, however, did not consider tear gas to be a chemical weapon and therefore did not consider its use to be banned by international law. Many others outside of government disagreed, using as evidence the fact that those who used tear gas wore protective masks. The soldiers shown here are wearing the little-known M28 protective mask. This lightweight (and perhaps more comfortable) mask was designed to be worn in situations in which the threat was not from nerve agents, and the heavy-duty protection offered by the standard masks was not necessary. Photograph: Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

In the United States, the congress has debated the chemical agent issue over several years, with much of the debate focused on the morality of the weapon. [63–65] Congress decided in 1988 to approve the production of the binary nerve gas weapon, influenced then by increasing evidence that chemical weapons were in hand and appeared to be increasing in the arsenals of nonfriendly nations (see Exhibit 4-1 in Chapter 4, Medical Implications of the Chemical Warfare Threat). [66] The accuracy of such information can clearly be challenged, and the lists themselves vary from publication to publication. [66–68] Nonetheless, interest began to increase in a new United Nations treaty to ban chemical weapons. [69,70] In September 1996, the U.S. Senate considered the new treaty, which called for banning production of chemical weapons and for an inspection program. General John M. Shalikashvilli, Chairman of the Joint Chiefs of Staff, urged ratification. Public debate varied widely. [71–73] The U.S. Senate initially rejected the treaty, [74] but it has since been approved, not only by the U.S. Senate (24 April 1997), but also by the 65 member nations of the United Nations required for its enactment and enforcement. [75]

While it is true that there are residual effects— physical, physiological, and psychological—after every American war, [76] the chemical weapon has aroused persistent public interest, veterans complaints, and charges of medical indifference, coverup, and incompetence. After World War I, the issue was tuberculosis caused by pulmonary agents. [77] After World War II, there was the delayed discovery of cancer and cataracts in enlisted men who had been test subjects for chemical exposures. [78] After the Vietnam War, the herbicide Agent Orange (specifically its dioxin component) had been the assigned cause for a number of compensable diseases. [79] And, as of this writing (January 1997), some veterans of the Persian Gulf War have an unexplained Gulf War “syndrome,” with low-dose exposure to chemical agents being suggested as a possible cause. [80]

It is obvious that use of the chemical weapon remains possible. This textbook documents this concern on the part of the U.S. Army Medical Department. I therefore believe that it is the responsibility of the U.S. military medical community to prepare to operate in a chemical environment. Fighting a chemical war will markedly hinder our medical, tactical, and operational capacity (problems well discussed in this textbook), and cause long-term postexposure residual effects. Thus, students of this topic may still find relevance in the words that Sir Charles Bell (who was a surgeon at Waterloo in 1815) wrote in 1812:

> When the drum beats to quarters there is now a time of fearful expectation, and it is now the surgeon feels how much the nature of the wounds of those who may be brought to him ought to have occupied his mind in previous study.[81]

It is that "previous study" that is the purpose of this book: to educate our military and civilian medical communities about chemical warfare and their consequent medical responsibilities.

SUMMARY

The chemical weapon has a long and ancient history, especially in its presentation as flame and smoke. Modern chemistry made possible the use of chemical agents in a logistically and tactically feasible way in World War I. Most of what was known—and is still understood by the public—is based on the gas warfare of 1915–1918. Since then, "poison gas" has usually aroused public repugnance at its use as a weapon. Modest use in the 1930s against tribes and its lack of employment in World War II suggested that "gas warfare" had ended. The discovery of the German nerve gases after World War II, the Cold War, and the utility of tear gas in Vietnam maintained a military interest in the chemical weapon.

The use of gas by Iraq against Iranian troops and the threat of Iraqi use in the Persian Gulf War clearly document that chemical warfare remains possible.

(*This chapter was based on Dr. Joy's lecture, "Historical Aspects of Medical Defense Against Chemical Warfare." The figure legends were provided by the textbook editors.)*

REFERENCES

1. Brodie B, Brodie F. From *Crossbow to H-Bomb.* New York, NY: Dell Publishing; 1962: 35–36.
2. Thucydides; Crawley R, trans. *The Peloponnesian War*. New York, NY: Modern Library; 1934: 262.
3. Richardson BW. Greek fire: Its ancient and modern history. *Popular Sci Rev*. 1864;3:166–177.
4. Laffout R. *The Ancient Age of Warfare: 1300 BC–1650 AD*. Vol 1. New York, NY: Time-Life; 1966: 45–46.
5. Fries AA, West CJ. *Chemical Warfare*. New York, NY: McGraw-Hill; 1921: 2–4.
6. Poole JB. A sword undrawn: Chemical warfare and the Victorian age. *Army Q.* 1976;106:463–469.
7. Miles WD. Part I: Admiral Cochrane's plans for chemical warfare. *Armed Forces Chemical Journal*. 1957;11:22–23.
8. Miles WD. Part II: The chemical shells of Lyon Playfair (1854). *Armed Forces Chemical Journal.* 1957;11:23, 40.
9. Waitt AH. *Gas Warfare*. New York, NY: Duell, Sloan, and Pearce; 1942: 9–11.
10. Bruce RV. *Lincoln and the Tools of War*. New York, NY: Bobbs-Merrill; 1956: 247–248.
11. Johnson JA. *The Kaiser's Chemists*. Chapel Hill, NC: University of North Carolina Press; 1990.

12. Jaffe B. *Crucibles: The Story of Chemistry*. New York, NY: Hutchinson; 1949.

13. Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons*. Edgewood Arsenal, Md: Chemical Warfare School; 1928.

14. Trumpener U. The road to Ypres: The beginning of gas warfare in World War I. J *Modern Hist*. 1975;47:460–480.

15. Goran M. *The Story of Fritz Haber*. Norman, Okla: University of Oklahoma Press; 1957.

16. Watkins OS. *Methodist Report*. Cited in: Fries AA, West CJ. *Chemical Warfare.* New York, NY: McGraw-Hill; 1921: 13.

17. Haber LF. *The Poisonous Cloud*. Oxford, England: Clarendon Press; 1986.

18. Moore W. *Gas Attack*. London, England: Leo Cooper; 1987.

19. Cookson J, Nottingham J. *A Survey of Chemical and Biological Warfare*. London, England: Sheed and Ward; 1969.

20. Brown F. *Chemical Warfare: A Study in Restraints*. Princeton, NJ: Princeton University Press; 1968.

21. Prentiss AM. *Chemicals in War: A Treatise on Chemical Warfare*. New York, NY: McGraw-Hill; 1937.

22. Spiers EM. *Chemical Weapons*. New York, NY: St. Martin's Press; 1989.

23. Spiers EM. *Chemical Warfare*. Chicago, Ill: University of Illinois Press; 1986.

24. Haber LF. *Gas Warfare 1915–1945: The Legend and the Facts*. In: The Stevenson Lecture of 1975. London, England: Bedford College; 1976.

25. King CR. *A Review of Chemical/Biological Warfare During World War I*. Aberdeen Proving Ground, Md: 1979. AMSAA-TOAO Interim Note T-18.

26. Vedder EB. *The Medical Aspects of Chemical Warfare*. Baltimore, Md: Williams & Wilkins; 1925.

27. Winter D. *Death's Men*. London, England: Allen Lane; 1978: 124.

28. Jones DP. Chemical warfare research during World War I: A model of cooperative research. In: Parascandola J, Whorton J, eds. *Chemistry and Modern Society*. Washington, DC: American Chemical Society; 1983: 165–186.

29. Weed FW, ed. *Medical Aspects of Gas Warfare*. Vol 14. In: Ireland MW, ed. *The Medical Department of the United States Army in World War*. Washington, DC: US Department of the Army, Medical Department, Office of The Surgeon General, US Government Printing Office; 1926.

30. Brophy LP, Miles WD, Cochrane RC. *US Army in World War II: The Chemical Warfare Service, From Laboratory to Field.* Washington, DC: US Government Printing Office; 1959: 1–27.

31. Gordon MK, Sude BR, Overveck RA. Chemical testing in the Great War: The American University experiment station. *Washington Hist*. 1994:6;25–45, 106–107.

32. Gilchrist HL, Matz PB. *The Residual Effects of Wartime Gases*. Washington, DC: US Government Printing Office; 1933.

33. Haber F. Chemistry in war. *J Chem Education*. 1945;22:526–529, 553.

34. Richter D. *Chemical Soldiers*. Lawrence, Kan: University of Kansas; 1992.

35. Foulkes CH. *"Gas!" The Story of the Special Brigade.* Edinburgh, Scotland: Blackwood; 1936.

36. Leroux L. *La Guerre Chimique*. Paris, France: Editions Spes; 1932.

37. Langer WL. *Gas and Flame in World War I*. New York, NY: Knopf; 1965. Original title: *With "E" of the First Gas.* n.p: 1919.

38. Heller CE. *Chemical Warfare in World War I: The American Experience*. Fort Leavenworth, Kan: Combat Studies Institute; 1984. Leavenworth Paper 10.

39. Hartcup G. *The War of Invention: Scientific Developments, 1914–18.* London, England: Brassey; 1988: 94–117.

40. Med Hist of 3rd Div, p 117. Quoted in: Cochrane RC. The 3rd Division at Chateau Thierry July 1918. In: *US Army Chemical Corps Historical Studies: Gas Warfare in World War I*. Washington, DC: Office of the Chief Chemical Officer, US Army Chemical Corps Historical Office; 1959: 91. Study 14.

41. Gilchrist HL. *A Comparative Study of Warfare Gases*. Carlisle, Pa: Medical Field Service School; 1925. US Army Medical Bulletin 16.

42. Keller PE. First aid to gas casualties. *Chem Warfare Bull*. 1937:23;15–19.

43. Howe HE. The editor's point of view. *Indust Eng Chem*. 1932:24;121–122.

44. Slotten HR. Humane chemistry or scientific barbarism? American responses to World War I poison gas, 1915– 1930. *J Amer Hist.* 1990:77;476–498.

45. Haldane JSB. *Callinicus: A Defense of Chemical Warfare*. New York, NY: EP Dutton; 1926.

46. Gilman A, Philips FS. The biological actions and therapeutic applications of the B-chloroethyl amines and sulfides. *Science*. 1946:103;409–415.

47. Jacobson LO, et al. Nitrogen mustard therapy. *JAMA*. 1946:132;263–271.

48. Weisse AB. From trench warfare to war on cancer. *Hosp Pract*. 1992:27;141–143, 147–148.

49. *Protocol for Prohibition of the Use in War of Asphyxiating, Poisonous, or Other Gases, and of Bacteriological Methods of Warfare.*Geneva, Switzerland: 1925. (This protocol was signed at Geneva in 1925, but not ratified then by Japan or the United States. It was finally ratified by the United States in 1975, after herbicides and riot control agents were excluded.)

50. Barker AJ. *The Civilizing Mission: The Italo–Ethiopian War*. New York, NY: Dial Press; 1968: 241–244.

51. Parkhurst R, reviewer. *J Mil Hist.* 1996;60:783. Review of: Del Boca A. *I gas di Mussolini: Il fascismo e la guerra d' Etiopia* [in Italian]. Rome, Italy: Editori Riuniti; 1996.

52. Kleber BE, Birdsell D. *The Chemical Warfare Service: Chemicals in Combat*. Washington, DC: Office of the Chief of Military History, Department of the US Army, US Government Printing Office; 1966.

53. Brophy L, Fisher GJB. *The Chemical Warfare Service: Organizing for War*. Washington, DC: Office of the Chief of Military History, Department of the US Army, US Government Printing Office; 1959.

54. Brophy LP, Miles WD, Cochrane R. *The Chemical Warfare Service: From Laboratory to Field*. Washington, DC: Office of the Chief of Military History, Department of the US Army, US Government Printing Office; 1959.

55. Alexander SF. Medical report of the Bari Harbor mustard casualties. *Military Surgeon.* 1947;101:1–17.

56. Infield GB. *Disaster at Bari*. New York, NY: Macmillan; 1971.

57. Gilbert M. *The Holocaust.* New York, NY: Henry Holt; 1985.

58. Blair C. *The Forgotten War: America in Korea 1950–1953*. New York, NY: Times Books; 1987: 622.

59. Westmorland WC. *A Soldier Reports*. Garden City, NY: Doubleday; 1994: 278–279.

60. United Nations Security Council. *Report of the Specialists Appointed by the Secretary-General to Investigate Allegations by Islamic Republic of Iran Concerning the Use of Chemical Weapons.* New York, NY: The United Nations. S/ 16433; 26 March 1984.

61. *Time*. 22 Aug 1988.

62. *Washington Post.* 25 Sep 1988.

63. Hearing Before the US House of Representatives Committee on Foreign Affairs. *Chemical-Biological Warfare: U.S. Policies and International Effects,* 91st Cong, 1st Sess (1969). Washington, DC: Government Printing Office; 1969.

64. Dryden J. *Deadly Allies: Canada's Secret War*, 1937–1947. Toronto, Ontario, Canada: McClelland and Stewart; 1989.

65. Meselson M, Robinson JP. Chemical warfare and chemical disarmament. *Sci Am.* 1980;242:1–10.

66. *US News and World Report.* 16 Jan 1989;30–31.

67. *Washington Post.* 25 Aug 1985.

68. *Time.* 16 Jan 1989:22.

69. *Washington Post.* 14 May 1991.

70. *Washington Post.* 14 Jan 1993.

71. Perry WJ, Reno J. A treaty in the US interest. *Washington Post*. 11 Sep 1996.

72. Krauthammer C. Peace through paper. *Washington Post.* 12 Sep 1996.

73. Weymouth L. Chemical weapons fraud. *Washington Post.* 12 Sep 1996.

74. *Washington Post.* 13 Sep 1996.

75. *Washington Post.* 2 Nov 1996.

76. Hyams KC, Wignall S, Roswell R. War syndromes and their evaluation: From the US Civil War to the Persian Gulf War. *Ann Intern Med*. 1996;125:398–405.

77. Gilchrist HL, Matz, PB. *The Residual Effects of Warfare Gases.* Washington, DC: US Government Printing Office; 1933.

78. Institute of Medicine; Committee to Survey the Health Effects of Mustard Gas and Lewisite. *Veterans at Risk.* Washington, DC: National Academy Press; 1993.

79. Institute of Medicine. *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam.* Washington, DC: National Academy Press; 1994.

80. Institute of Medicine. *Health Consequences of Service During the Persian Gulf War.* Washington, DC: National Academy Press; 1996.

81. Bell C. *A Dissertation on Gunshot Wounds.*London, England: Longman; 1814.

Chapter 4

THE CHEMICAL WARFARE THREAT AND THE MILITARY HEALTHCARE PROVIDER

ERNEST T. TAKAFUJI, M.D., M.P.H.*; AND ALLART B. KOK, M.S.†

INTRODUCTION

THE CHEMICAL THREAT AND ENEMY CAPABILITY

THE STATUS OF CHEMICAL PROLIFERATION
- Chemical Warfare Capabilities of Nations
- International Agreements and Verification

MILITARY CHEMICAL AGENTS

TACTICAL AND STRATEGIC USE OF CHEMICAL WEAPONS
- Chemical Agent Delivery Systems
- Physical Properties of Chemical Agents
- Choice of Agent and Delivery System
- Detection and Protection

RESPONDING TO THE THREAT: MANAGING CASUALTIES

FUTURE CONFLICTS IN A CHEMICAL ENVIRONMENT

SUMMARY

* *Colonel, Medical Corps, U.S. Army; Commander, Walter Reed Army Institute of*

Research, Walter Reed Army Medical Center, Washington, D.C. 20307-5011
† Science Applications International Corporation, Joppa, Maryland 21085

INTRODUCTION

Some military medical personnel view the specter of chemical warfare with fear and repugnance. The images of clouds of poisonous chemicals; contaminated terrain and equipment; and the need to work in protective masks and hot, cumbersome overgarments are intimidating even to well-trained soldiers. But for medical personnel there is an added factor: the fear of the unknown. The milieu of the chemical battlefield is especially alien since little in the normal professional practice of most military medical personnel has any resemblance to the management of casualties of chemical warfare.

Although military strategists might view chemical warfare agents as simply one of the means to immobilize or destroy an enemy force, others may view such weapons as abhorrent extensions of conventional warfare. Be that as it may, it is not the intent of this chapter to justify the use of chemical weapons in battle but rather to relate the use of chemical warfare to health issues. Although current policy of the United States government prohibits using chemical weapons against an adversary, this policy is not shared by all nations; therefore, to be effective, military medical personnel must be knowledgeable and trained.

To the military healthcare provider, chemical warfare crosses over the lines of strategic and tactical purposes, where victory may be viewed as full justification of

- the means;
- the difficult challenges associated with the identification, treatment, and prevention of specific injuries and illnesses in a deliberately contaminated and highly stressful environment;
- the psychologically demoralizing effect that chemical weapons may cause; and
- the personal ethical concerns that many medics may have about suffering resulting from the deliberate use of weapons specifically targeted to the human element.

Although healthcare providers are usually not involved in the political or military decisions surrounding use of chemical weapons, they must be

- prepared to deal with the military and civilian casualties resulting from use of such agents;
- cognizant of what constitutes a chemical threat and the military tactics that could be employed against the force, since they may be called on to render advice to their commanders from an individual and public health perspective;
- familiar with the acute and chronic medical effects of chemical agents in order to plan appropriate medical support; and
- fully knowledgeable of the diagnostic tools available to identify specific etiologic agents to which their patients may have been exposed and the most effective methods of intervention and prevention.

The “chemical threat” can be defined as a statement of the who, what, when, where, and how of chemical warfare. The threat may involve single or multiple chemical agents—not only the classic chemical agents specifically developed for military applications (ie, chemicals that had been weaponized by the 1950s), but also highly toxic industrial compounds that could achieve the same objective. Military medical care providers need to be well-informed of the current chemical warfare threat in environments in which they may be called to serve. They should also be familiar with sociological and psychological factors motivating the use of such weapons in a battlefield or terrorist scenario.

Chemical warfare agents do not need to be lethal to be disruptive. It is not difficult to envision a scenario where medical practitioners may be the first to observe and recognize the effects of chemical exposure—in the absence of warnings from the intelligence community. Few physical indicators of chemical attack may be evident, other than the initial observation of unusual signs and symptoms. This scenario could occur when new agents, for which there may be no environmental monitoring, are used. An increased incidence of symptoms consistent with nerve, vesicant, blood, or respiratory agent exposure should raise immediate suspicion of poisoning, even among presumably protected troops. The possibility of combined use of chemical and biological warfare agents should also be considered.

Offensive use of chemical agents continues to be attractive to some nations, for chemical agents can be dispersed over large areas and can eventually penetrate even the most well-defended positions. They can be employed against specific targets, including headquarters control centers and, depending on the agent or combination of agents used, the effects can be immediate or delayed incapacitation, disorientation, or death. The psychological impact is ever-present, even among well-seasoned and -trained troops equipped with full barrier protection. Many of the more common classic chemical agents—which are generally believed favored by possessor states—can be produced inexpensively and quietly, and they can be stored indefinitely. Their minimal cost has earned chemical warfare agents the appellation “the poor man’s atomic bomb.”

Although treaties dealing with control or elimination or both of classic chemical weapons may reduce the danger that chemical warfare agents will ever be used, difficulties in verification and in controlling the manufacturing, acquisition, and storage of precursor chemicals make chemical war a continuing concern for the U.S. government. Chemical proliferation has not decreased. Saddam Hussein’s use of chemical warfare against the Kurds in 1988 demonstrates how readily such weapons can be used, even within the confines of one’s own country. The 1994 and 1995 incidents involving the Aum Shinrikyo cult’s use of sarin (ie, the nerve gas GB) to cause fatalities and disruption in Matsumoto and in the Tokyo subway system demonstrate how easily a terrorist organization can quietly produce and use a classic chemical warfare agent.

THE CHEMICAL THREAT AND ENEMY CAPABILITY

The term “chemical threat” centers on enemy capability. The term *capability* encompasses[1]

- the availability and supply of specific agents;
- the delivery system or systems that would be used in different battle situations;
- the facilities to produce these agents and munitions;
- plans and procedures for the employment of such weapons, including training for the delivery and handling of such weapons;
- protection of a nation’s own forces against specific agents; and
- the national will to use such weapons.

Although international disapproval may discourage a country from using

chemical agents, the aggressor nation may finally decide that protecting its national interests and survivability is more important.

An active research and development program on agents and delivery mechanisms supports the notion of operational use. Chemical warfare munitions are particularly important, for weapon systems must deliver agent to the target and distribute that agent with maximal effective contact. Successful chemical warfare munition use must also be combined with meteorological assessment capabilities. For example, sarin, a highly volatile agent with little persistence on the ground, must be delivered under specific environmental conditions and in a timely manner that would allow greatest human contact for optimal effectiveness. Ideal conditions and carefully developed delivery systems are not always necessary, however: Iraq simply pushed containers of chemicals out of aircraft during the Iran–Iraq War.

Ordinarily, an enemy with chemical warfare capability will be well equipped for chemical warfare protection; they will have defined procedures on decontamination, individual and equipment protection, and detection and surveillance. Since chemical warfare agents are nonselective in their human targets and dangerous for the user as well as the enemy, they require that offensive and defensive programs be developed simultaneously. Special military teams (eg, logistical, medical, and chemical corps teams trained to operate in a chemical environment) and the ability to monitor meteorological conditions are characteristic of nations with offensive or defensive programs or both. In the assessment of enemy capability, chemical stockpiles, production capacities, and the control of use are evaluated when an offensive or a defensive posture is being determined. This is not easily accomplished, since industrial plants that are manufacturing products with peaceful applications may be capable of having their manufacturing processes redirected toward chemical agent production.

In a changing world, where the traditional East–West conflict has subsided in the face of steadily increased chemical agent proliferation among many Third World nations, the chemical threat appears to be increasing from smaller nations or political splinter groups with little or no sophisticated chemical warfare industrial capability. Hence we must be prepared for chemical agent attack from terrorist elements, and for crude delivery systems as well as highly developed offensive chemical warfare operations. Political instabilities and changes toward radicalism only heighten the dangers and concerns that defensive programs can be converted to offensive efforts, and, although classic chemical warfare agents are harder to produce and stockpile in large quantities without being noticed, small amounts of some older agents can be manufactured in relatively crude laboratories and used to create disruption.

THE STATUS OF CHEMICAL PROLIFERATION

Until 1987, when the Soviet Union admitted for the first time that it possessed an offensive chemical warfare capability, the United States was the only publicly declared state capable of conducting chemical warfare. In July 1988, long after a United Nations commission had confirmed Iraqi use of chemical agents (mustard and nerve agent) against Iran (a flagrant violation of the 1925 Geneva Protocol, which Iraq had signed in 1931), Iraq also declared an offensive capability. Apart from the three nations mentioned, estimates of countries that possess chemical agents, and the nature of the agents they possess, must rely on sources other than official statements. Given this, it is not surprising that published lists of states with chemical warfare capability have varied widely. However, a key trend in chemical warfare capability over the past 20 years is evident: an increasing number of states are likely to possess such weapons.

Chemical Warfare Capabilities of Nations

The magnitude of world interest in offensive chemical warfare capability was made evident during an open hearing of the U.S. Senate Committee on Governmental Affairs on 9 February 1989, when William H. Webster, former Director of Central Intelligence, presented a list of weapon states. The confirmed chemical weapon possessor states were the United States, the Soviet Union, Iraq, and Iran. France has subsequently declared a chemical warfare capability, bringing the number of possessor nations to five. Countries currently suspected of possessing chemical weapons or in the process of acquiring them are identified in Exhibit 4-1. Several other nations are being closely monitored for signs of an acquisition program.[1]

An article in the March 15, 1991, issue of *The Washington Post*[2]described the latest annual report of the Office of Naval Intelligence, listing 14 nations with “an offensive chemical-warfare capability”; the list included Egypt, Israel, Pakistan, and South Korea, 4 nations that receive large quantities of military aid from the United States. Four additional nations (Saudi Arabia, Indonesia, South Africa, and Thailand) were purported to possibly possess such a capability, and then more nations were believed to be in the process of developing or seeking to develop chemical weapons. Interestingly, this list conflicts with a U.S. Department of Commerce list that does not list the strong trade partners of South Korea, Indonesia, and Thailand as having definite offensive capability. In a 1993 U.S. House of Representatives Committee on Armed Services report, 31 nations were mentioned as possessing or having the ability to develop offensive chemical weapons.[3]

Offensive chemical warfare capabilities depend on such factors as

chemical agent quantities, types of agents weaponized, modes of delivery, doctrine for use, means of self-protection, and other considerations that together characterize the total threat posed by a chemical warfare-capable state. Such detailed analysis of the threat posed by each possessor state is beyond the scope of this chapter, and interested readers should review classified and unclassified sources for each nation.

A general idea of the classic agents was provided by the Soviets when chemical armaments were displayed for the first time to Western visitors in 1987, and later by Iraq when its arsenal was inspected. The Soviets showed a wide variety of chemical weapons delivery systems that could carry blistering agents (mustard and Lewisite), nerve agents (soman, sarin, and VX) or the riot control agent CS (2-chlorobenzalmalononitrile). Some of these agents were thickened to increase battlefield deposition and persistence (see Military Chemical Agents, below).

United Nations weapons inspectors, on gaining access to Iraq's arsenal in 1991, found primarily sulfur mustard, sarin (and a sarin analog, GF), and another nerve agent, tabun.[4]The Iraqi chemical warfare development program was well developed, and included experimentation with VX. Far beyond pushing barrels out of helicopters, the sophisticated Iraqi chemical weapons delivery systems now included aerial bombs, artillery rockets, artillery shells, cluster bombs, and mortars. Seventy-five chemical warfare ballistic missile warheads were also discovered, filled with sarin/GF mixtures or binary nerve agent (in binary systems, two individually less-toxic reagents are mixed in the weapon at the time of use to form the agent).

EXHIBIT 4-1
CHEMICAL WARFARE CAPABILITIES OF NATIONS

Nations With Confirmed or Strongly Suspected Capabilities		Nations Monitored for Capability or Aquisition
Balkan states	Israel	Argentina
Bulgaria	Laos	Brazil
Burma (Myanmar)	Libya	Chile
China	North Korea	India
Czechoslovakian states	Romania	Pakistan
Egypt	Russia	Peru

Ethiopia	South Africa	Saudi Arabia
France	Syria	South Korea
Hungary	Taiwan	Thailand
Indonesia	United States	
Iran	Vietnam	
Iraq		

Adapted with permission from Burck G, Flowertree CC. *International Handbook on Chemical Weapons Proliferation.* New York, NY: Greenwood Press; 1991: 164–165.

Prior to its destruction, Iraq's primary chemical warfare agent production facility at Al Muthanna, Iraq, produced thousands of tons of agent. The complex included well-built, underground, bunker-type complexes, billed as sites for pilot plants for pesticide production but apparently capable of VX production. Another facility served as an inhalation chamber for making lethality estimations. Two other sites were built at Fallujah, Iraq, to make chemical precursors for use at the Al Muthanna plant, although one of the Fallujah plants, while containing stored agent precursors (for VX, GB, and HD [mustard]), was adapted for malathion pesticide formulation. These examples illustrate why it can be difficult, with limited inspections or information from open declarations, to distinguish facilities designed for the chemical industry from those for munitions generation.

These chemical manufacturing facilities are only part of Iraq's chemical warfare capability. Iraq, like Libya, also invested heavily in the development of facilities for the production of indigenously generated delivery components.

The current development of offensive chemical warfare capabilities varies among the possessor states and is now particularly dynamic. First, the United States and Russia, which has inherited the chemical weapons arsenals of the Soviet Union, have embarked on a program to radically reduce the chemical agent stocks held by both sides by the late 1990s. Second, in January 1992, some 125 nations signed a treaty, formulated over 24 years, at the Chemical Weapons Convention in France, calling for the prohibition of the production, storage, use, and transfer of chemical weapons; and for the elimination of chemical warfare arms and production facilities.[5]By January 1993, 130 nations had signed the treaty, and more are expected to follow.

During the 1980s, some nations vastly increased their development of offensive chemical capabilities, particularly in the Middle East, where chemical warfare capability substitutes in some respects for a nuclear

weapons capability. A rapid transition from nascent development programs to full weaponization occurred in Iraq, Syria, and Libya, and Iraq unleashed chemical weapons against Iran in 1984. Iraqi use of mustard and nerve agents constituted the first full-scale use of chemical weapons in battle since World War I.

The effective elimination of the Warsaw Pact and the dissolution of the Soviet Union have greatly diminished and reconfigured the overall chemical threat from that part of the world. The exportation of Soviet technology has been a continuing process, and it is likely that the chemical weaponry and technological know-how was well disseminated to developing nations. The Russians have declared a chemical agent stockpile of about 40,000 tons (higher than the 30,000 of the United States).[6,7]As of 1996, it appears that all of the former Soviet Union's chemical weapons containers and munition components are maintained at seven sites in Russia. As is done in the United States, most of the agent is primarily stored in nonweaponized containers; the weaponizable portion appears to consist entirely of soman- and VX-like nerve agents, sulfur mustard, and Lewisite. Mixed preparations are common in the arsenal. With all chemical warfare agents under a single central control, the possibility of unauthorized weapons proliferation by former Soviet border nations, several of which are politically unstable, should be greatly diminished, although the possibility of chemical weapons proliferation through theft under a climate of economic turmoil remains real. Uncertainties regarding dispersal, and management procedures concerning that stockpile, suggest that some chemical weapons could be lost while awaiting destruction.[8]

The Soviet military appeared to have invested considerable effort toward the development of forces capable of operating in a chemical warfare environment.[9]According to the U.S. Defense Intelligence Agency, Soviet facilities associated with the production, testing, and storage of chemical or biological agents or both continued to expand through 1987.[10]Today, a variety of chemical warfare delivery systems are available to Russian military forces, including artillery, bombs, free rockets, ballistic missiles, and cruise missiles.

The origin and nature of the overall chemical agent exposure threat to U.S. troops changed considerably during the 1980s. Although the threat of chemical warfare confrontation with former Warsaw Pact nations appears eliminated, the proliferation and use of chemical warfare agents within unstable sectors of the Third World has raised great concern regarding the potential for future use of chemical warfare agents both with respect to open conflicts and to terrorist activities. For Third World nations, chemical weapons are less expensive and easier to acquire, and are a more

credible threat than nuclear weapons. The adaptation and incorporation of chemical agent-containing munitions to conventional or missile delivery systems can give a weaker nation a military threat with which to counterbalance that of neighbors that possess a greater conventional capability.

Nations may initially acquire a limited chemical warfare capability through the transfer or purchase of bombs or artillery-compatible chemical warfare shells. In some cases, unweaponized agent may have been transferred.[11]Alternatively, nations may invest in the development of chemical industries that involve the manufacture or acquisition of chemical precursors or intermediates. In this way, wealthier nations (eg, Iraq, Libya) or those under a strong, perceived threat (eg, Syria) may increase their chemical warfare potential by acquiring the technology and facilities to synthesize agents and incorporate them into munitions that are compatible with existing or newly acquired delivery systems. Industrial compounds such as organophosphates (pesticides), phosgene, chlorine, and cyanide are not difficult to obtain.

Economic factors such as wealth, profit incentives, international debt, and isolation can contribute to the proliferation of chemical warfare capabilities. For example, oil-rich nations ruled by dictators (eg, Libya, Iraq) have been able to use their profits to acquire expensive delivery systems such as ballistic missiles and long-range bombers, along with associated support aircraft.[12,13]When shunned by major arms-systems producers such as the United States, Britain, France, and Russia, the oil-rich nations have approached other Western sources or those in less-developed nations, some of them deeply in need of foreign capital, such as Brazil, Chile, Argentina, Yugoslavia, Israel, Egypt, North Korea, or the People's Republic of China.[12-16]

Inevitably, there is a trickle-down effect in the arms world, as aging munitions and weapons systems are replaced and move from the major weapons producers to their Third World client states, and from the latter to other nations. For example, the Soviet Union probably supplied a chemical warfare capability to Egypt,[11]which, in turn, first supplied Syria,[15]which, in turn, supplied Iran.[16]It should be noted that some weapons systems, especially from the former Eastern Bloc countries, were designed to operate in a chemical warfare theater.[9]As noted earlier, a defensive capability is generally held to be prerequisite to an effective offensive chemical warfare capability.

The profit motive has driven many private industries to supply Iraq and Libya with technology, infrastructure, and chemical precursors for the synthesis of nerve and blister agents in large volume, and the manufacture of artillery shells and bombs required for their delivery. Until recently, West

German government export control was minimal, resulting in the involvement of some 86 German firms in Iraq's development of chemical and nuclear weapon capabilities and ballistic missile design. A considerable number of companies in Austria, Britain, France, Italy, Switzerland, and the United States were also involved in these efforts. Products included chemical agent production plants and precursor compounds, computer systems, machine tools, casting and milling technology and facilities, weapon and ammunition production facilities, missile technology and "super gun" components.[16]Commercial dissemination of chemical warfare capabilities will be a continuing problem in the years ahead.

International Agreements and Verification

Despite such uncertainties, a chemical weapons reduction agreement[17]was reached in 1990 between the United States and the Soviet Union that will (1) effectively stop chemical weapon production and (2) reduce each nation's chemical agent stocks to a value of 5,000 metric tons by the year 1999 (by the end of the year 2002, this number will fall to 500 tons). Destruction of the remainder is contingent on a commitment for similar, total chemical warfare stock elimination by other chemical warfare–capable nations.[18,19]

On May 13, 1991, U.S. President George Bush further advanced his 1989 plan before the United Nations to destroy 98% of the U.S. stockpile in the first 8 years under a new, proposed treaty. Under its conditions, he pledged (1) to destroy all U.S. chemical weapons within 10 years and (2) never to use chemical weapons again.[20](However, anticipated difficulties in chemical weapon demilitarization and destruction may prolong the presence of chemical weapon depots.) This message sent a clear challenge to other powers to eliminate chemical weapons. The United States ratified the treaty on 24 April 1997, which was a few days before the treaty went into effect. Although signed by nearly 160 nations, it must still be ratified by most of those nations. The treaty still leaves in doubt the development and use of chemical warfare agents by developing nations or nonsigners of such agreements (most notably Libya, Iraq, and North Korea). Chemical warfare treaty ratification by nations such as Iran, given the behavior of its neighbor, Iraq, may prove to be understandably difficult in the short term.

Reluctance by possessor states to employ chemical weapons, which could be termed "the chemical warfare threshold," has seemed to be relatively high since World War I. However, the Iraqi precedent, the ineffective world response to Iraq's use of chemical warfare, and the

perceived effectiveness of this use all suggest that the chemical warfare threshold has been substantially lowered. The growing list of states motivated, for reasons of offense or deterrence, to develop relatively low-technology, low-cost weapons of mass destruction greatly increases the likelihood that military personnel will have to contend with casualties of chemical warfare.

Finally, the problem of verification of treaty compliance continues to be difficult even with on-site inspections. The former Director of Central Intelligence, William H. Webster, stated on 15 October 1988:

> After all, any country with a petrochemical, pesticide, fertilizer, or pharmaceutical industry has the potential in terms of equipment, raw materials warfare, and technical expertise to produce some chemical agents. Without direct access to such facilities, it is nearly impossible to know whether activities being undertaken are of a commercial or a military nature.[21(p9)]

This concern was reiterated in congressional testimony following the Persian Gulf War.[18]

Terrorism

Finally, no threat assessment would be complete without addressing the terrorist dimension. Terrorism may derive from clandestine, state-directed initiatives[22]or, more commonly, from small splinter groups with special interests or agendas. Groups with training and financial backing need only to set up small laboratories to make chemical or biological warfare agents. For example, while investigating Red Army faction activities in 1980, French police uncovered in an apartment a clandestine laboratory capable of producing botulinum toxin.[23]This suggests that state-sponsored terrorism could serve as a conduit for the testing of the products of rapidly emerging biotechnology techniques. It also places healthcare providers in a position in which they may be the first to encounter and evaluate the dangers of new and emerging threats. Also notable is the successful manufacture of a military nerve agent by able university students recruited by Aum Shinrikyo. Psychological manipulation and religious zeal were combined to support the terrorist actions of this organization, which was well funded by its members.

Chemical and pharmaceutical industries continue to spread around the world, providing unsponsored terrorist groups access to precursors and chemicals. Compounds such as chlorine, phosgene, and cyanide are commonplace, and theft of such materials has been reported.[24]

In addition to terrorist actions, accidents will occur as manufacturing with potent industrial chemicals becomes widespread. Although industrial compounds are not traditionally classified as chemical agents, they are lethal and potent (eg, the disaster in Bhopal, India, which is discussed later in this chapter). Poor economic conditions may also promote theft of agents and their chemical precursors and illegal transfer of weapons—not only by international brokers but also by industrial workers.

MILITARY CHEMICAL AGENTS

Military chemical agents are characterized according to several features. Among them are the nature of their use, their persistency in the field, and their physiological action. Toxic chemical warfare agents are capable of producing incapacitation, serious injury, and death. These agents are further characterized by their physiological action and are discussed in detail in their individual chapters. Table 4-1 lists the major chemical warfare agents.

Nerve agents such as tabun (GA), sarin (GB), soman (GD), and VX inhibit acetylcholinesterase enzyme throughout the body, notably in the nervous system. This causes hyperactivation of cholinergic pathways, causing convulsive seizures and respiratory failure. VX differs from its “G” agent counterparts in its low volatility.

Vesicants, such as sulfur mustard (HD) and the arsenical Lewisite (L), cause irritation and vesication of the skin and mucous membranes, notably of the lungs. Mustard exposure to the skin is insidious, causing no immediate discernible effects to the skin for several hours; blistering occurs 12 to 24 hours after exposure.[25]Although mustard causes few deaths, its vesicating properties are incapacitating, and casualties require 1 to 4 months of hospitalization. Lewisite blisters heal within several weeks.

Pulmonary toxicants, such as phosgene (CG) and diphosgene (DP), injure the respiratory tract, causing suffocation. Phosgene intoxication rapidly leads to pulmonary edema. The initial effects of eye exposure resemble those of tear gas; severe pulmonary edema follows in about 4 hours, eventually leading to death. It is notable that both phosgene and elemental chlorine (an immediate phosgene precursor), which can cause pulmonary edema and hemorrhaging, are industrial compounds.

Finally, cyanides such as hydrogen cyanide (AC) and cyanogen chloride (CK) both release cyanide ions in the body. Lower doses cause headaches, weakness, disorientation, and nausea; higher doses cause circulatory effects, seizures, and respiratory and cardiac failure. While often attributed to its blockade of energy metabolism, the mechanisms of cyanide intoxication remain unclear and may include cellular targets more

sensitive to inhibition than cytochrome oxidase.

The most common agents in modern arsenals are vesicants and nerve agents. Cyanides and pulmonary toxicants are thought to be represented in some stockpiles, but are typically less toxic and more difficult to employ because of their physical characteristics. Some cyanides and pulmonary toxicants have specific characteristics that make them appropriate for military use, such as rapid rate of action, very low persistency, and the ability to penetrate or damage protective equipment.

Other chemicals present in military arsenals include incapacitating agents, which produce physiological or mental effects, or both, rendering individuals incapable of performing their assigned duties. Recovery may take several hours to several days, although intensive medical treatment may not be required. Riot control agents produce intense effects, such as irritation of the skin, eyes, and respiratory tract, but recovery is normally rapid when exposure is terminated. Unfortunately, little is known about the long-term effects of many of these agents, and this is an area of increasing medical concern.

Chemical smoke agents are used to obscure objects or areas from observation or from engagement by weapons with electro-optical control systems. They are usually not toxic in field concentrations, but may cause eye or respiratory irritation in higher concentrations. Some smokes have adverse chronic exposure effects.

TABLE 4-1
CHEMICAL WARFARE AGENTS

U.S. Army Code	Agent
Cyanide	
AC	Hydrogen cyanide
CK	Cyanogen chloride
Nerve Agents	
GA (Tabun)	Ethyl *N,N*-dimethyl-phosphoramidocyanidate
GB (Sarin)	Isopropyl-methylphosphonofluoridate
GD (Soman)	1,2,2-Trimethylpropyl methylphosphonofluoridate
GF	Cyclohexyl-methylphosphonofluoridate

VX	*o*-Ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate
Lung Agents	
CG (Phosgene)	Carbonyl chloride
DP (Diphosgene)	Trichloromethyl chloroformate
Vesicants	
HD (Mustard)	*bis*-2-Chloroethyl sulfide
L (Lewisite)	2-Chlorovinyl dichloroarsine
HL	Mustard–Lewisite mixture
Incapacitating Agents	
BZ	3-Quinuclidinyl benzilate (QNB)
Tear Gas	
CN	2-Chloro-1-phenylethanone
CS	2-Chlorobenzalmalononitrile
Vomiting Gas	
DM (Adamsite)	10-Chloro-5,10-dihydrophenarsazine

Other compounds with military applications include agents used in flame warfare, such as thickeners for napalm and incendiary materials, and herbicides (defoliants).

Thus far, discussion has centered on chemical compounds with a military application. Other highly toxic industrial chemicals also pose a potential risk to the military. The disaster in Bhopal, India, in December 1984, when an estimated 8,000 persons died and another 30,000 were injured from breathing methylisocyanate and chlorine released in an industrial accident, is just one of many examples of the devastating effect of poisonous gases.[26]

Chlorine and phosgene are industrial compounds that have been and could again be used as military weapons by an enemy with access to such

materials, and medical personnel should also be prepared for such emergencies should military missions be in close proximity to industrial plants. The first large-scale use of a chemical compound in Ypres, Belgium, on 22 April 1915, the beginning of chemical warfare as we know it today, involved the dispersal of 180 tons of chlorine from over 5,700 canisters by the German forces. During that war, the list of chemical agents was expanded to include mustard, phosgene, adamsite, and cyanide.

TACTICAL AND STRATEGIC USE OF CHEMICAL WEAPONS

Delivery of chemical agents can be accomplished by a full range of weaponry. Liquid agents may be dispensed from land mines and spray tanks to artillery projectiles, aerial bombs, rocket and missile warheads, or even cruise missiles. This means that all battlefield areas, from front lines to rear reserves, are vulnerable to chemical warfare attack, and that medical practitioners should be fully prepared to treat chemical warfare casualties from a variety of locations. It is also important to note that, while this section largely focuses on the use of chemical warfare agents on the battlefield, medical personnel must also be prepared for the possibility of isolated and spontaneous chemical attacks on both military personnel and civilians in areas subject to low-intensity conflict and isolated acts of terrorism.

To be effective, chemical agents must be efficiently dispersed over their intended targets. Most applications call for large-scale agent distribution over large target areas that are occupied by, or may be of interest to, military units. For example, documents recovered from the former German Democratic Republic called for Warsaw Pact forces to employ heavy chemical warfare attacks early in any conflict with the West.[23]Considerable quantities of an agent may have to be applied to ensure good coverage in the face of such factors as wind, heat, and agent volatility, and surprising the enemy so as to find them unprotected (eg, unmasked).

Chemical Agent Delivery Systems

The four methods of delivering chemical agents are explosive release, bulk release, base ejection, and spray delivery (Figure 4-1). The most common method is explosive release. Bursts from individual explosive munitions are, effectively, point sources for chemical warfare dissemination. Chemical warfare artillery shells, which serve as smaller point sources, could be laid down in a grid to cover a large area. The same could be accomplished with fewer missiles, which carry larger

payloads and have longer ranges. Agents can also be delivered from multiple explosive point sources using submunitions to cover a larger area or, if detonated in sequence, to lay down the agent along a trajectory line. Such line deliveries may be delivered directly over the target, or upwind of the target, preferably perpendicular to the wind.

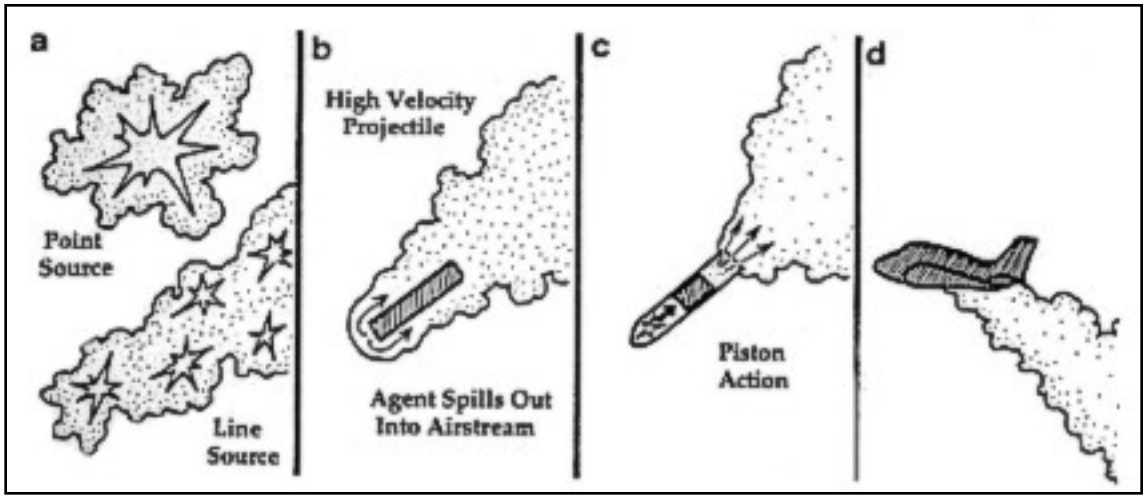


Fig. 4-1. Four modes of chemical agent release. **(a)**Explosive-release devices are predominantly represented among the major chemical warfare arsenals. While some agent is lost to decomposition, their simplicity makes these the weapons of choice. Point-source explosives are single detonation devices, while line-source munitions release a series of time-delayed explosions that lay agent toward the end of the trajectory. **(b)**Bulk-release munitions spill agent into the airstream of the projectile. **(c)**Base-ejection devices are relatively uncommon owing to their cost and complexity. Like explosives and bulk-release devices, these munitions can be carried on longer-range missiles. **(d)**Spray delivery can be used to achieve large-area coverage, such as that required for terrain denial. However, because of aircraft vulnerability, spray delivery is generally limited to application on undefended territory or against a poorly defended foe.

Bulk release, base ejection, and spray delivery also deliver chemical warfare agents along trajectory lines. In bulk release, the forward covering, or “skin,” of a warhead is blown off, exposing agent to aerodynamic breakup by high-speed air flow. In base ejection, an explosive charge causes an internal pistonlike action to force the agent out of the back of the warhead—either through small apertures, aerosolizing it, or into a high-speed airstream for aerodynamic breakup. Explosive, bulk release, and base ejection methods are primarily suited for the dispersal of liquid chemical agents. For the few solid agents such as the tear gas CS and the incapacitating agent BZ (3-quinuclidinyl benzilate), effective aerosolization is often achieved by pyrotechnic munitions.

Spray delivery is more efficient than the other three methods in providing a very fine aerosolization (average droplet diameter = <5 µm) of agent, which can be inhaled far down into the lungs. This method is

particularly suited to the delivery of toxins, which require deep inhalation and which differ from most chemical agents in that they are solids and do not vaporize. Spray delivery requires slow speeds and low altitudes, conditions that render aircraft particularly vulnerable to attack. Spray tanks could also be mounted on trucks or boats, and unmanned aircraft could be designed to perform the task. The increased vulnerability of spray-delivery systems makes their use more likely against unarmed or poorly equipped opponents, or on carefully targeted sites under cover of surprise. Spray delivery could also be applied to closed ventilation systems in more focal applications.

From a tactical military standpoint, explosive munitions, the dominant mode of chemical agent delivery, vary with respect to effective agent delivery. Disregarding differences among chemical warfare agents for now, Figure 4-2 describes chemical agent dissemination with respect to explosive munitions in further detail and illustrates important considerations regarding the chemical agent dynamics and toxicity. Explosion of a chemical agent shell, at ground level or some height over the target site, generates two products: vapor and droplets. Droplets (average diameter range = 100 µm to 1 mm for pure agents) will fall to the ground in a fine rain to coat the target surface with liquid.

Agent vapor, the greatest threat for inhalational intoxication, derives from three sources. First, agent vaporizes from explosive burst energy.

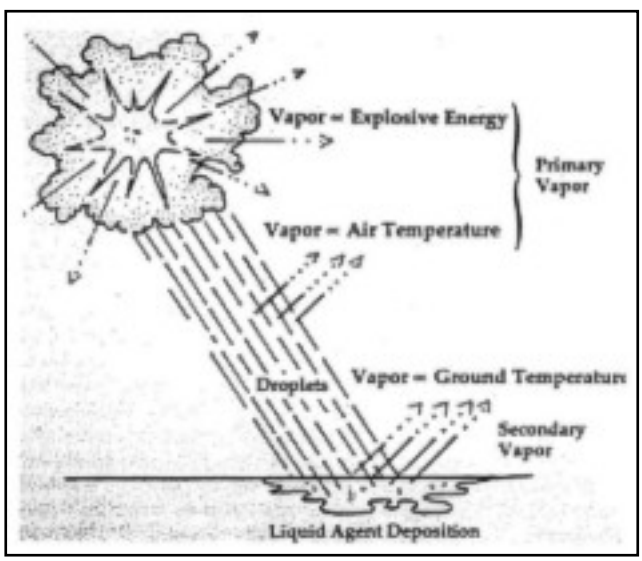


Fig. 4-2. Agent vaporization increases in proportion to energy sources such as heat from explosive charges or from ambient heat (as measured by air or surface temperatures). Vapor persistence is then determined by weather factors such as wind and humidity. Hydrolysis rates are affected by factors such as temperature and solubility. Agents show characteristic hydrolysis rates in water, and water vapor, as described by humidity, may cause significant hydrolysis of vaporized agent. The vesicant Lewisite, for example, shows relatively rapid hydrolysis in water vapor, while the nerve

agent VX is more resistant to hydrolysis.

This will vary with shell design and specific agent payload. Important shell design factors are shell casing thickness, shell casing material, and the agent-to-burster ratio. Second, additional vapor will be generated as falling droplets vaporize. Heat from the explosion dissipates quickly, and ambient air temperature is the most important factor in driving this volatilization. Third, the liquid coating of agent on the ground evaporates, ground temperature being an important factor. Vapor produced by the first two, explosive energy and droplet vaporization, is called primary vaporization, while that rising from the ground is secondary.

Considering these phenomena, we can appreciate, for example, the differences in agent threat (liquid persistence and deposition vs vaporization) in scenarios wherein chemical agent shells are dropped on a desert area during different times of the day. The influence of wide environmental temperature fluctuations over the 24-hour cycle, combined with the agent used (see below) can make a large difference: we can expect increased surface deposition and skin-contact threat during cool nights, and a considerably increased inhalational toxicity threat during the heat of the day.

TABLE 4-2 COMPARATIVE VOLATILITIES OF CHEMICAL WARFARE AGENTS

Agent	Volatility* (mg/m^3)
Hydrogen Cyanide	1,000,000
Sarin (GB)	22,000
Soman (GD)	3,900
Sulfur Mustard (HD)	900

Successful employment of chemical agents is influenced by many variables. Most notable among these is the weather, in that the agent is transported by the wind and air currents when released as a vapor or an aerosol. Unfavorable meteorological conditions frequently preclude successful agent deployment owing to the inordinately high number of weapons used. Once deployed, the persistence of liquid contamination is affected by temperature, sunlight, wind action, and rainfall.

Physical Properties of Chemical Agents

Toxicity mechanisms aside, the physical properties of the agent itself and its formulation also present similarly important threat

Tabun (GA)	610
VX	10

* Approximate amount of agent (in milligrams) that 1 m^3 of air can hold at 25°C.
Adapted from US Departments of the Army, Navy, and Air Force. *Potential Military Chemical/Biological Agents and Compounds.* Washington, DC: Headquarters, DA, DN, DAF; 12 Dec 1990: Appendix B, Table B, pp 95-97. Field Manual 3-9. Naval Facility Command P-467. Air Force Regulation 355-7.

considerations. Selection of agents and agent formulations can be used to effect differential impacts with respect to droplet size and liquid deposition, agent persistence, and agent volatility. The classic chemical warfare agents have a tremendous range of volatility (Table 4-2), and volatility can be a determinant in deciding which agents will be used.[27] Agents such as hydrogen cyanide and sarin are relatively volatile; they present an immediate, but short-lived, threat. These agents are referred to as nonpersistent (ie, they vaporize rapidly after delivery). Alternatively, agents such as VX and sulfur mustard tend to fall largely in droplets, with less vaporization, and remain on exposed surfaces for a long time. These agents are called *persistent.*

Formulation is also used to manipulate the fate of the agent. Soman, VX, Lewisite, and sulfur mustard can be mixed with high-molecular-weight thickeners to increase droplet size and thereby decrease primary vaporization. Such additives are generally used to promote efficient agent deposition on the target site. Thickeners can also increase agent persistence and may hamper decontamination efforts.

Nonpersistent Agents

In tactical use, the threat of nonpersistent, volatile agents such as hydrogen cyanide or sarin is greatest to the respiratory systems of unprotected soldiers. A sudden, heavy bombardment of these agents may effect many casualties if unmasked soldiers are caught by surprise. When used against an unprotected force, nonpersistent agents are particularly effective in generating casualties, thereby creating breakthrough points in enemy front lines. The successful use of nonpersistent nerve agent was demonstrated by Iraqi counterattacks against Iranian forces during 1988.[23] Nonpersistent agents can also be used to slow down the enemy by forcing him to wear protective equipment. Finally, they can also circumvent the enemy's protection against conventional high-explosive munitions and may be used in night attacks to harass the enemy.

Persistent Agents

Given favorable weather conditions, the use of persistent agents such as mustard and VX may pose a threat for many days. Such agents can deny or interfere with enemy occupation of terrain or use of equipment and, in theory, could be used defensively to protect vulnerable flanks. However, although they can slow down enemy movement, they can also hamper the movement of friendly forces through a contaminated area. Delayed casualties may occur even among protected troops operating in a contaminated area for an extended period. Hence, persistent agents may not be the agents of choice when occupation of territory by friendly forces is imminent.

Chemical land mines may be used in conjunction with military barrier systems to complicate breaching or clearing the barriers by dispersing persistent agent. The mines are typically based on high-explosive mine designs, with several pounds of agent being substituted for most of the explosive charge. High-explosive land mines will cause contaminated open wounds, primarily on lower extremities, that must be properly decontaminated; this could be more difficult when persistent agents are used.

Because of its action, sulfur mustard blistering agent offers strategic benefits besides those considered above. Sulfur mustard was used very effectively both during World War I and during the Iran-Iraq War to generate thousands of casualties. Although deaths among unprotected sulfur mustard exposure victims are relatively few,[28]mustard injuries can tie up medical treatment facilities with patients. While survivors of other agents stabilize relatively soon after exposure, mustard lesions demand months of medical care. This was the fate of many thousands of Iranian recruits, who were unprepared or poorly equipped when they were exposed to sulfur mustard agent.

Underscoring the importance of ambient temperature and climate, we should note that persistence can change greatly with temperature; sulfur mustard volatility increases nearly 40-fold between 0°C and 40°C: from 75 to 2,860 mg/m^3. Although always present, the threat of respiratory intoxication from sulfur mustard is considerably greater at higher temperatures, although its persistence is reduced.

Rapidity of action also factors into agent selection. Volatile agents such as cyanide and sarin can act very swiftly, primarily via the respiratory tract. In general, nerve agent effects follow immediately after exposure, culminating in seizures and death within a few minutes of inhalation, cutaneous dosing, or both. Other agents, such as mustard, Lewisite, and phosgene act only after a delay. For example, both the blistering and the

edematous effects of skin exposure to sulfur mustard occur only many hours after exposure.

Choice of Agent and Delivery System

By selecting the appropriate agents, formulations, and delivery systems, a well-equipped military will be in a better position to achieve its tactical objectives. U.S. Army Field Manual (FM) 3-10, *Employment of Chemical Agents*, discusses how chemical munitions could be used separately or integrated with conventional weapons. Chemical warfare agents can be used to cause casualties, harass the enemy, and hamper or restrict the use of terrain. Although an offensive capability no longer exists, FM 3-10 provides useful information on how chemical warfare agents can be used on the battlefield.[29]Brigadier General Augustin M. Prentiss, a Chemical Warfare Service officer, describes in his classic 1937 book, *Chemicals in War*, the offensive tactical uses of chemical agents that were in place following World War I.[30]

The most militarily significant effects of chemical agents are through inhalation, in that most agents are more toxic and faster acting by that route of exposure. Almost without exception, modern armies are equipped with protective equipment: masks to protect the eyes and respiratory tract, and protective clothing to prevent skin contamination. However, the very act of donning protective equipment is an encumbrance. In hot weather, remaining in protective clothing for more than a few minutes can itself produce casualties. The mission-oriented protective posture gear (MOPP) that the U.S. Army issues, which was designed for use in the European theater, can swiftly cause an active wearer to experience heat stress and dehydration under desert conditions.[31,32]The British protective counterpart, the MK 4 suit, keeps the wearer cooler by allowing perspiration to evaporate, although the heat stress problem remains.[33]

Detection and Protection

Adequate agent detection capabilities are essential for successful chemical agent defense. Although U.S. Army doctrine prescribes donning full MOPP 4 gear if an attack is imminent or in progress, detection capabilities permit recognition of the true agent threat and appropriate reductions to protective posture (ie, MOPP 3 or even MOPP 2). Detection capability for medical teams is essential to provide warning that casualties are contaminated, and to avoid inappropriate assumption of high levels of protective posture if they are not.

Well-equipped, well-trained troops who apply high levels of discipline in using protective equipment are not very likely to become chemical

casualties. Most casualties will have sustained respiratory injury due to failure to mask properly in time when under attack. We can speculate that as the length of time spent in protective posture increases, the percentages of casualties with skin effects will increase. Most of these effects will be from failures in procedure when donning protective clothing and removing contaminated protective clothing.

Among exposed populations, the range of agent intoxication effects can be expected to correlate with levels of protective equipment, training, and discipline. The healthcare provider should anticipate that poorly trained soldiers will show an increased incidence of skin contamination by vesicants, for example. There will also be a greater need for decontamination. Civilian populations will generally be the most vulnerable. Most will have little if any protective equipment, and no means of detecting the presence of agent.

Even with protective equipment, the threat of agent intoxication is greater for casualties with facial, neck, or chest wounds that may compromise the integrity of the protective mask seal. Based on wound descriptions, a retrospective analysis[34] of 2,021 casualties admitted to the Naval Support Hospital in Da Nang, Vietnam, found that mask failure could have been expected to affect 34% of these patients.

RESPONDING TO THE THREAT: MANAGING CASUALTIES

The medical management of casualties, including triage, decontamination, and specific therapy, is discussed in separate chapters. However, several points need emphasis under a discussion of concerns for the healthcare provider.

First, many of the early signs and symptoms produced by chemical warfare agents may resemble those of a variety of disorders, including stress. Among unseasoned troops, especially those with limited experience in a chemical environment, psychological withdrawal or physical complaints of palpitation, gastrointestinal distress, headaches, dizziness, and inattentiveness will present difficult diagnostic dilemmas for medical personnel on the battlefield. A clinical awareness of the early signs and symptoms is critically important, but so is an awareness of the medical problems associated with stress. The potential for mass hysteria is also high, even among troops with full individual protection, and the horror of dying from a chemical agent attack is widespread. Apprehension will be a major factor in the confusion of battle.

To minimize such problems, continuous training is required so that soldiers are comfortable donning their protective equipment and operating in a chemically contaminated environment. Similarly, soldiers must understand the rationale for taking the nerve agent pretreatment,

pyridostigmine, as an *added*protective measure, not as a replacement for masking. Such training should involve medical input.

Second, the risk of chemical contamination of medical equipment and medical treatment facilities is an added threat, and precautions need to be taken to ensure that patients are properly decontaminated before being brought into designated uncontaminated treatment areas. Frontline medics faced with many casualties can only be expected to administer lifesaving procedures, such as opening the airway or preventing further hemorrhage; decontamination can be expected to be minimal.

In rear areas, chemical decontamination of open wounds adds substantial complexity to otherwise conventional wounds, which then require procedures different from those ordinarily established for debridement. Some nations have adopted special irrigation–suction devices to irrigate and clean wounds, and air-flow protection methods to minimize the risk to hospital staff. The risk of wound contamination may be higher with low-velocity wounds, when pieces of contaminated clothing or debris may be carried into the wound and remain deeply imbedded for a time; this may be a greater problem with persistent agents. Therefore medical procedures must be well defined, and healthcare providers should regularly review the steps required in handling a casualty.

While chemical agents are an occupational hazard to the combat soldier, they are also a danger to the emergency room and surgical staff, who must rely on their hands and eyes to stabilize and treat the casualty. Standard surgical latex gloves are not sufficient protection against chemical agents, and they provide little protection against a vapor hazard. Should hospital-based medical personnel in critical specialties become casualties themselves, the healthcare delivery system will be significantly compromised. An improperly decontaminated casualty may also pose a risk to other patients.

Third, the patient flow pattern in a chemical environment will be substantially altered. Treatment rates can be expected to be reduced because of the decontamination procedures that must be in place. Injuries that will be seen will range from severe to minor, with the latter probably constituting the majority. With some agents, the effects of chemical injury may not be readily apparent until after a delay, and this must be considered in the disposition of the patient. Therefore, the process of medical evaluation and observation may tax the holding capability of a facility.

Finally, the medical logistical requirements will be increased. It has been stated that up to 40% more transport is required to move a typical field hospital in a chemical environment, and the fuel necessary to power air pumps, special filtration units, and air conditioners is an added

requirement.[35]Water requirements may also be increased in a chemical environment. Medical treatment facility planners should recognize the importance of environmental factors within a chemical warfare theater. For example, the MOPP gear may not be designed for the climatic conditions on the battlefield. Tests have shown that perspiration compromises the ability of the battledress overgarment to protect the wearer from chemical agents[36]and may actually predispose an individual to injury.[32]

FUTURE CONFLICTS IN A CHEMICAL ENVIRONMENT

From the standpoint of military strategy, two reasons are commonly cited for a combatant to employ chemical weapons. First, they can be highly effective when densely applied onto concentrated, largely immobile forces or populations. This factor largely promoted their use against entrenched troop positions during World War I. During the Cold War, military strategists anticipated similar intense chemical warfare bombardments from Warsaw Pact forces in the European theater. Second, chemical attacks can be initiated at lower levels to encumber an opponent with defensive equipment, or to create panic and disorder among poorly trained or unprepared troops. Application onto enemy troops or civilian populations can also have a strong demoralizing effect.

Two important influences on the decision to employ chemical attacks are weather patterns and user objectives. Gas dispersal depends on wind speed and direction. If the attacking force is in close proximity to the target area, it must use protective gear in the event of wind shift. This handicap can be avoided, if the situation allows, when agents can be delivered from a remote location by either air or long-range artillery. The objectives of an attacker may also determine whether chemical warfare will be employed and, if so, which agents are to be used. Thickened nerve agents and sulfur mustard deny free access to terrain and are not likely to be used by forces intent on occupation. Nonthickened nerve agents are not persistent and could be used by a mobile, advancing force.

Western powers had contemplated using chemical weapons during World War II. Sir Winston Churchill seriously contemplated resorting to chemical warfare should the defense of Britain have become desperate.[37]Later in the war, U.S. military commanders also contemplated the use of chemical agents to counter Japanese fanaticism, which, even during imminent defeat in 1945, caused exceedingly high losses on both sides during island warfare (nearly 110,000 Japanese died in the battle for Okinawa alone). These circumstances led the United States to resort to unconventional weaponry (the atomic bomb). With Germany out of the war and the death of President Roosevelt, who had opposed any first use of chemical weapons, the use of sulfur mustard and other agents was

seriously contemplated during the summer of 1945.[38]Based on recent events and decisions, however, it is unlikely that an offensive chemical warfare program would be initiated by Western powers. This does not negate the need for a strong defense posture, however, as long as chemical proliferation continues.

Fanaticism shown by Iranian Revolutionary Guard units may have precipitated Iraqi use of chemical warfare agents at the end of 1983. Throughout the Iran–Iraq War, Iraq generally used chemical weapons only when facing probable defeat with conventional weapons.[39]History suggests that a cornered and besieged enemy, confronting troops intent on inducing complete surrender, could employ chemical warfare agents as a final resort or act of vengeance. In 1937, Prentiss stated:

> In the last analysis, war is not a sport, but a grim contest between states for national existence. War cannot be conducted by any code of sportsmanship, but only by the law of military necessity, however much civilization may deplore the results.[31(p699)]

Therefore, the United States military must maintain a strong readiness posture in the face of a continuing chemical warfare threat.

SUMMARY

The military healthcare provider should be prepared to be the first to recognize military or civilian casualties of chemical warfare attack. This requires an informed understanding of the likelihood of chemical warfare agent use or threat, and it requires the ability to clearly recognize agent-exposure symptoms against a varying background of unrelated injury and stress behaviors. The healthcare provider should be informed, to the fullest extent possible, when to anticipate chemical warfare attack by hostile forces or terrorist activities. This requires consideration of an adversary with regard to political factors and motivation, chemical agent possession or access, chemical warfare offensive and defensive capabilities, and the strategic advantage to be realized through agent use.

When individuals suspected to have been exposed to chemical warfare agents are encountered, initial recognition of the type of agent used may be facilitated through an understanding of tactical agent use, modes of agent dissemination, likely routes of casualty exposure to agent, and physical agent properties and other factors determining the persistence of these toxicants in the operating environment. Finally, to protect both the injured and medical personnel, casualty care must take place within a

framework of decontamination both in the field and in forward medical support facilities.

REFERENCES

1. Burck G, Flowertree CC. *International Handbook on Chemical Weapons Proliferation.*New York, NY: Greenwood Press; 1991: 3-4, 164–171.

2. Smith J. Confusing data on chemical capability: US intelligence, diplomatic lists of armed nations differ. *Washington Post.*15 Mar 1991;A21.

3. Browder G, chairman. *Countering the Chemical and Biological Weapons Threat in the Post-Soviet World.*Report of the special inquiry into the chemical and biological threat of the Committee on Armed Services, US House of Representatives, 102nd Congress (2nd Session). Washington, DC: US Government Printing Office; 23 Feb 1993. ISBN 0-16-040163-1.

4. Evans D, Bailey K, Lambakis S, Rudney B, Victory B. *Iraqi Inspections: Lessons Learned.*Washington, DC: Defense Nuclear Agency; 1992. Unclassified Technical Report. DTIC B170991.

5. Ember E. Chemical arms treaty ratification moves forward. *Chemical and Engineering News*. 1993;70(47):14–15.

6. Mathewson W. Soviets' chemical weapons. *Wall Street Journal*. 3 Aug 1990:A10.

7. Ember E. Russia seeks US expertise, money to destroy its chemical arms. *Chemical and Engineering News*. 1992;71(4):18–19.

8. Felten E. Amid discontent, weapons could fall into unsafe hands. *Washington Times (Insight)*. 29 Oct 1990;26–27.

9. Segal D. The Soviet Union's mighty chemical warfare machine. *Army*. Aug 1987;37(8):26–35, 38.

10. Mohr H. Chemical weapons out of control. *Washington Times.*17 Oct 1989;F3.

11. Livingstone NC, Douglass JD. *Chemical and Biological Weapons: The Poor Man's Atomic Bomb.*Cambridge, Mass: Institute for Foreign Policy Analysis; 1 Feb 1984. National Security Paper 1.

12. Nolan JE, Wheelon AD. Third World ballistic missiles.*Sci Am.*1990;263(2):34–40.

13. Kempster N. For the Middle East, arms control is no longer unthinkable. *Los Angeles Times*. 1 May 1990;H5.

14. Windrem R. Iraq's most lethal weapons. *Popular Science*. Feb 1991:238(2):51–55, 84.

15. Rathmell A. Chemical weapons in the Middle East: Syria, Iraq, and Libya. *Marine Corps Gazette*. July 1990;59.

16. Timmerman KR. The poison gas connection: Western suppliers of unconventional weapons and technologies to Iraq and Libya. Los Angeles, Calif: Simon Weisenthal Center; 1990. Special Report.

17. Felton J. Approval seen on chemical weapons. *Congressional Quarterly Weekly Report.*9 June 1990;48 (23):1800.

18. The Committee on Armed Services, US House of Representatives. Countering the chemical and biological weapons threat in the post-Soviet world. Report of the Special Inquiry Into the Chemical and Biological Threat. Washington, DC: US Government Printing Office; 23 Feb 1993.

19. Towell P. In the bag: Chemical weapons pact. *Congressional Quarterly*. 26 May 1990;48(21):1664.

20. Smith RJ, Yang YE. Bush to scrap all US chemical arms. *Washington Post*. 14 May 1991;114(160)A-1, A-12.

21. Browder G, chairman. *Countering the Chemical and Biological Weapons Threat in the Post-Soviet World.*Report of the special inquiry into the chemical and biological threat of the Committee on Armed Services, US House of Representatives, 102nd Congress. Washington, DC: US. Government Printing Office; 23 Feb 1993: 9. ISBN 0-16-040163.

22. Wilkinson P. Terrorism, Iran and the Gulf region. *Jane's Intelligence Review*. 1992;4(5):222–226.

23. US Army Chemical School. World-wide NBC threat briefing. Fort McClellan, Ala: US Army Chemical School Threat Office; March 1992. Unclassified.

24. Agence France Presse. Cyanide stolen in Kyrgyzstan. *Agence Presse English Wire*. 25 September 1992.

25. Papirmeister B. A global picture of battlefield vesicants, I: A comparison of properties and effects. *Medical Chemical Defense.*1992;5(1):1–8.

26. Kurzman D. *A Killing Wind: Inside Union Carbide and the Bhopal Catastrophe.*New York, NY: McGraw-Hill; 1987.

27. US Departments of the Army, Navy, and Air Force.*Potential Military Chemical/Biological Agents and Compounds*. Washington, DC: Headquarters, DA, DN, DAF; 12 December 90. Field Manual 3-9. Naval Facility Command P-467. Air Force Regulation 355-7.

28. Gilchrist HL. Statistical consideration of gas casualties. In: Weed FW, ed. *Medical Aspects of Gas Warfare. Vol 14.*In: Ireland MW. *The Medical Department of the United States Army in the World War.*Washington, DC: US Government Printing Office. 1926: 273–279.

29. US Departments of the Army, Navy, and Air Force. *Employment of Chemical Agents.*Washington, DC: Headquarters, DA, DN, DAF; Mar 1966. Field Manual 3-10. Naval Warfare Information Publication 36-2. Air Force Manual 355-4. Fleet Marine Force Manual 11-3.

30. Prentiss AM. *Chemicals in War.*New York, NY: McGraw-Hill; 1937.

31. 60 Minutes. Likely effects of Iraqi chemical weapons attack. Columbia Broadcasting System. 26 Aug 1990.

32. Carter BJ, Cammermeyer M. Emergence of real casualties during simulated chemical warfare environment. *Milit Med.*1985;150(12):657–665.

33. Harrington L. US lightens up for chemical fight. *Chicago Tribune.*26 November 1990;14.

34. McCaughey BG, Garrick J, Kelly JB. Combat casualties in a conventional and chemical warfare environment. *Milit Med.*1988;153 (5):227–229.

35. Hammick M. The chemical question. In: The cutting edge: Battlefield casualty management.*International Defense Review.*Mar 1992;25(3):250–251.

36. Greve F. US troops ill-equipped for desert gas warfare. *Philadelphia Inquirer.*9 Aug 1990;1.

37. Taylor LB, Taylor CL. *Chemical and Biological Warfare*. New York, NY: Franklin Watts Press; 1985.

38. Van Courtland Moon JE. Project SPHINX: The question of the use of gas in the planned invasion of Japan. *Journal of Strategic Studies*. September 1989;12(3):303–323.

39. Morrison DC. Chemical fears exaggerated. *National Journal*. 8 September 1990;22(36):2133.

Chapter 5

NERVE AGENTS

FREDERICK R. SIDELL, M.D.*

INTRODUCTION

HISTORY

PHARMACOLOGY OF CHOLINESTERASE INHIBITORS
- Cholinesterase in Tissue
- Blood Cholinesterases
- Nerve Agents

EXPOSURE ROUTES
- Inhalational Exposure to Vapor
- Dermal Exposure to Liquid

EFFECTS ON ORGANS AND ORGAN SYSTEMS
- The Eye
- The Nose
- Pulmonary System
- Skeletal Musculature
- Central Nervous System and Behavior
- Cardiovascular System

GENERAL TREATMENT PRINCIPLES
- Terminating the Exposure
- Ventilatory Support
- Atropine Therapy
- Oxime Therapy
- Anticonvulsive Therapy
- Therapy for Cardiac Arrhythmias

SPECIFIC TREATMENT BY EXPOSURE CATEGORY
- Suspected Exposure
- Minimal Exposure
- Mild Exposure

Moderate Exposure
Moderately Severe Exposure
Severe Exposure

RETURN TO DUTY

SUMMARY

** Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014*

INTRODUCTION

Nerve agents are extremely toxic chemicals that were first developed in secrecy before and during World War II primarily for military use. Related substances are used in medicine, in pharmacology, and for other purposes, such as insecticides, but they lack the potency of the military agents. Much of the basic knowledge about the clinical effects of nerve agents comes from research performed in the decades immediately following World War II.

The military stockpiles of several major powers are known to include nerve agents, and the armamentaria of other countries are thought to contain them as well (see Chapter 4, The Chemical Warfare Threat and the Military Healthcare Provider). Because of the possibility of nerve agent use in future conflicts, military medical personnel should have some knowledge of these agents, their effects, and the proper therapy for treating casualties.

HISTORY

Possibly the earliest recorded use of a substance that works, like nerve agents, by inhibiting cholinesterase (ChE) is by native tribesmen of western Africa who used the Calabar bean as an “ordeal poison” in witchcraft trials.[1,2] An extract, “the elixir of the Calabar bean,” was later used medicinally,[3] and in 1864, the active principle was isolated by Jobst and Hesse and called physostigmine.[1] Vee and Leven independently isolated this same substance in 1865 and named it eserine,[1] hence its dual nomenclature.

The first organophosphorus ChE inhibitor was probably tetraethyl pyrophosphate (TEPP), synthesized by Wurtz and tasted (with no ill results) by Clermont in 1854.[4] During the next 80 years, chemists (such as Michaelis, Arbusow, and Nylen) made numerous advances in organophosphorus chemistry, but generally they did not realize the toxicity of the substances with which they were working.[4]

In the early 1930s, interest in both physostigmine-type (reversible) and organophosphorus-type (irreversible) ChE inhibitors increased. (The terms “reversible” and “irreversible” refer to the duration of binding of the compound with the enzyme ChE; see the Mechanism of Action section below.) The reversible type, most of which are carbamates, were developed for treating conditions such as intestinal atony, myasthenia gravis, and glaucoma; for example, treating gastric atony with neostigmine was described in 1931.[1]

Five organophosphorus compounds are generally regarded as nerve agents. They are commonly known as tabun (North Atlantic Treaty Organization [NATO] military designation, GA), sarin (GB), and soman (GD); and GF and VX (also NATO military designations; these compounds have no common names). The agents in the “G” series allegedly were given the code letter G because they originated in Germany; the “V” allegedly stands for venomous. GF is an old agent, previously discarded by the United States as being of no interest. During the Persian Gulf War, it was believed that Iraq might have GF in its arsenal; however, interest has waned again and GF has retreated to obscurity.

Lange and Krueger reported on the marked potency of organophosphorus compounds in 1932 after noting the effects of the vapors of dimethyl and diethyl phosphorofluoridate on themselves.[1,4] Shortly thereafter, the German company I. G. Farbenindustrie developed an interest in organophosphorus compounds as insecticides. On 23 December 1936, Gerhard Schrader, who headed the company’s research effort, synthesized what today is known as tabun.[5,6] Like Lange and Krueger, he noted the toxicity (miosis and discomfort) of the vapors of the substance in himself.

Over a year later, Schrader synthesized a second organophosphorus compound and named it sarin in honor of those who were instrumental in its development and production: Schrader, Ambros, Rudriger, and van der Linde.[5] Because the German Ministry of Defense required that substances passing certain toxicity tests be submitted to the government for further investigation, these compounds were examined for possible military use.

The potential of tabun and sarin as weapons was soon realized. A large production facility was built in Dyhernfurth and production of tabun was begun in 1942.[5,6] Sarin was also produced in Dyhernfurth and

possibly at another plant in Falkenhagen.[6] Late in World War II, Soviet troops captured the Dyhernfurth facility (then in Germany, now in Poland), dismantled it, and moved it, along with key personnel, to the former Soviet Union, where production of the agents commenced in 1946.[6]

About 10,000 to 30,000 tons of tabun and smaller quantities of sarin were produced and put into munitions by the Germans during World War II, but these weapons were never used.[6] Why they were not remains a matter of conjecture.

In the waning days of World War II, troops of the United States and the United Kingdom captured some of these munitions, which were being stored at Raubkammer, a German testing facility. The munitions, which contained an agent unknown to scientists in the United Kingdom and the United States, were taken to the two countries for examination. Over a single weekend, a small group of scientists at the U.K. Chemical Defence Establishment, working despite miosis caused by accidental exposure to the agent vapor, elucidated the pharmacology and toxicity of tabun and documented the antidotal activity of atropine.[7]

Thus, during the latter part of World War II, Germany possessed chemical weapons against which its foes had little protection and no antidotes. Use of these weapons probably would have been devastating and might have altered the outcome of that conflict. The Germans had tested nerve agents on inmates of concentration camps, not only to investigate their intoxicating effects but also to develop antidotes.[8] Many casualties, including some fatalities, were reported among the plant workers at Dyhernfurth; the medical staff there eventually developed antidotal compounds.[5] The Allies were unaware of these German experiments until the close of the war, months after the initial U.K. studies.[7]

Soman was synthesized in 1944 by Richard Kuhn of Germany, again in a search for insecticides.[6] Small amounts were produced, but development had not proceeded far by the end of the war. The nerve agent VX was first synthesized by an industrial concern in the United Kingdom in the early 1950s[6] and was given to the United States for military development.

Other potential nerve agents were synthesized by scientists in the United States and United Kingdom but were not developed for military use. For example, GF, which may have been first synthesized about 1949 by a chemist in another country in the search for other nerve agents, was studied in both the United States and the United Kingdom. It was then discarded for reasons that are not entirely clear. Possible explanations are that it was too expensive to manufacture or that there was no perceived need for an agent with its properties. The manufacturing process for GF is

apparently similar to that for GB. During the Persian Gulf War (1990–1991), Iraq was believed to have switched from the manufacture of GB to the manufacture of GF when the precursors of GB, but not those of GF, were embargoed.

The United States began to produce sarin in the early 1950s, and VX in the early 1960s, for potential military use; production continued for about a decade.[6] The U.S. munitions inventory today contains these two nerve agents in 30- to 45-year-old M55 rockets; land mines; 105-mm, 155-mm, and 8-in. projectiles; 500-lb and 750-lb bombs; wet-eye bombs (one of a family of “eye” bombs, which has liquid chemical [wet] contents); spray tanks; and bulk containers.[9] These munitions are stored at six depots within the continental United States (CONUS) and one outside the continent; the locations of these depots are public knowledge.[10] The six CONUS depots are near Tooelle, Utah; Umatilla, Oregon; Anniston, Alabama; Pine Bluff, Arkansas; Newport, Indiana; and Richmond, Kentucky; the seventh depot is on Johnston Island in the Pacific Ocean.

Sarin has also been used in terrorist attacks. In June 1994, members of a Japanese cult released sarin in an apartment complex in Matsumoto, Japan. Although there were almost 300 casualties, including 7 dead, this event was not well publicized. On 20 March 1995, sarin was released on Tokyo subways. More than 5,500 people sought medical care; about 4,000 had no effects from the agent but 12 casualties died. This incident required a major expenditure of medical resources to triage and care for the casualties. (Also see Chapter 1, Overview: Defense Against the Effects of Chemical and Biological Warfare Agents).

PHARMACOLOGY OF CHOLINESTERASE INHIBITORS

Cholinesterase in Tissue

Nerve agents are compounds that exert their biological effects by inhibition of the enzyme acetyl-cholinesterase (AChE), according to the current, widely accepted explanation. Some other compounds cause similar effects by the same mechanism and, in a broad sense, can also be considered nerve agents.

Acetylcholinesterase belongs to the class of enzymes called esterases, which catalyze the hydrolysis of esters. ChEs, the class of esterases to which AChE belongs, have high affinities for the esters of choline. Although there are several types of choline esters, acetylcholine (ACh), the neurotransmitter of the cholinergic portion of the nervous system, is most relevant to nerve agent activity.

The enzyme AChE, found at the receptor sites of tissue innervated by the cholinergic nervous system, hydrolyzes ACh very rapidly: it has one of

the highest enzyme turnover numbers (number of molecules of substrate that it turns over per unit time) known.[11] A similar enzyme having ACh as its preferred substrate is found in or on erythrocytes (red blood cells, RBCs) and is known as erythrocyte, or true, ChE (RBC-ChE). Butyrocholinesterase (BuChE, also known as serum or plasma cholinesterase, and as pseudocholinesterase), another enzyme of the ChE family, has butyrylcholine as its preferred substrate. Butyrylcholine is present in plasma or serum and in some tissues. BuChE and RBC-ChE are discussed in the Blood Cholinesterases section below.

Cholinesterase-Inhibiting Compounds

Most ChE-inhibiting compounds are either carbamates or organophosphorus compounds. Among the carbamates is physostigmine (eserine; elixir of the Calabar bean), which has been used in medicine for more than a century.[3] Neostigmine (Prostigmin, manufactured by ICN Pharmaceuticals, Costa Mesa, Calif.) was developed in the early 1930s for management of myasthenia gravis; ambenonium was developed later for this same purpose. Pyridostigmine bromide (Mestinon, manufactured by ICN Pharmaceuticals, Costa Mesa, Calif.) has been used for decades for the management of myasthenia gravis. The military of the United States and several other nations also field pyridostigmine bromide (manufactured by Phillips Duphar, Holland), known as PB or NAPP (nerve agent pyridostigmine pretreatment), as a pretreatment, or antidote-enhancing substance, to be used before exposure to certain nerve agents (see Chapter 6, Pretreatment for Nerve Agent Exposure). Today these carbamates are mainly used for treating glaucoma and myasthenia gravis. Other carbamates, such as Sevin (carbaryl, manufactured by Techne, St. Joseph, Mo.), are used as insecticides.

Most commonly used insecticides contain either a carbamate or an organophosphorus compound. The organophosphorus insecticide malathion has replaced parathion, which was first synthesized in the 1940s. The organophosphorus compound diisopropyl phosphorofluoridate (DFP) was synthesized before World War II and studied by Allied scientists before and during the war, but was rejected for use as a military agent. For a period of time, this compound was used topically for treatment of glaucoma but later was rejected as unsuitable because it was found to produce cataracts. It has been widely used in pharmacology as an investigational agent.

Mechanism of Action

Nerve agents inhibit ChE, which then cannot hydrolyze ACh. This

classic explanation of nerve agent poisoning holds that the intoxicating effects are due to the excess endogenous ACh. This explanation, however, may not account for all nerve agent effects.

Research suggests that other nerve agent actions may contribute to toxicity. For example, ChE inhibitors inhibit enzymes other than ChE; the effect of this inhibition of additional enzymes on nerve agent toxicity may be significant.[12] Concentrations of ChE inhibitors that are severalfold higher than lethal concentrations produce direct effects on receptor sites by blocking conductance through the ion channel or by acting as agonists at the channel complex.[13] While these findings offer hope that better means of therapy will be developed in the future, their relevance to clinical effects is not clear at this time.

A detailed discussion of the chemistry of ChE inhibition is beyond the scope of this chapter and can be found in most textbooks of pharmacology (eg, see Koelle[11]). The relevant aspects are summarized here.

The efferents of the human nervous system can be subdivided according to the neurotransmitter released. The adrenergic nervous system, for which the neurotransmitter is adrenaline (epinephrine) or, more correctly, noradrenaline (norepinephrine), comprises one large subsection. Other, less prominent efferent tracts have γ-aminobutyric acid (GABA), dopamine, or some other substance as the neurotransmitter. The cholinergic nervous system, a second major subdivision, has acetylcholine as the neurotransmitter. Acetylcholine is the neurotransmitter of the neurons to skeletal muscle, of the preganglionic autonomic nerves, and of the post-ganglionic parasympathetic nerves. Exogenous ACh causes stimulation of the muscles and other structures innervated by these fibers.

This portion of the cholinergic nervous system can be further subdivided into the muscarinic and nicotinic systems, because the structures that are innervated have receptors for the alkaloids muscarine (mAChR) and nicotine (nAChR), respectively, and can be stimulated by these compounds. Muscarinic sites are innervated by postganglionic parasympathetic fibers. These sites include glands (eg, those of the mouth and the respiratory and gastrointestinal systems), the musculature of the pulmonary and gastrointestinal systems, the efferent organs of the cranial nerves (including the heart via the vagus nerve), and other structures. Nicotinic sites are at the autonomic ganglia and skeletal muscles.

Fig. 5-1 is not shown due to copyright restrictions, please refer to the textbook.

Fig. 5-1. Diagram of neuromuscular conduction. **(a)** Nerve fiber with axon terminal in synaptic trough of muscle. **(b)** Close-up of axon terminal in trough, with synaptic vesicles indicated. **(c)** Acetylcholine synthesis from acetate and choline and storage of acetylcholine in synaptic vesicles. **(d)** Release of acetylcholine from synaptic vesicles after an action potential. **(e)** Acetylcholine stimulation of endplate at receptor for site. **(f)** Hydrolysis of acetylcholine by membrane-bound acetylcholinesterase.

The production of a response in an organ to a neuromediated impulse consists of several stages. First, the impulse travels down a nerve to the axonal terminal, or presynaptic area, creating an action potential. (This action potential consists of a change in the resting potential of the polarized nerve membrane.) At the prejunctional area, the action potential stimulates the release of the neurotransmitter ACh from storage in synaptic vesicles. The ACh diffuses across the synaptic cleft and combines with specialized areas—the receptor sites—on the postsynaptic membrane to produce a postsynaptic potential, which may be either a depolarization or a hyperpolarization of the membrane. The postsynaptic activity thus initiated is a contractile response in muscle or secretion in a gland. (Events in the central nervous system [CNS] are less clear.) Following each impulse, the neurotransmitter is destroyed to prevent further postsynaptic potentials (Figure 5-1).

In the cholinergic portion of the nervous system, ChE hydrolyzes the neurotransmitter ACh to terminate its activity at the receptor site (Figure 5-2). Acetylcholine attaches to two sites on the ChE enzyme: the choline moiety to the anionic site and the acetyl group to the esteratic site. The choline splits off, leaving the acetylated esteratic site, which then reacts very quickly with water to form acetic acid and regenerated, or reactivated, enzyme.

Fig. 5-2 is not shown due to copyright restrictions, please refer to the textbook.

If AChE were absent from the site, or if it were unable to function, ACh would accumulate and would continue to produce postsynaptic action potentials and activity in the organ. The nerve agents and other

ChE-inhibiting substances produce biological activity by disabling (or inhibiting) AChE, an action that leads to an accumulation of ACh. The biological activity, or toxicity, of ChE inhibitors is due to this excess endogenous ACh, which is not hydrolyzed.

Fig. 5-2. This schematic ribbon diagram shows the structure of *Torpedo californica* acetylcholinesterase. The diagram is color-coded; green: the 537-amino acid polypeptide of the enzyme monomer; pink: the 14 aromatic residues that line the deep aromatic gorge leading to the active site; and gold and blue: a model of the natural substrate for acetylcholinesterase, the neurotransmitter acetylcholine, docked in the active site.

The compounds in the two major categories of AChE inhibitors, carbamates and organophosphorus compounds, also attach to the ChE enzyme. There are some differences, however, between them and the natural substrate ACh. Carbamates attach to both the esteratic and the anionic sites. A moiety of the carbamate is immediately split off, leaving the enzyme carbamoylated at the esteratic site. Instead of hydrolysis occurring at this site within microseconds, as it does with the acetylated enzyme, hydrolysis does not occur for minutes to hours, and the enzyme remains inactive or inhibited for about 1 hour after reacting with physostigmine and 4 to 6 hours after reacting with pyridostigmine.

Most organophosphorus compounds combine with the ChE enzyme only at the esteratic site, and the stability of the bond (ie, the interval during which the organophosphorus compound remains attached) depends on the structure of the compound. Hydrolytic cleavage of the compound from the enzyme may occur in several hours if the alkyl groups of the organophosphorus compound are methyl or ethyl, but if the alkyl groups are larger, cleavage may not occur. Thus, the phosphorylated form of the enzyme may remain indefinitely; in this case, return of enzymatic activity occurs only with the synthesis of new enzyme.

Since most of these compounds attach to the esteratic site on AChE, a second binding compound cannot attach on that site if the site is already occupied by a molecule. Thus a previously administered ChE inhibitor will, in a manner of speaking, protect the enzyme from a second one.[14,15] This activity forms the pharmacological basis for administering a carbamate (pyridostigmine) before expected exposure to some nerve agents to provide partial protection (lasting 6–8 h) against the more permanently bound nerve agents. (This mode of protection is described in more detail in Chapter 6, Pretreatment for Nerve Agent Exposure). Because of the different lengths of time required for carbamates and organophosphorus compounds to be hydrolyzed from the enzyme, they are sometimes referred to, respectively, as reversible and irreversible inhibitors.

After inhibition by irreversibly bound inhibitors, recovery of the

enzymatic activity in the brain seems to occur more slowly than that in the blood ChE.[16,17] However, one individual severely exposed to sarin was alert and functioning reasonably well for several days while ChE activity in his blood was undetectable (Exhibit 5-1).[18] This case study and other data suggest that tissue function is restored at least partially when ChE activity is still quite low.

EXHIBIT 5-1
CASE REPORT: SARIN EXPOSURE OF A MAN IN FULL PROTECTIVE GEAR

This 52-year-old man [who worked at Edgewood Arsenal, Edgewood, Maryland] had been cleaning a sarincontaminated area and was wearing full protective gear, including a protective mask, which was later shown to have a crack in the voicemitter diaphragm, which should have been noted when he donned and tested the mask. After complaining of an increase in oral and nasal secretions and difficulty breathing, he left the area. Within minutes he was in marked respiratory distress and had copious secretions. He arrived at the emergency room 5-10 min after the first symptom.

On arrival, the patient was cyanotic and convulsing; his breathing was labored; muscular fasciculations, miosis, and marked salivation and rhinorrhea were evident. Because of the urgency of the situation, treatment was begun before he was examined more thoroughly.

Atropine sulfate, 2 mg i.v. and 2 mg i.m., was given immediately; then an intravenous infusion of pralidoxime chloride (2 gm in 150 mL of normal saline), was begun and oxygen was given through a face mask. An additional 2 mg of atropine (i.v.) was given several minutes later. In 2-3 min his respirations were less labored and the cyanosis had decreased. Blood pressure was 190/110mm Hg. The heart was regular at 130 beats per min.... Marked muscular fasciculations were still present, but bronchoconstriction and secretions had diminished.

Twenty minutes after admission, the first dose of pralidoxime chloride had been absorbed and another 2 gm was started. Because of the return of copious secretions more atropine (2 mg, i.v.) was given. About 30 min after admission he was awake, but

intermittently irrational. Pertinent findings on examination were muscular fasciculations, bilateral wheezes, S_4 cardiac gallop, and marked miosis. He became nauseated and vomited a small amount.

Over the next 20 min his respiration diminished in frequency and amplitude; 50 min after admission, he again vomited and then became more irrational, fought assistance (including the oxygen mask), and became cyanotic. More atropine (2 mg, i.m.) was given and a third dose of pralidoxime chloride (2 gm) was begun. At 60 min after admission he again became comatose and totally apneic. Although rhonchi and wheezes were present throughout his pulmonary fields, adequate aeration was established by assisted respiration and his color improved. A nasogastric tube was passed because of gastric distention and repeated vomiting. An additional dose of atropine sulfate (3 mg) was given slowly, intravenously, because of increasing bronchoconstriction.

After 50 min of assisted ventilation feeble respiratory efforts returned, but air exchange was poor and intermittent respiratory assistance was continued for an hour more (until 3 hr after admission). At 2.25 hr after admission (1.25 hr after atropine), bronchoconstriction increased, but diminished rapidly when additional atropine sulfate (1 mg, i.v.) was given.

At about 2.5 hr after admission his sensorium slowly began to clear, he began to breathe spontaneously with adequate aeration, and his color remained good, although there were several more episodes of vomiting. At 9 hr after admission he felt well enough to walk around the ward although he was very weak and areflexic.

During a restless night he complained of numerous muscular pains and vomited twice more. The following morning (18 hr after admission) he had small but reactive pupils, clear lung fields, no cardiac gallop, and reactive deep tendon reflexes.

Red blood cell cholinesterase (RBC-ChE), in blood drawn after part of the first dose of pralidoxime chloride had been absorbed, was 0.36 µ moles/mL/min (or less than 7% of the subsequent value). REC-ChE in a second sample drawn after pralidoxime administration was 5.59 µ moles/mL/min (within the normal range of 4.08- 8.06 by the method[1] used).

An electrocardiogram (EKG) taken an hour after admission showed sinus tachycardia and marked depression of the ST segment in all leads. A second EKG, taken 18 hr after admission, showed elevation of the ST segment in leads I, aVL, and V_{1-3} . At 24 hr after admission the ST segment recorded from the anterior chest leads was elevated, and the T wave in leads V_{4-6} was inverted. The following day, 42 hr after admission, the ST segment was elevated (leads I, aVL, and V_{2-6}) and the T wave was inverted (leads 1, II, aVL, and V_{2-6}). During this period the patient complained of generalized muscular pain and soreness, but even in retrospect did not describe a pattern typical of cardiac ischemia. The EKG pattern stabilized over the next few days. Then the ST segment became isoelectric, but the T-wave inversions persisted for the next 4 weeks.

Because of these EKG changes he was hospitalized elsewhere. During the first two to three days he was very labile emotionally and had one episode of hysterical voice loss. He also complained of minimal, migratory chest pains and a slight productive cough. From the fourth day onward the patient was asymptomatic, and he had an uneventful recovery. After two weeks of bed rest his physical activity was gradually increased, and by the time of his discharge two weeks later (four weeks post exposure), he was ambulatory and doing well. An EKG taken four months after exposure was entirely within normal limits.

At four months he was rehospitalized for complaints of easy fatigability, dyspnea on exertion, restlessness, and poorly localized pains in his chest and abdomen. No physical causes were found for these complaints, but he was noted to have a marked depression associated with anxiety, crying spells, and restlessness. At six months, a psychiatrist felt that the patient had a depressive reaction with a great deal of anxiety about the intactness of his body arising from worry over his cardiac status. The evaluation was hindered by only meager knowledge of the patient's pre-exposure personality. He received psychiatric assistance throughout these months, but was lost to follow up when he retired and moved from the area. Approximately a year later (18 months after his exposure) a casual inquiry revealed that he had died suddenly. The autopsy diagnosis was acute myocardial infarction, involving the posterior portions of both ventricles, the posterior part of the septum, and the anterolateral

portion of the left ventricle; and marked sclerosis of the coronary arteries, with reduction of the lumen of the left coronary artery to about 30% and complete occlusion of the right coronary artery.

Blood Cholinesterases

To review, there are two forms of ChE in the blood: BuChE, which is found in plasma or serum, and RBC-ChE, which is associated with erythrocytes. Neither enzyme is identical to the tissue enzyme with the corresponding substrate specificity (butyrylcholine and ACh, respectively). However, because blood can be withdrawn, the activities of each of these enzymes can be assayed by standard, relatively simple laboratory techniques, whereas tissue enzyme is unavailable for assay. The measurements obtained from the blood assay can be used as an approximation of tissue enzyme activity in the event of a known or possible exposure of an animal, such as man, to an AChE inhibitor.

Persons who are occupationally exposed to ChE-inhibiting substances are periodically monitored for asymptomatic exposure by assays of blood-ChE activity. Those at risk include crop sprayers and orchard workers who handle ChE-inhibiting insecticides, and chemical agent–depot workers or laboratory scientists who handle nerve agents. To be meaningful, such monitoring must include knowledge of physiological variation in the blood enzymes.

Individuals who work with or around nerve agents, such as laboratory investigators and depot or storage-yard personnel, have their RBC-ChE activity monitored periodically. Before the individuals begin work, two measures of RBC-ChE, drawn within 14 days but not within 24 hours of each other, are averaged as a “baseline.” At periodic intervals, the frequency of which depends on the individuals’ jobs, blood is drawn for measuring cholinesterase activity (for further discussion, see Chapter 17, Healthcare and the Chemical Surety Mission). If the activity is 75% or more of their baseline, no action is taken. If the activity is below 75% of their baseline, they are considered to have had an asymptomatic exposure and they are withdrawn from work. Investigations are undertaken to find how they were exposed. Although asymptomatic, they are not permitted to return to a work area around nerve agents until their RBC-ChE activity is higher than 80% of their baseline activity.[19] If an individual has symptoms from a possible nerve agent exposure or if an accident is known to have occurred in his area, his RBC-ChE activity is immediately measured and the criteria noted above, as well as signs and symptoms, are used for exclusion from and return to work. The values of 75% and 80% were

selected for several reasons, including (a) the normal variation of RBC-ChE in an individual with time (discussed below), (b) laboratory reproducibility in analysis of RBC-ChE activity, and (c) the lower tolerance to nerve agents with a low RBC-ChE as demonstrated in animals (discussed below). This topic is also discussed in Chapter 14, Pesticides, in *Occupational Health: The Soldier and the Industrial Base*, another volume in the *Textbook of Military Medicine* series.

Butyrocholinesterase

The enzyme BuChE is present in blood and throughout tissue. Its physiological role in man is unclear[20]; however, it may be important in canine tracheal smooth muscle,[21] the canine ventricular conducting system,[22] and rat atria.[23]

BuChE is synthesized in the liver and has a replacement time of about 50 days. Its activity is decreased in parenchymal liver disease, acute infections, malnutrition, and chronic debilitating diseases, and is increased in the nephrotic syndrome.[20] This enzyme has no known physiological function in blood, but may assist in hydrolyzing certain cho-line esters.

Persons who have a prolonged paralysis caused by succinylcholine, a muscle relaxant, usually are found to have low BuChE activity.[20] The structure of BuChE is determined by two autosomal alleles. The frequency of occurrence of the gene responsible for abnormal ChE is about 1 in 2,000 to 1 in 4,000 people. Thus, about 96% of the population have the usual phenotype, close to 4% have the heterozygous phenotype, and about 0.03% have the homozygous abnormal phenotype.[20] In addition to having low BuChE activity, which results from this genetic abnormality, in the usual assay, persons with abnormal ChE have low dibucaine numbers (the enzyme activity in an assay in which dibucaine is used as the ChE substrate). The mean dibucaine number for the normal phenotype is about 79%, that for the heterozygote is 62%, and that for the homozygous abnormal phenotype is 16%.[24]

The relationship of BuChE activity and succinylcholine can be somewhat different, however. One author[25] reports on an individual whose BuChE activity was 3-fold higher than normal. His dibucaine number was normal, and he was found to be relatively resistant to succinylcholine. His sister and daughter also had high BuChE activities. The author of this report suggests that this abnormality is autosomal dominant and that it represents another genetic abnormality of BuChE.

Erythrocyte Cholinesterase

RBC-ChE is synthesized with the erythrocyte, which has an average life of 120 days. The activity of this enzyme is decreased in certain diseases involving erythrocytes (such as pernicious anemia) and is increased during periods of active reticulocytosis (such as recovery from pernicious anemia) because reticulocytes have higher ChE activity than do mature cells. No other disease states are known to affect RBC-ChE activity,[20] but one report[26] describes three members of one family who had decreased RBC-ChE activity, suggesting that differences in this enzyme are genetic.

The physiological role of the enzyme in (or on the stroma of) the erythrocyte is unknown. Recovery of RBC-ChE activity after irreversible inhibition takes place only with the synthesis of new erythrocytes, or at a rate of approximately 1% per day.

Variation in Cholinesterase Activities

Butyrocholinesterase. In longitudinal studies[27,28] lasting 3 to 250 weeks, the coefficient of variation (standard deviation divided by the mean) for an individual's BuChE activity ranged from 5% to 11.8% in men and women. Of the ranges (range is defined as the difference between the highest and lowest activities divided by the mean) for individuals in the study, the lowest was 24% and the highest was 50% over 1 year.[28]

BuChE activity does not vary with age in women[29,30] until the age of 60 years, when higher BuChE activities are seen.[30] BuChE activities in men have been reported in some studies to increase with age and in other studies to decrease with age.[20] In matched age groups, BuChE activity was higher in men than in women,[20,30] and higher in women not taking oral contraceptives than in those taking them.[30–32]

Erythrocyte Cholinesterase. RBC-ChE activity is more stable than the activity of the BuChE.[28,33,34] In a study[28] that lasted 1 year, the coefficients of variation were 2.1% to 3.5% in men and 3.1% to 4.1% in women, with ranges of 7.9% to 11.4% in men and 12.0% to 15.9% in women. This variation was less than that observed for the hematocrits of these individuals.

It is unclear whether age affects RBC-ChE activity. In one study,[29] RBC-ChE activity was unchanged with age, while in another,[30] enzyme activity increased with age from the third to the sixth decades in men, with a less marked increase through the fifth decade in women.

Inhibition of Blood Cholinesterases

Some ChE-inhibiting substances inhibit BuChE preferentially, and some inhibit RBC-ChE preferentially. Large amounts of ChE inhibitors will completely inhibit both enzymes.

The blood enzymes appear to act as buffers for the enzymes in the tissue. There is little inhibition of tissue enzyme until much of the blood enzyme is inhibited. The RBC-ChE appears to be more important than the plasma enzyme in this regard. In two studies,[35,36] a small dose of DFP in humans inhibited about 90% of the plasma enzyme activity but only 15% to 20% of RBC-ChE activity. Symptoms correlated with depression of RBC-ChE, but not with depression of BuChE (see the Central Nervous System and Behavior section below). In humans, some pesticides, such as parathion,[37–39] systox,[37] and malathion,[20] also preferentially inhibit the plasma enzyme, while others, such as dimefox[39] and mevinphos,[40] initially bind with the RBC enzyme. In animals, there appears to be a species difference, inasmuch as parathion preferentially inhibits RBC-ChE in rats and the plasma enzyme in dogs.[20]

The nerve agent VX preferentially inhibits RBC-ChE; in two studies,[41,42] a small amount caused a 70% or greater decrease in the activity of this enzyme, whereas the activity of BuChE was inhibited by no more than 20%. Sarin also preferentially inhibits the RBC-ChE; 80% to 100% inhibition of RBC-ChE activity was observed in two studies,[35,43] while BuChE was inhibited by 30% to 50%. Therefore, estimation of the RBC-ChE activity provides a better indicator of acute nerve agent exposure than does estimation of the plasma enzyme activity. When the blood enzymes have been irreversibly inhibited, recovery of ChE activity depends on production of new plasma enzymes or production of new erythrocytes. Hence, complete recovery of BuChE activity that has been totally inhibited by sarin will occur in about 50 days, and recovery of the RBC-ChE, in 120 days (about 1% per day).[44] In humans, after inhibition by VX, the RBC-ChE activity seems to recover spontaneously at the rate of about 0.5% to 1% per hour for a few days, but complete recovery depends on erythrocyte production.[41,42]

Time Course of Inhibition. After very large amounts of nerve agent (multiple LD_{50} s [ie, multiples of the dose that is lethal to 50% of the exposed population]) are placed on the skin, signs and symptoms occur within minutes, and inhibition of blood ChE activities occurs equally quickly. However, with smaller amounts of agent, the onset is not so rapid. In studies in which small amounts of VX were applied on the skin of humans, the onset of symptoms and the maximal inhibition of blood ChE activity were found to occur many hours after application of the agent. In

one study[42] in which equipotent amounts of VX were applied to the skin in different regions, the time to maximal inhibition was 5 hours for the head and neck, 7 hours for the extremities, and 10 hours for the torso. In a similar study,[45] the average time from placing VX on the skin to the onset of nausea and vomiting and maximal drop of blood ChE activity was 10.8 hours.

TABLE 5-1
RELATION OF EFFECTS OF NERVE AGENT EXPOSURE TO ERYTHROCYTE CHOLINESTERASE ACTIVITY

Effect	Patients Affected	Range of RBC-ChE Activity (% of Baseline*)
Miosis alone (bilateral)	22	0-100
Miosis alone (unilateral)	7	3-100
Miosis and tight chest	12	28-100
Miosis and rhinorrhea	9	5-90
Miosis, rhinorrhea, and tight chest	9	20-92
Rhinorrhea and tight chest	3	89-90

*Cholinesterase activity before nerve agent exposure RBC-ChE: erythrocyte cholinesterase

In a third study,[46] VX was applied to the cheek or forearm at environmental temperatures ranging from 0°F to 124°F, and 3 hours later the subjects were decontaminated and taken to a recovery area (about 80°F). In all temperature groups, the RBC-ChE activity continued to decline after decontamination, and maximal inhibition occurred at 5.6 hours after exposure at 124°F, 8.5 hours after exposure at 68°F, 10.4 hours after exposure at 36°F, and 12.2 hours after exposure at 0°F. At the two lowest temperatures, the rates of agent penetration and of decline in RBC-ChE activity increased after the subjects were taken from the cold environment and decontaminated. These results suggest that agent absorption through the skin is more rapid and complete at higher temperatures, and that even after thorough decontamination, a considerable amount of agent remains in the skin to be absorbed.

Inhalation of nerve agent vapor inhibits blood ChE activity and produces signs and symptoms of exposure more rapidly than does dermal contact. Although there is no correlation between ChE activity and clinical effects after exposure to small amounts of vapor, both clinical effects and ChE inhibition occur within minutes. In one study,[41] both the maximal inhibition of RBC-ChE activity and the appearance of signs and symptoms occurred about 1 hour after intravenous administration of small amounts of VX. After ingestion of VX, the interval was 2 to 3 hours.

Relation to Signs and Symptoms. The local signs and symptoms in the eye, nose, and airways caused by small amounts of vapor are due to the direct effect of the vapor on the organ; no correlation between the severity of these effects and the blood ChE activity seems to exist. These early experimental data[47–49] indicating the lack of correlation were supported by a retrospective analysis of 62 individuals seen at the Edgewood Arsenal Toxic Exposure Aid Station between 1948 and 1972. Although all individuals had physical signs or definite symptoms (or both) of nerve agent vapor exposure, there was no correlation between local effects from vapor exposure and RBC-ChE activity (Table 5-1).[50]

Minimal systemic effects, such as vomiting, occur in half the population when the RBC-ChE is inhibited to 25% of its control activity.[41,42] In a study[42] in which VX was placed on the skin, no vomiting occurred in 30 subjects whose minimal RBC-ChE activities were 40% of control or higher. Vomiting occurred in 9 (43%) of 21 subjects whose minimal RBC-ChE activities were 30% to 39% of control, in 10 (71%) of 14 subjects whose minimal enzyme activities were 20% to 29% of control, and in 3 (60%) of 5 subjects whose minimal RBC-ChE activities were 0% to 19% of control. In other instances, patients had an RBC-ChE activity of 0% without the expected symptoms; this inhibition was acutely induced (personal observation).

TABLE 5-2
RELATION OF CHOLINESTERASE ACTIVITY TO VOMITING AFTER EXPOSURE TO VX

Min. RBC-ChE (% of Baseline*)	Patients	Patients Vomiting	Percentage Vomiting
>50	166	1	0.6
40-49	24	2	8.3
30-39	27	9	33.3
20-29	42	19	45.2
<20	24	16	66.7

*Cholinesterase activity before nerve agent exposure RBC-ChE: erythrocyte cholinesterase

Table 5-2 categorizes data from 283 individuals (data are from published sources[41,42] and unpublished research) who received VX by various routes; the numbers of subjects, the activity ranges of RBC-ChE, and the numbers and percentages of those who vomited are shown. The degree of inhibition needed to cause vomiting in these 283 people corresponds to that found in experimental data from other sources, which indicate that "to exert significant actions in vivo, an anti-ChE must inhibit from 50% to 90% of the enzyme present."[11(p446)]

Nerve Agents

Molecular models of the nerve agents tabun, sarin, soman, and VX are shown in Figure 5-3. Table 5-3 summarizes the chemical, physical, environmental and biological properties of these compounds.

Nerve agents differ from commonly used ChE inhibitors primarily because they are more toxic (ie, a smaller amount is needed to cause an effect on an organism). For example, an in vitro study[43] with ChE from human erythrocytes, brain, and muscle showed that sarin had about 10-fold more inhibitory activity than TEPP, 30-fold more than neostigmine, 100-fold more than DFP, and 1,000-fold more than parathion.

The vapor or aerosol exposure (the product of concentration [C] and time [*t*]) needed to cause death in 50% of the exposed population is known as the LCt_{50} (Exhibit 5-2); the estimated LCt_{50}s for humans for these four agents are as follows:

- for tabun vapor, 400 mg•min/m^3,
- for sarin vapor, 100 mg•min/m^3,
- for soman vapor, 50 mg•min/m^3, and
- for VX vapor, 10 mg•min/m^3.

In comparison, the estimated LCt_{50} for hydrogen cyanide is 2,500 to 5,000 mg•min/m^3.

The estimated percutaneous LD_{50}s for the four compounds are as follows:

- for tabun, 1,000 mg,
- for sarin, 1,700 mg,
- for soman, 350 mg, and
- for VX, 6 to 10 mg.

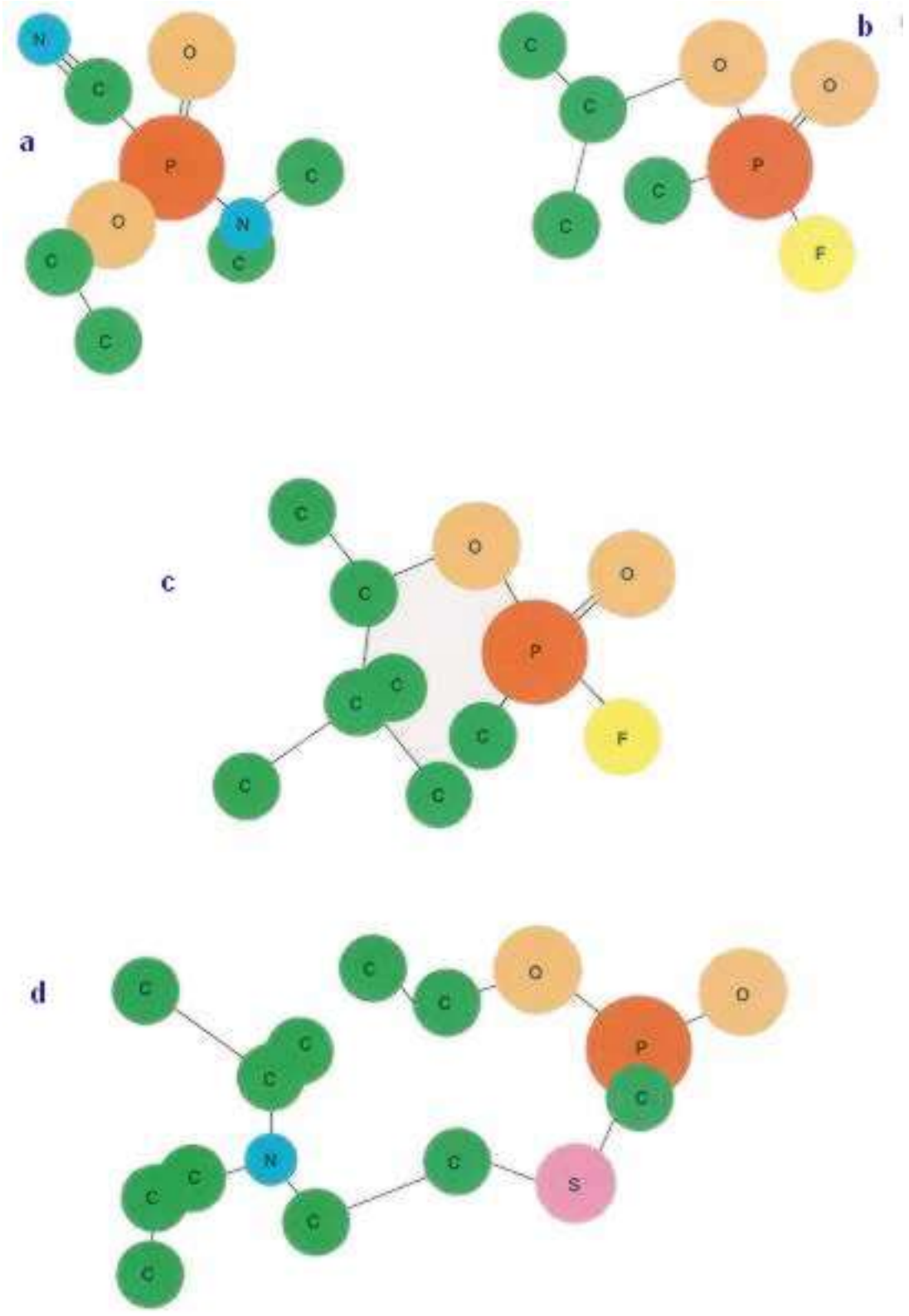


Fig. 5-3. Molecular models of **(a)** tabun (GA), **(b)** sarin (GB), **(c)** soman (GD), and **(d)** VX. Molecular models: Courtesy of Offie E. Clark, US Army Medical Research Institute of Chemical Defense, Aberdeen, Md.

TABLE 5-3
CHEMICAL, PHYSICAL, ENVIRONMENTAL, AND BIOLOGICAL PROPERTIES OF NERVE AGENTS

Properties	Tabun (GA)	Sarin (GB)	Soman (GD)	VX
Chemical and Physical				
Boiling Point	230° C	158°C	198°C	298°C
Vapor Pressure	0.037mm Hg at 20°C	2.1 mm Hg at 20°C	0.40 mm Hg at 25°C	0.0007 mm Hg at 20°C
Density:				
Vapor (compared to air)	5.6	4.86	6.3	9.2
Liquid	1.08 g/mL at 25°C	1.10 g/mL at 20°C	1.02 g/mL at 25°C	1.008 g/mL at 20°C
Volatility	610 mg/m^3 at 25°C	22,000 mg/m^3 at 25°C	3,900 mg/m^3 at 25°C	10.5 mg/m^3 at 25°C
Appearance	Colorless to brown liquid	Colorless liquid	colorless liquid	Colorless to straw-colored liquid
Odor	Fairly fruity	No odor	Fruity; oil of camphor	Odorless
Solubility:				
In Water	9.8 g/100 g at 25°C	Miscible	2.1 g/100 g at 20°C	Miscible <9.4°C
In Other Solvents	Soluble in most organic solvents	Soluble in all solvents	Soluble in some solvents	Soluble in all solvents
Environmental and Biological				
Detectability:				

Vapor	M8A1, M256A1, CAM, ICAD	M8A1, M256A1, CAM, ICAD	M8A1, M256A1, CAM, ICAD	M8A1, M256A1, CAM, ICAD
Liquid	M8, M9 Paper	M8, M9 Paper	M8, M9 Paper	M8, M9 Paper
Persistency:				
In Soil	Half-life 1-1.5 d	2-24 h at 5°C-25°C	Relatively persistent	2-6 d
On Materiel	Unknown	Unknown	Unknown	Persistent
Decontamination of skin	M258A1, diluted hypochlorite, soap and water, M291 kit	M258A1, diluted hypochlorite, soap and water, M291 kit	M258A1, diluted hypochlorite, soap and water, M291 kit	M258A1, diluted hypochlorite, soap and water, M291 kit
Biologically Effective Amount:				
Vapor	LCt_{50}: 400 mg • min/m^3	LCt_{50}: 100 mg • min/m^3	LCt_{50}: 50 mg • min/m^3	LCt_{50}: 10 mg • min/m^3
Liquid	LD_{50} (skin): 1.0 g/70-kg man	LD_{50} (skin): 1.7 g/70-kg man	LD_{50} (skin): 350 mg/70-kg man	LD_{50} (skin): 10 mg/70-kg man

CAM: chemical agent monitor; ICAD: individual chemical agent detector; LCt_{50}: vapor or aerosol exposure necessary to cause death in 50% of the population exposed; LD_{50} : dose necessary to cause death in 50% of the population with skin exposure; M8A1: chemical alarm system; M256A1: detection card; M258A1: self-decontamination kit; M291: decontamination kit; M8 and M9: chemical detection papers

VX has a much lower LD_{50} because it is much less volatile and remains intact on the skin, whereas the other nerve agents will evaporate unless covered (eg, by clothing).[6,8] Different sources provide different estimates for these LD_{50} and LCt_{50} values; however, those noted above seem to be the most commonly accepted.

The four nerve agents are liquid at moderate temperatures; thus, the term “nerve gas” is a misnomer. In their pure state, they are clear, colorless, and, at least in dilute solutions of distilled water, tasteless. Tabun has been reported to have a faint, slightly fruity odor, and soman, to have an ill-defined odor; sarin and VX are apparently odorless.

The G agents are volatile; VX has very low volatility. Sarin, the most volatile, is somewhat less volatile than water; tabun and soman are less volatile than sarin. The G agents present a definite vapor hazard; VX is much less likely to unless the ambient temperature is high.

EXHIBIT 5-2
DEFINITIONS OF C*t*, LCt_{50} AND LD_{50}

For comparative purposes, the terms *Ct* and L*Ct* 50 are often used to express the dose of a vapor or aerosol. However, the terms do not describe inhaled doses; they actually describe the amount of compound to which an organism is *exposed*.

• The term *Ct* is used to describe an estimate of dose. C represents the concentration of the substance (as vapor or aerosol) in air (usually expressed as mg/m 3) and *t* represents time (usually expressed in minutes).

• The *Ct* value is the product of the concentration (C) to which an organism is exposed multiplied by the time(t) during which it remains exposed to that concentration. *Ct* does not express the amount retained within an organism; thus, it is not an inhalational dose.

• Since *Ct* is a product of C • t, a particular value can be produced by inversely varying the values of C and t. The *Ct* to produce a given biological effect is usually constant over an interval of minutes to several hours (Haber’s Law). Thus, an effect that is produced by an exposure to 0.05 mg/m 3 for 100 minutes is also produced by an exposure to 5 mg/m 3 for 1 minute (*Ct* = 5 mg•min/m 3 in both cases). This generalization usually is not valid for very short or very long times, however. The organism may hold its breath for several seconds and not actually inhale the vapor; over many hours, some detoxification may occur in the organism.

• The term L*Ct* 50 is often used to denote the vapor or aerosol exposure (*C*t) necessary to cause death in 50% of the population exposed (L denotes lethal, and 50 denotes 50% of the population). In the same manner, the term LD 50 is used to denote the dose that is lethal for 50% of the population exposed by other routes of administration.

EXPOSURE ROUTES

Inhalational Exposure to Vapor

The effects produced by nerve agent vapor begin in seconds to minutes after the onset of exposure, depending on the concentration of vapor. These effects usually reach maximal severity within minutes after the individual is removed from or protected from the vapor or may continue to worsen if the exposure continues. There is no delay in onset as there is after liquid exposure.

TABLE 5-4
EFFECTS OF EXPOSURE TO NERVE AGENT VAPOR

Amount of Exposure	Effects*
Small (Local effects)	Miosis; rhinorrhea; slight bronchoconstriction; secretions (slight dyspnea)
Moderate (Local effects)	Miosis; rhinorrhea; slight bronchoconstriction; secretions (moderate to marked dyspnea)
Large	Same as for moderate exposure, plus: loss of consciousness; convulsions (seizures); generalized fasciculations; flaccid paralysis; apnea; involuntary micturition/defecation possible with seizures

*Onset of effects occurs within seconds to several minutes after exposure onset

At low C*t*s, the eyes, nose, airways, or a combination of these organs are usually affected. The eyes and nose are the most sensitive organs; the eyes may be affected equally or unequally. There may be some degree of miosis (with or without associated conjunctival injection and pain) with or without rhinorrhea, or there may be rhinorrhea without eye involvement

(Table 5-4).

As exposure increases slightly, the triad of eye, nose, and lung involvement is usually seen. The casualty may or may not notice dim vision and may complain of “tightness in the chest.” “Tightness in the chest” may occur in the absence of physical findings. At higher exposures, the effects in these organs intensify. Marked miosis, copious secretions from the nose and mouth, and signs of moderate-to-severe impairment of ventilation are seen. The casualty will complain of mild-to-severe dyspnea, may be gasping for air, and will have obvious secretions.

In severe exposures, the casualty may not have time to report the initial effects before losing consciousness, and may not remember them on awakening. One severely exposed individual later recalled that he noticed an increase in secretions and difficulty in breathing, and another said he felt giddy and faint before losing consciousness. In both instances, the casualties were unconscious within less than a minute after exposure to agent vapor. When reached (within minutes) by rescuers, both were unconscious and exhibited convulsive jerking motions of the limbs; copious secretions from the mouth and nose; very labored, irregular, and gasping breathing; generalized muscular fasciculations; and miosis. One developed flaccid paralysis and apnea a minute or two later. The other received immediate, vigorous treatment, and his condition did not progress (personal observation).

TABLE 5-5
EFFECTS OF DERMAL EXPOSURE TO LIQUID NERVE AGENTS

Level of Exposure	Effects
Mild	
Effects may be precipitant in onset after an asymptomatic interval of up to 18 h	Increased sweating at the site Muscular fasciculations at site
Moderate	
Effects may be precipitant in onset after an asyptomatic interval of up to 18 h	Same as for mild exposure, plus: Nausea Diarrhea Generalized weakness

Severe	
Effects may be precipitant in onset after a 2-30 min asyptomatic interval	Same as for moderate exposure, plus: Loss of consciousness Convulsions (seizures) Generalized fasciculations Flaccid paralysis Apnea Generalized secretions Involuntary micturition/defecation possible with seizures

Dermal Exposure to Liquid

The early effects of a drop of nerve agent on the skin and the time of onset of these effects depend on the amount of nerve agent and several other factors, such as the site on the body, the temperature, and the humidity. After a delay during which the individual is asymptomatic, localized sweating occurs at the site of the droplet; less commonly, there are localized fasciculations of the underlying muscle (Table 5-5). Unless the amount of the nerve agent is in the lethal range, the next effects (or perhaps the first effects, if the sweating and fasciculations do not occur or are not noticed) are gastrointestinal: nausea, vomiting, diarrhea, or a combination of these symptoms. The casualty may notice generalized sweating and complain of tiredness or otherwise feeling ill. There may be a period of many hours between exposure and the appearance of symptoms and signs. These signs and symptoms might occur even if the casualty has been decontaminated.[46]

After large exposures, the time to onset of effects may be much shorter than for smaller exposures and decreases as the amount of agent increases. For instance, two individuals were decontaminated within minutes of exposure to a drop of nerve agent. There was a 15- to 20-minute, asymptomatic interval before the precipitant onset of effects: collapse, loss of consciousness, convulsive muscular jerks, fasciculations, respiratory embarrassment, and copious secretions. Within several minutes, flaccid paralysis and apnea occurred in both (personal observation).

EFFECTS ON ORGANS AND ORGAN SYSTEMS

Most of the information on the effects of nerve agents on organ systems in humans is derived from studies done in the post–World War II period, from reports of people exposed to pesticides, or from clinical evaluations of accidental exposures of people who worked in nerve agent–research laboratories, manufacturing facilities, or storage areas or depots (Table 5-6). Some organ systems have been studied more intensively than others; for some organ systems there are few human data. For example, for the musculoskeletal system, there is a plethora of data from animal studies and studies in isolated neuromuscular preparations, but study results are difficult to apply to a human clinical situation.

The Eye

Nerve agents in the eye may cause miosis, conjunctival injection, pain in or around the eye, and dim or blurred vision (or both). Reflex nausea and vomiting may accompany eye exposure. These effects are usually local, occurring when the eye is in direct contact with nerve agent vapor, aerosol, or liquid, but exposure by other routes (such as on the skin) can also affect the eyes. Because eyes often react late in the course of intoxication in the latter case (exposure on the skin), they cannot be relied on as an early indication of exposure.

Systemic (such as skin or peroral) exposure to a nerve agent might be large enough to produce moderate symptoms (nausea, vomiting) without miosis. In studies[41,42,45] in which VX was placed on the skin, administered intravenously, or given orally, a significant number of subjects experienced nausea, vomiting, sweating, or weakness, but none had miosis. In 47 patients with parathion poisoning, all of the 14 severe cases had miosis, whereas 6 of 11 patients with moderate poisoning and only 5 of 22 patients with mild effects had miosis.[51] On the other hand, a vapor or aerosol exposure might cause miosis without other signs or symptoms and an exposure in one eye will cause miosis in that eye (a local effect because of a mask leak in one eyepiece or similar causes) without affecting the other eye.

If the eye exposure is not associated with inhalation of the nerve agent, there is no good correlation between severity of the miosis and inhibition of RBC-ChE activity. The latter may be relatively normal or may be inhibited by as much as 100% (see Table 5-1), so the severity of the miosis cannot be used as an index of the amount of systemic absorption of agent or amount of exposure. On the other hand, an early study[52] demonstrated a relationship between the C*t* of sarin and pupil size at the time of maximal miosis, and the investigator suggested that the pupil size might be used as an index of the amount of exposure.

Unilateral miosis is sometimes seen in workers handling nerve agents or insecticides and usually occurs because of a small leak in the eyepiece of the protective mask. Again, the RBC-ChE may or may not be inhibited (see Table 5-1). The unilateral miosis has no prognostic medical significance. However, there may be problems with judging distances (that is, depth perception). This impairment may cause difficulty in activities such as driving a car or piloting an airplane, which require stereo-visual coordination (the Pulfrich stereo effect).[20]

TABLE 5-6
EFFECTS OF NERVE AGENTS IN HUMANS

Organ or System	Effect
Eye	Miosis (unilateral or bilateral), conjunctival injection; pain in or around the eye; complaints of dim or blurred vision
Nose	Rhinorrhea
Mouth	Salivation
Pulmonary tract	Bronchoconstriction and secretions, cough; complaints of tight chest, shortness of breath; on exam: wheezing, rales, rhonchi
Gastrointestional tract	Increase in secretions and motility; nausea, vomiting, diarrhea; complaints of abdominal cramps, pain
Skin and sweat glands	Sweating
Muscular	Fasciculations ("rippling"), local or generalized; twitching of muscle groups, flaccid paralysis; complaints of twitching, weakness
Cardiovascular	Decrease or increase in heart rate; usually increase in blood pressure

Central nervous system	Acute effects of severe exposure: loss of consciousness, convulsion (or seizures after muscular paralysis), depression of respiratory center to produce apnea Acute effects of mild or moderate exposure or lingering effects (days to weeks) of any exposure: forgetfulness, irritability, impaired judgement, decreased comprehension, a feeling of tenseness or uneasiness, depression, insomnia, nightmares, difficulties with expression

The onset of miosis may be within seconds to minutes of the start of exposure; if the concentration of agent vapor or aerosol is quite low, maximal miosis may not occur until an hour or longer following exposure. The duration varies according to the amount of agent. The pupils may regain their ability to react to normal levels of indoor lighting within several days after exposure, but their ability to dilate maximally in total darkness may not return for as long as 9 weeks (Figure 5-4 and Exhibit 5-3). [18,53]

The effects of nerve agents on vision have been studied for decades. Characteristically, an unprotected individual exposed to nerve agent will have the signs discussed above and may complain of dim vision, blurred vision, or both.

Light Reduction

Dim vision is generally believed to be related to the decrease in the amount of light reaching the retina because of miosis. In a study[54] in which miosis was induced in one eye by instillation of sarin, the decrease in visual sensitivity correlated with the reduction in the area of pupillary aperture. Fifty-three subjects accidentally exposed to G agents reported improvements in dim vision before the miosis improved, which suggests that factors other than a small pupil are responsible for the high light threshold.[55] In another study,[56] however, no change in visual threshold was measured after miosis was induced by instillation of sarin onto the eye; the light threshold increased after systemic administration of sarin vapor with the eyes protected, so that no miosis occurred. The threshold was reduced to normal following systemic administration of atropine sulfate (which enters the CNS), but not after administration of atropine methylnitrate (which does not enter the CNS).[57] The authors suggested that the dimness of vision was due to neural mechanisms in the retina or

elsewhere in the CNS.

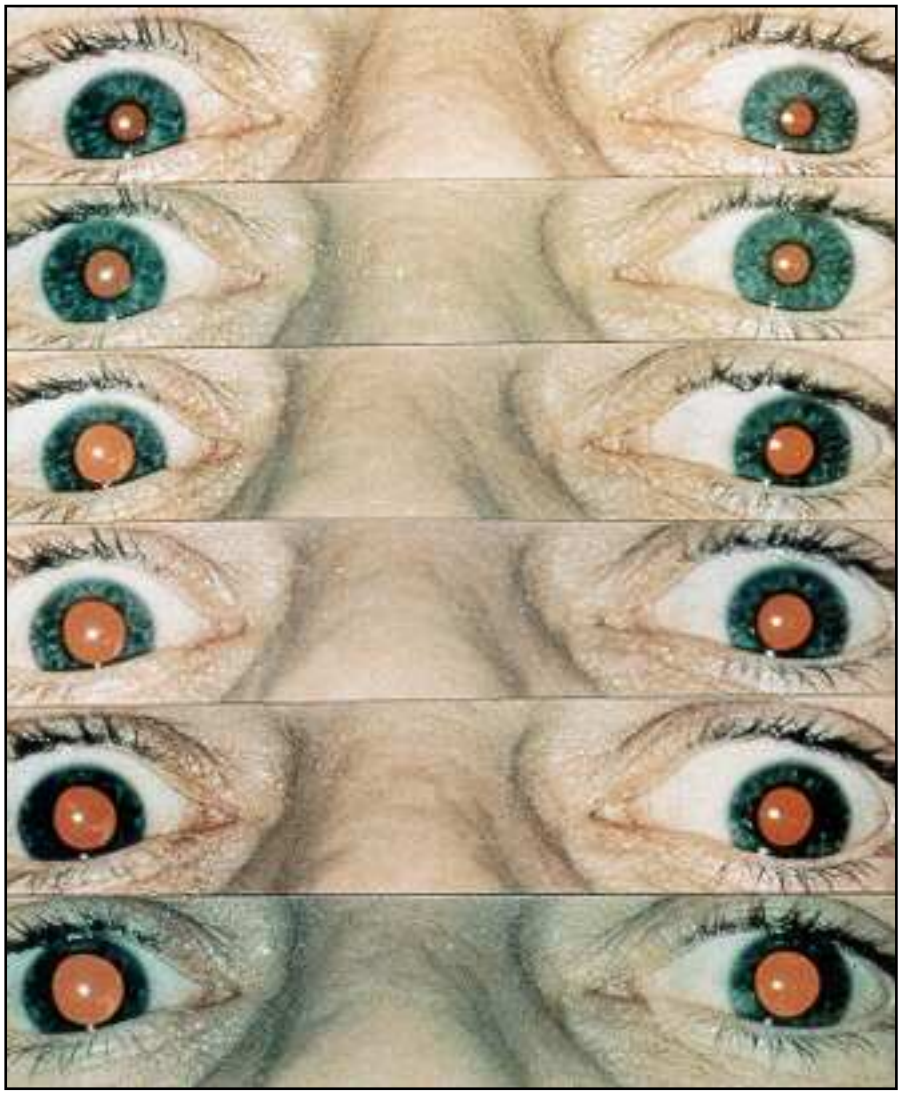


Fig. 5-4. This man was accidentally exposed to an unknown amount of nerve agent vapor. The series of photographs shows his eyes gradually recovering their ability to dilate. All photographs were taken with an electronic flash (which is too fast for the pupil to react) after the subject had been sitting in a totally dark room for 2 minutes. These photographs were taken (from top to bottom) at 3, 6, 13, 20, 41, and 62 days after the exposure. Subsequent photographs indicate that the eyes did not respond fully to

darkness for 9 weeks; maximal dilation was reached on day 62 after the exposure.

Although the dim vision reported by persons exposed to nerve agent vapor is generally ascribed to miosis, the above accounts suggest that more-central neural mechanisms may have equal or greater importance. In the case of the carbamate physostigmine, an increase in light sensitivity (a decreased threshold) after intramuscular administration of the drug has been reported.[58] However, carbamates may differ from nerve agents in their effects on vision. Regardless of its cause, reduction in visual sensitivity impairs those who depend on vision in dim light: individuals who watch a tracking screen, monitor visual displays from a computer, or drive a tank in the evening or at night. As a practical matter, anyone whose vision has been affected by exposure to a nerve agent should not be allowed to drive in dim light or in darkness.

Visual Acuity

Persons exposed to nerve agents sometimes complain of blurred as well as dim vision. In one study,[59] visual acuity was examined in six subjects before and after exposure to sarin vapor at a Ct of 15 mg•min/m^3. Near visual acuity was not changed in any of the six after exposure and was worsened after an anticholinergic drug (cyclopentolate) was instilled in the eyes. Far visual acuity was unchanged after sarin exposure in five of the six subjects and was improved in the sixth, who nonetheless complained that distant vision was blurred after sarin. Two presbyopic workers who were accidentally exposed to sarin had improved visual acuity for days after exposure. As the effects of the agent decreased, their vision returned to its previous state; in each case, this took about 35 days.[53] The author suggested, as others have previously, that miosis accounted for the improvement in visual acuity (the pinhole effect).

EXHIBIT 5-3
CASE REPORT: EXPOSURE OF THREE MEN TO SARIN

Three men [who worked at Edgewood Arsenal, Edgewood, Maryland], ages 27, 50, and 52 years, were brought to the emergency room because of sudden onset of rhinorrhea and slight respiratory discomfort. At the onset of symptoms they were working in a large room in which some containers of sarin were stored. Although there were other workers in the room, the three patients were together at one end where a leak was later found in one of the containers.

On examination all three patients had essentially the same signs and symptoms: very mild respiratory distress, marked miosis and slight eye pain, rhinorrhea, a moderate increase in salivation, and scattered wheezes and rhonchi throughout all lung fields. No other abnormal findings were noted.

All three patients reported that their respiratory distress had decreased since its onset about 20 min before they arrived at the emergency room. The men were kept under observation for the next 6 hr, but no therapy was administered. They continued to improve and at the time of discharge from the ward they were asymptomatic except for a slight irritation in the eyes and decreased vision in dim light.

The patients were seen the next day and at frequent intervals thereafter for a period of four months. Each time they were seen, their [blood cholinesterase activities (both erythrocyte cholinesterase and butyrylcholine esterase)] were measured …and photographs were taken of their eyes [see Figure 5-4]. The first photographs were taken the day of the exposure, but the patients were not dark adapted. On each visit thereafter a photograph was taken by electronic flash after the man had been in a completely dark room for 2 min. ... About 60- 70% of the lost ability to dark adapt returned in two weeks, but complete recovery took two months.

Eye Pain

Eye pain may accompany miosis, but the reported incidence varies. A sharp pain in the eyeball or an aching pain in or around the eyeball is common. A mild or even severe headache (unilateral if the miosis is unilateral) may occur in the frontal area or throughout the head. This pain is probably caused by ciliary spasm and is worsened by looking at bright light, such as the light from a match a person uses to light a cigarette (the "match test"). Sometimes this discomfort is accompanied by nausea, vomiting, and malaise.

Local instillation of an anticholinergic drug such as atropine or homatropine usually brings relief from the pain and systemic effects (including the nausea and vomiting), but because these drugs cause blurring of vision, they should not be used unless the pain is severe.[59]

The Nose

Rhinorrhea is common after both local and systemic nerve agent exposure. It may occur soon after exposure to a small amount of vapor and sometimes precedes miosis and dim vision, or it may occur in the absence of miosis. Even a relatively small exposure to vapor may cause severe rhinorrhea. One exposed worker compared the nasal secretions to the flow from a leaking faucet, and another said that they were much worse than those produced by a cold or hay fever (personal observation).

Rhinorrhea also occurs as part of an overall, marked increase in secretions from glands (salivary, pulmonary, and gastrointestinal) that follows a severe systemic exposure from liquid on the skin and, under this circumstance, becomes a secondary concern to both the casualty and the medical care provider.

Pulmonary System

After exposure to a small amount of nerve agent vapor, individuals often complain of a tight chest (difficulty in breathing), which is generally attributed to spasm or constriction of the bronchiolar musculature. Secretions from the goblet and other secretory cells of the bronchi also contribute to the dyspnea. Exposure to sarin at a Ct of 5 to 10 mg•min/m^3 will produce some respiratory discomfort in most individuals, with the discomfort and severity increasing as the amount of agent increases.

Several decades ago, investigators attempted to characterize pulmonary impairment caused by exposure to nerve agents by performing pulmonary function studies (such as measurements of vital capacity and maximal breathing capacity) on subjects exposed to small amounts of

sarin vapor (the C*t* values for sarin ranged up to 19.6 mg•min/m^3).[60] Some observers found increases in airway resistance[61] and other changes, while other researchers did not.[62]

Although these studies yielded conflicting results, clinical practitioners have found that the inhalation of nerve agent vapor or aerosol causes dyspnea and pulmonary changes that usually are audible on auscultation. These changes are noticeable after low C*t* exposures (5–10 mg•min/m^3) and intensify as the C*t* increases. The pulmonary effects begin within seconds after inhalation. If the amount inhaled is large, the effects of the agent include severe dyspnea and observable signs of difficulty with air exchange, including cyanosis.

If the amount of the inhaled agent is small, a casualty may begin to feel better within minutes after moving into an uncontaminated atmosphere, and may feel normal in 15 to 30 minutes. It was not uncommon, for example, for individuals who had not received atropine or other assistance to arrive at the Edgewood Arsenal Toxic Exposure Aid Station about 15 to 20 minutes after exposure and report that their initial, severe trouble in breathing had already decreased markedly (personal observation). If the exposure was larger, however, relief was likely to come only after therapeutic intervention, such as administration of atropine.

Attempts to aid ventilation in severely poisoned casualties can be greatly impeded by constriction of the bronchiolar musculature and by secretions. One report[63] mentions thick mucoid plugs that hampered attempts at assisted ventilation until the plugs were removed by suction. Atropine may contribute to the formation of this thicker mucus because it dries out the thinner secretions.

A severely poisoned casualty becomes totally apneic and will die as a result of ventilatory failure, which precedes collapse of the circulatory system. Many factors contribute to respiratory failure, including obstruction of air passages by broncho-constriction and secretions; weakness followed by flaccid paralysis of the intercostal and diaphragmatic musculature, which is needed for ventilation; and a partial or total cessation of stimulation to the muscles of respiration from the CNS, indicating a defect in central respiratory drive.

Older data on the relative contributions of each of these factors in causing death were summarized in a report[64] describing original studies in nine species. The authors concluded that central respiratory failure appeared to dominate in most species, but its overall importance varied with the species, the agent, and the amount of agent. For example, under the circumstances of the studies, failure of the central respiratory drive appeared to be the major factor in respiratory failure in the monkey, whereas bronchoconstriction appeared early and was severe in the cat.

The authors of another report[65] suggest that the presence of anesthesia, which is used in studies of nerve agent intoxication in animals, and its type and depth are also factors in establishing the relative importance of central and peripheral mechanisms.

In another study,[66] bronchoconstriction seen in the dog after intravenous sarin administration was quite severe compared with that found in the monkey (however, the dog is known to have thick airway musculature). Differences in circulatory and respiratory effects were seen between anesthetized and unanesthetized dogs given sarin.[67] Convulsions and their associated damage were not seen in the anesthetized animals. In this study, there were no significant differences in the cardiovascular and respiratory effects when the agent was given intravenously, percutaneously, or by inhalation. In a study[68] of rabbits poisoned with sarin, bronchoconstriction appeared to be a minor factor, while neuromuscular block (particularly at the diaphragm) and central failure were the primary factors in respiratory failure.

In a recent review[69] describing studies in anesthetized cats given tabun, sarin, soman, or VX, the loss of central respiratory drive was found to be the predominant cause of respiratory failure with each of the agents, and the contribution of bronchoconstriction was apparently insignificant (in contrast to the severe bronchoconstriction noted in the earlier study[64]). Thus, respiratory failure was the predominant cause of death in the species studied inasmuch as significant cardiovascular depression occurred only after cessation of respiration.[68,69] When atropine was administered in adequate amounts before the failure of circulation, it reversed the central depression and bronchoconstriction but not the neuromuscular block, a finding that might be expected, because the neuromuscular effects of poisoning with these nerve agents occur at a nicotinic site.[64,68]

In a recent study,[70] pyridostigmine, a drug currently fielded as a pretreatment, was administered to primates, which then were exposed to a nerve agent and given the standard therapeutic drugs, atropine and 2-pyridine aldoxime methyl chloride (2-PAM Cl, also called 2-pralidoxime chloride; pyridine-2-aldoxime methyl chloride; 2-formyl-1- methylpyridinium chloride; Protopam chloride, manufactured by Wyeth-Ayerst Laboratories, Philadelphia, Pa; see section below on oximes). Pyridostigmine does not appear to enter the CNS because it is a quaternary compound and thus would not be expected to protect central sites of respiratory stimulation against the effects of a nerve agent. The pretreated animals continued to breathe, however, in contrast to controls that did not receive pyridostigmine pretreatment but were otherwise treated in the same manner.

The results of this study suggest that pyridostigmine protects against the cessation of respiration. Since pyridostigmine appears not to enter the CNS, this suggests that peripheral mechanisms of breathing (skeletal muscles and airways) must predominate in sustaining breathing. Alternatively, the blood–brain barrier may change in the presence of a nerve agent (as with other types of poisoning or hypoxia) to allow the penetration of drugs it otherwise excludes. For example, when 2-PAM Cl, which is also a quaternary compound, is administered to animals poisoned with a ChE inhibitor, it can be found in their CNS, but it is not found in the brains of normal animals after they receive 2-PAM Cl.[71]

Skeletal Musculature

The neuromuscular effects of nerve agents have been the subject of hundreds of studies since nerve agents were first synthesized in 1936. Much of our information on the mechanism of action of nerve agents and potential therapeutic measures has come from these studies. Because this chapter is primarily concerned with clinical effects of nerve agent poisoning, a comprehensive review of these studies is not presented here.

The effects of nerve agent intoxication on skeletal muscle are caused initially by stimulation of muscle fibers, then by stimulation of muscles and muscle groups, and later by fatigue and paralysis of these units. These effects on muscle may be described as fasciculations, twitches (or jerks), and fatigue.

Fasciculations are the visible contractions of a small number of fibers innervated by a single motor nerve filament. They appear as ripples under the skin. They can occur as a local effect at the site of a droplet of agent on the skin before enough agent is absorbed to cause systemic effects. They also can appear simultaneously in many muscle groups after a large systemic exposure. A casualty who has sustained a severe exposure will have generalized fasciculations, a characteristic sign of poisoning by a ChE inhibitor; typically, fasciculations will continue long after the patient has regained consciousness and has voluntary muscle activity.

After a severe exposure, there are intense and sudden contractions of large muscle groups, which cause the limbs to flail about or momentarily become rigid or the torso to arch rigidly in hyperextension. Whether these movements, which have been described as convulsive jerks, are part of a generalized seizure or originate lower in the nervous system is unclear. Occasionally, these disturbances may be a local effect on the muscle groups below or near the site of exposure—for instance, the marked trismus and nuchal rigidity in an individual who pipetted soman into his mouth (Exhibit 5-4).[18] After several minutes of hyperactivity (fasciculations or twitching), the muscles fatigue and flaccid paralysis occurs. This, of

course, stops convulsive activity and respiration.

Central Nervous System and Behavior

Behavioral and psychological changes in humans exposed to ChE-inhibiting substances have been discussed in numerous reports. The incidence of psychological effects is higher in individuals who have had more severe exposures to nerve agents, but they may occur—probably more frequently than is commonly recognized—in individuals who have received a small exposure and have no or minimal physical signs or symptoms. Although the effects may begin as late as 1 day after exposure, they usually start within a few hours and last from several days to several weeks. Common complaints include feelings of uneasiness, tenseness, and fatigue. Exposed individuals may be forgetful, and observers may note that they are irritable, do not answer simple questions as quickly and precisely as usual, and generally display impaired judgment, poor comprehension, decreased ability to communicate, or occasional mild confusion. Gross mental aberrations, such as complete disorientation or hallucinations, are not part of the symptom complex.

EXHIBIT 5-4
CASE REPORT: ACCIDENTAL EXPOSURE OF A MAN TO LIQUID SOMAN

This 33-year-old man [who worked at Edgewood Arsenal, Edgewood, Maryland] had been working with small amounts of soman in solution [25% (V/V) concentration, total volume <1 mL] when a syringe-needle connection broke, splashing some of the solution into and around his mouth.... He immediately washed his face and rinsed his mouth with water and was brought to the emergency room about 9 AM, 5-10 min after the accident. He was asymptomatic until he arrived at the ER when, as he later said, he felt "the world was caving in on me," and he collapsed. His past medical history was noncontributory. Physical examination showed him to be comatose and mildly cyanotic with slightly labored respirations. Intravenous atropine sulfate (2 mg) was given and may have been partially responsible for his initial blood pressure of 180/80 and heart rate of 150. He had miosis (1-2 mm, bilaterally), markedly injected conjunctiva, marked oral and nasal secretions, moderate trismus and nuchal rigidity, prominent muscular fasciculations, and hyperactive deep-tendon reflexes. Except for tachycardia, his heart, lungs, and abdomen were normal.

Within a minute after he collapsed (about 10 min after exposure) he was given intravenous atropine sulfate and in the ensuing 15 min he received a total of 4 mg intravenously and 8 mg intramuscularly, and pralidoxime chloride (2-PAMCl) was administered (2 gm over a 30 min period in an intravenous drip). Supportive care in the first 30 min consisted of oxygen by nasal catheter and frequent nasopharyngeal suction. Bronchoconstriction and a decreased respiratory rate and amplitude were prominent; the former was more responsive to atropine therapy. He became cyanotic and attempts to insert an endotracheal tube were unsuccessful because of trismus. Since spontaneous respiration did not cease, a tracheostomy was not performed.

After the initial therapy his cyanosis cleared and his blood pressure and heart rate remained stable. He began to awaken in about 30 min and thereafter was awake and alert. Migratory involuntary muscular activity (fasciculations and tremor) continued through the day.

He improved throughout the day, but was generally uncomfortable and restless with abdominal pain and nausea throughout the day and night. Atropine (4 mg, i.v.) was required again at 11 PM (14-hr post exposure) after several episodes of vomiting. About 4 AM, he was catheterized because of urinary retention.

His restlessness and intermittent nausea continued, and about 5 AM (20 hr after exposure) he again vomited. Because the previous atropine had apparently caused urinary retention, this emesis was treated with a small dose (5 mg, i.m.) of prochlorperazine, although phenothiazines have been reported to be deleterious in anticholinesterase compound poisoning.[1,2] His general condition, including his discomfort, did not change. He vomited twice more between 7:30-8 AM (22-23 hr post exposure) and was again given atropine (4 mg, i.m.). He voided small amounts several times, but catheterization was necessary several hours later.

Several EKGs recorded on admission and during the first day showed sinus tachycardia. On the second day (25 hr after exposure and about 2 hr after atropine administration), his cardiac rhythm was irregular, and an EKG showed atrial fibrillation with a ventricular rate of 90-100 beats per min. This persisted throughout the day and evening, but his cardiac rhythm was again regular sinus the next morning.

During the second evening (about 36 hr after exposure), he again

became nauseated and had recurrent vomiting. Because of the occurrences of urinary retention and arrhythmia, presumably due to atropine, he was again given prochlorperazine (5 mg, i.m.) at 10 PM and again at 2 AM. Half an hour after the first he complained of transient "tingling" feelings over his body, but there were no objective changes. After the second he rested comfortably and slept soundly for 3-4 hr, his first restful sleep since the exposure. At 11 AM the next morning, he was restless and had an expressionless face, torticollis, and athetoid movements. Diphenylhydramine hydrochloride (50 mg, i.v.) promptly relieved these symptoms and signs, which are characteristic of the extrapyramidal side effects of a phenothiazine. Throughout the remainder of his hospitalization, the patient's physical condition improved although he was treated with sulfisoxazole for three weeks for a urinary tract infection that developed after catheterization.

His psychiatric condition did not improve as rapidly as his physical condition. As the complications of the treatment for the physical effects subsided, evidence of lingering mental effects began to appear. A psychiatrist ..., who saw the subject frequently, recorded that he seem depressed, was withdrawn and subdued, admitted to antisocial thoughts, slept restlessly and fitfully, and had bad dreams. On the third day [after the exposure] the patient was given scopolamine hydrobromide (5 μg/kg, or 330 μg, i.m.) as a therapeutic trial. Psychiatric evaluation at the time of maximum scopolamine effect showed a slight but distinct improvement in mental status as he seemed more comfortable and performed better on several mental function tests (eg, serial 7s) than before scopolamine. That evening he was given 1.8 mg of scopolamine (orally) at bedtime and slept much better for most of the night.

This nighttime benefit from scopolamine may have occurred because of its sedative properties, but the improvement in mental status during the day suggested a more specific action, as scopolamine in this dose produces a slight decrease in intellectual functioning in normal subjects.[3] [Thereafter, scopolamine and methscopolamine (which does not enter the central nervous system) were administered on randomly assigned days. The patient did better mentally (by examination) and on a written arithmetic test after receiving scopolamine than after methscopolamine.]

There was no detectable RBC-ChE until about the tenth day after exposure.... Apparently neither the RBC nor plasma ChE was

significantly reactivated by the initial oxime therapy, which reflects the rapid irreversible phosphorylation and hence refractoriness of the soman-inhibited enzyme to reactivation by oxime.

Hematocrit, hemoglobin, white blood cell count, prothrombin time, blood urea nitrogen, bilirubin, creatinine, calcium, phosphorus, serum glutamic oxaloacetic transaminase, alkaline phosphatase, sodium, potassium, chloride, and carbon dioxide were all within normal limits the day of admission and on repeated measurements during his hospitalization.

About five weeks after his admission, the subject again received scopolamine (5 mg/kg, i.m.) and had a decrement in mental functioning, including a 25-30% reduction in NF [Number Facility] scores, which are the findings in normal subjects. This contrasts with the paradoxical improvement in mental status seen earlier.

About a week later, the psychiatrist noted that "he is probably close to his premorbid level intellectually and there is no evidence of any serious mood or thinking disorder."

A battery of standard psychological tests was given the subject 16 days, 4 months, and 6 months after the accident. He scored well on the Wechsler-Bellevue IQ test with a slight increase in score on the arithmetic section at the later testings. He had high Hs (hypochondriasis) and Hy (hysteria) scales on the Minnesota Multiphasic Personality Inventory (MMPI) on the early test and their later improvement indicated to the examiner that he had a decreased concern about bodily function. He did poorly on a visual retention task (the object of which was to remember and then reproduce a simple drawing) on first testing as he attempted to improve already correct drawings, made several major errors, and showed poor motor control; his later tests were normal. On word association, proverbs, and the ink blot he was slow and sometimes used delaying tactics, had difficulty generating verbal associations, and failed the harder proverbs, responses that in the examiner's opinion were not consistent with his IQ. The results of his later tests were faster, imaginative, and indicated full use of his intellectual facilities.

When last seen, six months after his exposure, the patient was doing well.

Studies of Behavioral and Psychological Changes

In one of the earliest studies of the effects of ChE-inhibiting substances,[36] behavioral and psychological changes were reported in 49 of 60 subjects (of whom 50 were normal and 10 had myasthenia gravis) after daily intramuscular doses (1.5–3.0 mg) of DFP. Changes were reported about 1 hour after dose administration. The most prominent CNS effects reported were excessive dreaming (33 subjects); insomnia (29 subjects); and jitteriness, restlessness, increased tension, emotional lability, and tremulousness (29 subjects). The authors noted, without comment, that one subject reported visual hallucinations. Hallucinations are not mentioned elsewhere as an effect of ChE inhibitors. Later, similar effects were reported as sequelae of accidental exposure to nerve agent poisoning.[72]

One report[63] suggests that several workers accidentally exposed to sarin had some behavioral effects. Another report[73] lists “weakness” (actually tiredness), nervousness, and drowsiness as complaints from 16 of 40 workers accidentally exposed to small amounts of nerve agent vapor.

In a series[55] of 49 workers who were accidentally exposed to sarin or tabun (a total of 53 exposures), 13 workers reported sleep disturbances, 12 reported mood changes, and 10 reported easy fatigability. Overall, 51% had CNS effects. The authors pointed out that the complex of CNS symptoms may not fully develop until 24 hours after exposure. The data on blood ChE activities (both RBC-ChE and BuChE) in these workers were scanty. The individual with the greatest ChE inhibition, however, had an RBC-ChE activity of 33% of his personal control value, which suggests that the exposures were not severe. No correlation between the presence or severity of symptoms and the degree of ChE inhibition was seen, and most of the effects of exposure disappeared within 3 days. Systemic atropine was not given to any of these individuals, which suggests that therapy is unnecessary if a paucity of physical signs exists. The authors concluded that mild intoxication by nerve agents may cause psychological disturbances and that these disturbances might have serious consequences to the individuals and to those dependent on their judgment.[55]

In a series[74] of 72 workers exposed to sarin, 2 reported difficulty in concentration, 5 reported mental confusion, 5 reported giddiness, and 4 reported insomnia. All but 2 of these individuals were considered to have been exposed to a small amount of sarin; these 2 were given 2 mg of atropine intramuscularly, and 12 others received atropine orally (0.4–0.8 mg). RBC-ChE ranged from less than 9% to more than 100% of the

individual's control activity.

Behavioral changes and whole-blood ChE activities were reported in another study[75] in which VX was placed on the skin of volunteers. Since VX preferentially inhibits RBC-ChE and has relatively little effect on BuChE, the decreases in whole-blood ChE activities were assumed to indicate mainly inhibition of RBC-ChE. In subjects with whole-blood ChE activities of 10% to 40% of control (RBC-ChE activities ≤ 20% of control), 30% reported anxiety, 57% had psychomotor depression, 57% had intellectual impairment, and 38% had unusual dreams. Of those with whole-blood ChE activities of 41% to 80% of control (RBC-ChE activities of 20%–40% of control), 8% reported anxiety, 4% had psychomotor depression, 4% had intellectual depression, and 33% had unusual dreams. Nausea and vomiting were the other symptoms noted. Some subjects had both psychological and gastrointestinal effects, with onsets often separated by several hours. Some subjects had symptoms related to only one organ system.

Overall, the onset of signs and symptoms occurred 3.5 to 18 hours after percutaneous exposure, and maximal depression in blood ChE occurred 3 to 8 hours after exposure. However, no measurements were taken between 8 and 24 hours, and the maximal inhibition might have been in this period. (It is not often recognized that there may be a long delay between exposure on the skin and onset of signs or symptoms.) The authors stressed that psychological impairment might occur before the onset of other signs or symptoms or might occur in their absence.[75]

Although the frequency, onset, and duration of each reaction were not noted, some of the behavioral effects reported in the VX subjects were fatigue, jitteriness or tenseness, inability to read with comprehension, difficulties with thinking and expression, forgetfulness, inability to maintain a thought trend, a feeling of being mentally slowed, depression, irritability, listlessness, poor performance on serial 7s and other simple arithmetic tests, minor difficulties in orientation, and frightening dreams. Illogical or inappropriate trends in language and thinking were not noted, nor was there evidence of conceptual looseness. The investigators found no evidence of perceptual distortion resulting in delusions or hallucinations.

A severe, accidental exposure to soman caused one person to become depressed, withdrawn, and subdued, have antisocial thoughts, and sleep restlessly with bad dreams for several days immediately after the exposure (see Exhibit 5-4).[18] He received oral doses of scopolamine hydrobromide on 3 of the following 6 days and was given scopolamine methylbromide, which does not enter the CNS, on the other days to mimic the peripheral effects of the hydrobromide salt, such as dry mouth. On the hydrobromide days, the subject was more spontaneous and alert, less depressed, and slept better; his performance on a simple arithmetic test

also improved. Because scopolamine hydrobromide is more effective in the CNS than the methylbromide salt of scopolamine or atropine, it seemed likely that the drug reversed the CNS effects, at least temporarily. The subject's performance on standard psychological tests 16 days after exposure was below that expected for one of his intellectual capabilities, but it improved to his expected level of functioning when he was tested 4 months later and again at 6 months later when he was discharged from further care. The author suggested that the use of scopolamine hydrobromide deserves further evaluation in patients who have these lingering effects while recovering from nerve agent poisoning.

Changes in the ability to perform certain laboratory or field tests after exposure to sarin have been reported. Generally, at the exposures used (C*t*s of 4–14.7 mg•min/m^3), there was some impairment on tasks requiring vision, hand–eye coordination, dexterity, response time, comprehension, and judgment.[76,77] No decrements were found on physical tasks[78] (at a C*t* of 14.7 mg•min/m3). On a military field exercise,[79] most tasks were performed satisfactorily, if suboptimally, in the daylight. Nighttime performance, however, was difficult, if not downright hazardous.

Electroencephalographic Effects

Information is scanty on the electroencephalographic (EEG) effects in humans who have been severely poisoned by ChE-inhibiting substances. In an early study,[80] DFP, administered intramuscularly daily, caused EEG changes in 19 of 23 subjects (19 normal, 4 with myasthenia gravis). The changes were

- greater-than-normal variations in potential;
- increased frequency, with increased beta rhythm; and
- more irregularities in rhythm and the intermittent appearance of abnormal waves (high-voltage, slow waves; these were most prominent in the frontal leads).

These changes usually followed the onset of CNS symptoms, they could be correlated with decreases of RBC-ChE activity (but not with BuChE decreases), and they were decreased or reversed by atropine 1.2 mg, administered intravenously.

In another study,[81] the EEG of a subject who was severely intoxicated with sarin was recorded after the loss of consciousness but before the onset of convulsions. The recording showed marked slowing of activity, with bursts of high-voltage, 5-Hz waves in the temporofrontal leads. These

waves persisted for 6 days despite atropine administration.

In one study[43] in which subjects were exposed to smaller amounts of sarin, the EEG changes coincided with severity of symptoms. With mild symptoms, there was a slight diminution of voltage. Irregularities in rhythm, variation in potential, and intermittent bursts of abnormal waves (slow, elevated-voltage waves) occurred with moderate symptoms. These changes persisted for 4 to 8 days after the disappearance of symptoms and decreased somewhat (decreases in voltage, in irregular frequency and potential, and in slow waves) after administration of atropine 1 mg, administered intravenously.

Long-Term Effects

Long-term effects on the human CNS after poisoning with nerve agents or organophosphate insecticides have been reported.[18,82,83] These reports are based on clinical observations, which occasionally are supported by psychological studies. In general, the behavioral effects have not been permanent but have lasted weeks to several months, or possibly several years.[84]

Necropsy findings from animal studies suggest that there are long-lasting or permanent CNS effects after exposures to lethal or near-lethal amounts of nerve agents. In one study,[85] 264 rats were given approximately 1 LD_{50} of soman. Some animals died shortly after agent administration; others were sacrificed at intervals up to 39 days. In those surviving for 39 days, no neurological sequelae were reported. On microscopic examination of the brains of the deceased animals, neuronal changes similar to those seen after hypoxic encephalopathy were found in some animals, but only in those that had exhibited respiratory distress and repeated or prolonged convulsions.

In another study[86] with a similar protocol, brains of rats were examined 15 to 28 days after the animals were given a single dose of soman. Lesions were seen in all of the animals that had convulsions, in three of four that had tremors but not convulsions, and in none of the others. The author concluded that the convulsions were not necessary for the lesions to occur.[86]

The neuronal degeneration and necrosis seen in the brains of soman-poisoned rats in another study[87] suggested a “hypoxic pattern” or the type of lesion seen after status epilepticus. The lesions were seen only in animals that survived prolonged convulsions, and the authors surmised that the brain damage was seizure mediated.

Although most studies have been with soman, similar damage has been reported after exposure to sarin.[88] Also, in one elegant study,[89] VX was

micro-injected into specific brain regions. The investigators concluded that neuropathology was not due to a direct neurotoxic effect of the agents on brain neurons, that systemic hypoxia is probably not a mechanism for their toxicity, and that the brain damage produced by nerve agents is probably seizure mediated.

In addition to having morphologically detectable brain lesions, animals surviving severe soman intoxication have been shown to have decrements in performance, as measured on a variety of behavioral tests.[90–92] These decrements lasted for about 4 months, when the last survivors were sacrificed.

There is conflicting evidence regarding the possible role of hypoxia as an etiologic factor in brain damage following seizure activity—whether this activity is caused by nerve agents or other factors. Rats given bicuculline convulsed for 2 hours under controlled conditions. Those given a lower percentage of oxygen in their inspired air to keep the partial pressure of arterial oxygen (PaO_2) close to 50 mm Hg did not have brain lesions, whereas those with normal air intake and PaO_2 higher than 128 mm Hg developed brain lesions.[93] Although this evidence does not eliminate the possibility of localized hypoxic areas in the brain as a factor in nerve agent-induced damage, it does suggest that systemic hypoxia is not a factor. On the other hand, a similar study[94] (hypoxic rats with bicuculline-induced convulsions that lasted 2 h) suggested that there were slightly more brain lesions in the hypoxic animals than in normoxic animals.

In studies in which cynomolgus[86] or rhesus[95] monkeys were given nearly lethal amounts of soman, the animals that convulsed were later found to have morphologically detectable brain damage. Monkeys that were pretreated with pyridostigmine, given soman, and treated with atropine and 2-PAM Cl in another study[70] had severe and prolonged tremors and convulsions. Although the survival rate was much higher than that for the control group, one might expect that more of the survivors would have brain lesions because of the prolonged seizure activity.

In general, in untreated or inadequately treated nerve agent–poisoned animals, convulsive (and seizure) activity usually stops very shortly after respiration ceases. Often these animals die. Occasionally they recover after some degree of apnea, but in either case, the duration of convulsive and seizure activity is brief. In a few reported cases of severe nerve agent intoxication in humans,[18,63,81] convulsive activity has also been brief. Animals given adequate therapy and, in particular, those given pyridostigmine before exposure to the agent are more likely to recover. They also are more likely to have long and recurrent convulsive episodes, since respiration does not stop. The chance of survival increases but, possibly, at the cost of prolonged or permanent CNS damage.

Therapy

Diazepam, an anticonvulsant of the benzodiazepine family, has been shown to control soman-induced convulsions in monkeys[96] and convulsions induced by other ChE inhibitors in the rabbit.[97,98] There have also been anecdotal reports of its effectiveness in controlling convulsions induced by organophosphate insecticides. In rats, diazepam has been reported to decrease the frequency of convulsions and brain lesions (although, when given without atropine, it did not decrease mortality).[99] When given with 2-PAM Cl, with or without atropine, diazepam reduced the severity, but not the incidence, of brain lesions in soman-poisoned rats.[100]

In another study,[101] three groups of monkeys were given pyridostigmine before soman exposure and were treated with atropine and 2-PAM Cl after exposure. One group was also given diazepam, the second was given midazolam, and the third (the control group) was given water. The incidence of tremors decreased in the anticonvulsant-treated animals, and convulsions were absent in these groups. Overall, the brain lesions in the treatment groups in this study were less severe than those seen in animals in a previous study[95] in which animals had received neither pretreatment nor treatment. Although the occurrence of brain lesions in most brain areas was lower in the anticonvulsant-treated groups than in the control group, the difference was statistically significant ($P < .05$) for only one of these five brain areas studied. Lesions occurred more frequently ($P < .05$) in the frontal cortex in the diazepam-treated group than in the other groups. The reason for this finding and its physiological significance were not apparent to the investigators.

At a workshop[102] on this topic, many investigators in the field reviewed and discussed findings and future avenues for research. It was generally agreed that brain lesions did not occur if convulsions lasted less than 45 minutes, and that brain damage was found if convulsions lasted longer than 45 minutes. This distinction is not apparent in most reports, which specify only that convulsions were “prolonged” or “repeated.”

A report[103] notes that brain damage after convulsions was first reported over a century ago, and that the relationship between seizure activity and brain damage has been the subject of numerous studies in past decades. For example, well-oxygenated rats had lesions after 30 minutes of flurothyl-induced convulsions[104] ; similar results were found using another volatile convulsant gas in rats.[105] Similarly, baboons that had convulsions for 82 to 299 minutes after bicuculline administration had brain damage.[106] These and other studies, such as that by McDonough and associates,[89] suggest that the brain damage is not caused by the agent per se, but rather by the

prolonged seizure and associated disturbances.

The efficacy of diazepam in stopping seizures is generally accepted, and there is evidence that the drug also reduces brain damage. Rats given bicuculline had convulsions for 1 hour; the convulsions were then terminated with diazepam. Rats sacrificed 5 minutes later had fewer brain lesions than did those sacrificed immediately after the cessation of the convulsion. Rats that recovered longer and were sacrificed 2 hours after termination of the convulsion had even less brain damage.[107]

Most reports in the clinical literature recommend stopping the convulsion within 1 hour, using drastic measures, such as hypothermia and barbiturate coma, if necessary.[108] The mortality of status epilepticus (usually defined as a convulsion lasting 60 min, or a series of convulsions lasting 60 min without consciousness intervening) is said to be 6% to 30%. Moreover, twice that number of victims acquire irreversible neurological deficits as a result of status epilepticus.[108] (In a study of children, permanent deficits were found to occur in 34%.[109]) These data emphasize the need for an effective anticonvulsant.

Soldiers are issued three MARK I kits (Figure 5-5). Each MARK I kit contains two autoinjectors: an AtroPen containing 2 mg of atropine in 0.7 mL of diluent, and a ComboPen containing 600 mg of 2-PAM Cl in 2 mL of diluent (autoinjectors and their contents manufactured by Survival Technology, Rockville, Md.). During the Persian Gulf War, the U.S. military also issued an autoinjector containing 10 mg of diazepam to all military personnel. This ComboPen was not intended for self-use, but rather for use by a buddy when a soldier exhibited severe effects from a nerve agent. The buddy system was used because any soldier able to self-administer diazepam does not need it. Medics and unit lifesavers were issued additional diazepam autoinjectors and could administer two additional doses at 10-minute intervals to a convulsing casualty. Current policy states that diazepam is given following the third MARK I when three MARK I kits are given at one time. The contents and use of MARK I kits are further described throughout this chapter.

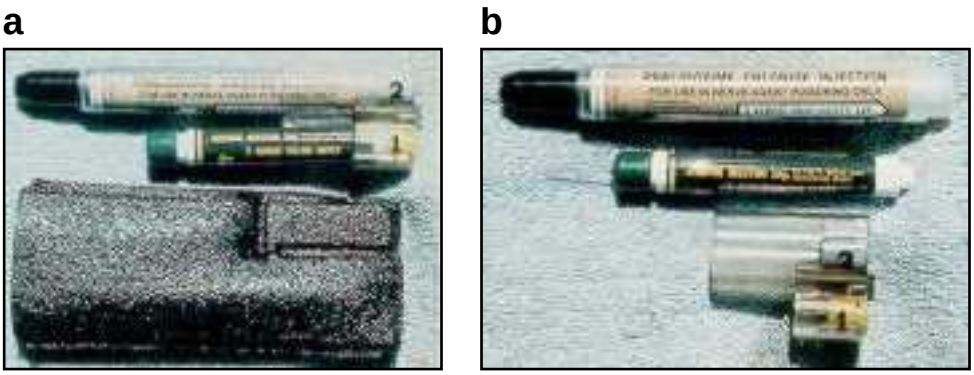


Fig. 5-5. The MARK I kit with its two autoinjectors: the AtroPen (containing atropine), labeled 1, and the ComboPen (containing 2-pyridine aldoxime methyl chloride, 2-PAM Cl), labeled 2. **(a)** The two unused injectors in the safety clip, and the black carrier. **(b)** The used injectors and empty safety clip.

Cardiovascular System

Few data on the cardiovascular effects of nerve agents in humans exist. In mild-to-moderate intoxication from nerve agents, blood pressure may be elevated, presumably because of cholinergic stimulation of ganglia or other factors, such as stress reaction.

Arrhythmias

After nerve agent exposure, the heart rate may decrease and some atrial-ventricular (A-V) heart block (first-, second-, or third-degree) with bradycardia (personal observation) may occur because of the stimulation of the A-V node by the vagus nerve. In some cases, an increase in heart rate may occur because of stress, fright, or some degree of hypoxia. Since the initiation of treatment is of great urgency in severely intoxicated patients, electrocardiograms (ECGs) have not been done before administration of atropine. However, if possible, an ECG should be done before drugs are given if the procedure will not delay therapy. In normal

subjects, atropine may cause a very transient A-V dissociation before the onset of bradycardia (which precedes the familiar tachycardia), and ChE-inhibiting substances may cause bradycardia and A-V block. For reasons noted above, these transient rhythm abnormalities have not been recorded in patients with nerve agent intoxication. These rhythm disturbances are probably not important clinically.

Reports of patients exposed to pesticides and the results of animal studies provide additional information about cardiovascular reactions to nerve agents. In one study,[110] dogs exposed to lethal amounts of sarin vapor had idioventricular rhythms within minutes after exposure; following atropine therapy, some of the dogs had third- and first-degree heart blocks before a normal rhythm returned. In another study,[111] conscious dogs had few cardiac rhythm changes after sublethal doses (0.25–0.5 LD_{50} , administered subcutaneously) of VX. Four of five anesthetized dogs receiving a 1-LD_{50} dose had arrhythmias, including first-degree heart block and premature ventricular complexes; one had torsade de pointes (a type of ventricular tachycardia). Cardiac arrhythmias are not uncommon in humans after organophosphate pesticide poisoning.[112]

Dogs were instrumented to examine the cardiac changes occurring for a month after intravenous administration of 2 LD_{50} of soman.[113] Atropine and diazepam were administered shortly after soman exposure to control seizure activity. During the study period, there was an increased frequency of episodes of bradycardia with ventricular escape, second- and third-degree heart block, and independent ventricular activity (single premature beats, bigeminy, or runs of ventricular tachycardia).

In a similar study,[114] rhesus monkeys were given the standard military regimen of pyridostigmine before exposure to soman (1 LD_{50} , administered intramuscularly), and atropine and 2-PAM Cl after the agent. The monkeys were monitored continuously for 4 weeks. Except for the period immediately after agent administration, the incidence of arrhythmias was the same as or less than that observed during a 2-week baseline period.

Torsade de pointes has been reported after nerve agent poisoning in animals[111] and after organophosphate pesticide poisoning in humans.[115] Torsade de pointes is a ventricular arrhythmia, usually rapid, of multifocal origin, which on ECGs resembles a pattern midway between ventricular tachycardia and fibrillation. It is generally preceded by a prolongation of the QT interval, it starts and stops suddenly, and it is refractory to commonly used therapy. It was first described as a clinical entity in the late 1960s; undoubtedly it was seen but called by another name in experimental studies with nerve agents before then.

Ventricular fibrillation, a potentially fatal arrhythmia, has been seen

after administration of a ChE inhibitor and atropine. It can be precipitated by the intravenous administration of atropine to an animal that has been rendered hypoxic by administration of a ChE inhibitor.[116,117] Although this complication has not been reported in humans, atropine should not be given intravenously until the hypoxia has been at least partially corrected.

Heart Rate

Although it is frequently stated that a patient intoxicated with a nerve agent will have bradycardia, this is not borne out by clinical data. In a review of the records of 199 patients seen at the Edgewood Arsenal Toxic Exposure Aid Station for mild-to-moderate nerve agent exposure (one or more definite signs or symptoms of nerve agent intoxication, such as miosis or a combination of miosis with dim vision or a tight chest), 13 presented with heart rates less than 64 beats per minute. There were 13 patients with heart rates of 64 to 69 beats per minute, 63 with heart rates of 70 to 80, 41 with heart rates of 81 to 89, 38 with heart rates of 90 to 99, and 31 with heart rates higher than 100. A heart rate of 64 to 80 beats per minute is considered normal in adults.[118] Thus, 13 patients (6.5%) had low heart rates, and 110 patients (55%) had high heart rates; (69 of these patients [35%] had heart rates = 90). Reports of the heart rates of patients severely intoxicated by insecticides vary. In a report[119] describing 10 patients (9 of whom had moderate-to-severe impairment of consciousness), 7 presented with heart rates over 100 and the other 3 had heart rates over 90 (5 had a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or both). In another report,[120] the heart rates of 3 unconscious patients were slow (1 had cardiac arrest). In a comprehensive review of organophosphate poisoning,[51] 2 acutely ill, unconscious patients were described; 1 had a heart rate of 108 beats per minute and the other, 80. The authors of the study pointed out that cardiovascular function is usually maintained until the terminal stage and that blood pressure and heart rate increase in the acute stage but may decline later. Heart rate was not listed in their tabulation of signs and symptoms.

GENERAL TREATMENT PRINCIPLES

The principles of treatment of nerve agent poisoning are the same as they are for any toxic substance exposure: namely, terminate the exposure; establish or maintain ventilation; administer an antidote, if one is available; and correct cardiovascular abnormalities.

Most importantly, medical care providers or rescuers must protect themselves from contamination. If the caregiver becomes contaminated, there will be one more casualty and one fewer rescuer. Protection of the rescuer can be achieved by physical means, such as masks, gloves, and aprons, or by ensuring that the casualty has been thoroughly decontaminated. The importance of casualty decontamination should be obvious, but, unfortunately, it is often forgotten or overlooked. There were reports that in several instances during the Iran–Iraq War (1981–1988), incompletely decontaminated mustard casualties who were transported to European medical centers for further care caused contamination of others, who then also became casualties.

This section discusses the general principles of treating nerve agent poisoning. The specific treatment of casualties in the six exposure categories (suspected, minimal, mild, moderate, moderately severe, and severe) is addressed in the next section.

Terminating the Exposure

Decontamination is performed for two reasons:

1. to prevent further absorption of the agent by the casualty or further spread of the agent on the casualty, and
2. to prevent spread of the agent to others, including medical personnel, who must handle or who might come into contact with the casualty.

Because of the small amount of nerve agent needed to cause death and because of the short time (10–15 min) in which a lethal amount will cause severe effects in an untreated casualty, it is unlikely that a living nerve agent–poisoned casualty with nerve agent on his skin will be brought to a medical care facility. To successfully reduce damage to the casualty, decontamination must be performed within minutes after exposure. The only decontamination that prevents or significantly reduces damage from a chemical agent, whether a nerve agent or another agent, is that done within the first several minutes: *self-decontamination*.

The importance of rapid self-decontamination cannot be

overemphasized and must be clearly understood by anyone who might be exposed to chemical agents. Because the skin absorbs most chemical agents rapidly and because of evaporation (even “persistent” agents, such as VX and mustard, evaporate from skin rather quickly), it is unlikely that there will be a significant amount of agent on the skin by the time the casualty reaches a medical treatment facility. However, agent may be in areas, such as in hair or on clothing, where it will not be readily absorbed percutaneously. *Skin decontamination is not necessary after exposure to nerve agent vapor*.

If vapor is the only exposure source, the exposure can be terminated by putting a protective mask on the casualty or by moving him to an environment free of toxic vapor (eg, by moving the casualty outside and sealing the doors if the vapor is in a room or building). The standard M40 (or M17A2) protective mask will protect against any likely field concentration of nerve agent vapor for days. Liquid agent on the skin or clothing should be physically removed and detoxified by chemical degradation or neutralization. Nerve agents penetrate clothing; mere removal of contaminated apparel is not adequate since some agents may reach the skin before the clothing is removed. If the contamination is localized, cutting out the affected section of clothing (leaving very wide margins) may be adequate. If there is doubt, however, all clothing should be removed. The underlying skin should then be decontaminated thoroughly.

The M291 decontamination kit contains charcoal and sorptive resins; the agent is physically removed and adsorbed. The M258A1 is a standard decontamination kit that contains two moistened towelettes. One is intended for use with the G agents, and the other with VX and mustard. Since the agent probably would not be identified at the time of exposure in the field, both towelettes should be used to physically remove the chemical agent by blotting, not wiping, it; the towelettes also aid in decontamination by chemically neutralizing the agent, although this chemical reaction is slow. (See Chapter 15, Decontamination, and Chapter 16, Chemical Defense Equipment, for details about the decontamination kits.)

A solution that releases chlorine, such as household bleach (5% sodium hypochlorite) or a solution that is sufficiently alkaline to neutralize the agent, such as dilute hydroxide, can also be used for physical removal and chemical neutralization of a chemical agent. Because of the potential for skin damage from 5% hypochlorite, the current military procedure is to use 0.5% hypochlorite for skin decontamination. Dilute hydroxide and the contents of the M258A1 kit are damaging to the skin, however, and should be thoroughly rinsed off.

Water is also a decontaminant since, when used in large amounts, it

physically removes and dilutes chemical agents. (Most agents hydrolyze to some degree in water, but hydrolysis usually takes hours to days.) If used alone, water is not ideal; however, if nothing else is available, flushing with large amounts of water to physically remove the chemical agent is satisfactory. Water should be used to wash off the other decontaminants. Commonly available products (such as tissue paper and flour) that can help remove or adsorb the agent should be used if other decontaminants are not available.[121]

Ventilatory Support

Ventilatory support is a necessary aspect of therapy if a casualty with severe respiratory compromise is to be saved. Antidotes alone may be effective in restoring ventilation and saving lives in some instances; in animal studies,[122,123] antidotes alone, given intramuscularly at the onset of signs, were adequate to reverse the effects of agent doses of about 3 LD_{50} , but their effectiveness was greatly increased with the addition of ventilation. Pyridostigmine, given as pretreatment and followed by the current therapy after challenges with higher amounts of two agents, appears to prevent apnea (see Chapter 6, Pretreatment for Nerve Agent Exposure).

Impairment of breathing is an early effect of exposure to nerve agent vapor or aerosol. When the exposure is small, the casualty may have mild to severe dyspnea, with corresponding physical findings, and the impairment will be reversed by the administration of atropine. If the distress is severe and the casualty is elderly or has pulmonary or cardiac disease, the antidote may be supplemented by providing oxygen by inhalation. In most other circumstances, supplementation with oxygen is unnecessary.

Severely exposed casualties lose consciousness shortly after the onset of effects, usually before any signs of respiratory compromise. They have generalized muscular twitching or convulsive jerks and may initially have spontaneous but impaired respiration. Breathing ceases completely within several minutes after the onset of exposure in a severely poisoned person who has not been pretreated with pyridostigmine.

Assisted ventilation may be required to supplement gasping and infrequent attempts at respiration, or it may be required because spontaneous breathing has stopped. In addition to a decrease in central respiratory drive, weakness or paralysis of thoracic and diaphragmatic muscles, and bronchospasm or constriction, there are copious secretions throughout the airways. These secretions tend to be thick, mucoid, and “ropy,” and may plug up the airways. Postural drainage can be used, and

frequent and thorough suctioning of the airways is necessary if ventilation is to be successful. In one instance, efforts to ventilate a severely apneic casualty were markedly hindered for 30 minutes until adequate suction was applied to remove thick mucoid plugs.[63]

Initially, because of the constriction or spasm of the bronchial musculature, there is marked resistance to attempts to ventilate. Pressures of 50 to 70 cm H_2O or greater may be needed. After the administration of atropine, resistance decreases to 40 cm H_2O or lower, and the secretions diminish (although they may thicken), creating less obstruction to ventilatory efforts.

There are numerous mechanical devices, including sophisticated ventilators, that can be used to provide ventilatory assistance in an apneic casualty. None of these is available to the soldier or his buddy, and only a few—the mask-valve-bag ventilation device, the RDIC (resuscitation device, individual, chemical), and a simple ventilator—are available at the battalion aid station. Whatever device is used, it must be able to overcome the initial high resistance in the airways. If a casualty is apneic or has severe respiratory compromise and needs assisted ventilation, then endotracheal intubation, which will enable better ventilation and suction of secretions, can and should be attempted.

Mouth-to-mouth ventilation might be considered by a soldier who wants to assist an apneic buddy when no aid station is nearby. A major drawback is the likelihood of contamination. Before even considering this method, the rescuer should be sure that there is no vapor hazard, which is not always possible, and that there is no liquid contamination on the individual to be ventilated. The expired breath of the casualty is a lesser hazard. Studies[124–126] involving sarin have shown that only 10% or less of inspired nerve agent is expired, and that the toxicant is expired immediately after inspiration of the agent.

The Schäfer method of assisted ventilation (ie, gentle, intermittent pressure applied to the lower part of the thorax of a prone person to mimic breathing) was formerly used in severely poisoned individuals until other means became available. Generally, this is not a reliable method of ventilation even in an individual with normal airways.

In summary, spontaneous respiration will stop within several minutes after onset of effects caused by exposure to a lethal amount of nerve agent. Antidotes alone are relatively ineffective in restoring spontaneous respiration. Attempts at ventilation are hindered by the high resistance of constricted bronchiolar muscles and by copious secretions, which may be thick and plug the bronchi. Ventilatory assistance may be required briefly (20–30 min) or for a much longer period. In several instances, assistance was required for 3 hours[18,63] ; this seems to be the longest reported use

of ventilation.

Atropine Therapy

The antagonism between the ChE-inhibiting substance physostigmine and a cholinergic blocking substance has been recognized for well over a century.[127] In the early 1950s, atropine was found to reduce the severity of effects from ChE-inhibitor poisoning, but it did not prevent deaths in animals exposed to synthetic ChE-inhibiting insecticides.[128]

Cholinergic blocking substances act by blocking the effects of excess acetylcholine at muscarinic receptors. Acetylcholine accumulates at these receptors because it is not hydrolyzed by ChE when the enzyme is inactivated by an inhibitor. Thus, cholinergic blocking substances do not block the direct effect of the agent (ChE inhibition); rather, they block the effect of the resulting excess ACh. Many cholinergic blocking substances have been tested for antidotal activity. Among the findings are the following:

- Almost any compound with cholinergic blocking activity has antidotal activity.
- Atropine and related substances reduce the effects of the ChE inhibitors, primarily in those tissues with muscarinic receptor sites.
- Antidotal substances with higher lipoid solubility, which penetrate the CNS more readily, might be expected to have greater antidotal activity, since some of the more severe effects of ChE inhibitor poisoning (such as apnea and seizures) are mediated in the CNS.

  For example, the combination of benactyzine and atropine was shown to be more effective than atropine alone in reducing lethality from sarin[128] ; 3-quinuclidinyl benzilate (BZ, also called QNB; see Chapter 11, Incapacitating Agents) is an excellent antidote. Benactyzine was part of a mixture (plus atropine and *N,N'*-trimethyl-enebis-[pyridine-4-aldoxime bromide] [TMB4]) fielded as a nerve agent antidote (known as TAB) for several years in the late 1970s. However, these substances have CNS effects in the absence of nerve agents; this was one of the reasons that this mixture was withdrawn.

In the late 1940s, atropine was chosen as the standard antidote; despite extensive searches for other antidotes since, it has remained the standard. A dose of 2 mg was chosen for self- or buddy-administration (the AtroPen automatic injector included in the MARK I kit contains 2 mg) because it reverses the effects of nerve agents, the associated side

effects of a dose this size can be tolerated, and reasonably normal performance can be maintained by the individual receiving it. The rationale for this choice of dose was expressed (in the unclassified portion of a classified document) as follows:

> The dose of atropine which the individual serviceman can be allowed to use must be a compromise between the dose which is therapeutically desirable and that which can be safely administered to a nonintoxicated person. Laboratory trials have shown that 2 mg of atropine sulfate is a reasonable amount to be recommended for injection by an individual and that higher doses may produce embarrassing effects on troops with operational responsibilities.

When given to a normal individual (one without nerve agent intoxication), a dose of 2 mg of atropine will cause an increase in heart rate of about 35 beats per minute (which usually is not noticed by the recipient), a dry mouth, dry skin, mydriasis, and some paralysis of accommodation. Most of these effects will dissipate by 4 to 6 hours, but near vision may be blurred for 24 hours, even in healthy young men. The decrease in sweating caused by 2 mg of atropine is a major, potentially harmful side effect that may cause some people who work in the heat to become casualties. For example, when 35 soldiers were given 2 mg of atropine and asked to walk for 115 minutes at 3.3 mph at a temperature of about 83°F (71°F wet bulb), more than half dropped out because of illness or were removed from the walk because of body temperature of 103.5°F or above; on another day, without atropine, they all successfully completed the same march.[129]

The 6 mg of atropine contained in the three injectors given each soldier may cause mild mental aberrations (such as drowsiness or forgetfulness) in some individuals if administered in the absence of nerve agent intoxication. Atropine given intravenously to healthy young people causes a maximal increase in the heart rate in 3 to 5 minutes, but other effects (such as drying of the mouth and change in pupil size) appear later. In one study,[130] when atropine was administered with the AtroPen, the greatest degree of bradycardia occurred at 2.5 minutes (compared with 4.3 min when administered by standard needle-and-syringe injection); a heart rate increase of 10 beats per minute occurred at 7.9 minutes (vs 14.7 min with needle-and-syringe injection); and maximal tachycardia (an increase of 47 beats per min) occurred at 34.4 minutes (compared with an increase of 36.6 beats per min at 40.7 min with needle-and-syringe injection).

Thus, the autoinjector is not only more convenient to use than the needle and syringe, but its use causes more rapid absorption of the drug.

Needle-and-syringe delivery produces a “glob” or puddle of liquid in muscle. The AtroPen, on the other hand, sprays the liquid throughout the muscle as the needle goes in. The greater dispersion of the AtroPen deposit results in more rapid absorption. It has not been determined whether the onset of beneficial effects in treating nerve agent intoxication corresponds to the onset of bradycardia, the onset of tachycardia, or to other factors.

When administered in an adequate amount, atropine reverses the effects of the nerve agent in tissues that have muscarinic receptor sites. It decreases secretions and reverses the spasm or contraction of smooth muscle. The mouth dries, secretions in the mouth and bronchi dry, bronchoconstriction decreases, and gastrointestinal musculature will be less hyperactive. However, unless given in very large doses, intravenous or intramuscular atropine does not reverse miosis caused by nerve agent vapor in the eyes. A casualty with miosis alone should not be given atropine, therefore, and pupil size should not be used to judge the adequacy of atropine dosage. Whether atropine controls convulsions in humans is unclear.

The amount of atropine to administer is a matter of judgment. In a conscious casualty with mild-to-moderate effects who is not in severe distress, 2 mg of atropine should be given intramuscularly at 5- to 10-minute intervals until dyspnea and secretions are minimized. Usually no more than a total dose of 2 to 4 mg is needed. In an unconscious casualty, atropine should be given (a) until secretions are minimized (those in the mouth can be seen and those in the lungs can be heard by auscultation) and (b) until resistance to ventilatory efforts is minimized (atropine decreases constriction of the bronchial musculature and airway secretions). If the casualty is conscious, he will report less dyspnea, and if assisted ventilation is underway, a decrease in airway resistance will be noted. Secretions alone should not be the reason for administering more atropine if the secretions are diminishing and are not clinically significant. Mucus blocking the smaller airways may remain a hindrance despite adequate amounts of atropine. In severe casualties (unconscious and apneic), 5 to 15 mg of atropine has been used before spontaneous respiration resumed and the casualty regained consciousness (which occurred 30 min to 3 h after exposure).[18,63] Several recovering casualties have had non–life-threatening adverse effects (such as nausea and vomiting) 24 to 36 hours after exposure for which atropine was administered (personal observation).[18] However, there would appear to be no reason to give atropine routinely in this period.

In contrast, much larger amounts of atropine (500–1,000 mg) have been required in the initial 24 hours of treatment of individuals severely poisoned by organophosphorus pesticides.[131–133] *Medical care providers*

must recognize that the amount of atropine needed for treatment of insecticide poisoning is different from the amount needed for treatment of nerve agent poisoning. Pesticides may be sequestered in the body or metabolized at a slower rate than nerve agents; whatever the reason, they continue to cause acute cholinergic crises for a much longer period (days to weeks).

The goal of therapy with atropine should be to minimize the effects of the agent (ie, to remove the casualty from a life-threatening situation and to make him comfortable), which may not require complete reversal of all of the effects (such as miosis). However, in a casualty with severe effects, it is better to administer too much atropine than too little. Too much atropine does far less harm than too much unantagonized nerve agent in a casualty suffering severe effects. However, a moderately dyspneic casualty given atropine 2 mg, administered intramuscularly, will report improvement within 5 minutes. A caregiver should resist the temptation to give too much atropine to a walking, talking casualty with dyspnea. In general, the correct dose of atropine for an individual exposed to a nerve agent is determined by the casualty's signs and symptoms, the route of exposure (vapor or liquid), and the amount of time elapsed since exposure.

Atropine Therapy After Inhalational Exposure to Vapor

After vapor exposure, the effects of nerve agents appear very quickly and reach their maximum activity within seconds or minutes after the casualty is removed from or protected against the vapor. In what were apparently high concentrations of nerve agent vapor, two individuals collapsed, unconscious, almost immediately after taking one or two breaths, and 4 to 5 minutes later they were flaccid and apneic.[18,63] Even at very low concentrations, maximal effects occur within minutes of termination of exposure. Because effects develop so rapidly, antidotal therapy should be more vigorous for a casualty seen during or immediately after exposure than for a casualty seen 15 to 30 minutes later. For example, if a soldier's buddy in the field or a coworker in a laboratory suddenly complains of dim vision in an environment suspected of containing nerve agent vapor, the buddy or worker should immediately administer the contents of one MARK I antidote kit. There may be continuing exposure before the casualty can exit the environment or don a mask, or the effects from the exposure already absorbed may continue to develop for several minutes. On the other hand, if the casualty is seen at the medical aid station (installation or field) 15 to 30 minutes after the vapor exposure has terminated, an antidote is not needed if miosis is the only sign (atropine given intramuscularly has very little effect on miosis).

Effects caused by nerve agent vapor will not progress after this time.

As a general rule, if a casualty is seen immediately after exposure from vapor only, the contents of one MARK I kit should be given if miosis is the only sign, the contents of two kits should be administered immediately if there is any dyspnea, and the contents of three kits should be given for severe dyspnea or any more-severe signs or symptoms. When seen 15 to 30 minutes after an exposure to vapor alone, the casualty should receive no antidote if miosis is the only sign, the contents of one MARK I kit for mild or moderate dyspnea, the contents of two kits for severe dyspnea (obvious gasping), and the contents of three kits and diazepam (with additional atropine, but no more oxime) if there are more serious signs (such as collapse or loss of consciousness). If dyspnea is the most severe symptom, relief should begin within 5 minutes, and the drugs should not be repeated until this interval has passed. Remember that the aggressive therapy given immediately after the onset of effects is not for those early effects per se (eg, atropine is relatively ineffective against miosis), but is in anticipation of more-severe effects within the following minutes.

Atropine Therapy After Dermal Exposure to Liquid

The therapy for an individual whose skin has been exposed to nerve agent is less clear. The onset of effects is rarely immediate; they may begin within minutes of exposure or as long as 18 hours later. As a general rule, the greater the exposure, the sooner the onset; and the longer the interval between exposure and onset of effects, the less severe the eventual effects will be. Effects can begin hours after thorough decontamination; the time of onset may be related to the duration of time the agent was in contact with the skin before decontamination.

The problem with treating dermal exposure is not so much *how* to treat a symptomatic casualty as *whether* to treat an asymptomatic person who has had agent on the skin. Medical personnel usually have little or no information about the exposure incident, because the casualty often does not know the duration or amount of exposure.

The first effects of agent on the skin are localized sweating and fasciculations of underlying musculature (rippling), which usually are not observed. If these effects are noted, however, the casualty should immediately self-administer or be given the contents of one MARK I kit. These signs indicate that the chemical agent has penetrated the skin layers.

In general, an asymptomatic person who has had skin contact with a nerve agent should be kept under medical observation, because effects may begin precipitately hours later. Caregivers should not administer the contents of a MARK I kit to an asymptomatic person, but should wait for

evidence of agent absorption. However, if an individual is seen minutes after a definite exposure to a large amount of nerve agent on the skin ("large" is relative; the LD_{50} for skin exposure to VX is only 6–10 mg, which is equivalent to a single drop 2–3 mm in diameter), there may be some benefit in administering antidotes before the onset of effects. When the occurrence of exposure is uncertain, the possible benefits of treatment must be weighed against the side effects of antidotes in an unpoisoned individual.

Antidotes should be administered until ventilation is adequate and secretions are minimal. In a mildly to moderately symptomatic individual who is complaining of dyspnea, relief is usually obtained with 2 or 4 mg of atropine (the amount of atropine in one or two MARK I kits). In a severely exposed person who is unconscious and apneic or nearly apneic, at least 6 mg of atropine (the amount in three MARK I kits), and probably more, should be administered initially, and ventilatory support should be started. Atropine should be continued at appropriate intervals until the casualty is breathing adequately with a minimal amount of secretions in the mouth and lungs. The initial 2 or 4 mg has proven adequate in conscious casualties. Although 6 to 15 mg has been required in apneic or nearly apneic casualties, the need for continuing atropine has not extended beyond 2 to 3 hours (although distressing but not life-threatening effects, such as nausea and vomiting, have necessitated administering additional atropine in the following 6–36 h). This is in contrast to the use of atropine to treat intoxication by organophosphorus insecticides, which may cause cholinergic crises (such as an increase in secretion and bronchospasm) for days to weeks after the initial insult.[131–133]

Oxime Therapy

Oximes are nucleophilic substances that reactivate the organophosphate-inhibited ChE (the phosphorylated enzyme) by removing the organophosphoryl moiety. There are limitations to oxime therapy, however.

Mechanism of Action

After the organophosphorus compound attaches to the enzyme to inhibit it, one of the following processes may occur:

- the enzyme may be spontaneously reactivated by hydrolytic cleavage, which breaks the organophosphoryl–ChE bond, reactivating the enzyme; or

- the organophosphoryl–ChE bond may “age,” or become resistant to reactivation by water or oxime.

Both of these processes are related to the size of the alkyl group attached to the oxygen of the organo-phosphorus compound, the group attached to the first carbon of this alkyl group, and other factors. Once the organophosphoryl–enzyme complex ages, it cannot be broken by an oxime. (Further discussion of the chemical process can be found elsewhere; for a brief discussion, see Chapter 6, Pretreatment for Nerve Agent Exposure; for more detailed information, see Koelle.[11]) Oxime therapy is not effective after aging occurs.

Because the nerve agents differ in structure, their rates of spontaneous reactivation and aging differ. For example, when complexed with VX, RBC-ChE spontaneously reactivates at a rate of roughly 0.5% to 1% per hour for about the first 48 hours; the VX– enzyme complex ages very little during this period.[42,45,83] The soman–enzyme complex does not spontaneously reactivate; the half-time for aging is about 2 minutes. The half-time for aging of the sarin–RBC-ChE complex is about 5 hours, and a small percentage (5%) of the enzyme undergoes spontaneous reactivation.[83] The half-time for aging of the tabun–enzyme complex is somewhat longer. (See Table 6-1 in Chapter 6, Pretreatment for Nerve Agent Exposure, for nerve agent–aging times.)

In the mid 1950s, Wilson and coworkers reported that hydroxamine reactivated organophosphoryl-inhibited ChE faster than water did,[134] and later reported that an oxime (pyridine-2-aldoxime meth-iodide [2-PAMI]) was far more effective than hydroxamine in reactivating the enzyme.[135]

The oximes differ in their required doses, their toxicity, and their effectiveness (for example, TMB4 is more effective against tabun poisoning than is 2-PAM Cl). After thorough study of many of these compounds, 2-PAM Cl was chosen for use in the United States. The choice was made because of research in both the civilian and military sectors and also because of the demonstrated efficacy of 2-PAM Cl in treating organophosphorus insecticide poisoning.[136–142] At present, the only oxime approved by the Food and Drug Administration for use in the United States is 2-PAM Cl. The methanesulfonate salt of pralidoxime (P2S) is the standard oxime in the United Kingdom, whereas TMB4 and Toxogonin (obidoxime) are used in other European countries.

Since oximes reactivate the ChE inhibited by a nerve agent, they might be expected to completely reverse the effects caused by nerve agents. However, because nerve agents possibly produce biological activity by mechanisms other than inhibition of ChE or because of reasons not understood, oximes are relatively ineffective in reversing effects in organs

with muscarinic receptor sites. They are much more effective in reversing nerve agent–induced changes in organs with nicotinic receptor sites. In particular, when oximes are effective (ie, in the absence of aging), they decrease the abnormality in skeletal muscle, improving strength and decreasing fasciculations.

Dosage

The therapeutic dosage of 2-PAM Cl has not been established, but indirect evidence suggests that it is 15 to 25 mg/kg. The effective dose depends on the nerve agent, the time between poisoning and oxime administration, and other factors. An early study[143] showed that a plasma concentration of about 4 µg/mL in blood reversed the sarin-induced neuromuscular block in anesthetized cats; for years this concentration was generally accepted as being therapeutic for sarin. There are few data to support or disprove this contention. The 2-PAM Cl administered with the ComboPen autoinjector (600 mg) produces a maximal plasma concentration of 6.5 µg/mL when injected intramuscularly in the average soldier (8.9 mg/kg in a 70-kg man).[130]

Different doses of 2-PAM Cl were administered (with atropine) in several studies. In sarin-poisoned rabbits, the protective ratio (PR: the ratio of the LD_{50} with treatment to the LD_{50} without treatment) increased from 25 to 90 when the intravenous dose of 2-PAM Cl increased from 5 mg/kg to 10 mg/kg[144]; the PR increased from 1.6 to 4.2 when the intramuscular dose of 2-PAM Cl increased from 30 mg/kg to 120 mg/kg in sarin-poisoned rats[122]; and the PR increased from 1.9 to 3.1 when the intramuscular dose of 2-PAM Cl increased from 11.2 mg/kg to 22.5 mg/kg in VX-poisoned rabbits.[123] (In the first two studies, the antidote was given immediately after the nerve agent; in the third, it was given at the onset of signs. No ventilatory support was used.) In humans, when 2-PAM Cl was administered intravenously 1 hour after sarin, a dose of 10 mg/kg reactivated 28% of the RBC-ChE, and doses of 15 or 20 mg/kg reactivated 58% of the enzyme. When given 3 hours after sarin, 5 mg/kg of 2-PAM Cl reactivated only 10% of the inhibited RBC-ChE, and 10 mg/kg or more reactivated more than 50%. When 2-PAM Cl was given at times from 0.5 to 24 hours after VX, doses of 2.5 to 25 mg/kg were found to reactivate 50% or more of the inhibited enzyme.[83]

For optimal therapy, 2-PAM Cl should be given intravenously, but usually this is not possible in the field. Even at small doses (2.5–5.0 mg/kg), the drug, when given intravenously in the absence of nerve agent poisoning, may cause transient effects, such as dizziness and blurred vision, which increase as the dose increases. Transient diplopia may

occur at doses higher than 10 mg/kg. These effects, if they occur, are insignificant in a casualty poisoned with a ChE-inhibiting substance. Occasionally, nausea and vomiting may occur. The most serious side effect is hypertension, which is usually slight and transient at intravenous doses of 15 mg/kg or less, but may be marked and prolonged at higher doses.[145] 2-PAM Cl is commercially available as the cryodesiccated form (Protopam Chloride, manufactured by Wyeth-Ayerst Laboratories, Philadelphia, Pa.) in vials containing 1 g, or about 14 mg/kg for a 70-kg person. Blood pressure elevations greater than 90 mm Hg systolic and 30 mm Hg diastolic may occur after administration of 45 mg/kg, and the elevations may persist for several hours.[145] Giving the oxime slowly (over 30–40 min) may minimize the hypertensive effect, and the hypertension can be quickly but transiently reversed by phentolamine 5 mg, administered intravenously (Figure 5-6).

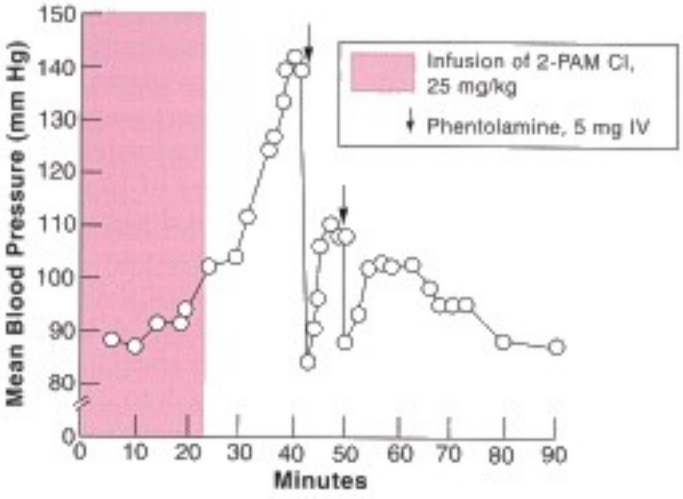


Fig. 5-6. An infusion of 25 mg/kg of pralidoxime chloride (2-PAM Cl) over about 25 minutes produces marked hypertension, which is rapidly but transiently reversed by phentolamine 5 mg. The mean blood pressure is the diastolic plus one third of the difference between the systolic and the diastolic.

2-PAM Cl is rapidly and almost completely excreted unchanged by the kidneys: 80% to 90% of an intramuscular or intravenous dose is excreted in 3 hours,[146] probably by an active tubular excretory mechanism (its renal clearance is close to that of paminohippurate[147]), with a half-time of about 90 minutes.[146] Both clearance and amount excreted are decreased by heat, exercise, or both.[148] Thiamine also decreases excretion (presumably by blocking tubular excretion), prolongs the plasma half-life, and increases the plasma concentration for the duration of thiamine activity[147–150]; some[151] question the therapeutic benefit of thiamine, however.

An early clinical report[152] on the use of 2-PAM Cl in insecticide-poisoned persons indicated that the oxime reversed the CNS effects of the poison (eg, patients regained consciousness and stopped convulsing shortly after the oxime was given). However, other early investigators found no oxime in the brain of animals[153,154] or the cerebrospinal fluid of humans[155] after experimental administration of 2-PAM Cl. Other

investigators[71,156] found small amounts of 2-PAM Cl or reversal of the brain ChE inhibition in brains of animals poisoned with organo-phosphorus compounds.

Administration

Initially, an oxime should be administered with atropine. In cases of severe exposure, the contents of three MARK I kits should be administered; if the kits are not available, then oxime 1 to 1.5 g should be administered intravenously over a period of 20 to 30 minutes or longer. Additional atropine should be given to minimize secretions and to reduce ventilatory problems, thereby relieving the casualty's distress and discomfort.

Since an improvement in the skeletal muscle effects of the agent (ie, an increase or decrease in muscle tone and reduced fasciculations) may be seen after oxime administration, medical personnel may be tempted to repeat the oxime along with atropine. Because of side effects, however, no more than about 2.5 g of oxime should be given within 1 to 1.5 hours. If the oxime is effective, it can be repeated once or twice at intervals of 60 to 90 minutes.

2-PAM Cl can be administered intravenously, intramuscularly, and orally. Soon after it became commercially available, 2-PAM Cl was administered orally both as therapy and as a pretreatment for those in constant contact with organophosphorus compounds (eg, crop dusters). At one time, the United Kingdom provided its military personnel with a supply of oxime tablets for pretreatment use, but it no longer does so. Enthusiasm for this practice waned for a number of reasons:

- erratic absorption of the drug from the gastrointestinal tract, leading to large differences (both between individuals and in the same person at different times) in plasma concentration;
- the large dose required (5 g to produce an average plasma concentration of 4 µg/mL);
- the unpopularity of the large, bitter 0.5-g or 1.0-g tablets; and
- the relatively slow absorption compared with that for administration by other routes.

In addition, the frequent administration (every 4–6 h) required by workers at risk caused gastrointestinal irritation, including diarrhea. It is also no longer common practice for crop workers to be given 2-PAM Cl as a pretreatment, the rationale being that crop workers who take the medication might have a false sense of security and therefore might tend

to be careless with safety measures.

Despite these drawbacks, 2-PAM Cl tablets might be the best alternative in certain cases, such as a depot worker exposed to a nerve agent who shows no effects except for an inhibition of RBC-ChE activity. An oxime might be given to restore his RBC-ChE activity to 80% of his baseline value, which is necessary for his return to work. (See Blood Cholinesterases section, above, for discussion of monitoring RBC-ChE activity.) Administration by the oral route might be considered preferable (although less reliable) to administration by a parenteral route because tablets can be self-administered and taking tablets avoids the pain of an injection.

Intramuscular administration of 2-PAM Cl with the ComboPen results in a plasma concentration of 4 µg/kg at 7 minutes versus 10 minutes for conventional needle-and-syringe injection.[130] (A maximal plasma concentration of 6.9 µg/kg occurs at 19 min vs 6.5 µg/kg at 22 min for the needle-and-syringe method.) About 80% to 90% of the intact drug is excreted unmetabolized in the urine; the half-life is about 90 minutes. When a 30% solution of 2-PAM Cl was injected intramuscularly at doses ranging from 2.5 to 30 mg/kg, the drug caused no change in heart rate or any signs or symptoms (except for pain at the injection site, as expected after an injection of 2 mL of a hypertonic solution).[146,147] When given intramuscularly, 30 mg/kg caused an elevation in blood pressure and minimal ECG changes, but no change in heart rate.[146]

Because of the very rapid aging of the soman–AChE complex, oximes are often considered ineffective in treating soman poisoning. Experimental studies in animals have shown that oximes are not nearly as effective in treating soman intoxication as in sarin intoxication, but they do provide some therapeutic benefit (a 5%–10% reactivation of the inhibited enzyme).[157,158] Suggested reasons for this benefit are that an oxime acts as a cholinergic blocking drug at the nicotinic sites, analogous to atropine at the muscarinic sites[157] or that it causes the circulation to improve, possibly by stimulating the release of catecholamines.[158]

Anticonvulsive Therapy

Convulsions occur after severe nerve agent exposure. In reports[18,63,81] of severe cases, convulsions (or what were described as “convulsive jerks” or “spasms”) started within seconds after the casualty collapsed and lost consciousness, and persisted for several minutes until the individual became apneic and flaccid. The convulsions did not recur after atropine and oxime therapy and ventilatory support were administered. In these instances, no specific anticonvulsive therapy was needed nor was it given.

Laboratory studies indicate that the convulsive period lasts much longer (hours) in animals, even those given therapy. However, the antidotes are given in a standard dose to experimental animals rather than titrated to a therapeutic effect as they are in human patients; this difference may account for the greater duration of convulsions in animals. In animals, convulsions occur more frequently and are more severe when the animal is pretreated with pyridostigmine and given nerve agent followed by standard therapy than when no pyridostigmine pretreatment is used. For these reasons, it is anticipated that humans pretreated with pyridostigmine would also have more frequent and more severe convulsions when pretreated with pyridostigmine than when not pretreated with pyridostigmine.

Diazepam has been used successfully to terminate convulsions caused by organophosphate insecticide poisoning (see discussion of behavioral effects above) and has been fielded in the U.S. military. As discussed earlier, each soldier is issued one autoinjector (ComboPen) containing 10 mg of diazepam in 2 mL of diluent. When a soldier exposed to a nerve agent is unable to help himself, a buddy should administer diazepam as well as the contents of three MARK I kits—whether or not there are indications of seizure activity. In fact, it is preferable to administer diazepam before the onset of seizure activity. The medic carries additional diazepam injectors and is authorized to administer two additional injectors to a convulsing casualty at 10-minute intervals. Current military doctrine is for the buddy to administer the diazepam immediately following the administration of the third MARK I when the three MARK I kits are given together. This is not only military doctrine, it is sound medical advice, and this action should be taken automatically when assisting a casualty with severe exposure to organophosphate nerve agents.

Therapy for Cardiac Arrhythmias

Transient arrhythmias occur after nerve agent intoxication and after atropine administration in a normal individual. However, the irregularities generally terminate after the onset of atropine-induced sinus tachycardia

(see discussion of cardiac effects, above).

Experimental studies[117,159] have shown that when animals are poisoned with ChE inhibitors and then allowed to become cyanotic, rapid intravenous administration of atropine will cause ventricular fibrillation. Ventricular fibrillation after rapid intravenous administration of atropine has not been reported in humans.

After severe intoxication from exposure to an organophosphate insecticide, a 20-year-old patient was stabilized with atropine and ventilatory support, but her ECG showed depression of the ST segment and flattening of the T wave, presumably because of persistent sinus tachycardia secondary to large doses of atropine (287 mg in 4 days; total of 830 mg). She was given a ß-adrenergic blocking agent (propranolol), which slowed the heart rate to 107 beats per minute, normalizing the ST-T changes. The normal ECG pattern and heart rate of 107 beats per minute persisted despite repeated doses of atropine. In effect, a pharmacologically isolated heart (with both cholinergic and adrenergic blockade) was produced; the authors reporting on the case suggested that propranolol might be of value in protecting against the effects of atropine and organophosphorus intoxication.[160]

SPECIFIC TREATMENT BY EXPOSURE CATEGORY

The goals of medical therapy are, in most cases, fairly straightforward: to minimize the patient's discomfort, to relieve distress, and to stop or reverse the abnormal process. These goals are the same in the treatment of a patient with nerve agent intoxication.

If a patient has severe dyspnea or vomiting (or retching), he or she may be unable to vocalize, but it can be assumed that the discomfort is severe. Therapy should be titrated against the complaints of dyspnea and objective manifestations such as retching; administration of the contents of MARK I kits (or atropine alone) should be continued at intervals until relief is obtained. Seldom are more than two to three MARK I kits required to provide relief. Because eye or head pain is not relieved by MARK I injections, a patient with severe eye or head pain from miosis will complain when he has no other injury that causes more overwhelming discomfort. Topical application of atropine or homatropine is quite effective in relieving this severe pain.

The signs of severe distress in a fellow soldier, such as twitching, convulsions, gasping for breath, and apnea, can be recognized by even a relatively untrained observer. A casualty's buddy will usually act appropriately, but because a buddy's resources are few, the level of assistance is limited: a buddy can administer three MARK I kits and diazepam and then seek medical assistance. In a more sophisticated

setting, adequate ventilation is the highest priority, but even the best ventilators help little in the presence of copious secretions and high resistance in the airways. Atropine must be given until secretions (nose, mouth, airways) are decreased and resistance to assisted ventilation is minimal.

The goals of therapy must be realistic. Current drugs will not immediately restore consciousness or respiration or completely reverse the skeletal muscle abnormalities, nor will intramuscular or intravenous drug therapy reverse miosis. Muscular fasciculations and small amounts of twitching may continue in a conscious patient long after adequate ventilation is restored and the patient is walking and talking.

Although in practice exposure categories are never clear-cut, different therapeutic measures are recommended for treating nerve agent casualties who have different degrees of exposure severity. Treatment is based on the signs and symptoms caused by the particular exposure (Table 5-7). The following suggested exposure categories are based on the casualty's presenting signs and symptoms.

Suspected Exposure

Suspected, but unconfirmed, exposure to a nerve agent sometimes occurs in an area where liquid agent was present. A person without signs or symptoms may be unsure whether he is contaminated. In such cases, the suspected casualty should be thoroughly and completely decontaminated and kept under close medical observation for 18 hours. If a laboratory facility is available, blood should be drawn for measurement of RBC-ChE activity.

An individual working with nerve agent in an industrial or laboratory environment will have a baseline RBC-ChE activity value on record. If this value is still at baseline after a possible exposure, then no significant absorption has occurred, and the new value provides confirmation of the baseline. (See Blood Cholinesterases section, above, on RBC-ChE activity monitoring.) If the activity is decreased, however, then absorption of the agent has occurred, but the decision to begin therapy should be based on signs or symptoms, not on the RBC-ChE activity (with one possible exception: an asymptomatic worker with decreased ChE activity; see Oxime Therapy section, above). The medical care provider must remember that the nadir of RBC-ChE activity may not occur for 18 to 24 hours, and if there has been no oxime therapy, then the final sample for analysis must be drawn during that time period.

Since the onset of effects caused by nerve agent exposure may occur as long as 18 hours after skin contact, prolonged observation is prudent. The longer the interval until the onset of signs and symptoms, the less

severe they will be, but medical assistance will still be necessary. Since vapor (or inhaled aerosol) causes effects within seconds or minutes, it is extremely unlikely that a “suspected” asymptomatic casualty would be produced by this route.

TABLE 5-7
RECOMMENDED THERAPY FOR CASUALTIES OF NERVE AGENTS

Exposure Route	Exposure Category	Signs and Symptoms	Therapy
Inhalational (Vapor)			
	Minimal	Miosis with or without rhinorrhea; reflex nausea and vomiting	< 5 min of exposure: 1 MARK I kit > 5 min of exposure * : observation
	Mild	Miosis; rhinorrhea; mild dyspnea; reflex nausea and vomiting	< 5 min of exposure: 2 MARK I kits > 5 min of exposure: 0 or 1 MARK I kit, depending on severity of dyspnea
	Moderate	Miosis; rhinorrhea; moderate to severe dyspnea; reflex nausea and vomiting	< 5 min of exposure: 3 MARK I kits + diazepam > 5 min of exposure: 1–2 MARK I kits

	Moderately severe	Severe dyspnea; gastrointestinal or neuromuscular signs	3 MARK I kits; standby ventilatory support; diazepam
	Severe	Loss of consciousness; convulsions; flaccid paralysis; apnea	3 MARK I kits; ventilatory support, suction; diazepam
Dermal (Liquid on Skin)			
	Mild	Localized sweating, fasciculations	1 MARK I kit
	Moderate	Gastrointestinal signs and symptoms	1 MARK I kit
	Moderately severe	Gastrointestinal signs plus respiratory or neuromuscular signs	3 MARK I kits; standby ventilatory support
	Severe	Same as for severe vapor exposure	3 MARK I kits; ventilatory support, suction; diazepam

*Casualty has been out of contaminated environment during this time.

Minimal Exposure

Miosis, with accompanying eye symptoms, and rhinorrhea are signs of a minimal exposure to a nerve agent, either vapor or vapor and liquid. This distinction is quite important in the management of this casualty. There are many situations in which one can be reasonably certain that exposure was by vapor alone (if the casualty was standing downwind from a munition or container, for example, or standing across a laboratory or storeroom from a spilled agent or leaking container). On the other hand, if an unprotected individual is close to an agent splash or is walking in areas where liquid

agent is present, exposure may be by both routes. Effects from vapor exposure occur quickly and are at their maximum within minutes, whereas effects from liquid agent on the skin may not occur until hours later.

Atropine (and oxime) should not be given for miosis because it is ineffective in the usual doses (2 or 4 mg). If eye pain (or head pain) is severe, topical atropine or homatropine should be given. However, the visual blurring caused by atropine versus the relatively small amount of visual impairment caused by miosis must be considered. If the rhinorrhea is severe and troublesome, atropine (the 2 mg contained in one MARK I kit) may give some relief.

If liquid exposure is suspected, the patient must be kept under observation, as noted above. If liquid exposure can be excluded, there is no reason for prolonged observation.

Mild Exposure

An individual with mild or moderate dyspnea and possibly with miosis, rhinorrhea, or both can be classified as having a mild exposure to nerve agent. The symptoms indicate that the casualty has been exposed to a nerve agent vapor and may or may not have been contaminated by a liquid agent.

If an exposed person in this category is seen within several minutes after exposure, he should receive the contents of two MARK I kits immediately. If 5 to 10 minutes have passed since exposure, the contents of only one kit should be given immediately. If no improvement occurs within 5 minutes under either circumstance, the casualty should receive the contents of another MARK I kit. The contents of an additional kit may be given if the casualty’s condition worsens 5 to 10 minutes later, but it is unlikely that it will be needed. Only three oxime autoinjectors should be given; further therapy should be with atropine alone.

A person having mild exposure to a nerve agent should be thoroughly decontaminated (exposure to vapor alone does not require decontamination) and have blood drawn for measurement of RBC-AChE activity prior to MARK I administration if facilities are available for the assay. As noted above, if there is reason to suspect liquid exposure, the casualty should be observed longer.

Moderate Exposure

A casualty who has had moderate exposure to either a nerve agent vapor alone or to vapor and liquid will have severe dyspnea, with accompanying physical signs, and probably also miosis and rhinorrhea. The casualty should be thoroughly decontaminated (REMEMBER:

exposure to vapor alone does not require decontamination) and blood should be drawn for assay of RBC-ChE activity if assay facilities are available. The contents of three MARK I kits and diazepam should be given if the casualty is seen within minutes of exposure. If seen later than 10 minutes after exposure, the casualty should receive the contents of two kits. Additional atropine should be given at 5- to 10-minute intervals until the dyspnea subsides. No more than three MARK I kits should be used; however, additional atropine alone should be administered if the contents of three kits do not relieve the dyspnea after 10 to 15 minutes. If there is reason to suspect liquid contamination, the patient should be kept under observation for 18 hours.

Nausea and vomiting are frequently the first effects from liquid contamination; the sooner after exposure they appear, the more ominous the outlook. Therapy should be more aggressive when these symptoms occur within an hour after exposure than when there is a longer delay in onset. If the onset is about an hour or less from the known time of liquid exposure, the contents of two MARK I kits should be administered initially, and further therapy (the contents of MARK I kits to a total of three, then atropine alone) given at 5- to 10-minute intervals, with a maximum of three oxime injections. If the onset is several hours after the time of known exposure, the contents of one MARK I kit should be given initially, and additional MARK I kits as needed to a total of three. Atropine alone should be used after the third MARK I. If the time of exposure is unknown, the contents of two MARK I kits should be administered.

Nausea and vomiting that occur several hours after exposure have been treated successfully with 2 or 4 mg of atropine, and the symptoms did not recur. However, the exposure was single-site exposure (one drop at one place). It is not certain that this treatment will be successful if exposure is from a splash or from environmental contamination with multiple sites of exposure on the skin. Therefore, casualties with this degree of exposure should be observed closely for at least 18 hours after the onset of signs and symptoms.

Moderately Severe Exposure

In cases of moderately severe exposure, the casualty will be conscious and have one or more of the following signs and symptoms: severe respiratory distress (marked dyspnea and objective signs of pulmonary impairment such as wheezes and rales), marked secretions from the mouth and nose, nausea and vomiting (or retching), and muscular fasciculations and twitches. Miosis may be present if exposure was by vapor, but it is a relatively insignificant sign as a guideline for therapy in this context.

The contents of three MARK I kits should be administered immediately. Preferably, if the means are available, 2 or 4 mg of atropine should be given intravenously, and the remainder of the total amount of 6 mg of atropine, along with the three oxime injections, should be given intramuscularly. The anticonvulsant diazepam should always be given when the contents of three MARK I kits are administered together. The casualty should be thoroughly decontaminated and have blood drawn for AChE assay before oxime is given.

Again, knowledge of the route of exposure is useful in planning further treatment. If the exposure was by vapor only and the casualty is seen in a vapor-free environment some minutes later, drug therapy should result in improvement. If the casualty has not lost consciousness, has not convulsed, and has not become apneic, he should improve. If the exposure was the result of liquid agent or a combination of liquid and vapor, there may be a reservoir of unabsorbed agent in the skin; despite the initial therapy, the casualty's condition may worsen. In either case, medical care providers should be prepared to provide ventilatory assistance, including adequate suction, and additional drug therapy (atropine alone) if there is no improvement within 5 minutes after intravenous administration of atropine, or 5 to 10 minutes after intramuscular administration of atropine.

The triad of consciousness, lack of convulsive activity, and spontaneous respiration is an indicator of a good outcome, provided adequate therapy is given early.

Severe Exposure

A casualty who is severely exposed to a nerve agent will be unconscious. He may be apneic or gasping for air with marked cyanosis, and may be convulsing or postictal. The casualty will have copious secretions from the mouth and nose and will have generalized fasciculations in addition to convulsive or large-muscle twitching movements. If the casualty is postictal, he may be flaccid and apneic.

If the casualty shows no movement, including no signs of respiration, the initial response should be to determine if the heart is beating. This is not an easy task when the rescuer and the casualty are both in full mission-oriented protective posture (MOPP 4) gear, but it must be accomplished because a nonmoving, nonbreathing casualty without a heartbeat is not a candidate for further attention on the battlefield. In a medical treatment facility, the medical personnel may be slightly more optimistic and proceed with aggressive therapy. After the sarin release in the Tokyo, Japan, subways, several casualties who were not breathing and who had no cardiac activity were taken to a hospital emergency department. Because

of very vigorous and aggressive medical management, one or two of these casualties were able to walk out of the hospital several days later.

Despite the circumstances, self-protection from contamination from the patient is important. Since decontamination of the patient may not be the first priority, caregivers must wear appropriate protective equipment until they have an opportunity to decontaminate the casualty and to remove him and themselves from the contaminated area.

The success of therapy under these circumstances is directly proportional to the viability of the casualty's cardiovascular system. If the heart rate is very slow or nonexistent or if there is severe hypotension, the chances for success are poor, even in the best possible circumstances.

First, medical personnel must provide oxygenation and administer atropine by a technique that ensures it will be carried to the heart and lungs. If ventilatory assistance is not immediately available, the best treatment is to administer the contents of three MARK I kits and diazepam. If ventilatory assistance will be forthcoming within minutes, the contents of the three MARK I kits should be administered whether the circulation is intact or not. When there is no chance of rapid ventilatory assistance, little is gained by MARK I therapy, but an attempt at treatment should be made anyway.

In the case of a failed or failing cardiovascular system, routes of atropine administration other than intramuscular should be considered. The intravenous route generally provides the fastest delivery of the drug throughout the body, but it is not without danger in an apneic and cyanotic patient. Whether or not concomitant ventilatory support can be provided, military medical personnel might want to consider administering atropine intratracheally by needle and syringe, if available, or with the atropine autoinjector (the AtroPen). Even if the casualty's systemic blood pressure is low, the peribronchial circulation may still have adequate blood flow to carry the drug to vital areas. If an endotracheal tube can be inserted, atropine could be injected into the tube either by needle and syringe or with the injector.

For severely exposed casualties, the initial dose of atropine should be at least the 6 mg from the three autoinjectors, but an additional 2 mg or 4 mg should also be given intravenously—if the capability is available and if the casualty is not hypoxic (ventilatory support must be started before intravenous atropine is given). If additional atropine cannot be given intravenously, then the amount should be given intramuscularly. The total initial dose of atropine can be as much as 10 mg, but this dose should not be exceeded without allowing at least several minutes for a response. Further atropine administration depends on the response. If secretions decrease or if there are attempts at breathing, it might be prudent to wait even longer before administering additional atropine. All three injectors of

2-PAM Cl should be given with the initial 6 mg of atropine, but no more oxime should be given for an hour.

Possibly the most critical factor in treatment of severely exposed casualties is restoration of oxygenation. Atropine alone might restore spontaneous breathing in a small number of apneic individuals. Ideally, an apparatus that delivers oxygen under positive pressure will be available. Even an RDIC or a mask-valve-bag apparatus used with ambient air will provide some assistance.

When the contents of three MARK I kits are administered together to a severely poisoned casualty, diazepam should be administered with the contents of the third MARK I—whether or not there are indications of seizure activity. The risk of respiratory depression from this amount of diazepam given intramuscularly is negligible.

Hypotension per se need not be treated, at least initially. Generally the restoration of oxygenation and the increase in heart rate caused by atropine, aided perhaps by the hypertensive effects of 2-PAM Cl, will cause the blood pressure to increase to an acceptable level.

Even with adequate oxygenation and large amounts of atropine, immediate reversal of all of the effects of the nerve agent will not occur. The casualty may remain unconscious, without spontaneous respiration and with muscular flaccidity or twitching, for hours. Even after respiration is at least partly spontaneous, secretions are minimized, and the casualty is partly alert, close monitoring is necessary. Muscular fasciculations may continue for hours after the casualty is alert enough and has strength enough to get out of bed.

RETURN TO DUTY

Various factors should be considered before an individual who has been a nerve agent casualty is returned to duty. In an industrial setting (depot or laboratory), the criteria for reactivation are that the individual's RBC-ChE activity must have returned to within 80% of its baseline value and that the individual is otherwise symptom- and sign-free.

In a military field setting, however, ChE-activity measurements are not available, and the need to return the fighting soldier to duty may be more acute. The decision is largely a matter of judgment and should include the following considerations:

- If exposed to nerve agent again, will the soldier be in greater danger because of the previous exposure?
- How well can the soldier function?
- What is the military need for the soldier?

In the absence of blood ChE measurements, it is difficult to predict whether a soldier would be at greater risk from a second nerve agent exposure. Even an individual with rather mild effects (miosis and rhinorrhea) may have marked ChE inhibition. On the other hand, if an oxime (contained in the MARK I kit) was given and the agent was one susceptible to oxime therapy, then the enzyme activity may be restored. In a field setting, neither the identity of the agent nor the degree of ChE inhibition or restoration will be known. In any case, proper use of MOPP 4 gear should protect against further exposure. If the soldier is able and needed, he should be returned to duty.

A soldier who has had signs of severe exposure, with loss of consciousness, apnea, and convulsions, may have milder CNS effects for many weeks after recovery from the acute phase of intoxication. Except in dire circumstances, return to duty during this period should not be considered for such casualties.

An individual with relatively mild effects (miosis, dyspnea, rhinorrhea) may be returned to duty within 1 to several days or even hours, depending on the assignment and the military need. However, the soldier may experience visual problems in dim light and may have mental lapses for as long as 6 to 8 weeks[18] (and personal observation); these factors must be considered before returning a soldier to duty. Several observations support this conclusion. In one case, troops who were symptomatic (miosis, rhinorrhea, dyspnea) as a result of nerve agent exposure carried out maneuvers (including firing weapons) in a satisfactory, although suboptimal, manner. They did not do nearly as well at night because of visual problems.[79]

In another instance, workers in an industrial operation learned the effects of the agent after they had accidentally been exposed several times. They also learned that it was a bigger problem to seek medical aid (with the ensuing administrative processes) than to continue working in the presence of symptoms. They stopped going to the aid station if they noted the onset of only mild effects. These workers were generally not in positions requiring acute vision or complex decisions; it is not known how well they performed while symptomatic. However, they could continue to perform their jobs, and their supervisors apparently did not notice a decrement (personal observation).

The need for soldiers in a frontline military operation may require that every walking casualty be returned to duty. In an otherwise asymptomatic casualty, the primary limiting factors will be (1) the soldier’s visual acuity compared with the visual demands of the job and (2) the soldier’s mental status compared with the intellectual demands of the job. Prolonged mental changes can be subtle and may require a careful examination to

detect.

SUMMARY

Nerve agents are the most toxic chemical warfare agents known. They cause effects within seconds and death within minutes. These agents are in the military stockpiles of several countries but have been used in only one war. They can be manufactured by terrorist groups and have been used in terrorist attacks.

Nerve agents cause biological effects by inhibiting the enzyme AChE, causing an excess of the neurotransmitter to accumulate. Hyperactivity in those organs innervated by cholinergic nerves results, with increased secretions from exocrine glands, hyperactivity of skeletal muscles leading to fatigue and paralysis, hyperactivity of smooth muscles with bronchoconstriction, and CNS changes, including seizure activity and apnea.

Therapy is based on the administration of atropine, which interferes with receptor binding of acetylcholine at muscarinic but not nicotinic receptors, and the oxime 2-PAM Cl, which breaks the agent–enzyme bond formed by most agents. Assisted ventilation and other supportive measures are also required in severe poisoning.

REFERENCES

1. Koelle GB. Anticholinesterase agents. In: Goodman LS, Gilman A, eds. *The Pharmacological Basis of Therapeutics*. 5th ed. New York, NY: Macmillan; 1975: 445.

2. Davis W. *The Serpent and the Rainbow*. New York: Warner Books Inc; 1985: 36–37.

3. Fraser TR. On the characters, actions, and therapeutic use of the ordeal bean of Calabar. *Edinb Med J*. 1863;9:124–132.

4. Holmstedt B. Structure–activity relationships of the organophosphorus anticholinesterase agents. In: Koelle GB, ed. *Cholinesterases and Anticholinesterase Agents*. Berlin, Germany: Springer Verlag; 1963: 429.

5. Harris R, Paxman J. *A Higher Form of Killing*. New York, NY: Hill and Wang; 1982: 53.

6. Robinson JP. *The Rise of CB Weapons*. Vol 1. In: *The Problem of Chemical and Biological Warfare.* New York, NY: Humanities Press; 1971: 71.

7. Kenneth W. Wilson, Directorate of Medical Research, Edgewood Arsenal, Md. Personal communications, mid to late 1960s.

8. Wills JH, DeArmon IA. *A Statistical Study of the Adamek Report*. Army Chemical Center, Md: Medical Laboratories; 1954. Medical Laboratory Special Report 54.

9. Program Executive Officer—Program Manager for Chemical Demilitarization. *Chemical Stockpile Disposal Program: Final Programmatic Environmental Impact Statement*. Aberdeen Proving Ground, Md: Program Manager for Chemical Demilitarization; Jan 1988. Publication A3, vol 3.

10. Smith RJ. Army poison gas stockpile raises worries in Kentucky. *Washington Post*. 1989;Jan 22:1, 9.

11. Koelle GB. Anticholinesterase agents. In: Goodman LS, Gilman A, eds. *The Pharmacological Basis of Therapeutics*. 4th ed. New York, NY: Macmillan; 1970: 446.

12. O'Neill JJ. Non-cholinesterase effects of anticholinesterases. *Fundam Appl Toxicol*. 1981;1:154–160.

13. Albuquerque A, Alaike APS, Rickett DL. The interaction of anticholinesterase agents with acetylcholine receptor-ionic channel complex. *Fundam Appl Toxicol*. 1984;4:527–533.

14. Koelle GB. Protection of cholinesterase against irreversible inactivation by di-isopropyl fluorophosphate in vitro. *J Pharmacol Exp Ther*. 1946;88:232–237.

15. Koster R. Synergisms and antagonisms between physostigmine and di-isopropyl fluorophosphate in cats. *J Pharmacol Exp Ther*. 1946;88:39–46.

16. Freedman AM, Willis A, Himwich HE. Correlation between signs of toxicity and cholinesterase level of brain and blood during recovery from di-isopropyl fluorophosphate (DFP) poisoning. *Am J Physiol*. 1948;157:80–87.

17. Oberst FW, Christensen MK. Regeneration of erythrocyte and brain cholinesterase activity in rats after sub-lethal exposures to GB vapor. *J Pharmacol Exp Ther*. 1956;116:216–219.

18. Sidell FR. Soman and sarin: Clinical manifestations and treatment of accidental poisoning by organophosphates. *Clin Toxicol*. 1974;7:1–17.

19. Evans ES Jr, Olds KL, Weyandt TB. Pesticides. In: Deeter DP, Gaydos JC, eds. *Occupational Health: The Soldier and the Industrial Base*. In: Zajtchuk R, Bellamy RF, eds. *Textbook of Military Medicine*. Washington, DC: US Department of the Army, Office of The Surgeon General, and Borden Institute; 1993: 532–536.

20. Hayes WJ. Organic phosphorus pesticides. In: Hayes WJ, ed. *Pesticides Studied in Man*. Baltimore, Md: Williams & Wilkins; 1982: 284–435.

21. Adler M, Filbert MG. Role of butyrylcholinesterase in canine tracheal smooth muscle function. *FEBS Lett*. 1990;267:107–110.

22. Kent KM, Epstein SE, Cooper T, Jacobowitz DM. Cholinergic innervation of the canine and human ventricular conducting system. *Circulation*. 1974:50:948–955.

23. Slavkov J, Vik J, Hlavickov V. Acetylcholinesterase and butyrylcholinesterase activity in the atria of the heart of adult albino rats. *Physiol Bohemoslov*. 1982;31:407–414.

24. Kalow W, Genest K. A method for the detection of atypical forms of human serum cholinesterase: Determination of dibucaine numbers. *Can J Biochem Physiol*. 1957;35:339–346.

25. Neitlich H. Increased plasma cholinesterase activity and succinylcholine resistance: A genetic variant. *J Clin Inves*t. 1966;45:380–386.

26. Johns RJ. Familial reduction in red cell cholinesterase. *N Engl J Med*. 1962;267:1344–1348.

27. Wetstone HJ, LaMotta RV. The clinical stability of serum cholinesterase activity. *Clin Chem*. 1965;11:653–663.

28. Sidell FR, Kaminskis A. Temporal intrapersonal physiological variability of cholinesterase activity in human plasma and erythrocytes. *Clin Chem*. 1975;21:1961–1963.

29. Shanor SP, van Hees GR, Baart N, Erdos EEG, Foldes FF. The influence of age and sex on human plasma and red cell cholinesterase. *Am J Med Sci*. 1961;242:357–361.

30. Sidell FR, Kaminskis A. Influence of age, sex, and oral contraceptives on human blood cholinesterase activity. *Clin Chem*. 1975;21:1393–1395.

31. Robertson GS. Serum protein and cholinesterase changes in association with contraceptive pills. *Lancet*. 1967;i:232–235.

32. Whittaker M. Charlier AR, Ramaswamy S. Changes in plasma cholinesterase isoenzyme due to oral contraceptives. *J Reprod Fertil*. 1971;26:373–375.

33. Callaway S, Davies DR, Rutland JP. Blood cholinesterase levels and range of personal variation in a healthy adult population. *Br Med J*. 1951;ii:812–816.

34. Augustinsson K. The normal variation of human blood cholinesterase activity. *Acta Physiol Scand*. 1955;35:40–52.

35. Ketchum JS, Sidell FR, Crowell EB Jr, Aghajanian GK, Hayes AH Jr. Atropine, scopolamine, and Ditran: Comparative pharmacology and antagonists in man. *Psychopharmacology (Berlin)*. 1973;28:121–145.

36. Grob D, Lilienthal JL Jr, Harvey AM, Jones BF. The administration of di-isopropyl fluorophosphate (DFP) to man, I: Effect on plasma and erythrocyte cholinesterase; general systemic effects; use in study of hepatic function and erythropoieses; and some properties of plasma cholinesterase. *Bull Johns*

Hopkins Hosp. 1947;81:217–244.

37. Rider JA, Moeller HC, Puletti EJ, Swader JI. Toxicity of parathion, systox, octamethyl pyrophosphoramide, and methyl parathion in man. *Toxicol Appl Pharmacol*. 1969;14:603–611.

38. Hayes GR, Funckes AJ, Hartwell WV. Dermal exposure of human volunteers to parathion. *Arch Environ Health*. 1964;8:829–833.

39. Edson EF. No-effect levels of three organophosphates in the rat, pig, and man. *Food Cosmet Toxicol*. 1964;2:311–316.

40. Rider JA, Puletti EJ, Swader JI. The minimal oral toxicity level for mevinphos in man. *Toxicol Appl Pharmacol*. 1975;32:97–100.

41. Sidell FR, Groff WA. The reactivatibility of cholinesterase inhibited by VX and sarin in man. *Toxicol Appl Pharmacol*. 1974;27:241–252.

42. Sim VM. *Variability of Different Intact Human Skin Sites to the penetration of VX*. Edgewood Arsenal, Md: Medical Research Laboratory; 1962. Chemical Research and Development Laboratory Report 3122.

43. Grob D, Harvey JC. Effects in man of the anticholinesterase compound sarin (isopropyl methyl phosphonofluoridate). *J Clin Invest*. 1958;37:350–368.

44. Grob D, Harvey AM. The effects and treatment of nerve gas poisoning. *Am J Med*. 1953;14:52–63.

45. Sim VM, Stubbs JL. *VX Percutaneous Studies in Man*. Edgewood Arsenal, Md: Medical Research Laboratory; 1960. Chemical Research and Development Laboratory Report 3015.

46. Craig FN, Cummings EG, Sim VM. Environmental temperature and the percutaneous absorption of a cholinesterase inhibitor, VX. *J Invest Dermatol*. 1977;68:357–361.

47. Ministry of Defence. *Cholinesterase as an Aid in the Early Diagnosis of Nerve Gas Poisoning. Part II: The Variation of Blood Cholinesterase in Man Before and After the Administration of Very Small Quantities of G Vapor by Inhalation*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

48. Harvey JC. *Clinical Observations on Volunteers Exposed to Concentrations of GB*. Edgewood Arsenal, Md: Medical Research Laboratory; 1952. Medical Laboratory Research Report 144.

49. Craig AB, Woodson GS. Observations on the effects of exposure to nerve gas, I: Clinical observations and cholinesterase depression. *Am J Med Sci*. 1959;238:13–17.

50. Sidell RF. Clinical considerations in nerve agent intoxication. In: Somani SM, ed. *Chemical Warfare Agents*. New York, NY: Academic Press; 1992: 163.

51. Namba T, Nolte CT, Jackrel J, Grob D. Poisoning due to organophosphate

insecticides. *Am J Med*. 1971;59:475–492.

52. Johns RJ. *The Effects of Low Concentrations of GB on the Human Eye*. Edgewood Arsenal, Md: Medical Research Laboratory; 1952. Medical Laboratory Research Report 100.

53. Rengstorff RH. Accidental exposure to sarin: Vision effects. *Arch Toxicol*. 1985;56:201–203.

54. Stewart WC, Madill HD, Dyer AM. Night vision in the miotic eye. *Can Med Assoc J*. 1968;99:1145–1148.

55. Craig AB Jr, Freeman G. *Clinical Observations on Workers Accidentally Exposed to "G" Agents*. Edgewood Arsenal, Md: Medical Research Laboratory; 1953. Medical Laboratory Research Report 154.

56. Rubin LS, Krop S, Goldberg MN. Effect of sarin on dark adaptation in man: Mechanism of action. *J Appl Physiol*. 1957;11:445–449.

57. Rubin LS, Goldberg MN. Effect of tertiary and quaternary atropine salts on absolute scotopic threshold changes produced by an anticholinesterase (sarin). *J Appl Physiol*. 1958;12:305–310.

58. Trussov MS. Effects of eserine upon light sensitivity and dark adaption. *Oftalmol J Ukraine*. 1962;17:366–371.

59. Moylan-Jones RJ, Thomas DP. Cyclopentolate in treatment of sarin miosis. *Br J Pharmacol*. 1973;48:309–313.

60. Ministry of Defence. *An Evaluation of the Functional Changes Produced by the Inhalation of GB Vapour*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

61. Ministry of Defence. *Air-Way Resistance Changes in Men Exposed to GB Vapour*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

62. Clements JA, Moore JC, Johnson RP, Lynott J. *Observations on Airway Resistance in Men Given Low Doses of GB by Chamber Exposure*. Edgewood Arsenal, Md: Medical Research Laboratory; 1952. Medical Laboratory Research Report 122.

63. Ward JR. Case report: Exposure to a nerve gas. In: Whittenberger JL, ed. *Artificial Respiration: Theory and Applications*. New York, NY: Harper & Row; 1962: 258–265.

64. De Candole CA, Douglas WW, Evans CL, et al. The failure of respiration in death by anticholinesterase poisoning. *Br J Pharmacol Chemother*. 1953;8:466–475.

65. Ministry of Defence. *The Predominantly Peripheral Effects of Acute GB Poisoning in Anaesthetised Animals*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

66. Johnson RP, Gold AJ, Freeman G. Comparative lung-airway resistance and cardiovascular effects in dogs and monkeys following parathion and sarin intoxication. *Am J Physiol*. 1958;192:581–584.

67. Fredriksson T, Hansson C, Holmstedt B. Effects of sarin in the anaesthetized and unanaesthetized dog following inhalation, percutaneous absorption and intravenous infusion. *Arch Int Pharmacodyn Ther*. 1960;126:288–302.

68. Wright PG. An analysis of the central and peripheral components of respiratory failure produced by anticho-linesterase poisoning in the rabbit. *J Physiol (Lond)*. 1954;126:52–70.

69. Rickett DL, Glenn JF, Beers ET. Central respiratory effects versus neuromuscular actions of nerve agents. *Neurotoxicology*. 1986;7:225–236.

70. Kluwe WM, Chinn JC, Feder P, Olson C, Joiner R. Efficacy of pyridostigmine pretreatment against acute soman intoxication in a primate model. In: *Proceedings of the Sixth Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute for Chemical Defense; 1987: 227–234. Report AD B121516.

71. Firemark H, Barlow CF, Roth LC. The penetration of 2-PAM-Cl^{14} into brain and the effects of cholinesterase inhibitors on its transport. *J Pharmacol Exp Ther*. 1964;145:252–265.

72. Brown EC Jr. *Effects of G Agents on Man: Clinical Observations*. Edgewood Arsenal, Md: Medical Laboratory; 1948. Medical Division Report 158.

73. Craig AB Jr, Cornblath M. *Further Clinical Observations in Workers Accidentally Exposed to G Agents*. Edgewood Arsenal, Md: Medical Research Laboratory; 1953. Medical Laboratory Research Report 234.

74. Brody BB, Gammill JF. *Seventy-Five Cases of Accidental Nerve Gas Poisoning at Dugway Proving Ground*. Dugway Proving Ground, Utah: Medical Investigational Branch; 1954. Medical Investigational Branch Special Report 5.

75. Bowers MB, Goodman E, Sim VN. Some behavioral changes in man following anticholinesterase administration. *J Nerv Ment Dis*. 1964;138:383–389.

76. Ministry of Defence. *Psychological Effects of a G-Agent on Men*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

77. Ministry of Defence. *Psychological Effects of a G-Agent on Men: 2nd Report*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

78. Ministry of Defence. *The Effects of a Single Exposure to GB (Sarin) on Human Physical Performance*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

79. Ministry of Defence. *The Effects of a Minor Exposure to GB on Military Efficiency*. United Kingdom: Ministry of Defence. Unpublished report, n.d.

80. Grob D, Harvey AM, Langworthy OR, Lilienthal JL Jr. The administration of di-isopropyl fluorophosphate (DFP) to man, III: Effect on the central nervous system with special reference to the electrical activity of the brain. *Bull Johns Hopkins Hosp*. 1947;81:257–266.

81. Grob D. The manifestations and treatment of poisoning due to nerve gas and other organic phosphate anticholinesterase compounds. *Arch Intern Med*. 1956;98:221–239.

82. Levin HS, Rodnitzky RL. Behavioral effects of organophosphate pesticides in man. *Clin Toxicol*. 1976;9:391–405.

83. Karczmar AG. Acute and long lasting central actions of organophosphorus agents. *Fundam Appl Toxicol*. 1984;4:S1–S17.

84. Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. Long-term effects of an organophosphate upon the human electroencephalogram. *Toxicol Appl Pharmacol*. 1979;47:161–176.

85. Lemercier G, Carpentier P, Sentenae-Roumanou H, Morelis R. Histological and histochemical changes in the central nervous system of the rat poisoned by an irreversible anticholinesterase organophosphorus compound. *Acta Neuropathol*. 1983;61:123–129.

86. Petras JM. Brain pathology induced by organophosphate poisoning with the nerve agent soman. In: *Proceedings of the Fourth Annual Chemical Defense Bioscience Review.* Aberdeen Proving Ground, Md: US Army Medical Research Institute for Chemical Defense; 1985: 407–414.

87. McLeod CG Jr, Singer AW, Harrington DG. Acute neuropathology in soman poisoned rats. *Neurotoxicology*. 1984;5:53–58.

88. Singer AW, Jaax NK, Graham JS, McLeod CG Jr. Cardiomyopathy in soman and sarin intoxicated rats. *Toxicol Lett*. 1987;36:243–249.

89. McDonough JH Jr, McLeod CG Jr, Nipwoda T. Direct microinjection of soman or VX into the amygdala produces repetitive limbic convulsions and neuropathology. *Brain Res*. 1987;435:123–137.

90. Raffaele K, Hughey D, Wenk G, Olton D, Modrow H, McDonough J. Long-term behavioral changes in rats following organophosphonate exposure. *Pharmacol Biochem Behav*. 1987;27:407–412.

91. McDonough JH Jr, Smith RF, Smith CD. Behavioral correlates of soman-induced neuropathology: Deficits in DRL acquisition. *Neurobehav Toxicol Teratol*. 1986;8:179–187.

92. Modrow HE, Jaax NK. Effect of soman exposure on the acquisition of an operant alternation task. *Pharmacol Biochem Behav*. 1989;32:49–53.

93. Blennow G, Brierley JB, Meldrum BS, Siesjo BK. Epileptic brain damage: The

role of systemic factors that modify cerebral energy metabolism. *Brain*. 1978;101:687–700.

94. Soderfeldt B, Blennow G, Kalimo H, Olsson Y, Siesjo BK. Influence of systemic factors on experimental epileptic brain injury. *Acta Neuropathol*. 1983;60:81–91.

95. Wall HG. Brain lesions in rhesus monkeys after acute soman intoxication. In: *Proceedings of the Sixth Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute for Chemical Defense; 1987: 155–162.

96. Lipp JA. Effect of benzodiazepine derivatives upon soman induced seizure activity and convulsions in the monkey. *Acta Int Pharmacodyn*. 1973;202:241–251.

97. Rump S, Grudzinska E, Edelwein Z. Effects of diazepam on abnormalities of bioelectrical activity of the rabbit's brain due to fluostigmine. *Act Nerv Super*. 1972;14:176–177.

98. Rump S, Grudzinska E, Edelwein Z. Effects of diazepam on epileptiform patterns of bioelectrical activity of the rabbit brain induced by fluostigmine. *Neuropharmacology*. 1973;12:815–819.

99. Martin LJ, Doebler JA, Shih T, Anthony A. Protective effect of diazepam pretreatment on soman-induced brain lesion formation. *Brain Res*. 1985;325:287–289.

100. McDonough JH Jr, Jaax NK, Crowley RA, Mays MZ, Modrow HE. Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. *Fundam Appl Toxicol*. 1989;13:256–276.

101. Hayward IJ, Wall HG, Jaax NK, Wade JV, Marlow DD, Nold JB. *Influence of Therapy With Anticonvulsant Compounds on the Effects of Acute Soman Intoxication in Rhesus Monkeys*. Aberdeen Proving Ground, Md: US Army Medical Research Institute for Chemical Defense; 1988. Technical Report 88-12.

102. *Proceedings of Workshop on Convulsions and Related Brain Damage Induced by Organophosphorus Agents*. Aberdeen Proving Ground, Md: US Army Medical Research Institute for Chemical Defense; 1990. USAMRICD-SP-90-02.

103. Meldrum BD. Metabolic factors during prolonged seizures and their relation to nerve cell death. In: Delgado-Escueta AV, Wasterlain CG, Treiman DM, Porter RJ, eds. *Advances in Neurology*. 34th ed. New York, NY: Raven Press; 1983: 261–275.

104. Nevander G, Ingvar M, Auer R, Siesjo BK. Status epilepticus in well-oxygenated rats causes neuronal necrosis. *Ann Neurol*. 1985;18:281–290.

105. Nevander G, Ingvar M, Auer R, Siesjo BK. Irreversible neuronal damage after short periods of status epilepticus. *Acta Physiol Scand*. 1984;120:155–157.

106. Meldrum BS, Brierley JB. Prolonged epileptic seizures in primates. *Arch Neurol*. 1973;28:10–17.

107. Soderfeldt B, Kalimo H, Olsson Y, Siesjo BK. Bicuculline-induced epileptic brain injury. *Acta Neuropathol*. 1983;62:87–95.

108. Orlowski JP, Erenberg G, Lueders H, Cruse RP. Hypothermia and barbiturate coma for refractory status epilepticus. *Crit Care Med*. 1984;12:367–372.

109. Aicardi J, Chevrie J. Consequences of status epilepticus in infants and children. In: Delgado-Escueta AV, Wasterlain CG, Treiman DM, Porter RJ, eds. *Advances in Neurology*. 34th ed. New York, NY: Raven Press; 1983: 115–124.

110. Oberst FW, Ross RS, Christensen MK, Crook JW, Cresthull P, Umland CW. Resuscitation of dogs poisoned by inhalation of the nerve gas GB. *Milit Med*. 1956;119:377–386.

111. Pazdernik TL, Cross RS, Giesler M, Samson FE, Nelson SR. Changes in local cerebral glucose utilization induced by convulsants. *Neuroscience*. 1985;14:823–835.

112. Kiss Z, Fazekas T. Arrhythmias in organophosphate poisoning. *Acta Cardiol*. 1979;5:323–330.

113. Hassler CR, Moutvic RR, Hamlin RL. Studies of the action of chemical agents on the heart. In: *Proceedings of the Sixth Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute for Chemical Defense; 1987: 551–554.

114. Hassler CR, Moutvic RR, Hobson DW, et al. Long-term arrhythmia analysis of primates pretreated with pyridostigmine, challenged with soman, and treated with atropine and 2-PAM. In: *Proceedings of the 1989 Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute for Chemical Defense; 1989: 479–482.

115. Ludomirsky A, Klein HO, Sarelli P, et al. Q-T prolongation and polymorphous (“torsade de pointes”) ventricular arrhythmias associated with organophosphorus insecticide poisoning. *Am J Cardiol*. 1982;49:1654–1658.

116. Freeman G. Ludemann H, Cornblath M, et al. *Cardiovascular and Respiratory Effects of Acute Parathion Poisoning in Dogs with Particular Regard to Ventricular Fibrillation*. Army Chemical Center, Md: Medical Laboratories; 1954. Medical Laboratory Research Report 303.

117. Kunkel AM, O’Leary JF, Jones AH. *Atropine-Induced Ventricular Fibrillation During Cyanosis Caused by Organophosphorus Poisoning*. Edgewood Arsenal, Md: Medical Research Laboratory; 1973. Edgewood Arsenal Technical Report 4711.

118. Bellet S. *Clinical Disorders of the Heart Beat*. Philadelphia, Pa: Lea & Febiger; 1963: 110.

119. Ganendran A. Organophosphate insecticide poisoning and its management. *Anaesth Intensive Care*. 1974;2:361–368.

120. Willems J, Vermeire P, Rolly G. Some observations on severe human poisonings with organophosphate pesticides. *Arch Toxicol*. 1971;28:182–191.

121. van Hooidonk C, Ceulen BI, Bock J, van Genernen J. CW agents and the skin: Penetration and decontamination. In: *Proceedings of the International Symposium on Protection Against Chemical Warfare Agents*. Stockholm, Sweden: FOA Reports; 1983: 153–160.

122. Davies DR, Green AL, Willey GL. 2-Hydroxyiminomethyl-N-methylpyridinium methanesulphonate and atropine in the treatment of severe organophosphate poisoning. *Br J Pharmacol*. 1959;14:5–8.

123. Sidell FR, Mershon MM, Savola RH, Schwartz HN, Wiles JS, McShane WP. *Treatment of Percutaneous VX Intoxication in Rabbits Under Conditions Simulating Self-Therapy in the Field*. Edgewood Arsenal, Md: Medical Research Laboratory; 1968. Technical Memorandum 114–22.

124. Ainsworth M, Shephard RJ. The intrabronchial distribution of soluble vapours at selected rates of gas flow. In: Davies CN, ed. *Inhaled Particles and Vapours*. New York, NY: Pergamon Press; 1961: 233–247.

125. Oberst FW. Factors affecting inhalation and retention of toxic vapors. In: Davies CN, ed. *Inhaled Particles and Vapours*. New York, NY: Pergamon Press; 1961: 249–265.

126. Oberst FW, Koon WS, Christensen MK, Crook JW, Cresthull P, Freeman G. Retention of inhaled sarin vapor and its effect on red blood cell cholinesterase activity in man. *Clin Pharmacol Ther*. 1968;9:421–427.

127. Fraser TR. An experimental research on the antagonism between the actions of physostigma and atropia. *Trans R Soc Edinb*. 1870;26:259–713.

128. Barrett, et al. Unpublished observations. Cited by: Wills JH. Pharmacological antagonists of the anticholinesterase agents. In: Koelle GB, ed. *Cholinesterase and Anticholinesterase Agents*. Berlin, Germany: Springer Verlag; 1963: 897.

129. Robinson S, Magenis TP, Minter DI, Harper H. The effects of varying doses of atropine on temperature regulation of men and dogs. In: Robinson S, ed. *The Physiological Effects of Atropine and Potential Atropine Substitutes*. Edgewood Arsenal, Md: Medical Research Laboratories; 1953. Medical Laboratory Contract Report 15.

130. Sidell FR, Markis JE, Groff WA, Kaminskis A. Enhancement of drug absorption after administration by an automatic injector. *J Pharmacokinet Biopharm*. 1974;2:197–210.

131. Vale JA, Meredith TJ, Heath A. High dose atropine in organophosphorus poisoning. *Postgrad Med J*. 1990;66:881.

132. Chew LS, Chee KT, Yeo JM, Jayaratnam FJ. Continuous atropine infusion in the management of organophosphorus insecticide poisoning. *Singapore Med J*. 1971;12:80–85.

133. LeBlanc FN, Benson BE, Gilg AD. A severe organophosphate poisoning requiring the use of an atropine drip. *Clin Toxicol*. 1986;24:69–76.

134. Wilson IB. Acetylcholinesterase, XI: Reversibility of tetra ethylpyrophosphate inhibition. *J Biol Chem*. 1951;190:111–117.

135. Wilson IB, Ginsburg S. A powerful reactivator of alkyl phosphate-inhibited acetylcholinesterase. *Biochim Biophys Acta* . 1955;18:168–170.

136. Quinby GE. Further therapeutic experience with pralidoximes in organic phosphorus poisoning. *JAMA*. 1964;187:202–206.

137. Quinby GE, Clappison GB. Parathion poisoning. *Arch Environ Health*. 1961;3:538–542.

138. Quinby GE, Loomis TA, Brown HW. Oral occupational parathion poisoning treated with 2-PAM iodide (2-pyridine aldoxime methiodide). *N Engl J Med*. 1963;268:639–643.

139. Rosen FS. Toxic hazards: Parathion. *N Engl J Med*. 1960;262:1243–1244.

140. Jacobziner H, Raybin HW. Parathion poisoning successfully treated with 2-PAM (pralidoxime chloride). *N Engl J Med*. 1961;265:436–437.

141. Funckes AJ. Treatment of severe parathion poisoning with 2-pyridine aldoxime methiodide (2-PAM). *Arch Environ Health*. 1960;1:404–406.

142. Durham WF, Hayes WJ Jr. Organic phosphorus poisoning and its therapy. *Arch Environ Health*. 1962;5:21–47.

143. Sundwall A. Minimum concentrations of N-methylpyridinium-2-aldoxime methane sulphonate (P2S) which reverse neuromuscular block. *Biochem Pharmacol*. 1961;8:413–417.

144. O'Leary JF, Kunkel AM, Jones AH. Efficacy and limitations of oxime-atropine treatment of organophosphorus anticholinesterase poisoning. *J Pharmacol Exp Ther*. 1961;132:50–57.

145. Calesnick B, Christensen JA, Richter M. Human toxicity of various oximes. *Arch Environ Health*. 1967;15:599–608.

146. Sidell FR, Groff WA. Intramuscular and intravenous administration of small doses of 2-pyridinium aldoxime methochloride to man. *J Pharm Sci*. 1971;60:1224–1228.

147. Swartz RD, Sidell FR. Renal tubular secretion of pralidoxime in man. *Proc Soc Exp Biol Med*. 1974;146:419–424.

148. Swartz RD, Sidell FR. Effects of heat and exercise on the elimination of pralidoxime in man. *Clin Pharmacol Ther*. 1973;14:83–89.

149. Josselson J, Sidell FR. Effect of intravenous thiamine on pralidoxime kinetics. *Clin Pharmacol Ther*. 1978;24:95–100.

150. Josselson J, Sidell FR. *Dose-Response Effects of Intravenous Thiamine Hydrochloride on Pralidoxime Pharmacokinetics in Man*. Edgewood Arsenal, Md: Biomedical Laboratory; 1977. EB-TR 116117.

151. Jeevarathinam K, Ghosh AK, Srinivasan A, Das Gupta S. Pharmacokinetics of pralidoxime chloride and its correlation to therapeutic efficacy against diisopropyl fluorophosphate intoxication in rats. *Pharmazie*. 1988;43:114–115.

152. Quinby GE. Further therapeutic experience with pralidoximes in organic phosphorus poisoning. *JAMA*. 1964;187:114–118.

153. Kewitz H, Nachmansohn D. A specific antidote against lethal alkylphosphate intoxication, IV: Effects in brain. *Arch Biochem Biophys*. 1957;66:271–283.

154. Loomis T. Distribution and excretion of pyridine-2-aldoxime methiodide (PAM), atropine and PAM in sarin poisoning. *Toxicol Appl Pharmacol*. 1963;5:489–499.

155. Jager BV, Stagg GN, Green N, Jager L. Studies on distribution and disappearance of pyridine-2-aldoxime methiodide (PAM) and of diacetyl monoxime (DAM) in man and in experimental animals. *Bull Johns Hopkins Hosp*. 1958;102:225–234.

156. de la Manche IS, Verge DE, Bouchard C, Coq H, Sentenac-Roumanou H. Penetration of oximes across the blood brain barrier: A histochemical study of the cerebral cholinesterase reactivation. *Experientia*. 1979;35:531–532.

157. Fleisher JH. Directorate of Medical Research, Biomedical Laboratory, Edgewood Arsenal, Md. Personal communication, 1970s.

158. von Bredow J. Major, Medical Service Corps, US Army; Directorate of Medical Research, Biomedical Laboratory, Edgewood Arsenal, Md. Personal communication, 1970s.

159. Wills JH, McNamara BP, Fine EA. Ventricular fibrillation in delayed treatment of TEPP poisoning. *Fed Proc*. 1950;9:136.

160. Valero A, Golan D. Accidental organic phosphorus poisoning: The use of propranolol to counteract vagolytic cardiac effects of atropine. *Isr J Med Sci*. 1967;3:582–584.

Chapter 6

PRETREATMENT FOR NERVE AGENT EXPOSURE

MICHAEL A. DUNN, M.D., FACP*; BRENNIE E. HACKLEY, JR., PH.D.†; AND FREDERICK R. SIDELL, M.D.‡

INTRODUCTION

AGING OF NERVE AGENT–BOUND ACETYLCHOLINESTERASE

PYRIDOSTIGMINE, A PERIPHERALLY ACTING CARBAMATE COMPOUND
- Efficacy
- Safety
- Wartime Use
- Improved Delivery

CENTRALLY ACTING NERVE AGENT PRETREATMENTS

NEW DIRECTIONS: BIOTECHNOLOGICAL PRETREATMENTS

SUMMARY

* *Colonel, Medical Corps, U.S. Army; Director, Clinical Consultation, Office of the Assistant Secretary of Defense (Health Affairs), Washington, D.C. 20301-1200; formerly, Commander, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425*

† *Scientific Advisor, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425*

‡ *Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical*

Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014

INTRODUCTION

Nerve agents are rapidly acting chemical compounds that can cause respiratory arrest within minutes of absorption. Their speed of action imposes a need for rapid and appropriate reaction by exposed soldiers, their buddies, or medics, who must administer antidotes quickly enough to save lives. A medical defense against nerve agents that depends completely on postexposure antidote treatment, however, has two key limitations:

- In the stress of a chemical environment, even well-trained military personnel will not be uniformly successful in performing such tasks as self- and buddy-administration of nerve agent antidotes.[1]
- Aging, a change over time in the interaction of nerve agents with the target enzyme acetylcholinesterase (AChE), renders oxime therapy (an important component of nerve agent antidotes) much less effective.[2] As explained below, aging poses an especially difficult problem for treating effects from the nerve agent soman.

Because of these limitations of postexposure protection, military physicians have focused on the possibility of protecting soldiers from nerve agents by medical prophylaxis, or pretreatment, designed to limit the toxicity of a subsequent nerve agent exposure. A significant problem with pretreatments, however, has been their own potential for adverse effects. In general, the pharmacological pretreatments that protect humans from the toxic effects of nerve agents are themselves neuroactive compounds. Thus, their principal adverse actions are neurological as well and may impair physical and mental performance. A pretreatment must be administered to an entire force under a nerve agent threat. Any resulting performance decrement, even a comparatively minor one, would make pretreatment use unacceptable in battlefield situations requiring maximum alertness and performance for survival.

In the late 1980s, the United States, following the example of Great Britain, stocked the compound pyridostigmine for its combat units as a wartime contingency pretreatment adjunct for nerve agent exposure.[3] Several other Allies, including most members of the North Atlantic Treaty Organization (NATO), did so as well. At the recommended dose,

pyridostigmine is free of performance-limiting side effects. Unfortunately, pyridostigmine by itself is ineffective as a pretreatment against subsequent nerve agent exposure and thus it is not a true pretreatment compound. Pyridostigmine pretreatment does provide greatly improved protection against soman exposure, however, when combined with postexposure antidote therapy. For this reason, pyridostigmine is classified as a pretreatment adjunct.

Research workers have attempted to develop true nerve agent pretreatments whose own neurotoxicity is balanced or diminished by coadministration of a pharmacological antagonist to their undesirable properties (eg, the carbamate compound physostigmine, which is administered in combination with a cholinolytic compound, such as scopolamine). The potential and the problems of this pretreatment approach are considered in this chapter, along with a new pretreatment concept that involves inactivating or binding nerve agents with scavenger macromolecules in the circulation.

AGING OF NERVE AGENT–BOUND ACETYLCHOLINESTERASE

Organophosphate nerve agents inhibit the active site of AChE, a key enzymatic regulator of cholinergic neurotransmission. As noted in Chapter 5, Nerve Agents, agent-bound AChE can be reactivated by a class of antidote compounds, the oximes, which remove the nerve agent molecule from the catalytic site of AChE.

During the attachment of the agent with the enzyme, a portion of the agent—the leaving group—breaks off. During a second, later reaction, one of the nerve agent's alkyl groups leaves: this is the process known as *aging*. The rate at which this dealkylation of the AChE-bound nerve agent molecule proceeds depends on the nature of the nerve agent. Table 6-1 shows the aging half-time of each of the five chemical compounds commonly considered to be nerve agents: tabun (GA), sarin (GB), soman (GD), GF, and VX.

TABLE 6-1
AGING HALF-TIME OF NERVE AGENTS

Nerve Agent	RBC-ChE Source	Aging Half-Time
GA (Tabun)	Human (in vitro)	>14 h[1]
	Human (in vitro)	13.3 h[2]
GB (Sarin)	Human (in vivo)	5 h[3]
	Human (in vitro)	3 h[1]
GD (Soman)	Marmoset (in vivo)	1.0 min[4]
	Guinea pig (in vivo)	7.5 min[4]
	Rat (in vivo)	8.6 min[4]
	Human (in vitro)	2–6 min[1]
GF	Human (in vitro)	40 h[1]
	Human (in vitro)	7.5 h[5]
VX	Human (in vivo)	48 h[3]

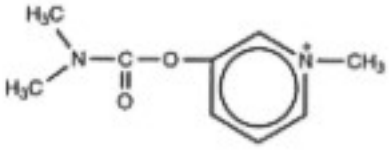
RBC-ChE: erythrocyte cholinesterase
Data sources: (1) Mager PP. *Multidimensional Pharmacochemistry*. San Diego, Calif: Academic Press; 1984: 52–53. (2) Doctor BP, Blick DW, Caranto G, et al. Cholinesterases as scavengers for organophosphorus compounds: Protection of primate performance against soman toxicity. *Chem Biol Interact*. 1993;87:285–293. (3) Sidell FR, Groff WA. The reactivatibility of cholinesterase inhibited by VX and sarin in man. *Toxicol Appl Pharm*. 1974;27:241–252. (4) Talbot BG, Anderson DR, Harris LW, Yarbrough LW, Lennox WJ. A comparison of in vivo and in vitro rates of aging of soman-inhibited erythrocyte acetylcholinesterase in different animal species. *Drug Chem Toxicol*. 1988;11:289– 305. (5) Hill DL, Thomas NC. R*eactivation by 2-PAM Cl of Human Red Blood Cell Cholinesterase Poisoned in vitro by Cyclohexylmethylphosphonofluoridate (GF)*. Edgewood Arsenal, Md: Medical Research Laboratory; 1969. Edgewood Arsenal Technical Report 43-13.

Aging is an irreversible reaction. After dealkylation, an AChE-bound nerve agent molecule can no longer be removed from the enzyme by an oxime. Thus, aging of enzyme-bound nerve agent prevents oxime antidotes from reactivating AChE. This is an extremely difficult problem in the case of poisoning with soman, which ages within 2 minutes.

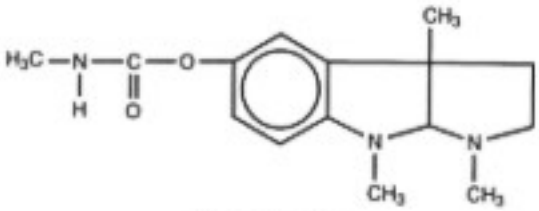
Aging appears to be a key limiting factor in the efficacy of postexposure oxime therapy for soman poisoning. One method for assessing the relative efficacy of antidotes and other countermeasures is the determination of their protective ratios. The protective ratio (PR) of an antidote is the factor by which it raises the LD_{50} or the LCt_{50} of a toxic nerve agent challenge. Readers will remember that LD_{50} is defined as the dose (D) of liquid or

solid nerve agent that is lethal (L) to 50% of the subjects exposed to it; LD_{50} is also described as the median lethal dose. LCt_{50} is the term used to describe the median lethal concentration for an aerosol or vapor agent, expressed as concentration (C) • time (*t*) of exposure (mg•min). For example, a PR of 1.0 would indicate a completely ineffective antidote, because it means that the LD_{50} or LCt_{50} is the same for subjects who received an antidote and those who did not. A PR of 5, on the other hand, indicates that the LD_{50} or LCt_{50} for subjects who received an antidote is 5-fold higher than that for subjects who did not receive one. A PR of 5 or greater is considered to represent a reasonable level of effectiveness for medical countermeasures against nerve agents. This value was determined through threat analysis of battlefield conditions and consideration of the fact that trained and equipped soldiers will be able to achieve at least partial protection against nerve agent attacks by rapid donning of masks and use of chemical protective clothing.

PYRIDOSTIGMINE, A PERIPHERALLY ACTING CARBAMATE COMPOUND



Pyridostigmine



Physostigmine

Pyridostigmine is one of a class of neuroactive compounds called carbamates. Its chemical structure and that of a related carbamate, physostigmine, are shown above. Like the nerve agents, carbamates inhibit the enzymatic activity of AChE. As a quaternary amine, pyridostigmine is ionized under normal physiological conditions and penetrates poorly into the central nervous system (CNS). Pyridostigmine has been used for many years in the therapy of neurological disorders, especially myasthenia gravis, a disease of neuro-muscular transmission. In patients with myasthenia gravis, inhibition of synaptic AChE is clinically beneficial.

As an inhibitor of AChE, pyridostigmine in large doses mimics the peripheral toxic effects of the organophosphate nerve agents. At first it might seem paradoxical that carbamate compounds should help protect against nerve agent poisoning, but two critical characteristics of the carbamate–enzyme bond explain the usefulness of the carbamates.

First, carbamoylation, the interaction between carbamates and the

active site of AChE, is freely and spontaneously reversible, unlike the normally irreversible inhibition of AChE by the nerve agents. No oxime reactivators are needed to dissociate, or decarbamoylate, the enzyme from a carbamate compound. Carbamates do not undergo the aging reaction of nerve agents bound to AChE.

Second, carbamoylated AChE is fully protected from attack by nerve agents because the active site of the carbamoylated enzyme is not accessible for binding of nerve agent molecules. Functionally, sufficient excess AChE activity is normally present in synapses so that carbamoylation of 20% to 40% of the enzyme with pyridostigmine does not significantly impair neurotransmission.

When animals are challenged with a lethal dose of nerve agent, AChE activity normally decreases rapidly, becoming too low to measure. In pyridostigmine-pretreated animals with a sufficient quantity of protected, carbamoylated enzyme, spontaneous decarbamoylation of the enzyme regenerates enough AChE activity to sustain vital functions, such as neuromuscular transmission to support respiration. Prompt postexposure administration of atropine is still needed to antagonize acetylcholine (ACh) excess, and an oxime reactivator must also be administered if an excess of nerve agent remains to attack the newly uncovered AChE active sites that were protected by pyridostigmine.

Efficacy

Exposure of humans to soman is virtually unknown in Western countries, with the exception of a single laboratory accident.[4] The decision to provide military forces with pyridostigmine is therefore based on a series of animal efficacy studies[5–7] conducted with several species in a number of countries that found evidence that pyridostigmine pretreatment strongly enhances postexposure antidote therapy for soman poisoning.

Data from one experiment are shown in Table 6-2. In this study[7] with male rhesus monkeys, pretreatment with orally administered pyridostigmine inhibited circulating red blood cell AChE (RBC-AChE) by 20% to 45%. (Inhibition of RBC-AChE by pyridostigmine is a useful index of its inhibition of AChE in peripheral synapses). Monkeys that had no pyridostigmine pretreatment were not well protected from soman by the prompt administration of atropine and 2-pyridine aldoxime methyl chloride (2-PAM Cl). The PR of 1.64 in these monkeys is typical of the most effective known postexposure antidote therapy in animals not given pretreatment before a soman challenge. In contrast to this low level of protection, however, the combination of pyridostigmine pretreatment and prompt post-challenge administration of atropine and 2-PAM Cl resulted in greatly improved protection (PR > 40 when compared with the control

group; PR = 24 when compared with the group given atropine and 2-PAM Cl).[7]

TABLE 6-2
EFFECT OF THERAPY ON LD_{50} IN MONKEYS EXPOSED TO SOMAN

Group	Mean LD_{50} (µg/kg) [95% CL]	Mean Protective Ratio [95% CL]
Control (no treatment)	15.3 [13.7–17.1]	—
Postexposure atropine + 2-PAM Cl	25.1 [22.0–28.8]	1.64 [1.38–19.5]
Pyridostigmine pretreatment + postexposure atropine + 2-PAM Cl	> 617	>40*

* Indeterminate because of small number of subjects; PR relative to the atropine plus 2-PAM Cl group > 24 (617 ÷ 25.1)
CL: confidence limit (based on a separate slopes model)
LD_{50} : the dose that is lethal to 50% of the exposed population
PR: factor by which the LD_{50} of a nerve agent challenge is raised (in this experiment, the LD_{50} for group given therapy divided by the LD_{50} for control group)
2-PAM Cl: 2-pyridine aldoxime methyl chloride
Adapted from Kluwe WM. Efficacy of pyridostigmine against soman intoxication in a primate model. In: *Proceedings of the Sixth Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987: 233.

Limitation of the number of animals available for soman challenge at extremely high doses made accurate calculation of a PR indeterminate in this experiment. The PR was well in excess of 40, clearly meeting the requirement for effectiveness of 5-fold improved protection. In a later study,[8] four of five rhesus monkeys receiving pyridostigmine pretreatment and postexposure therapy of atropine and 2-PAM Cl survived for 48 hours after being challenged with soman at a level 5-fold higher than its LD_{50} .

Pyridostigmine pretreatment shows its strongest benefit (compared with atropine and oxime therapy alone) in animals challenged with soman and tabun and provides no benefit against challenge by sarin or VX.[9–11] Table 6-3 shows the PRs obtained in animals given atropine and oxime

therapy after challenge with the five nerve agents with and withoutpyridostigmine pretreatment.[9] As shown, pyridostigmine pretreatment is essential for improved survival against soman and tabun challenge. With sarin or VX, depending on the animal system studied, pyridostigmine causes either no change or a minor decrease in PRs, which still indicate strong efficacy of atropine and oxime therapy for exposure to these agents. The data for GF show no benefit from pyridostigmine pretreatment for mice and a small benefit for guinea pigs. The only published data[8] on protection of primates from GF show a PR of more than 5 with pyridostigmine pretreatment and atropine/oxime therapy, but a control group treated with atropine/oxime alone for comparison was not included. Clinical experts from all countries who have evaluated pyridostigmine have concluded from these data that it is an essential pretreatment adjunct for nerve agent threats under combat conditions, where the identity of threat agents is virtually never known with certainty.

Pyridostigmine was used to protect soldiers from an actual nerve agent threat in the Persian Gulf War. NATO Allies using pyridostigmine followed their national policies on chemical protection. British soldiers, for example, were ordered to take pyridostigmine for over a month while they were positioned near the Iraqi border. U.S. forces followed the doctrine of only using pyridostigmine when a nerve agent threat was assessed to be imminent by the responsible division- or corps-level commander. Thus, soldiers of the U.S. XVIII Airborne Corps took pyridostigmine for several days in January 1991 until it was determined that SCUD missiles fired against them did not have chemical loads. Later, U.S. ground forces attacking into Iraq and Kuwait used pyridostigmine only as long as the corps-level commanders on the ground considered the Iraqi chemical capability a threat.

U.S. and Allied decisions to use pyridostigmine followed established doctrine, taking into account Iraqi capabilities and intentions. Iraq was known to have substantial stocks of sarin and VX, for which pyridostigmine pretreatment is unnecessary, as discussed above. However, Iraq was also known to be keenly interested in acquiring any compounds that might defeat Allied protection, such as soman. The security of Warsaw Pact stocks of soman, for example, was a growing concern in 1990.

In 1990, it was also known that Iraq had begun large-scale production of GF, a laboratory compound that had not earlier been manufactured in weapons quantity. International restrictions on the purchase of chemical precursors of the better-known nerve agents may have led Iraq to acquire cyclohexyl alcohol, which it then was able to use to produce GF. Very limited data on medical protection against GF were not reassuring. Although GF's aging time with AChE was reported to be relatively long (see Table 6-1), unpublished information from Allied countries suggested

that postexposure atropine/oxime therapy in rodents exposed to GF did not protect against the effects of GF poisoning. As confirmed by the later studies shown in Table 6-3, atropine/oxime therapy only provided rodents with PRs in the range of 1.4 to 2.7. The only primate data available showed that rhesus monkeys given pyridostigmine pretreatment and atropine/oxime therapy uniformly survived a 5-LD_{50} challenge with GF.[8] Concern about Iraq's new GF capability, added to its known interest in acquiring soman, made Allied use of pyridostigmine a reasonable course of action.

TABLE 6-3
EFFECT OF THERAPY WITH AND WITHOUT PYRIDOSTIGMINE PRETREATMENT ON PROTECTIVE RATIOS IN ANIMALS EXPOSED TO NERVE AGENTS

		Protective Ratio	
Nerve Agent	**Animal Tested**	**Atropine + Oxime**	**Pyridostigmine + Atropine + Oxime**
GA (Tabun)	Rabbit	2.4	3.9[1]
	Mouse	1.3	1.7/2.1[*2]
	Guinea pig	4.4	7.8/12.1[*2]
	Rabbit	4.2	>8.5[3]
GB (Sarin)	Mouse	2.1	2.2/2.0[*2]
	Guinea pig	36.4	34.9/23.8[*2]
GD (Soman)	Mouse	1.1	2.5[4]
	Rat	1.2	1.4[5]
	Guinea pig	1.5	6.4/5.0[*6]
	Guinea pig	2.0	2.7/7.1[*7]
	Guinea pig	1.9	4.9[8]
	Guinea pig	1.7	6.8[9]
	Rabbit	1.4	1.5[1]
	Rabbit	2.2	3.1[4]
	Rabbit	1.9	2.8[3]
	Rhesus monkey	1.6	>40[10]

GF	Mouse	1.4	1.4[11]
	Guinea pig	2.7	3.4[11]
	Rhesus monkey	—	>5[12]
VX	Mouse	7.8	6.0/3.9*[2]
	Rat	2.5	2.1[5]
	Guinea pig	58.8	47.1/45.3*[2]

*Two doses of pyridostigmine were used.

Data sources: (1) Joiner RL, Dill GS, Hobson DW, et al. Task 87-35: Evaluating the efficacy of antidote drug combinations against soman or tabun toxicity in the rabbit. Columbus, Oh: Battelle Memorial Institute; 1988. (2) Koplovitz I, Harris LW, Anderson DR, Lennox WJ, Stewart JR. Reduction by pyridostigmine pretreatment of the efficacy of atropine and 2-PAM treatment of sarin and VX poisoning in rodents. *Fundam Appl Toxicol*. 1992;18:102–106. (3) Koplovitz I, Stewart JR. A comparison of the efficacy of HI6 and 2- PAM against soman, tabun, sarin, and VX in the rabbit. *Toxicol Lett*. 1994;70:269–279. (4) Sultan WE, Lennox WJ. *Comparison of the Efficacy of Various Therapeutic Regimens, With and Without Pyridostigmine Prophylaxis, for Soman (GD) Poisoning in Mice and Rabbits.* Aberdeen Proving Ground, Md: US Army Chemical Systems Labororatory; 1983. ARCSL Technical Report 83103. (5) Anderson DR, Harris LW, Woodard CL, Lennox WJ. The effect of pyridostigmine pretreatment on oxime efficacy against intoxication by soman or VX in rats. *Drug Chem Toxicol*. 1992;15:285–294. (6) Jones DE, Carter WH Jr, Carchman RA. Assessing pyridostigmine efficacy by response surface modeling. *Fundam Appl Toxicol*. 1985;5:S242–S251. (7) Lennox WJ, Harris LW, Talbot BG, Anderson DR. Relationship between reversible acetylcholinesterase inhibition and efficacy against soman lethality. *Life Sci*. 1985;37:793–798. (8) Capacio BR, Koplovitz I, Rockwood GA, et al. *Drug Interaction Studies of Pyridostigmine With the 5HT3 Receptor Antagonists Ondansetron and Granisetron in Guinea Pigs*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1995. USAMRICD Training Report 95-05. AD B204964. (9) Inns RH, Leadbeater L. The efficacy of bispyridinium derivatives in the treatment of organophosphate poisoning in the guinea pig. *J Pharm Pharmacol*. 1983;35:427–433. (10) Kluwe WM. Efficacy of pyridostigmine against soman intoxication in a primate model. In: *Proceedings of the 6th Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987: 227–234. (11) Stewart JR, Koplovitz I. The effect of pyridostigmine pretreatment on the efficacy of atropine and oxime treatment of cyclohexylmethylphosphonofluoridate (CMPF) poisoning in rodents. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1993. Unpublished manuscript. (12) Koplovitz I, Gresham VC, Dochterman LW, Kaminskis A, Stewart JR. Evaluation of the toxicity, pathology, and treatment of cyclohexylmethlyphosphonofluoridate (CMFF) poisoning in rhesus monkeys. *Arch Toxicol*. 1992;66:622–628.

The fact that pyridostigmine inhibits AChE has raised one theoretical problem with its use: if 20% to 40% of AChE has been inhibited by pyridostigmine, would a subsequent low-level exposure to a nerve agent, which might be well tolerated with no pretreatment, be converted to a toxic dose if it raised the total percentage of AChE inhibition into a toxic range? In practice, it has not been possible to clearly demonstrate such additive toxicity in animal experiments, perhaps because the increase in nerve agent toxicity from initial signs to lethality rises very sharply over a narrow exposure range. A minor additive toxicity effect would therefore be difficult to detect. The signs of mild nerve agent exposure are easily managed with antidote therapy, and the benefit of a pretreatment in life-threatening exposures is so great as to clearly warrant pyridostigmine pretreatment for soldiers whose exact extent of nerve agent exposure is not predictable.

The fact that an ionized, hydrophilic carbamate compound such as pyridostigmine is effective as a pretreatment adjunct against soman suggests that its critical sites of action and, therefore, the critical sites where soman exerts its lethal effects, are outside the blood–brain barrier. As noted in Chapter 5, Nerve Agents, respiratory arrest after lethal nerve agent exposure appears to be a summation of the agent's effects on tracheobronchial secretions and bronchoconstriction with obstruction, impairment of neuromuscular transmission with respiratory muscle insufficiency, and direct depression of central respiratory drive. Electrophysiological monitoring suggests that of these processes, central respiratory drive may be the most susceptible to nerve agent toxicity.[12]

The effectiveness of pyridostigmine pretreatment may not be conclusive evidence against the importance of central mechanisms in respiratory arrest; it appears that there is at least partial permeability of the blood–brain barrier to polar compounds such as pyridostigmine, specifically in the regions of the fourth ventricle and brainstem, where respiratory centers are located. In addition, an increase in blood–brain barrier permeability occurs rapidly after soman administration.[13,14] The key observation remains that animals pretreated with pyridostigmine that receive atropine and oxime therapy promptly after an otherwise lethal soman exposure are able to maintain adequate respiration and survive.

The major deficiency of pyridostigmine pretreatment is also related to its poor penetration into the brain. Animals that survive challenge with a supralethal dose of nerve agent because of pyridostigmine pretreatment frequently show severe histological evidence of brain injury, prolonged convulsions, and long-lasting performance impairments.[15] Although centrally acting carbamate pretreatment compounds, such as physostigmine, offer a degree of protection against nerve agent–induced brain injury, pretreatment with known brain-protecting compounds such as physostigmine, the benzodiazepine anticonvulsants, and benactyzine has

not been acceptable because of their known decremental effects on performance. Postexposure anticonvulsant therapy appears to be the most practical, readily available approach to minimizing nerve agent–induced brain injury and promoting rapid recovery of normal function after severe nerve agent exposure (for further discussion, see Chapter 5, Nerve Agents).

Safety

Pyridostigmine has had a good safety record over the years of its administration to patients with myasthenia gravis. Known adverse reactions have been limited to infrequent drug rashes after oral administration and the complete set of signs of peripheral cholinergic excess, which have been seen only when the dosage in patients with myasthenia gravis was increased to AChE inhibition levels well beyond the 20% to 40% range desired for nerve agent pretreatment. The effects of excessive pyrido-stigminemiosis, sweating, intestinal hypermotility, and salivation—could clearly degrade soldiers' performance.

When the recommended adult dose of 30 mg of pyridostigmine bromide, one tablet orally every 8 hours, has been followed, no significant decrements have been found in the performance of a variety of military tasks. A review of British studies reported[16] that pyridostigmine caused no changes in memory, manual dexterity, vigilance, day and night driving ability, or in psychological tests for cognitive and psychomotor skills. No significant changes in sensory, motor, or cognitive functioning at ground level, at 800 ft, and at 13,000 ft were noted in 12 subjects in another study[17] after their fourth 30-mg dose of pyridostigmine.

The flight performance of subjects taking pyridostigmine in two studies[18,19] was not affected, no impairment in neuromuscular function was noted in a study[20] in which subjects took pyridostigmine for 8 days, and cardiovascular and pulmonary function were normal at high altitudes in pyridostigmine-treated subjects in another study.[21] However, one study[22] noted a slight decrement in performance in subjects taking pyridostigmine when they tried to perform two tasks at the same time; these subjects also had a slight decrement on a visual probability monitoring task. Two studies[23,24] found an increase in sweating and a decrease in skin blood flow in pyridostigmine-treated subjects subjected to heat/work stress.

Although there has been wide experience with long-term administration of pyridostigmine to patients with myasthenia gravis, until recently there was no comparable body of safety data in healthy young adults. Short-term pyridostigmine administration (one or two 30-mg doses) has been

conducted in peacetime in some countries, including the United States, to screen critical personnel, such as aircrew, for unusual or idiosyncratic reactions, such as drug rash. The occurrence of such reactions appears to be well below the 0.1% level, and no military populations are now routinely screened with administration of a test dose of pyridostigmine.

Pyridostigmine for military use by the United States is approved only as a wartime contingency measure. After the Persian Gulf War, there was much discussion about the use of pyridostigmine under an Investigational New Drug (IND) application.[25–32] The Food and Drug Administration (FDA) waived informed consent for its use to make the best medical treatment available in a specific combat situation.[26] The FDA based this waiver on *(a)* data from animal studies conducted in both the United States and other NATO countries that found that pyridostigmine increases survival when used as pretreatment against challenge by certain nerve agents (data on efficacy in humans challenged by nerve agents is not experimentally obtained), and *(b)* a long history of safety when the drug was used for approved indications at doses severalfold higher than the doses administered in the military. Rarely considered in postwar discussions was the ethical issue of nonuse: If pyridostigmine had not been used, and Iraq had used nerve agents causing large numbers of casualties, should the military have been held responsible for withholding this drug?

A limited number of animal studies of toxicological abnormalities and teratogenicity and mutagenicity in animals that were given pyridostigmine have had negative results (Hoffman-LaRoche, proprietary information).[33] In a study[34] in which pyridostigmine was administered to rats, either acutely or chronically, in doses sufficient to cause an average 60% AChE inhibition, ultrastructural alteration of a portion of the presynaptic mitochondria at the neuromuscular junction resulted, as well as alterations of nerve terminal branches, postsynaptic mitochondria, and sarcomeres. These morphological findings, which occurred at twice the AChE inhibition level desired in humans, have not been correlated with any evidence of functional impairment at lower doses, but they emphasize the need to limit enzyme inhibition to the target range of 20% to 40%. Pyridostigmine has been used by pregnant women with myasthenia gravis at higher doses and for much longer periods than it was used during the Persian Gulf War and has not been linked to fetal malformations.[35] Because safety in pregnancy has not been completely established, the Food and Drug Administration considers pyridostigmine a Class C drug (ie, the risk cannot be ruled out).

Several studies have sought information on pyridostigmine use under certain conditions: soldiers in combat who frequently take other medications; wounding and blood loss; and use while undergoing

anesthesia. The possible interaction of pyridostigmine with other commonly used battlefield medications was reviewed by Keeler.[36] There appears to be no pharmacological basis for expecting adverse interactions between pyridostigmine and commonly used antibiotics, anesthetics, and analgesic agents. In a study[37] of pyridostigmine-treated swine, for example, the autonomic circulatory responses to hemorrhagic shock and resuscitation appeared normal. One potentially important effect of pyridostigmine deserves consideration by field anesthesiologists and anesthetists using muscle relaxants for anesthesia induction: depending on the duration of muscle-relaxant administration, there may be either up- or down-regulation of postsynaptic ACh receptors.[36] Clinical assessment of the status of neuromuscular transmission using a peripheral nerve stimulator should provide a basis for adjusting the dose of both depolarizing and nondepolarizing muscle relaxants to avoid an undesirable duration of muscle paralysis.

Wartime Use

Pyridostigmine bromide tablets, 30 mg, to be taken every 8 hours, are currently maintained in warstocks of U.S. combat units. The compound is packaged in a 21-tablet blister pack called the nerve agent pyridostigmine pretreatment set (NAPPS, Figure 6-1). One NAPPS packet provides a week of pyridostigmine pretreatment for one soldier.[3]

The decision to begin pretreatment with pyridostigmine is made by commanders at army division level or the equivalent, based on assessment of the nerve agent threat by their chemical, intelligence, and medical staff officers.[3] Because of the lack of data on long-term administration of pyridostigmine to healthy adults, current doctrine calls for a maximum pretreatment period of 21 days, with reassessment at frequent intervals of the need for continued pretreatment. A senior commander's judgment about the severity of a nerve agent threat beyond 21 days determines whether pretreatment should continue.

Fig. 6-1. A pyridostigmine blister pack containing 21 30-mg tablets, along with the carrying sleeve. This is the nerve agent pyridostigmine pretreatment set (NAPPS) that was used by designated military personnel during the Persian Gulf War.

Pyridostigmine is poorly absorbed when taken orally; its bioavailability is 5% to 10%.[38] Ideally, two doses of pyridostigmine, taken 8 hours apart, should be administered prior to any risk of nerve agent exposure.[3] However, some benefit would be expected even if the first pyridostigmine dose is taken an hour before nerve agent exposure. Because excessive AChE inhibition can impair performance, no more than one 30-mg tablet should be taken every 8 hours. If a dose is forgotten or delayed, administration should simply be resumed on an 8-hour schedule as soon as possible, without making up missed doses.

In Operation Desert Storm in 1991, pyridostigmine was administered under combat conditions for the first time to U.S. and Allied soldiers thought to be at risk for nerve agent exposure. Data on safety and possible adverse responses were collected from the unit medical officers caring for the 41,650 soldiers of the XVIII Airborne Corps who took from 1 to 21 doses of pyridostigmine during January 1991.[39] Most major unit commanders continued the medication for 6 to 7 days, with over 34,000 soldiers taking it for that time. There was nearly total compliance with the regimen by these soldiers, who were fully aware of the nerve agent threat. They were able to perform their missions without any noticeable impairment, similar to findings with peacetime volunteers participating in studies.[16] However, they reported a higher than expected incidence of side effects, as noted in Table 6-4.

Gastrointestinal changes included flatus, loose stools, and abdominal cramps that were noticeable but not disabling. Together with urinary urgency, many soldiers reported a sense of awareness that they were taking a medication. In most soldiers, these changes were noticed within hours of taking the first tablet. In many, the effects subsided after a day or two of administration, and in others they persisted as long as pyridostigmine was administered. Some units adopted a routine of taking pyridostigmine with meals, which was thought to minimize gastrointestinal symptoms.

TABLE 6-4
EFFECTS OF PYRIDOSTIGMINE PRETREATMENT * ON U.S. SOLDIERS IN THE PERSIAN GULF WAR

Effect	Incidence (%) N=41,650
Gastrointestinal symptoms	≤ 50
Urinary urgency and frequency	5–30
Headaches, rhinorrhea, diaphoresis, tingling of extremities	<5
Need for medical visit	<1

Soldiers taking

Discontinuation on medical advice	<0.1

* Dose was 30 mg pyridostigmine bromide, administered orally every 8 h for 1 to 7 d. Adapted from Keeler JR, Hurst CG, Dunn MA. Pyridostigmine used as a nerve agent pretreatment under wartime conditions. *JAMA*. 1991;266:694.

pyridostigmine during this period were also experiencing a wide range of other wartime-related stresses, such as repeatedly donning and removing their chemical protective suits and masks in response to alarms, sleep deprivation, and anticipation of actual combat. Because there was no comparable group of soldiers undergoing identical stresses without taking pyridostigmine, it is not clear to what extent pyridostigmine itself was responsible for the symptoms noted above. The findings are thus a worst-case estimate for effects attributable to pyridostigmine use in wartime.

Among these soldiers, fewer than 1% sought medical attention for symptoms possibly related to pyridostigmine administration (483 clinic visits). Most of these had gastrointestinal or urinary disturbances. Two soldiers had drug rashes; one of them had urticaria and skin edema that responded to diphenhydramine. Three soldiers had exacerbations of bronchospasm that responded to bronchodilator therapy. Because the units of the XVIII Airborne Corps had been deployed to a desert environment for 5 months before pyridostigmine was used, most soldiers with significant reactive airways disease had already developed symptoms and had been evacuated earlier. The consensus among medical personnel more recently arrived was that they saw more pyridostigmine-related bronchospasm in their soldiers, who had not been present in theater as long.

Because of increased exposure to the work-of-breathing requirements of being masked, as well as inhaled dust, smoke, and particles, it was unclear whether pyridostigmine was a major causative factor in those who had bronchospasm at the onset of hostilities. Two soldiers from the XVIII Airborne Corps had significant blood pressure elevations, with diastolic pressures of 110 to 120 mm Hg, that manifested as epistaxis or persistent bleeding after a cut and subsided when pyridostigmine was stopped. Another soldier who took two pyridostigmine tablets together to make up a missed dose experienced mild cholinergic symptoms, self-administered an atropine autoinjector, and recovered fully after several hours. There were no hospitalizations or medical evacuations attributable to pyridostigmine among XVIII Airborne Corps soldiers. In other units, at least two female soldiers, both weighing approximately 45 to 50 kg, noted increased salivation, muscular twitching, severe abdominal cramps, and sweating that prompted medical observation. The symptoms subsided after pyridostigmine was stopped. This experience suggests that cholinergic

symptoms may occur in a small number of persons of relatively low body weight.

Later in the Persian Gulf War, more than 200,000 service members took pyridostigmine for 1 to 4 days during the ground attack into Iraq and Kuwait. Their medical experience, as personally reported to us by many unit medical officers, was similar to that reported above. It is now clear that pyridostigmine can be used effectively in large military populations under combat conditions without impairing mission performance. Soldiers must have a clear understanding of the threat and the need for this medication, however. Otherwise, it seems unlikely that they would have the same degree of willingness to accept the gastrointestinal and urinary symptoms noted above or to comply with an 8-hour dosage schedule.

In a group of 213 soldiers in Israel who took pyridostigmine (30 mg every 8 h), 75% reported at least one symptom. Included among these symptoms were excessive sweating (9%), nausea (22.1%), abdominal pain (20.4%), diarrhea (6.1%), and urinary frequency (11.3%). In a smaller group of 21 soldiers, pseudocholinesterase (also called butyrocholinesterase, which is discussed later in this chapter) activity was the same in the 12 who were symptomatic and the 9 who were not symptomatic.[40]

An Israeli soldier who developed cholinergic symptoms after taking pyridostigmine was reported[41] to have a genetic variant of serum butyrocholinesterase. The variant enzyme has low binding affinity for pyridostigmine and other carbamates. The authors of the report suggested that persons who are homozygous for the variant enzyme could therefore show exaggerated responses to anticholinesterase compounds. The soldier had a history of prolonged apnea after receiving succinylcholine premedication for surgery. Persons with similar histories of severe adverse responses to cholinergic medications should be carefully assessed concerning their potential deployability to combat, where they might face either a nerve agent threat or the potential need for resuscitative surgery involving emergency induction of anesthesia[36] using cholinergic medications.

Since the Persian Gulf War, veterans of that conflict have experienced a range of illnesses in themselves, in their spouses, and in children conceived after the conflict. Combinations of symptoms have included fatigue, skin rash, muscle and joint pain, headache, loss of memory, shortness of breath, and gastrointestinal and respiratory symptoms, which could be explained by a variety of conditions, but do not fit readily into a single diagnostic pattern.[42]

The possible interaction of multiple, potentially toxic compounds has generated interest in the context of these problems. With respect to pyridostigmine, one report[43] was published of neurotoxicity in chickens

that received pyridostigmine together with large parenteral doses of the insect repellent DEET (diethyltoluamide) and the insecticide permethrin. The relevance of this report is doubtful, because systemic administration of the two interacting compounds to the chickens was at least 10,000-fold in excess of their maximum potential absorption from skin or clothing of soldiers.

Both the National Institutes of Health and the National Institute of Medicine of the National Academy of Sciences established expert panels to evaluate these problems and to suggest an etiology or etiologies. Both panels held public hearings, which included testimony from veterans with the symptoms. The initial reports[44,45] of these panels found no evidence to suggest that pyridostigmine use was related to the problems reported.

Improved Delivery

The currently stocked 30-mg pyridostigmine bromide tablets were purchased for wartime contingency use because of their ready availability. Clearly, the need to maintain an 8-hour schedule of pyridostigmine pretreatment under the conditions of actual or anticipated combat stress is a major practical deficiency in our medical defense against nerve agents.

The United States is considering the development of sustained-release forms of pyridostigmine that would permit maintenance of an adequate level of AChE inhibition with once-daily oral administration. To date, however, no sustained-release preparation has shown sufficient promise to warrant advanced testing. Unfortunately as well, efforts to provide transdermal delivery of pyridostigmine with skin patches have had disappointing results, as would be expected because of the polar nature of the compound.

CENTRALLY ACTING NERVE AGENT PRETREATMENTS

The inability of pyridostigmine to provide protection against nerve agent–induced CNS injury has led to two different pharmacological approaches to protection. The first involves improving postexposure treatment with brain-protecting anticonvulsant compounds, such as benzodiazepines. While these compounds have a clear-cut, intrinsic potential for functional impairment and incapacitation, their administration to casualties who are already incapacitated by nerve agents will not increase the total number of casualties. In fact, clinical observation of nonhuman primates suggests that postexposure therapy with the benzodiazepines diazepam and midazolam actually decreases the time to recovery of consciousness after soman intoxication.[46]

An alternative to postexposure therapy is protection of the CNS with

pretreatment compounds that penetrate the blood–brain barrier, such as physostigmine, a tertiary amine that freely enters the CNS. Physostigmine is often used as a model compound for reproducing in laboratory animals the clinical signs of nerve agent intoxication. This nonpolar compound carbamoylates CNS AChE and protects experimental animals from nerve agent challenge more effectively than does pretreatment with pyridostigmine.[47] Another centrally acting carbamate compound, cui-xing-ning, with characteristics that are apparently similar to those of physostigmine, has been evaluated in China.[48]

Neuroactive compounds that penetrate the CNS generally cause some degree of performance impairment in experimental animals, as well as a variable incidence of symptoms, such as nausea and light-headedness, in humans. Even a slight degree of impaired performance of critical battlefield tasks would be life-threatening in itself and therefore would be unacceptable in a pretreatment to be administered to all combatants. A possible solution to this problem is antagonism of the undesirable effects of carbamates, which are generally cholinergic in nature, by simultaneous administration of a cholinolytic pretreatment adjunct, such as atropine, scopolamine, or trihexyphenidyl (Artane, manufactured by Lederle Laboratories, Wayne, NJ). Animals treated with what has been called a behavior-deficit-free combination of physostigmine and a cholinolytic compound, for example, show excellent protection against subsequent nerve agent challenge and rapid clinical recovery of normal function.[49]

In theory, it is possible to offset the side effects of physostigmine and achieve a performance-deficit-free effect by careful titration with a cholinergic blocking drug. The severely limiting factor in developing a physostigmine combination pretreatment for practical use is an unacceptable degree of interindividual variation in the bioavailability of this short half-life compound when administered to humans.[50,51] At present, it would appear necessary to define, for each recipient, an acceptable dose ratio for physostigmine and a cholinolytic adjunct to avoid performance deficits. The effort required for protecting a total force is clearly beyond our current capability. In the event of a technological breakthrough in individual drug delivery of a well-matched, centrally acting pair of carbamate and adjunct compounds, the possibility of developing centrally acting pretreatments would merit further study.

NEW DIRECTIONS: BIOTECHNOLOGICAL PRETREATMENTS

Until recently, medical defense against nerve agents has focused on preventing or reversing the binding of the agents to AChE, as well as on limiting the effects of the agents on neurotransmission by administration of pharmacological antagonists such as atropine. An intriguing new concept

for dealing with nerve agent toxicity involves taking advantage of naturally occurring macromolecules, such as a circulating nerve agent scavenger or a metabolizing enzyme, that would, respectively, bind to or catalyze the hydrolysis of nerve agents. These macromolecules have the potential of providing protection against all effects of nerve agents with minimal side effects, since they would stoichiometrically bind or metabolize a nerve agent before its distribution to the site of toxic effect.

The first evidence that circulating macromolecules have potential for protecting animals from nerve agents came from study of the remarkably broad range of toxic doses of the nerve agents in different animal species. For example, the LD_{50} of soman in mice and rats is about 10-fold higher than the LD_{50} in monkeys or guinea pigs.[52] An enzyme, plasma carboxylesterase, binds to and thus inactivates soman and other nerve agents in the G series (but not VX). The different amounts of this enzyme in the blood of various species can adequately explain their differential sensitivity to the G-series nerve agents.[53]

In addition to carboxylesterase, blood contains two forms of cholinesterase, AChE in the red cells (RBC-AChE) and butyrocholinesterase (BuChE; also called pseudocholinesterase and plasma cholinesterase) in the plasma. Both of these forms of cholinesterase bind and inactivate nerve agents. In preloading experiments in which exogenous AChE from fetal bovine serum or BuChE from equine or human sources was administered to animals (non-human primates, mice, or rats) intravenously or intramuscularly, a stoichiometric degree of protection against subsequent nerve agent challenge was provided. Investigators supported by the U.S. Army Medical Research Institute of Chemical Defense have recently cloned and expressed the genes for both human AChE and human BuChE.[58,59] Administration of either of these human bioproducts, with a potential plasma half-life of up to 12 days for BuChE, may be able to provide similar protection against nerve agent challenge for humans. The main obstacles to development of these products at the present time appear to be the high cost of production of the quantities involved and the possible need for frequent parenteral administration of a relatively short-lived product.

Another biotechnological protective strategy under active study is the production of monoclonal antibodies with high affinity for nerve agents.[60,61] If a human-derived monoclonal antibody of the immunoglobulin G (IgG) class could be produced, theoretically it would have the advantage of being able to bind and thus protect against a soman challenge in man after administration of about 2 g of antibody protein, similar to the amount of polyclonal antibody routinely administered in 10 mL of standard immune serum globulin. The 6-week plasma half-life

of IgG in man would make the use of such a product more acceptable.

Nerve agents, like other reactive small molecules, pass through a high-energy transition state during their reaction with water or with tissue targets such as AChE. By preparing antigens with a geometry that spatially mimics the transition states of these small molecules,[62] researchers have raised antibodies which not only bind to the nerve agent molecules but also catalyze their hydrolysis.[63] These catalytic antibodies have a major advantage over the other bioproducts noted above in that they could continue to inactivate multiple nerve agent molecules. For this reason, the preparation of catalytic antibodies to nerve agents, if successful, may result in the development of a superior, long-term nerve agent pretreatment.

Enzymes found in hepatocytes,[64] neuronal cells,[65] and plasma also hydrolyze nerve agents, albeit comparatively weakly. Study of the requirements for hydrolysis at the enzyme active sites could potentially lead to the design of more efficient hydrolytic enzymes that could be used as catalytic scavengers.[66]

The major reason for interest in biotechnologically derived nerve agent pretreatments lies in their unique mechanism of action as potential circulating nerve agent scavengers and hydrolytic catalysts. Animals protected against nerve agent challenge with these compounds have shown no evidence of toxicity or performance impairment from the nerve agents.[54–56] Thus, soldiers pretreated with these products might be able to function normally in a chemical environment contaminaed with levels of agent below the limits of their circulating protection without requiring the use of masks or protective clothing. The operational advantage that these soldiers would have over opponents encumbered by chemical protective equipment adds considerable appeal to exploring the potential of these newer nerve agent countermeasures.

SUMMARY

The inadequacy of postexposure therapy for nerve agent casualties, particularly those with potentially lethal exposures to soman, has been of great concern. Development of pyridostigmine, a peripherally active carbamate compound, as a nerve agent pretreatment adjunct has substantially improved the ability of the U.S. military to protect its soldiers from the lethal effects of nerve agents. A major deficiency of this pretreatment program—that it does not protect the CNS against nerve agent–induced injury—may be overcome by postexposure administration of anticonvulsants. While centrally acting pretreatments offer more effective protection than does pyridostigmine, their development is limited because of their potential for impairing soldier performance. New research

may provide a revolutionary advance in protection against nerve agents with biotechnologically derived pretreatments that bind or inactivate nerve agents in the circulation.

REFERENCES

1. *Soldier's Manual of Common Tasks. Skill Level 1*. Washington, DC: Department of the Army; 1994: 507–510. Report STP 21-1-SMCT.

2. Michel HO, Hackley BE Jr, Berkowitz L, et al. Aging and dealkylation of soman (pinocolylmethyl-phosphonofluoridate)-inactivated eel cholinesterase. *Arch Biochem Biophys*. 1967;121:29–34.

3. Dunn MA, Sidell FR. Progress in medical defense against nerve agents. *JAMA*. 1989;262:649–652.

4. Sidell FR. Soman and sarin: Clinical manifestations and treatment of accidental poisoning by organophosphates. *Clin Toxicol*. 1974;7:1–17.

5. Dirnhuber P, French MC, Green DM, Leadbeater L, Stratton JA. The protection of primates against soman poisoning by pretreatment with pyridostigmine. *J Pharm Pharmacol*. 1979;31:295–299.

6. Gordon JJ, Leadbeater L, Maidment MP. The protection of animals against organophosphate poisoning by pretreatment with a carbamate. *Toxicol Appl Pharmacol*. 1978;43:207–234.

7. Kluwe WM. Efficacy of pyridostigmine against soman intoxication in a primate model. In: *Proceedings of the 6th Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987:227–234.

8. Koplovitz I, Gresham VC, Dochterman LW, Kaminskis A, Stewart JR. Evaluation of the toxicity, pathology, and treatment of cyclohexylmethlyphosphonofluoridate (CMPF) poisoning in rhesus monkeys. *Arch Toxicol*. 1992;66:622–628.

9. Leadbeater L. When all else fails. *Chem Br*. 1988;24:684–687.

10. Inns RH, Leadbeater L. The efficacy of bispyridinium derivatives in the treatment of organophosphate poisoning in the guinea pig. *J Pharm Pharmacol*. 1983;35:427–433.

11. Koplovitz I, Harris LW, Anderson DR, Lennox WJ, Stewart JR. Reduction by pyridostigmine pretreatment of the efficacy of atropine and 2-PAM treatment of sarin and VX poisoning in rodents. *Fundam Appl Toxicol*. 1992;18:102–106.

12. DeCandole CA, Douglas WW, Lovatt-Evans C, et al. The failure of respiration in death by anticholinesterase poisoning. *Br J Pharmacol Chemother*. 1953;8:466–475.

13. Petrali JP, Maxwell DM, Lenz DE. A study on the effects of soman on rat blood–brain barrier. *Anat Rec*. 1985;211:351–352.

14. Petrali JP, Maxwell DM, Lenz DE, Mills KR. Effect of an anticholinesterase compound on the ultrastructure and function of the rat blood–brain barrier: A review and experiment. *J Submicrosc Cytol Pathol*. 1991;23:331–338.

15. McLeod CG Jr. Pathology of nerve agents: Perspectives on medical management. *Fundam Appl Toxicol*. 1985; 5:S10–S16.

16. Gall D. The use of therapeutic mixtures in the treatment of cholinesterase inhibition. *Fundam Appl Toxicol*. 1981;1:214–216.

17. Schiflett SG, Stranges SF, Slater T, Jackson MK. Interactive effects of pyridostigmine and altitude on performance. In: *Proceedings of the 6th Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987:605–607.

18. Whinnery JE. Flight testing of pyridostigmine bromide in the tactical fighter aircraft operational environment. Kelly Air Force Base, Tex; 1993. Unpublished.

19. Schiflett SG, Miller JC, Gawron VJ. Pyridostigmine bromide effects of performance of tactical transport air-crews. In: *Proceedings of the 6th Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987:609–611.

20. Glickson M, Achiron A, Ram Z, et al. The influence of pyridostigmine administration on human neuromuscular functions—studies in healthy human subjects. *Fundam Appl Toxicol*. 1991;16:288–298.

21. Krutz RW Jr, Burton RR, Schiflett S, Holden R, Fisher J. Interaction of pyridostigmine bromide with mild hypoxia and rapid decompression. In: *Proceedings of the 6th Medical Chemical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987:601–604.

22. Graham C, Cook MR. *Effects of Pyridostigmine on Psychomotor and Visual Performance*. Wright-Patterson Air Force Base, Ohio: Final report, contract F33615-80-C-0606, MRI; 1984.

23. Stephenson LA, Kolka MA. Acetylcholinesterase inhibitor, pyridostigmine bromide, reduces skin blood flow in humans. *Am J Physiol*. 1990;258:R951–R957.

24. Kolka MA, Stephenson LA. Human temperature regulation during exercise after oral pyridostigmine administration. *Aviat Space Environ Med*. 1990;61:220–224.

25. Annas GJ. Changing the consent rules for Desert Storm. *N Engl J Med*. 1992;326:770–773.

26. Nightingale SL. Medicine and war. *N Engl J Med*. 1992;326:1097–1098. Letter.

27. Howe EG. Medicine and war. *N Engl J Med*. 1992;326:1098. Letter.

28. Annas GJ. Medicine and war. *N Engl J Med*. 1992;326:1098. Letter.

29. Berezuk GP, McCarty GE. Investigational drugs and vaccines fielded in support of Operation Desert Storm. *Milit Med*. 1992;157:404–406.

30. Howe EG, Martin ED. Treating the troops. *Hastings Center Report*. March-April 1991:21–24.

31. Annas GJ, Grodin MA. Commentary. *Hastings Center Report*. March-April 1991:24–27.

32. Levine RJ. Commentary. *Hastings Center Report*. March-April 1991:27–29.

33. Levine BS, Parker RM. Reproductive and developmental toxicity studies of pyridostigmine bromide in rats. *Toxicology*. 1991;69:291–300.

34. Hudson CS, Foster RE, Kahng MW. Neuromuscular toxicity of pyridostigmine bromide in the diaphragm, extensor digitorum longus and soleus muscles of the rat. *Fundam Appl Toxicol*. 1985;5:S260–S269.

35. Briggs GC, Freeman RK, Yaffe SJ. *Drugs in Pregnancy and Lactation*. Baltimore, Md: Williams & Wilkins; 1990: 543–544.

36. Keeler JR. Interactions between nerve agent pretreatment and drugs commonly used in combat anesthesia. *Milit Med*. 1990;155:527–533.

37. Wade CE, Waring PP, Trail DS, Gildengorin VL, Williams BF, Bonner GD. Effects of atropine, 2-PAM, or pyridostigmine in euvolemic or hemorrhagic conscious swine. *Milit Med*. 1988;153:470–476.

38. Aquilonius SM, Eckernas SA, Hartvig P, Lindstrom B, Osterman PO. Pharmacokinetics and oral bioavailabliity of pyridostigmine in man. *Eur J Clin Pharmacol*. 1980;18:423–428.

39. Keeler JR, Hurst CG, Dunn MA. Pyridostigmine used as a nerve agent pretreatment under wartime conditions. *JAMA*. 1991;266:693–695.

40. Sharabi Y, Danon YL, Berkenstadt H, et al. Survey of symptoms following intake of pyridostigmine during the Persian Gulf War. *Isr J Med Sci*. 1991;27:656–658.

41. Loewenstein-Lichtenstein Y, Schwarz M, Glick D, Norgaard-Pedersen B, Zakut H, Soreq H. Genetic predisposition to adverse consequences of anticholinesterases in “atypical” BCHE carriers. *Nature Medicine*. 1995;1:1082–1085.

42. NIH Technology Assessment Workshop Panel. The Persian Gulf experience and health. *JAMA*. 1994;272:391–396.

43. Abou-Donia M, Wilmarth KR, Jensen KF, Oehme FW, Kurt TL. Neurotoxicity

resulting from coexposure to pyridostigmine bromide, DEET, and permethrin. *J Toxicol Environ Health*. 1996;48:35–56.

44. Institute of Medicine. *Health Consequences of Service During the Persian Gulf War: Initial Findings and Recommendations for Immediate Action*. Washington DC: National Academy Press; 1995.

45. National Institutes of Health Technology Assessment Workshop. *The Persian Gulf Experience and Health*. Bethesda, Md: National Institutes of Health; 1994.

46. Hayward IJ, Wall HG, Jaax NK, Wade JV, Marlow DD, Nold JB. *Influence of Therapy with Anticonvulsant Compounds on the Effects of Acute Soman Intoxication in Rhesus Monkeys*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1988. Technical Report 88-12.

47. Solana RP, Gennings C, Carter WH Jr, et al. Efficacy comparison of two cholinolytics, scopolamine and azaprophen, when used in conjunction with physostigmine and pyridostigmine for protection against organophosphate exposure. *J Am Coll Toxicol*. 1991;10:215–222.

48. Lieske CN, Koplovitz I, Wade JV, et al. 5-(1,3,3-trimethylinodolinyl) N,N-dimethylcarbamate, a Chinese drug with multiple uses. In: *Proceedings of the 1989 Medical Defense Bioscience Review*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1989: 483–486.

49. Harris LW, Talbot BG, Lennox WJ, Anderson DR, Solana RP. *Physostigmine and Adjunct Pretreatment (Alone and Together With Therapy) Against Nerve Agent Intoxication*. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1988. Technical Report 88-18.

50. Whelpton R, Hurst P. Bioavailability of oral physostigmine. *N Engl J Med*. 1985;313:1293–1294.

51. Aquilonius SM, Hartvig P. Clinical pharmacokinetics of cholinesterase inhibitors. *Clin Pharmacokinet*. 1986;11:236–249.

52. Maxwell DM, Brecht KM, O'Neill BL. The effect of carboxylesterase inhibition on interspecies differences in soman toxicity. *Toxicol Lett*. 1987;39:35–42.

53. Maxwell DM, Wolfe AD, Ashani Y, Doctor BP. Cholinesterase and carboxylesterase as scavengers for organophosphorus agents. In: Massoulie J, Bacou F, Barnard E, Chatonnet A, Doctor B, Quinn DM, eds. *Cholinesterases: Structure, Function, Mechanism, Genetics, and Cell Biology*. Washington, DC: American Chemical Society; 1991: 206–209.

54. Doctor BP, Blick DW, Caranto G, et al. Cholinesterases as scavengers for organophosphorus compounds: Protection of primate performance against soman toxicity. *Chem Biol Interact*. 1993;87:285–293.

55. Maxwell DM, Castro CA, DeLaHoz DM, et al. Protection of rhesus monkeys against soman and prevention of performance decrement by pretreatment with

acetylcholinesterase. *Toxicol Appl Pharmacol*. 1992;115:44–49.

56. Broomfield CA, Maxwell DM, Solana RP, Castro CA, Finger AV, Lenz DE. Protection by butyrylcholinesterase against organophosphorus poisoning in nonhuman primates. *J Pharmacol Exp Ther*. 1991;259:633–638.

57. Raveh L, Grunwald J, Marcus D, Papier Y, Cohen E, Ashani Y. Human butyrylcholinesterase as a general prophylactic antidote for nerve agent toxicity. *Biochem Pharmacol*. 1993;45:2465–2474.

58. Velan B, Kronman C, Grosfeld H, et al. Recombinant human acetylcholinesterase is secreted from transiently transfected 293 cells as a soluble globular enzyme. *Cell Mol Neurobiol*. 1991;11:143–156.

59. Masson P, Adkins S, Govet P, Lockridge O. Recombinant human butyrylcholinesterase G390V, the fluoride-2 variant, expressed in Chinese hamster ovary cells, is a low affinity variant. *J Biol Che*m. 1993;268:14329–14341.

60. Lenz DE, Brimfield AA, Hunter KW Jr, et al. Studies using a monoclonal antibody against soman. *Fundam Appl Toxicol*. 1984;4:S156–S164.

61. Lenz DE, Yourick JJ, Dawson JS, Scott J. Monoclonal antibodies against soman: Characterization of soman stereoisomers. *Immunol Lett*. 1992;31:131–135.

62. Moriarty RM, Hiratake J, Liu K. New synthetic route to unsymmetrically substituted pentacoordinated phosphorus. Hydrolytically stable chiral monocyclic oxyphosphoranes. *J Am Chem Soc*. 1990;112:8575–8577.

63. Brimfield AA, Lenz DE, Maxwell DM, Broomfield CA. Catalytic antibodies hydrolysing organophosphorus esters. *Chem Biol Interact*. 1993;87:95–102.

64. Little JS, Broomfield CA, Fox-Talbot MK, Boucher LJ, MacIver B, Lenz DE. Partial characterization of an enzyme that hydrolyzes sarin, soman, tabun, and diisopropyl phosphofluoridate (DFP). *Biochem Pharmacol*. 1989;38:23–29.

65. Ray R, Boucher LJ, Broomfield CA, Lenz DE. Specific soman-hydrolyzing enzyme activity in a clonal neuronal cell culture. *Biochim Biophys Acta*. 1988;967:373–381.

66. Broomfield CA. A purified recombinant organophosphorus acid anhydrase protects mice against soman. *Pharmacol Toxicol*. 1992;70:65–66.

Chapter 7

VESICANTS

FREDERICK R. SIDELL, M.D.*; JOHN S. URBANETTI, M.D., FRCP(C), FACP, FCCP†; WILLIAM J. SMITH, PH.D.‡; AND CHARLES G. HURST, M.D.§

INTRODUCTION

MUSTARD
- Military Use
- Properties
- Toxicity
- Biochemical Mechanisms of Injury
- Metabolism
- Clinical Effects
- Diagnosis
- Laboratory Tests
- Patient Management
- Long-Term Effects

LEWISITE
- Military Use
- Properties
- Toxicity
- Biochemical Mechanisms of Injury
- Clinical Effects
- Diagnosis
- Laboratory Tests
- Patient Management
- Long-Term Effects

PHOSGENE OXIME
- Military Use
- Properties
- Biochemical Mechanisms of Injury
- Clinical Effects
- Patient Management

SUMMARY

* *Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014*
† *Assistant Clinical Professor of Medicine, Yale University School of Medicine, New Haven, Connecticut 06510*
‡ *Supervisor, Cellular Pharmacology Team, Pharmacology Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425*
§ *Colonel, Medical Corps, U.S. Army; currently, Special Assistant for Medical Programs, Office of the Deputy Assistant Secretary of Defense, Counterproliferation and Chemical/Biological Matters, Room 3E808, 3050 Defense Pentagon, Washington, D.C. 20301-3050; formerly, Commander, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425*

INTRODUCTION

A *vesicant* (ie, an agent that produces vesicles or blisters) was first used as a chemical weapon on the battlefields of World War I[1–3] ; that same vesicant—sulfur mustard—is still considered a major chemical agent. In the intervening years between World War I and today, there have been a number of recorded and suspected incidents of mustard use, culminating with the Iran–Iraq War in the 1980s. During this conflict, Iraq made extensive use of mustard against Iran. Popular magazines and television brought the horrors of chemical warfare to the public's attention with graphic images of badly burned Iranian casualties. When, in the fall of 1990, the U.S. military joined the United Nations forces in preparation to liberate Kuwait, one of the major concerns was the threat that Iraq would again use mustard. Fortunately, chemical agents were not used in the short ground phase of the Persian Gulf War; however, the threat of an enemy's using chemical weapons against U.S. forces is ever present. Although mustard is the most important vesicant militarily, the vesicant category includes other agents, such as Lewisite and phosgene oxime (Table 7-1). The clinical differences among the vesicants discussed in this chapter are shown in Table 7-2.

TABLE 7-1
CHEMICAL, PHYSICAL, ENVIRONMENTAL, AND BIOLOGICAL PROPERTIES OF VESICATING AGENTS

Properties	Impure Sulfur Mustard (H)	Distilled Sulfur Mustard (HD)	Phosgene Oxime (CX)	Lewisite(L)
Chemical and Physical				
Boiling Point	Varies	227°C	128°C	190°C
Vapor Pressure	Depends on purity	0.072 mm Hg at 20°C	11.2 mm Hg at 25°C (solid) 13 mm Hg at 40°C (liquid)	0.39 mm Hg at 20°C
Density:				
Vapor	approx 5.5	5.4	<3.9?	7.1
Liquid	approx 1.24 g/mL at 25°C	1.27 g/mL at 20°C	ND	1.89 g/mL at 20°C
Solid	NA	Crystal: 1.37 g/mL at 20°C	NA	NA
Volatility	approx 920 mg/m^3 at 25ºC	610 mg/m^3 at 20ºC	1,800 mg/m^3 at 20ºC	4,480 mg/m^3 at 20ºC
Appearance	Pale yellow to dark brown liquid	Pale yellow to dark brown liquid	Colorless, crystalline solid or a liquid	Pure: colorless, oily liquid As agent: amber to dark brown liquid
Odor	Garlic or mustard	Garlic or mustard	Intense, irritating	Geranium
Solubility:				

In Water	0.092 g/100 g at 22°C	0.092 g/100 g at 22°C	70%	Slight
In Other Solvents	Complete in CCl_4, acetone, other organic solvents	Complete in CCl_4, acetone, other organic solvents	Very soluble in most organic solvents	Soluble in all common organic solvents
Environmental and Biological				
Detection	Liquid: M8 paper	Liquid: M8 paper	M256A1 ticket or card	Vapor, M256A1 ticket or card, ICAD
	Vapor: CAM	Vapor: CAM, M256A1 kit, ICAD		
Persistence:				
In Soil	Persistent	2 wk–3 y	2 h	Days
On Materiel	Temperature-dependent; hours to days	Temperature-dependent; hours to days	Nonpersistent	Temperature-dependent; hours to days
Skin Decontamination	M2581 kit Dilute hypochlorite Water M291 kit	M258A1 kit Dilute hypochlorite Soap and water M291 kit	Water	Dilute hypochlorite M258A1 kit Water M291 kit
Biologically Effective Amount:				

Vapor (mg•min/m^3)	LCt_{50}: 1,500	LCt_{50}: 1,500 (inhaled) 10,000 (masked)	Minimum effective Ct: approx 300; LCt_{50}: 3,200 (estimate)	Eye: <30 Skin: approx 200 LCt_{50}: 1,200–1,500 (inhaled) 100,000 (masked)
Liquid	LD_{50} : approx 100 mg/kg	LD_{50} : 100 mg/kg	No estimate	40–50 mg/kg

CAM: chemical agent monitor
ICAD: individual chemical agent detector
LD_{50}: dose that is lethal to 50% of the exposed population (liquid, solid)
LCt_{50}: (concentration • time of exposure) that is lethal to 50% of the exposed population (vapor, aerosol)
NA: not applicable
ND: not determined

TABLE 7-2
CLINICAL DIFFERENCES AMONG VESICANTS

	Onset		
Chemical Agent	**Pain**	**Tissue Damage**	**Blister**
Mustard	Hours later	Immediate; onset of clinical effects is hours later	Fluid filled
Lewisite	Immediate	Seconds to minutes	Fluid filled
Phosgene Oxime	Immediate	Seconds	Solid wheal

There are two types of mustard: sulfur mustard and nitrogen mustard. An impure sulfur mustard was probably synthesized by Despretz in 1822, but it was not identified. Riche, in 1854, and Guthrie, several years later, repeated Despretz's reaction to obtain the same product. Guthrie described the product as smelling like mustard, tasting like garlic, and causing blisters after contact with the skin. Niemann, in 1860, also synthesized the compound. In 1886, Meyer prepared a much purer mustard but discontinued his research because

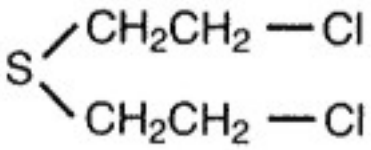
of the hazards involved. During World War I, the Germans used Meyer's method of synthesis to manufacture mustard.[3]

Nitrogen mustard (or more correctly, the nitrogen mustards) was first synthesized in the late 1930s; and although the properties of nitrogen mustard were only slightly different from those of sulfur mustard, none was found to be suitable for use as a weapon. However, a nitrogen mustard (HN_2, Mustargen, manufactured by Merck & Co., West Point, Pa.) was found useful for chemotherapy of certain neoplasms[4–7]; for years, it was a mainstay in cancer therapy until it was replaced by other compounds.

A second group of vesicants is the arsenicals. The major compound in this group is Lewisite. It was synthesized and developed in the United States during the late stages of World War I[1] and was manufactured for battlefield use. The shipment of Lewisite was on its way to Europe when the war ended, so it was destroyed at sea. There are no data on Lewisite from battlefield use. Lewisite has some advantages and disadvantages over mustard that are discussed later in this chapter.

The third compound considered to be a vesicant by the U.S. military is phosgene oxime. This is not a true vesicant because, unlike mustard and Lewisite, it does not produce fluid-filled blisters; rather, it produces solid lesions resembling urticaria. There has been no verified battlefield use of this compound, and there has been little study of it in the western world.

MUSTARD



HD

Mustard [bis-(2-chloroethyl) sulfide; also called 2,2'-dichlorethyl sulfide; is one of the two most important known chemical agents (the group of nerve agents is the other). Although mustard was introduced late in World War I (July 1917), it caused more chemical casualties than all the other agents combined: chlorine, phosgene, and cyanogen chloride. While lethality from mustard exposure was low, casualties filled the medical facilities. Despite 75 years of research, there is still no antidote for mustard. This fact is especially crucial when we consider that probably at least a dozen countries have mustard in their arsenals today.

Allegedly, mustard received its name from its smell or taste (onion, garlic, mustard)[3,8] or its color (which varies from yellow, to light tan, to dark brown). When mustard was first used by the Germans, the Allies called it Hun Stoffe (German stuff), abbreviated HS; later, it became known as H. Mustard manufactured by the Levinstein process is also known as H; it contains about 20% to 30% impurities (mostly sulfur). Distilled, or nearly pure, mustard is known as HD. Both forms of mustard, H and HD, can still be found today in

munitions manufactured over 50 years ago. Sulfur mustard has also been called LOST or S-LOST (for the two German chemists who suggested its use as a chemical weapon: *Lo*mmell and *St*einkopf); “yellow cross” (for the identifying mark on the World War I shells); and yperite (for the site of its first use).

Nitrogen mustard has not been used on the battlefield and is not thought to be an important military agent. There are three forms of this compound (HN_1, HN_2, HN_3); for several reasons, the nitrogen mustards were not suitable as military agents. These agents are similar to sulfur mustard in many ways, but they seem to cause more severe systemic effects, particularly in the central nervous system (CNS): they regularly caused convulsions when administered intravenously to animals.[9] Because nitrogen mustards have not been used militarily, they will not be discussed further. Unless stated otherwise, in this chapter the term “mustard” refers to sulfur mustard.

Military Use

Mustard has been contained in the arsenals of various countries since it was first used on July 12, 1917, when the Germans fired shells containing mustard at British troops entrenched near Ypres, Belgium.[1,2] Soon both sides were using mustard.

When a single agent was identified as the source of injury, it was estimated that mustard caused about 80% of the chemical casualties in World War I; the remaining 20% were caused by other agents such as chlorine and phosgene (see Chapter 9, Toxic Inhalational Injury). The British had 180,983 chemical casualties; the injuries of 160,970 (88%) were caused solely by mustard. Of these casualties, 4,167 (2.6%) died. Of the 36,765 single-agent U.S. chemical casualties, the injuries of 27,711 (75%) were caused solely by mustard. Of the casualties who reached a medical treatment facility (MTF), 599 (2.2%) died.[10]

Although mustard caused large numbers of casualties during World War I, very few of these casualties died. Most of those who did eventually die had been hospitalized for several days. Mustard survivors, likewise, required lengthy hospitalization: the average length of stay was 42 days. Combine this length of hospitalization with the vast number of casualties caused by mustard and we can easily see how the use of mustard can greatly reduce an enemy’s effectiveness.

Since the first use of mustard as a military weapon, there have been a number of isolated incidents in which it was reportedly used. In 1935, Italy probably used mustard against Abyssinia (now Ethiopia); Japan allegedly used mustard against the Chinese from 1937 to 1944; and Egypt was accused of using the agent against Yemen in the mid 1960s.[11]

Chemical agents were not used during World War II: it is thought that Germany did not use mustard because Hitler had been a mustard victim during World War I and was loath to use it. However, in December 1943, the SS *John Harvey*, which was carrying a large number of mustard bombs, was attacked while docked in Bari, Italy. There were 617 U.S. mustard casualties (83 fatal) from exploded shells in the water and from the smoke of the burning mustard. In addition, an unknown number of Italian civilians were casualties from the smoke.[12,13] (The incident at Bari is discussed in greater detail in this volume in Chapter 3, Historical Aspects of Medical Defense Against Chemical Warfare, and in *Occupational Health: The Soldier and the Industrial Base*,[14] another volume in the *Textbook of Military Medicine* series.)

Iraq employed mustard against Iran during the Iran–Iraq War (1982–1988). One source[15] estimates that there were 45,000 mustard casualties. In 1989, the journal *Annales Medicinae Militaris Belgicae* published a monograph by Jan L. Willems[16] that reported the western European experience treating a selected population of Iranian casualties of mustard. Willems reports that in March 1984, February 1985, and March 1986, Iranian casualties were sent to hospitals in Ghent, Belgium, and other western European cities for treatment. More casualties arrived in 1987. Because the hospital physicians lacked clinical experience in treating chemical warfare casualties, treatment policies varied.

In an attempt to establish whether chemical warfare agents had been used during the war, three United Nations missions (in 1984, 1986, and 1987) conducted field inspections, clinical examination of casualties, and laboratory analyses of chemical ammunition. The missions concluded that[16]

- aerial bombs containing chemical weapons were used in some areas of Iran,
- sulfur mustard was the primary chemical agent used, and
- there was some use of the nerve agent tabun.

Since mustard was introduced, a number of nonbattlefield exposures have occurred. Several occurred in the North Sea, where fishermen were exposed to mustard after dredging up munitions dumped there after World War II.[17–20] Others occurred when children found and played with mustard shells; the children were injured when the shells exploded, and several of the children died.[21,22] There have also been reported incidents of laboratory workers[23] and, in one instance, of soldiers in their sleeping quarters[24] who were accidentally exposed to mustard. In yet another

incident, a souvenir collector unearthed a mustard shell.[25]

Properties

Mustard is an oily liquid and is generally regarded as a “persistent” chemical agent because of its low volatility. In cool weather there is little vapor; however, mustard’s evaporation increases as the temperature increases. At higher temperatures, such as those in the Middle East during the hot season, 38°C to 49°C (100°F–120°F), mustard vapor becomes a major hazard. For example, the persistency of mustard (in sand) decreased from 100 hours to 7 hours as the temperature rose from 10°C to 38°C (50°F–100°F).[26] Although heat increases the vapor hazard, the rapid evaporation decreases the task of decontamination.

World War I data[27] suggest that the warming of the air after sunrise caused significant evaporation of mustard from the ground. Mustard attacks were frequently conducted at night, and the liquid agent did not readily evaporate in the cool night air. Several hours after daybreak, however, the sunwarmed air would cause the mustard to vaporize. By this time, thinking the danger from the attack was over, the soldiers had removed their masks; thus they fell victim to the evaporating mustard. This combination of events produced a significant number of casualties among the soldiers. Because of these nighttime shellings, it soon became standard policy not to unmask for many hours after daybreak.

Mustard vapor has a density 5.4-fold greater than that of air, causing it to hug the ground and sink into trenches and gullies. When mustard slowly evaporates, a detector held 3 to 6 feet above the ground may indicate no agent in the air; but closer to the ground, at 6 to 12 inches, the concentration might range from 1 to 25 mg/m^3. Despite this low volatility, more than 80% of the mustard casualties during World War I were caused by vapor, not the liquid form of mustard.[27]

The freezing temperature for mustard is 57°F. This high freezing point makes mustard unsuitable for delivery by aircraft spraying or for winter dispersal. Therefore, to lower the freezing point, mustard must be mixed with another substance. During World War I, mustard was mixed with chloropicrin, chlorobenzene, or carbon tetrachloride to lower its freezing point.[1] Today, mustard can be mixed with Lewisite to increase its volatility in colder weather.

Mustard’s high freezing point made it useful during those times of the year when the nighttime temperature was about 10°C (50°F) and the daytime temperature was in the 15°C to 21°C (60°F–70°F) range. In warm weather, mustard is 7- to 8-fold more persistent than Lewisite; therefore, it is highly desirable for use in such geographical areas as the Middle East.

Toxicity

For liquid mustard on the skin, the dose that is lethal to 50% of the exposed population (LD_{50}) is about 100 mg/kg, or about 7.0 g for a person weighing 70 kg. This is about 1.0 to 1.5 teaspoons of liquid; this amount will cover about 25% of the body surface area. An area of erythema with or without blisters caused by liquid mustard that covers this or a larger area of skin suggests that the recipient has received a lethal amount of mustard. A 10-µg droplet will produce vesication.

On the other hand, exposure to a vapor or aerosol in air is usually described as the product of the concentration (C, expressed as milligrams per cubic meter) and the time the exposure lasted (*t*, expressed as minutes):

$$Ct = \text{mg}\bullet\text{min/m}^3$$

Thus, the effect produced by an aerosol or vapor exposure to 0.05 mg/m^3 • 100 minutes is equal to the effect produced by an exposure to 5 mg/m^3 • 1 minute; in either case, $Ct = 5\ \text{mg}\bullet\text{min/m}^3$. (Ct, and particularly its relation to LD, are discussed in greater detail in Chapter 5, Nerve Agents; see Exhibit 5-1.)

Eye damage was produced by a Ct of 10 mg•min/ m^3 or less under laboratory conditions[28]; other estimates[29] for the eye damage threshold under field conditions range from 12 to 70 $mg\bullet min/m^3$. The estimated Ct for airway injury ranges from 100 to 500 $mg\bullet min/m^3$. The threshold for skin damage is highly dependent on skin site, heat, sweating, and other factors (localized sweating will lower the threshold on the portion of the skin that is sweating[30]); the threshold is generally in the range of 200 to 2,000 $mg\bullet min/m^3$.

Biochemical Mechanisms of Injury

Although mustard has been considered a major chemical weapon for 75 years, there is still no clear understanding of its biochemical mechanism of action; therefore, no specific therapy for its effects exists. While the chemistry of mustard interaction with cellular components is well defined, the correlation of this interaction with injury has not been made. Over the past few decades, scientists have made major advances in understanding the cellular and biochemical consequences of exposure to mustard and have put forth several hypotheses, two of which are discussed below, to account for mustard injury (Figure 7-1).[29,31,32]

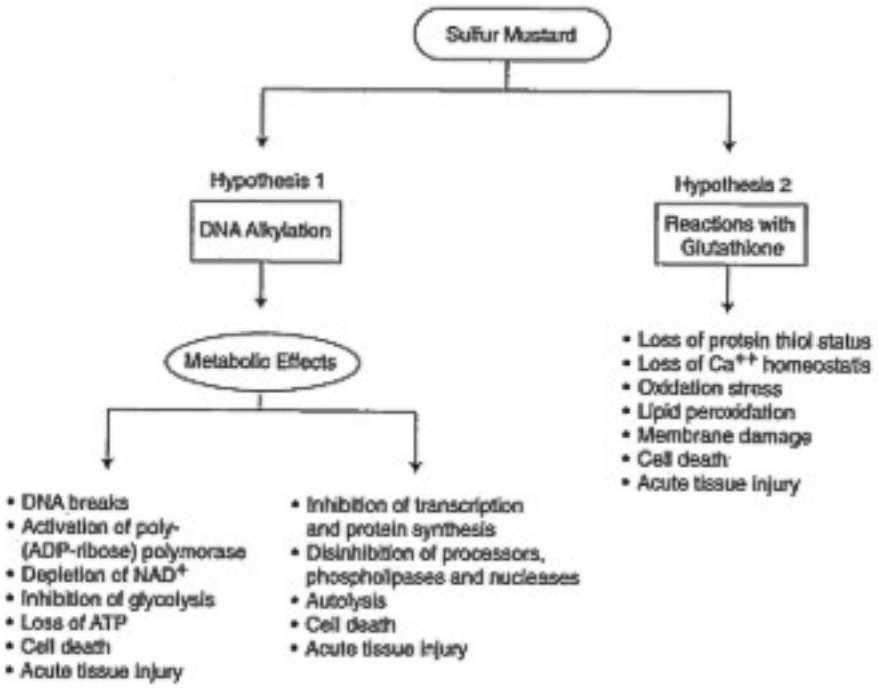


Fig. 7-1. The putative mechanisms by which sulfur mustard causes tissue damage. Adapted from US Army Medical Research Institute of Chemical Defense. A global picture of battlefield vesicants, I: A comparison of properties and effects. *Med Chem Def*. 1992;5(1):6.

The mustards—both sulfur and nitrogen—are alkylating agents that act through cyclization of an ethylene group to form a highly reactive sulfonium or immonium electrophilic center. This reactive electrophile is capable of combining with any of the numerous nucleophilic sites present in the macromolecules of cells. The products of these reactions are stable adducts that can modify the normal function of the target macromolecule. Because nucleophilic areas exist in peptides, proteins, ribonucleicacid (RNA), deoxyribonucleic acid (DNA), and membrane components, researchers have tried to identify the most critical biomolecular reactions leading to mustard injury.

Due to the highly reactive nature of mustard, it is conceivable that the injury following tissue exposure may result from a combination of effects described below in both hypotheses; or injury may result from additional changes not yet described in a formal hypothesis. Whether the initiating event is alkylation of DNA or modification of other cellular macromolecules, however, these steps would disrupt the epidermal–dermal junction. Once the site of tissue injury is established, the pathogenic process leading to formation of fully developed blisters must involve an active inflammatory response and altered fluid dynamics in the affected tissue.

Mustard also has cholinergic action stimulating both muscarinic and

nicotinic receptors.[33]

Alkylation of Deoxyribonucleic Acid

The first proposed hypothesis for the possible mechanism of injury for mustard links alkylation of DNA with the cellular events of blister formation.[34] According to this proposal, alkylation of DNA by sulfur mustard results in strand breaks. The strand breaks trigger activation of a nuclear DNA repair enzyme, poly(ADP-ribose) polymerase (PADPRP). Excessive activity of this enzyme depletes cellular stores of nicotinamide adenine dinucleotide (NAD^+), a critical cofactor and substrate needed for glycolysis.[35–37] Inhibition of glycolysis would cause a buildup of glucose-6-phosphate, a substrate in the hexose monophosphate shunt.[38] Stimulation of the hexose monophosphate shunt results in activation of cellular proteases.[39] Since a principal target of mustard in the skin is the basal epidermal cell,[40] protease from these cells could account for the cleavage of the adherent fibrils connecting the basal epidermal cell layer to the basement membrane.

Thus far, data in animal and cellular systems are consistent with many aspects of this hypothesis, which has DNA damage as the initiating step and PADPRP activation as a critical event. Studies in human skin grafts,[35] epidermal keratinocytes,[41] and leukocytes in culture[36] ; and in the euthymic hairless guinea pig[42] have shown decreases in cellular NAD^+ as a consequence of PADPRP activation following sulfur mustard–induced DNA damage. Niacinamide and other inhibitors of the PADPRP can ameliorate the pathology developing in both living animal and cellular models.[35,36,42,43] Unfortunately, while niacinamide has some beneficial actions, the protection it affords is never complete and is limited in duration.[41,42] No evidence currently shows activation of the hexose monophosphate shunt following mustard exposure, but significant metabolic disruptions in human keratinocytes have been reported after mustard exposure.[44] Protease activity is increased in human cells exposed in vitro to mustard.[45–47]

While many aspects of the PADPRP hypothesis have been verified, and there is good linkage between proposed steps of this pathway and mustard-induced cytotoxicity, no direct correlation with the full range of tissue pathologies seen following mustard exposure has yet been established. Even though DNA is an important macromolecular target of mustard alkylation in the cell, several other hypotheses of mustard toxicity have been developed that are based on mustard’s reaction with other cellular components. For a review of all such hypotheses, see *Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological*

Implications[29]; only those undergoing active investigation are discussed here.

Reactions With Glutathione

The second major hypothesis to explain the effects of mustard is that it reacts with the intracellular free radical scavenger glutathione, GSH, thereby depleting it, resulting in a rapid inactivation of sulfhydryl groups and the consequent loss of protection against oxygen-derived free radicals, specifically those causing lipid peroxidation.[48] In 1987, Orrenius and Nicotera[49] established that menadione-induced depletion of GSH resulted in loss of protein thiols and inactivation of sulfhydryl-containing enzymes. Included in this class of thiol proteins are the calcium and magnesium adenosine triphosphatases, which regulate calcium homeostasis. With the inactivation of the enzymes that control thiol proteins, intracellular calcium levels would increase. High calcium levels within the cell trigger activation of protease, phospholipases, and endonucleases, which could give rise to the breakdown of membranes, cytoskeleton, and DNA that would result in cell death.

A report[50] suggested that this mechanism could be activated by mustards and might be the mechanism of mustard injury. While several aspects of the thiol–calcium hypothesis (eg, release of arachidonic acid and decrease in membrane fluidity) have been observed in cell cultures following sulfur mustard exposure,[51] no definitive studies have drawn an association between calcium disruptions and mustard-induced pathology.

Another proposed consequence of the mechanism—based on the depletion of GSH following mustard exposure—is lipid peroxidation.[52,53] According to this hypothesis, depletion of GSH allows the formation of oxygen-derived free radicals. The oxidizing compounds thus formed will react with membrane phospholipids to form lipid peroxides that could, in turn, lead to membrane alterations, changes in membrane fluidity, and eventual breakdown of cellular membranes.

As previously mentioned, studies[51] have shown changes in membrane fluidity following sulfur mustard exposure. In addition, in 1989, Elsayed and colleagues[54] demonstrated the presence of lipid peroxidation indicators in the tissue of mice exposed to subcutaneous butyl mustard. However, as with the thiol–calcium hypothesis, no studies have directly linked lipid peroxidation with the mustard-induced injury.

Metabolism

The mechanism or mechanisms by which mustard is thought to cause tissue damage are described above. As the first step in any of the theories, mustard cyclizes to a sulfonium electrophilic center. This highly reactive moiety, in turn, combines with peptides, proteins, DNA, or other substances. After a few minutes in a biological milieu, intact mustard is no longer present; the reactive electrophile has attached to another molecule and is no longer reactive. The rapidity of this reaction also means that within a few minutes mustard has started to cause tissue damage. The clinical relevance is that intact mustard or its reactive metabolic product is not present in tissue or biological fluids, including blister fluid, a few minutes after the exposure; however, clothing, hair, and skin surfaces may still be contaminated hours later.

Several studies[29,31,32,55] support the observation that intact or active mustard is not present in tissue or biological fluids after a few minutes. Occluding the blood supply to areas of the intestinal tract or to selected bone marrow for a few minutes protected these organs from the effects of a lethal amount of intravenously administered mustard. Approximately 85% of S-labeled mustard[36] disappeared from the blood of humans after several minutes,[56] and the half-life for intravenously administered mustard to disappear from the blood of piglets was about 2 minutes.[57] Mustard blister fluid did not produce a reaction when instilled into the eyes of animals or humans[58] or onto the skin of humans.[59] A continuing outbreak of smaller vesicles near a source of blister fluid is probably the result of these areas having received an additional amount of exposure and not from contamination by the blister fluid.[58,60]

Clinical Effects

The organs most commonly affected by mustard are the skin, eyes, and airways (Table 7-3): the organs with which mustard comes in direct contact. After a significant amount of mustard has been absorbed through the skin or inhaled, the hemopoietic system, gastrointestinal tract, and CNS are alsodamaged. Mustard may also affect other organs but rarely do these produce clinical effects.

During World War I, 80% to 90% of U.S. mustard casualties had skin lesions, 86% had eye involvement, and 75% had airway damage.[61] These percentages are somewhat different from those seen in Iranian casualties, however. Of a group of 233 severely injured Iranian soldiers sent to western European hospitals by the Iranian government for treatment during the Iran–Iraq War, 95% had airway involvement, 92% had eye signs and symptoms, and 83% had skin lesions.[62] In a series of 535

Iranian casualties, including civilians, admitted to a dermatology ward, 92% had skin lesions and 85% had conjunctivitis; of the total number of patients, 79% had erythema and 55% had blisters. (Casualties with more serious problems, including injury to the pulmonary tract, were admitted to other wards).[63]

The slightly higher percentage of airway and eye involvement in Iranian soldiers versus U.S. World War I casualties is perhaps attributable to the higher ambient temperature in the area (compared with Europe), which caused more vaporization; it might also have been because Iranian protective equipment was not as good as that used during World War I, or the masks may not have been completely sealed because of facial hair. In 1984, the year the first Iranian casualties were treated in Europe, protective clothing and gas masks were not commonly worn by Iranian soldiers. Later, when gas masks became available, they probably were not fully effective; it is not known whether masking drills were carefully performed by the soldiers.[16]

Mustard-related death occurs in about 3% of the

TABLE 7-3
INITIAL CLINICAL EFFECTS FROM MUSTARD EXPOSURE

Organ	Severity	Effects	Onset of First Effect
Eyes	Mild	Tearing Itchy Burning Gritty feeling	4–12 h
	Moderate	Above effects, plus: Reddening Lid edema Moderate pain	3–6 h
	Severe	Marked lid edema Possible corneal damage Severe pain	1–2 h
Airways	Mild	Rhinorrhea Sneezing Epistaxis Hoarseness Hacking cough	6–24 h
	Severe	Above effects, plus: Productive cough Mild-to-severe dyspnea	2–6 h
Skin	Mild	Erythema	2–24 h

Severe	Vesication

casualties who reach an MTF; of those who die, most die 4 or more days after exposure. Table 7-4 illustrates the breakdown, in percentages, of British troops who died after exposure to mustard during World War I.[61] Of the casualties who died, 84% required at least 4 days of hospitalization. The causes of death are usually pulmonary insufficiency from airway damage, superimposed infection, and sepsis. Rarely, the amount of mustard will be overwhelming and cause death within 1 to 2 days; in these circumstances, death might be due to neurological factors[9,22] or massive airway damage.

TABLE 7-4
WORLD WAR I DEATHS AFTER EXPOSURE TO MUSTARD *

Day of Death (After Exposure)	Percentage of Deaths
≤1	1
2	2
3	5
4	8
5	22
≥6	62

* In 4,167 fatal mustard casualties among British troops
Data source: Gilchrist HL. A *Comparative Study of WWI Casualties From Gas and Other Weapons.* Edgewood Arsenal, Md: US Chemical Warfare School; 1928: Chart 3, p 14.

Willems's report[16] on Iranian casualties treated in western European hospitals gives some idea of the effect of medical advances since World War I on the management of mustard casualties. Clinical files of 65 of these casualties were studied in detail. Eight patients died between 6 and 15 days after exposure. One patient died 185 days after exposure: he had received ventilatory support for an extended period because of severe bronchiolitis complicated by a series of loculate pneumothoraces. Most patients returned to Iran in fairly good condition after 2 to 10 weeks of treatment. Their lesions were nearly completely healed, although some lesions remained. The duration of hospitalization was determined mainly by the time needed for healing of the deeper skin lesions.

Skin

The threshold amount of mustard vapor required to produce a skin lesion (erythema) is a C*t* of about 200 mg•min/m^3 . This varies greatly depending on a number of factors, including temperature, humidity, moisture on the skin, and exposure site on the body. Warm, moist areas with thin skin such as the perineum, external genitalia, axillae, antecubital fossae, and neck are much more sensitive. As was stated earlier, a liquid droplet of about 10 µg will produce vesication. About 80% of this 10 µg evaporates and 10% enters the circulation, leaving about 1 µg to cause the vesicle. Evaporation of small droplets is rapid and nearly complete in 2 to 3 minutes; amounts larger than several hundred milligrams may remain on the skin for several hours.[64] Mustard vapor rapidly penetrates the skin at the rates of 1.4 µg/cm^2/min at 70°F, and 2.7 µg/cm^2/min at 88°F.[26] Liquid mustard penetrates the skin at 2.2 µg/cm^2/min at 60°F and at 5.5 µg/cm^2/min at 102°F. Once mustard penetrates the skin, it is “fixed” to components of tissue and cannot be extracted.[64]

In one group of people, large differences in skin sensitivity to mustard were noted; some individuals were much more sensitive than others, although their skin pigment appeared to be equal. Darkly pigmented individuals were much more resistant than lightly pigmented people. Repeated exposures caused an increase in sensitivity. The horse was the most sensitive among eight nonhuman species tested; the guinea pig and monkey were the least sensitive; the dog most closely matched the sensitivity of humans.[30]

The mildest and earliest form of visible skin injury is erythema, which resembles sunburn (Figure 7-2). It is usually accompanied by pruritus, burning, or stinging. After a small exposure, this might be the extent of the lesion. More commonly, small vesicles will develop within or on the periphery of the erythematous areas (like a string of pearls); these vesicles will later coalesce to form larger blisters (Figure 7-3). Erythema begins to appear 1 to 24 hours after the skin is exposed to mustard, although onset can be later. The effects from liquid mustard appear more rapidly than the effects from mustard vapor. Characteristically, the onset of erythema is about 4 to 8 hours after mustard exposure. Vesication begins about 2 to 18 hours later and may not be complete for several days.

Fig. 7-2. Erythema of the chest of an Iranian casualty as it appeared 5 days after his exposure to mustard. He also had a pulmonary injury with an associated broncho-pneumonia due to infection with *Haemophilus influenzae*. The presence of a nasal oxygen catheter is indicative of the pulmonary insufficiency. Photograph: Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:13.

Fig. 7-3. The back of an Iranian casualty seen 16 hours after exposure to mustard. Note the small vesicles in proximity to the large bullae. Photograph: Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:8.

The typical bulla is dome-shaped, thin-walled, superficial, translucent, yellowish, and surrounded by erythema. Generally, it is 0.5 to 5.0 cm in diameter, although it can be larger (Figure 7-4). The blister fluid is initially thin and clear or slightly straw-colored; later it turns yellowish and tends to coagulate.[16,64,65] The blister fluid does not contain mustard and is not itself a vesicant. Vapor injury is generally a first- or second-degree burn; liquid mustard may produce deeper damage comparable to a third-degree burn.

After exposure to extremely high doses, such as those resulting from exposure to liquid mustard, lesions may be characterized by a central zone of coagulation necrosis, with blister formation at the periphery. These lesions are more severe, take longer to heal, and are more prone to secondary infection.[29] Necrosis and secondary inflammation, which were the expected prominent pathophysiological characteristics of a deep burn in the preantibiotic era, are evident.

Fig. 7-4. Large and extensive bullae on **(a)** the hands and **(b)** the feet of Iranian casualties as they appeared 5 days after exposure to mustard. **(c)** Some of the bullae are disrupted and have a purulent base. Note the extensive edema that afflicts the surrounding skin. The whitish material is an antimicrobial salve. Photographs: Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:14, 15.

The major change at the dermal–epidermal junction, visualized by light microscopy, is liquefaction necrosis of epidermal basal cell keratinocytes (Figure 7-5). Nuclear swelling within basal cells starts as early as 3 to 6 hours after exposure,[66] and progresses to pyknosis of nuclei and disintegration of cytoplasm. The pathological process can be described as follows (Figure 7-6

illustrates this process further):

> By a coalescence of neighboring cells undergoing the process of swelling, vacuolar, or hydropic degeneration ("liquefaction necrosis") and rupture, spaces of progressively increasing size are formed. This usually involves dissolution of cells of the basal layer, resulting in defects in the basal portion of the epidermis and separation of the upper layers of the epidermis from the corium....At first, there are multiple focal areas of such microvesicle formation, with septa of as yet uninvolved epidermal cells. Progressive dissolution of the cells of such septa follows, and although intact or partially degenerated basal cells may remain in the floor of the microvesicles at first, these also soon disintegrate as the vesicles enlarge.[67]

An electron microscopy study[68] published in 1990, of mustard lesions in human skin grafted onto nude mice, confirmed that damage to the basal cells (nucleus, plasma membrane, anchoring filaments) resulted in the separation of epidermis from dermis and the formation of a subepidermal microblister.

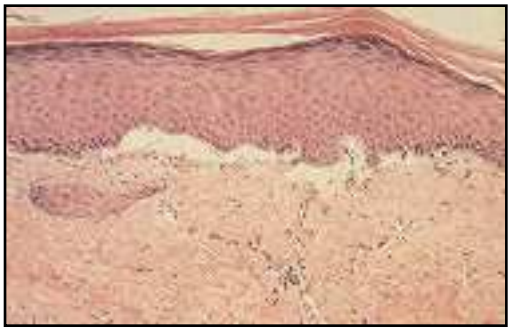
a  **b**

Fig. 7-5. The spectrum of cutaneous mustard injury as seen on light microscopy extends from superficially intact skin to sloughing of the epidermis. **(a)** A skin biopsy taken from an Iranian casualty on the 11th day following exposure to mustard. The gross appearance was of erythema. A cleavage plane is apparent between the dermis and epidermis, with edema extending into the stratum spinosum. (Note the enlarged spaces between individual cells.) Changes in cells of the stratum germinativum are difficult to ascertain at this level of magnification, but nuclei of cells on the extreme right of the figure appear to be pyknotic (shrunken and dark). **(b)** The biopsy was taken at the site of an erosion. The epidermis has sloughed, and the superficial dermis is necrotic. White blood cells have infiltrated the deeper layers of the dermis. Part of an intact hair follicle is seen; the epidermis will ultimately regenerate from such structures. Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:19.

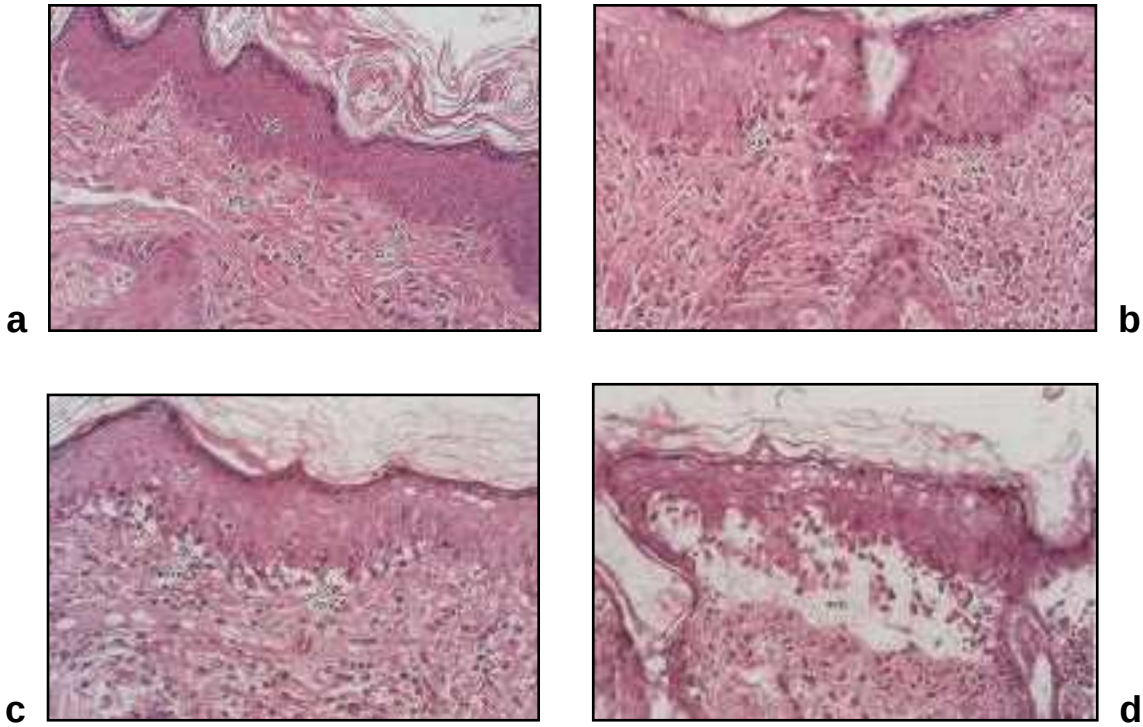


Fig. 7-6. Light and electron microscopic analysis of hairless guinea pig skin exposed to sulfur mustard vapor reveals that the epithelial basal cell of the stratum germinativum is selectively affected to the exclusion of other epidermal cells. Following an apparent latency period of 4 to 6 hours, the basal cell pathology progresses to include extensive hydropic vacuolation, swollen endoplasmic reticulum, coagulation of monofilaments, nuclear pyknosis, and cell death. At 12 to 24 hours, characteristic microvesicles/microblisters form at the dermal–epidermal junction, which cleave the epidermis from the dermis. The cavity formed within the lamina lucida of the basement membrane as a consequence of basal cell pathology—and perhaps as the result of disabling of adherent basement membrane proteins—is infiltrated with cellular debris, inflammatory cells, fibers, and tissue fluid. **(a)** This hairless guinea pig perilesional skin site not exposed to mustard (HD) vapor serves as the control. Epidermis (ep); dermis (d); basement membrane (arrows); basal cells of stratum germinativum (bc). **(b)** At 9 hours after exposure to HD vapor, degenerating basal cells with karyorrhectic and pyknotic nuclei (pyk) can be seen. **(c)** At 12 hours after HD exposure, microvesicles (mv) are forming at the basement membrane zone in association with degenerating basal cells. **(d)** At 24 hours after HD exposure, microvesicles have coalesced to form a characteristic microblister (mb), which separates the epidermis from the dermis. Original magnification × 220. Photographs: Courtesy of John P. Petrali, Ph.D., U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Md.

The healing time for mustard skin lesions depends on the severity of the lesion. Erythema heals within several days, whereas severe lesions may require several weeks to several months to heal, depending on the anatomical site, the total area of skin surface affected, and the depth of the lesion (Figure 7-7).[16]

Fig. 7-7. Healing of a deep erosive mustard burn of the hand. **(a)** The appearance on day 49. Epithelialization occurred by ingrowth of cells from patches of less injured skin. **(b)** The appearance on day 66, by which time complete epithelialization had occurred. The thin and fragile nature of the new skin is clearly apparent. Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:36.

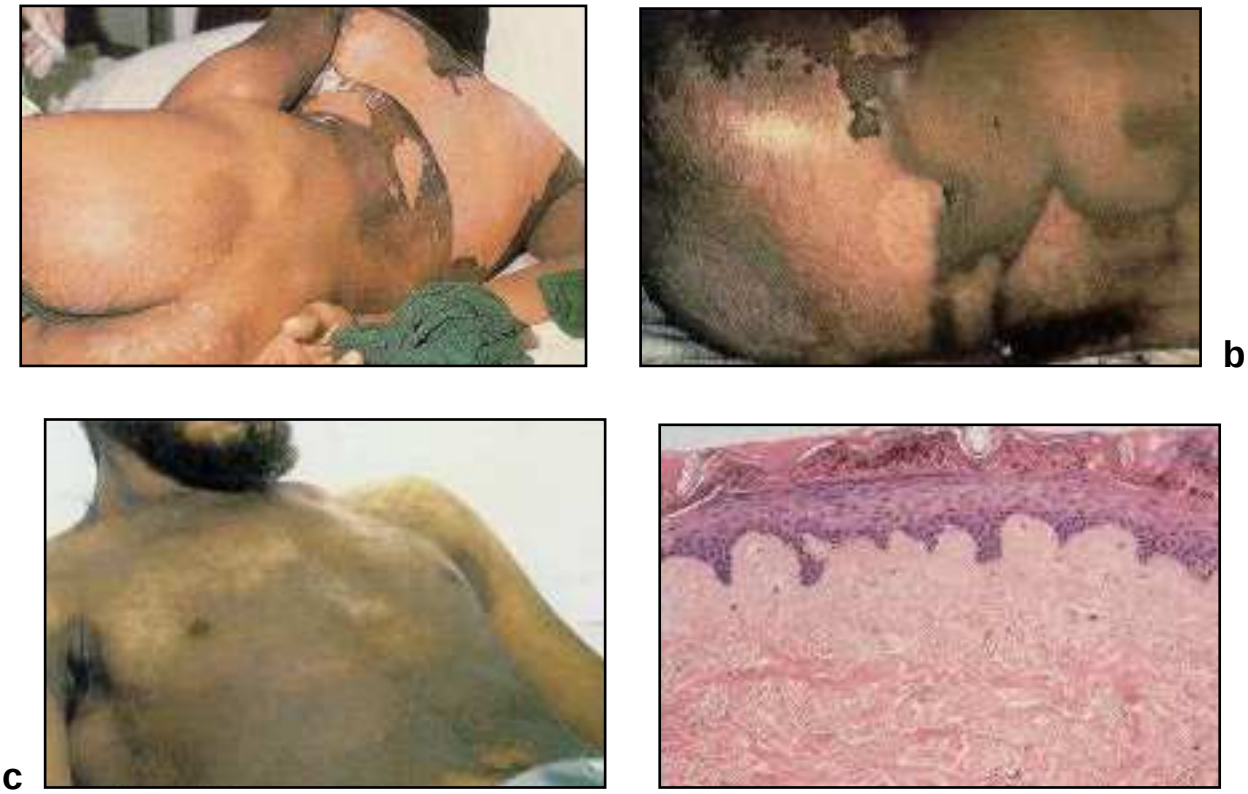


Fig. 7-8. Transient hyperpigmentation of the injured skin is observed frequently following mustard exposure. It is caused by the collection of melanin from dead melanocytes at the base of the soon-to-desquamate epidermis and disappears when the involved skin desquamates. Hyperpigmentation is not dependent on the formation of bullae. **(a)** An Iranian casualty as he appeared 5 days following exposure to mustard. Note the extensive desquamation of hyperpigmented skin on his back and the normal appearance of the underlying skin. This casualty developed a profound leukopenia (400 cells per µL) and a bronchopneumonia of 10 days' duration. Resolution of these problems required a 5-week hospitalization. **(b)** A different Iranian casualty, seen 12 days after exposure to mustard, has darkening of the skin, desquamation, pink areas showing regeneration of the epidermis, and yellow-white areas of deeper necrosis. **(c)** Another casualty's blackening of the skin and beginning desquamation of the superficial layer of the epidermis is seen 15 days after mustard exposure. Note the prominence of these changes in the skin of the axilla. **(d)** The appearance on light microscopy of a hyperpigmented area. Note the melanin in the necrotic epidermal layer under which is found a layer of regenerating epidermis. Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg.* 1989;3S:13, 18, 29, 30.

One of the interesting characteristics of the cutaneous mustard injury that Willems[16] reported in the Iranian casualties was the transient blackening, or hyperpigmentation, of the affected skin (Figure 7-8). When the hyperpigmented skin exfoliated, epithelium of normal color was exposed. Vesication was not necessary for hyperpigmentation to occur. The syndrome of hyperpigmentation and exfoliation was commonly recognized in World War I casualties, but less commonly in laboratory experiments in which liquid mustard was used.[16] A punctate hyperpigmentation—possibly due to postinflammatory changes—may be apparent in healed, deep mustard burns (Figure 7-9).

Fig. 7-9. By 32 days after exposure, this Iranian casualty has punctate hyperpigmentation in a healing deep mustard burn. This condition is perhaps indicative of postinflammatory changes in the epidermis that has regenerated from hair follicles. Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:34.

Eye

The eye is the organ most sensitive to mustard. The C*t* required to produce an eye lesion under field conditions is 12 to 70 mg•min/m^3 .[29] The effective C*t* for conjunctivitis, or slightly more severe damage, was just under 10 mg/m^3 in 13 subjects; several subjects had lesions at C*t*s of 4.8 to 5.8 mg•min/m^3 .[69] One subject had no symptoms after several hours; however, by 12 hours after the

exposure, marked blepharospasm and irritation were apparent.

Generally, the asymptomatic period varies with the concentration of mustard vapor (or the amount of liquid) and individual sensitivity. The latent period for eye damage is shorter than that for skin damage. Eye irritation within minutes after exposure has been reported,[16,69] but the authors of these reports speculate that the irritation might have been due to other causes.

After a low C*t* exposure, a slight irritation with reddening of the eye may be all that occurs (Figure 7-10). As the C*t* increases, the spectrum of injury is characterized by progressively more severe conjunctivitis, blepharospasm, pain, and corneal damage.[29,65] Photophobia will appear and, even with mild exposures, may linger for weeks.

Fig. 7-10. An eye injury of lesser severity in an Iranian casualty (shown 7 d after exposure) caused by exposure to mustard. The characteristic findings were edema of the lid and conjunctival injection. Corneal ulcerations were found with more severe exposure. Reprinted with permission from Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:12.

Corneal damage consists of edema with clouding (which affects vision), swelling, and infiltration of polymorphonuclear cells. Clinical improvement occurs after approximately 7 days with subsiding edema. Corneal vascularization (pannus development, which causes corneal opacity) with secondary edema may last for weeks. Vision will be lost if the pannus covers the visual axis. Severe effects from mustard exposure may be followed by scarring between the iris and the lens, which restricts pupillary movements and predisposes the individual to glaucoma.[29,70]

The most severe eye damage is caused by liquid mustard, which may be delivered by an airborne droplet or by self-contamination.[60] Symptoms may become evident within minutes after exposure.[65] Severe corneal damage with possible perforation of the cornea can occur after extensive eye exposure to liquid mustard. The patient may lose his vision or even his eye from panophthalmitis, particularly if drainage of the infection is blocked, such as by adherent lids.[65] Miosis sometimes occurs, probably due to the cholinergic activity of mustard.

During World War I, mild conjunctivitis accounted for 75% of the eye injuries; complete recovery took 1 to 2 weeks. Severe conjunctivitis with minimal corneal involvement, blepharospasm, edema of the lids and conjunctivae, and orange-peel roughening of the cornea accounted for 15% of the cases; recovery occurred in 2 to 5 weeks. Mild corneal involvement with areas of corneal

erosion, superficial corneal scarring, vascularization, and iritis accounted for 10% of the cases; convalescence took 2 to 3 months. Lastly, severe corneal involvement with ischemic necrosis of the conjunctivae, dense corneal opacification with deep ulceration, and vascularization accounted for about 0.1% of the injuries; convalescence lasted more than 3 months. Of 1,016 mustard casualties surveyed after World War I, only 1 received disability payments for defective vision.[10]

Studies conducted on rabbit eyes indicate that mustard injury to the cornea is characterized by initial degeneration of the epithelial cells, with changes ranging from nuclear swelling and nuclear vacuolization to pyknosis and nuclear fragmentation. Epithelial loosening and sloughing occurs either by separation of the basal cells from the basement membrane or by shearing of the cell just above its attachment to the basement membrane.[71,72]

Mustard initially causes vasodilation and increased vascular permeability in the conjunctiva, which lead to progressive edema. Secretion of mucus occurs within minutes of exposure. Pyknosis of epithelial cells begins concurrently with or shortly after these changes, leading to desquamation of the epithelium. In the later stages, inflammatory infiltration of connective tissue and exudation are present.[71,72] Medical personnel have reported seeing delayed keratitis in humans months to years after mustard exposure.[28,73]

Within approximately 5 minutes, liquid mustard dropped into the eyes of rabbits was absorbed, had disappeared from the eye's surface, had passed through the cornea and the aqueous, and had produced hyperemia of the iris. Likewise, damage to other structures (eg, Descemet's membrane) also occurred within a similar length of time.[28] Decontamination must be performed immediately after liquid mustard contaminates the eye because absorption and ocular damage occur very rapidly; after a few minutes, there will be no liquid remaining on the surface of the eye to decontaminate.

Airways

Mustard produces dose-dependent damage to the mucosa of the respiratory tract, beginning with the upper airways and descending to the lower airways as the amount of mustard increases. The inflammatory reaction varies from mild to severe, with necrosis of the epithelium. When fully developed, the injury is characterized by an acute inflammation of the upper and lower airways, with discharge in the upper airway, inflammatory exudate, and pseudomembrane formation in the tracheobronchial tree. The injury develops slowly, intensifying over a period of days.

After a low-dose, single exposure, casualties might notice a variety of catarrhal symptoms accompanied by a dry cough; on examination, they might have pharyngeal and laryngeal erythema. Hoarseness is almost

always present, and the patient often presents with a barking cough. Typically, this hoarseness may progress to a toneless voice, which appears to be particularly characteristic of mustard exposure. Patients characteristically note a sense of chest oppression. All of these complaints typically commence approximately 4 to 6 hours after exposure, with sinus tenderness appearing hours later. Vapor concentrations sufficient to cause these symptoms typically produce reddened eyes, photophobia, lacrimation, and blepharospasm. There may be loss of taste and smell. Patients occasionally experience mild epistaxis and sore throat. In individuals with abnormal sensitivity (smokers and patients with irritable airways or acute viral illness), prominent wheezing and dyspnea may be present.[58]

Exposures to higher concentrations of vapor result in an earlier onset and greater severity of the above effects. Hoarseness rapidly progresses to aphonia. Severe tachypnea and early radiological infiltrates may appear. More-intense respiratory exposures create necrotic changes in the respiratory epithelium that result in epithelial sloughing and pseudomembrane formation. There may be substantial airway occlusion from the inflammatory debris or from pseudomembranes, which can obstruct the upper airways as they form or can break off and obstruct lower airways.[16,58,60]

The initial bronchitis is nonbacterial. White blood cell elevation, fever, pulmonary infiltrates seen on radiograph, and colored secretions may all be present to mimic the changes of a bacterial process. This process is sterile during the first 3 to 4 days; bacterial superinfection occurs in about 4 to 6 days. Careful assessment of the sputum by Gram's stain and culture should be done daily.[60]

Mustard has little effect on lung parenchyma. Its damage is confined to the airways and the tissue immediately surrounding the airways, except after an overwhelming exposure to mustard and as a terminal event.[74] These changes are most intense in the upper airways and decrease in the trachea, bronchi, and smaller bronchioles—presumably reflecting a differential disposition of vapor on the mucosal surface.[71,75]

Fig. 7-11. A surgically excised lung from an Iranian mustard casualty showing bronchiectasis and severe chronic infection. Reprinted with permission from Freitag L, Firusian N, Stamatis G, Greschuchna D. The role of bronchoscopy in pulmonary complications due to mustard gas inhalation. *Chest.* 1991;100:1438.

Pulmonary edema is not a feature; however, it may occur in the terminal stages.[60,74]

The lungs of animals exposed to mustard show alternating areas of atelectasis and emphysema. Atelectasis is thought to be caused by the clogging of bronchioles with mucus, and the emphysema is compensatory.[76] These findings were confirmed when lungs resected at thoracotomy from Iranian casualties from the Iran–Iraq War showed similar effects.[77] As seen in Figure 7-11, the lungs showed bronchiectasis and severe chronic inflammation. The bronchiectasis was due to full-thickness injury of the airways. In some casualties, this injury healed by scarring of such intensity that severe and unrelenting tracheobronchial stenosis developed.

Gastrointestinal Tract

Nausea and vomiting are common within the first few hours after mustard exposure, beginning at about the time the initial lesions become apparent. The early nausea and vomiting, which are generally transient and not severe, may be caused by the cholinergic activity of mustard,[9,33] by a general reaction to injury, or because of the unpleasant odor.[33] Nausea and vomiting that occur days later are probably due to the generalized cytotoxic activity of mustard and damage to the mucosa of the gastrointestinal tract.

Diarrhea is not common, and gastrointestinal bleeding seems to be even less common. Animals that were given approximately 1 LD_{50} of mustard (administered either intravenously or subcutaneously) had profuse diarrhea, which was frequently bloody[60,78]; however, this was unusual when mustard was administered percutaneously or by inhalation. (Diarrhea was more common after nitrogen mustard.[9])

Diarrhea and gastrointestinal bleeding do not seem to be common in humans. Of 107 autopsied cases, none had experienced diarrhea; and in the 57 cases in which the gastrointestinal tract was thoroughly examined, none had significant lesions.[75] In several reported series of Iranian casualties, totaling about 700 casualties, few had diarrhea and only a very few who died had bloody diarrhea.[16,62,79] Constipation was noted in casualties with mild exposure.[60]

Central Nervous System

Although the effects are not usually prominent clinically, mustard affects the CNS. Reports of World War I casualties described apathy, depression, intellectual dullness, and languor.[60] Of 233 Iranian casualties sent to various western European hospitals for medical care during the Iran–Iraq War, about 83% had CNS complaints; most complaints, however, were mild and nonspecific.[62]

Large amounts of mustard administered to animals (via the inhalational, intravenous, subcutaneous, or intramuscular routes) caused hyperexcitability, abnormal muscular movements, convulsions, and other neurological manifestations.[60,80] Animals died a “neurological death” a few hours after receiving a lethal amount of mustard.[9] Autopsies of these animals disclosed few abnormalities.[80]

After three children were accidentally exposed to a large amount of mustard, two of them presented with abnormal muscular activity, and the third alternated between coma and agitation. The first two children died 3 to 4 hours after exposure, possibly from neurological mechanisms.[22] Whether these CNS manifestations are from a cholinergic activity of mustard or from other mechanisms is unknown.

Death

Most casualties die of massive pulmonary damage complicated by infection (bronchopneumonia) and sepsis (resulting from loss of the immune mechanism). When exposure is not by inhalation, the mechanism of death is less clear. In studies with animals in which mustard was administered via routes other than inhalational, the animals died from 3 to 7 days after the exposure; they had no signs of pulmonary damage and often had no signs of sepsis. The mechanism of death was not clear, but autopsy findings resembled those seen after radiation.[81] (Mustard is considered to be a radio-mimetic because it causes tissue damage similar to that seen after radiation.)

Diagnosis

The differential diagnosis of mustard casualties on the battlefield after a known chemical attack is not difficult. The history of a chemical attack is useful, particularly if the chemical agent is known. Simply questioning the casualty about when the pain started—whether it started immediately after the exposure or hours later—is very helpful. Whereas pain from Lewisite (the other vesicant that causes blistering) begins seconds to minutes after exposure, pain from mustard does not begin until the lesion develops hours later.

Blisters appearing simultaneously in a large number of people, in the absence of a known chemical attack, should alert medical personnel to search the area with a chemical agent detector. Because naturally occurring organisms, both plants and insects, cause similar blisters, the appearance of one or more blisters in only a single individual makes exposure to a natural substance the more likely possibility.

Laboratory Tests

There is no specific laboratory test for mustard exposure. As inflammation and infection occur, signs of these (eg, fever and leukocytosis) will develop. Several investigational studies have demonstrated the presence of significant amounts of thiodiglycol, a major metabolite of mustard, in the urine of mustard casualties. In two studies,[82,83] Iranian casualties had higher amounts of thiodiglycol in their urine than did control subjects. In a third study, the urinary thiodiglycol secreted by a laboratory worker accidentally exposed to mustard was quantitatively measured for a 2-week period (his postrecovery urine was used as a control); the half-life of thiodiglycol was 1.18 days.[23] The procedure for analysis of thiodiglycol is described in Technical Bulletin Medical 296.[84]

Patient Management

Decontamination within 1 or 2 minutes after exposure is the only effective means of preventing or decreasing tissue damage from mustard. This decontamination is not done by medical personnel. It must be performed by the soldier himself immediately after the exposure. Generally, a soldier will not seek medical help until the lesions develop, hours later. By that time, skin decontamination will not help the soldier because mustard fixes to the skin within minutes, and tissue damage will already have occurred.[64]

If any mustard remains on the skin, late decontamination will prevent its spreading to other areas of the skin; but after several hours, spreading will probably already have occurred. Decontamination will, however, prevent mustard from spreading to personnel who handle the casualty.

By the time a skin lesion has developed, most of the mustard will already have been absorbed (and the chemical agent will have fixed to tissue); and, unless the site was occluded, the remaining unabsorbed agent will have evaporated. Mustard droplets disappear from the surface of the eye very quickly, so late flushing of the eye will be of no benefit, either.

However, all chemical agent casualties must be thoroughly

decontaminated before they enter a clean MTF. This should be done with the realization that by the time a contaminated soldier reaches an MTF, this decontamination will rarely help the casualty; it does, however, prevent exposure of medical personnel.

Mustard casualties generally fall into three categories. The first is the return to duty category. These individuals have a small area of erythema or one or more small blisters on noncritical areas of their skin; eye irritation or mild conjunctivitis; and/or late-onset, mild upper respiratory symptoms such as hoarseness or throat irritation and a hacking cough. If these casualties are seen long after exposure, so that there is good reason to believe that the lesion will not progress significantly, they can be given symptomatic therapy and returned to duty.

The second category includes casualties who appear to have non–life-threatening injuries but who are unable to return to duty. Casualties with the following conditions must be hospitalized for further care:

- a large area of erythema (with or without blisters),
- an extremely painful eye lesion or an eye lesion that hinders vision, and
- a respiratory injury with moderate symptoms that include a productive cough and dyspnea.

Some of these conditions may develop into life-threatening injuries, and these categories, therefore, should be used only to assess a casualty's *presenting* condition. For example, an area of erythema caused by liquid mustard that covers 50% or more of the body surface area suggests that the individual was exposed to 2 LD_{50} of the agent. Likewise, dyspnea occurring within 4 to 6 hours after the exposure suggests inhalation of a lethal amount of mustard.

The third category comprises those casualties who appear to have life-threatening injuries when they first present at an MTF. Life-threatening injuries include large skin burns caused by liquid mustard, and early onset of moderate-to-severe pulmonary symptoms. Most of the casualties in this category will die from their injuries.

Many mustard casualties will fall into the first category, the majority will fall into the second category, and only a very small percentage of casualties will fall into the third category. Data from World War I, in which only 3% of mustard injuries were lethal despite the unsophisticated medical care at that time (eg, no antibiotics), suggest that most mustard casualties are not severely injured and that most of them will survive.

Most casualties of mustard exposure will, however, require some form of medical care—from a few days to many weeks. Eye care and airway

care will promote healing within weeks; skin lesions take the longest to heal and may necessitate hospitalization for months.[16] Casualties with mild-to-moderate mustard damage will need supportive care. Pain control is extremely important. Fluids and electrolytes should be carefully monitored. Although there is not a great deal of fluid loss from mustard burns (compared with thermal burns), a casualty will probably be dehydrated when he enters the MTF; and a sick patient usually does not eat or drink enough. Parenteral fluid supplements and vitamins may be of benefit. Casualties who have lost their eyesight because of mustard exposure should be reassured that they will recover their vision.

Casualties who do become critically ill from their exposure to mustard will present with large areas of burns, major pulmonary damage, and immunosuppression. Some of the casualties may die from sepsis or from overwhelming damage to the airways and lungs. Medical officers should remember, however, that even with the limited medical care available in World War I, very few deaths were caused by mustard exposure.

Despite the attention given to mustard since World War I, research has not produced an antidote. Because casualties have been managed in different eras and, more recently, in different medical centers, there have been no standard methods of casualty management, nor have there been any controlled studies of one method compared to another. The following advice describes care by organ system. Most casualties will have more than one system involved, and many of these casualties will be dehydrated and have other injuries as well.

Skin

The general principles for managing a mustard skin lesion are to keep the casualty comfortable, keep the lesion clean, and prevent infection. The burning and itching associated with erythema can be relieved by calamine or another soothing lotion or cream such as 0.25% camphor and menthol. These lesions should heal without complication.

Small blisters (< 1 cm) should be left alone; however, the surrounding area should be cleaned (irrigated) at least once daily. An application of a topical antibiotic should immediately be applied to the blisters and the surrounding area. The blisters and the surrounding area do not need to be bandaged unless the casualty will be returning to duty.

Larger blisters (> 1 cm) should be unroofed and the underlying area should be irrigated (2 to 4 times daily) with saline, sterile water, or clean soapy water, and liberally covered (to a depth of 1 mm) with a topical antibiotic cream or ointment (silver sulfadiazine, mafenide acetate, bacitracin, or Neosporin [Burroughs Wellcome Co., Research Triangle Park, N. C.]). Dakin’s solution (hypochlorite) was used on patients in World

War I[60] and during the Iran–Iraq War[16] as an irrigating solution. It does not detoxify the chemical agent in the skin, as was once thought; however, it is an adequate antiseptic and keeps the area clean. Multiple or large areas of vesication necessitate hospitalization for frequent and careful cleaning; a whirlpool bath is a useful means of irrigation. In general, care of mustard skin lesions is the same as that of second-degree thermal burns, although the pathophysiology is different.

Systemic analgesics should be given liberally, particularly before manipulation of the burned area. Systemic antipruritics (eg, trimeprazine) may be useful. Fluid balance and electrolytes should be monitored. Fluids are lost into the edematous areas, but fluid replacement is of less magnitude than that required for thermal burns. Medical personnel accustomed to treating patients with thermal burns must resist the temptation to overhydrate mustard burn patients, which could lead to untoward consequences such as pulmonary edema.[16]

Skin healing can take weeks to months but usually is complete, although pigment changes may persist. Scarring is proportional to the depth of the burn. Skin grafting is rarely needed, but it was successful in one person who had a deep burn.[25]

Eyes

The basic principles of eye care are to prevent infection and to prevent scarring. Although it is unlikely that mustard will still be in the eye by the time the casualty is seen, the eye should be irrigated to remove any possible chemical agent that might be on the lashes and to remove any inflammatory debris that might be on the surface of the eye. Mild lesions (eg, conjunctivitis) can be treated three to four times daily with a soothing eye solution.

Casualties with more-severe eye lesions should be hospitalized. Care for these patients should consist of at least one daily irrigation, preferably more, to remove inflammatory debris; administration of a topical antibiotic three to four times daily; and administration of a topical mydriatic (atropine or homatropine) as needed to keep the pupil dilated (to prevent later synechiae formation). Vaseline or a similar material should be applied to the lid edges to prevent them from adhering to each other; this reduces later scarring and also keeps a path open for possible infection to drain. (When animals’ eyes were kept tightly shut, a small infection could not drain, and a panophthalmitis developed that completely destroyed the eyes.[65])

Topical analgesics may be used for the initial examination; however, they should not be used routinely as they might cause corneal damage. Pain should be controlled with systemic analgesics. The benefit of topical

steroids is unknown; however, some ophthalmologists feel that topical steroids may be helpful if used within the first 48 hours after the exposure (but not after that). In any case, an ophthalmologist should be consulted as early as possible on this and other questions of care. Keeping the casualty in a dim room or providing sunglasses will reduce the discomfort from photophobia.

The transient loss of vision is usually the result of edema of the lids and other structures and not due to corneal damage. Medical personnel should assure the patient that vision will return. Recovery may be within days for milder injuries, while those with severe damage will take approximately a month or longer to recover.

Airways

The therapeutic goal in a casualty with mild airway effects (eg, irritation of the throat, nonproductive cough) is to keep him comfortable. In a casualty with severe effects, the goal is to maintain adequate oxygenation. Antitussives and demulcents are helpful for persistent, severe, nonproductive cough. Steam inhalation might also be useful.

Hypoxia is generally secondary to the abnormalities in the ventilation–perfusion ratio caused by toxic bronchitis. Mucosal sloughing further complicates this abnormality. Underlying irritable airways disease (hyperreactive airways) is easily triggered; consequently, therapy with bronchodilators may be necessary. Casualties with hyperreactive airways may benefit from steroid treatment with careful attention to the added risk of superinfection. Oxygen supplementation may be necessary for prolonged periods; this will depend, primarily, on the intensity of mustard exposure and the presence of any underlying pulmonary disorder.

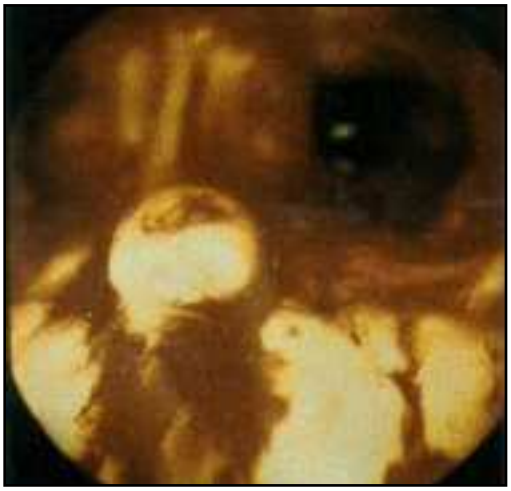
Hypercarbia may result from a previously unrecognized hyperreactive airways state or from abnormal central sensitivity to carbon dioxide, complicated by increased work of respiration (this state may result from bronchospasm). Bronchodilators are acceptable initial therapy. Ventilatory support may be necessary to assist adequate carbon dioxide clearance. The use of certain antibiotic skin creams (such as mafenide acetate) to treat skin lesions may complicate the acid–base status of the individual by inducing a metabolic acidosis. Steroids should be considered if a prior history of asthma or hyperreactive airways disease is obtained.

Initially, the bronchitis resulting from mustard exposure is nonbacterial. White blood cell elevation, fever, pulmonary infiltrates on a chest radiograph, and colored sputum may all be present; however, careful assessment of sputum by Gram’s stain and culture demonstrates that bacterial superinfection typically is not present during the first 3 to 4 days. Antibiotic therapy should be withheld until the identity of a specific

organism becomes available. Of particular importance is the patient's immune status, which may be compromised by a progressive leukopenia beginning about day 4 or 5. The development of leukopenia signals severe immune system dysfunction; massive medical support may become necessary for these patients. In these instances, sepsis typically supervenes, and despite combination antibiotic therapy, death commonly occurs.

A casualty with severe pulmonary signs should be intubated early, before laryngeal spasm makes it difficult or impossible. Intubation assists in ventilation and also allows suction of necrotic and inflammatory debris. Bronchoscopy may be necessary to remove intact pseudomembranes or fragments of pseudomembranes; one of the Iranian casualties treated in western European hospitals during the Iran–Iraq War died of tracheal obstruction by a pseudomembrane. Early use of positive end-expiratory pressure or continuous positive airway pressure may be beneficial. The need for continuous ventilatory support suggests a bad prognosis; of the Iranian casualties treated in western European hospitals who needed assisted ventilation, 87% died.[16]

An especially devastating pulmonary complication, severe and progressive stenosis of the tracheo-bronchial tree (Figure 7-12), was found in about 10% of the Iranian casualties treated in western European hospitals during the Iran–Iraq War. This complication was not recognized in World War I mustard casualties because the degree of exposure required to cause severe tracheobronchial injury resulted in early death from pneumonia: we must remember the primitive nature of early 20th-century medicine and its lack of antibiotics. With the Iranian casualties, bronchoscopy was of value when used both for diagnosis and for therapeutic dilation.[77] However, given the progressive nature of the scarring, unnaturally early death from respiratory failure is to be expected in all such casualties.

a  **c**

b

Fig. 7-12. (a) Bronchoscopic view of the trachea in an Iranian casualty 3 weeks after exposure to mustard. Severe hemorrhagic bronchitis, mucosal necrosis, and early scarring are apparent. **(b)** Bronchogram from an Iranian casualty 1 year after his exposure to mustard. The tip of a 10-mm rigid bronchoscope can be seen at the upper margin of the figure. Severe generalized narrowing of the entire tracheobronchial tree is apparent. The casualty presented with dyspnea, cough, hypoxia, and hypercarbia. **(c)** Bronchoscopic appearance of the carina of an Iranian casualty who had been exposed to mustard several years before. There is nearly total occlusion of the left main-stem bronchus. Reprinted with permission from Freitag L, Firusian N, Stamatis G, Greschuchna D. The role of bronchoscopy in pulmonary complications due to mustard gas inhalation. *Chest*. 1991;100:1437–1438.

Gastrointestinal Tract

The initial nausea and vomiting are rarely severe and can usually be relieved with atropine or common antiemetics. Later vomiting and diarrhea are usually indicative of systemic cytotoxicity and require fluid replacement.

Bone Marrow

Suppression of the hemopoietic elements cannot be predicted from the extent of skin lesions (eg, the lesions might be from vapor and therefore superficial, but significant amounts of mustard may have been absorbed by inhalation). Frequent counts of the formed blood elements must be done on a casualty who has significant skin lesions or airway damage. Mustard destroys the precursor cells, and cell elements in the blood are depressed. Because white blood cells have the shortest life span, their numbers decrease first; the red blood cells and the thrombocytes soon follow if the casualty lives long enough or does not start to recover. Typically, leukopenia begins at day 3 through day 5 after the exposure, and reaches a nadir in 3 to 6,[60] or 7 to 9,[16] days. Leukopenia with a cell count lower than 200 cells/mm^3 usually signifies a bad prognosis,[16] as does a rapid drop in the cell count; for example, from 30,000 to 15,000 cells/mm^3 in a day.[60]

Medical personnel should institute therapy that sterilizes the gut with nonabsorbable antibiotics at the onset of leukopenia.[16] Cellular replacement, either peripheral or marrow, may also be successful.

Other Treatment Modalities

A variety of antiinflammatory and sulfhydryl-scavenging agents (such as promethazine, vitamin E, heparin, and sodium thiosulfate) have been suggested as therapeutic drugs. Although animal studies suggest the value of these agents for prophylactic therapy (or therapy immediately after the exposure), there are no data to support their use after the lesions develop.[85–87]

Activated charcoal, administered orally, has been tried with unknown results[16]; however, it may provide some benefit if given immediately after mustard is ingested. Hemodialysis was not only without benefit, it appeared to have deleterious effects.[16] This is not surprising because mustard becomes fixed to tissue within minutes.

Long-Term Effects

Mustard burns may leave areas of hypopigmentation or hyperpigmentation, sometimes with scarring. Individuals who survive an acute, single mustard exposure with few or no systemic or infectious complications appear to recover fully. Previous cardiopulmonary disorders, severe or inadequately treated bronchitis or pneumonitis, a prior history of smoking, and advanced age all appear to contribute to long-term chronic bronchitis; there is no definitive way to determine whether these conditions

are the result of aging, smoking, or a previous mustard exposure. Casualties with severe airway lesions may later have postrecovery scarring and stenosis, which predisposes the individual to bronchiectasis and recurrent pneumonia.[58]

An important late sequela of mustard inhalation is a tracheal/bronchial stenosis that necessitates bronchoscopy and other procedures.[77] Mustard has been reported to create a long-term sensitivity to smoke, dust, and similar airborne particles, probably as a result of clinically inapparent broncho-spasm.[58,88]

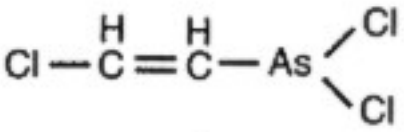
The relationship between mustard exposure and subsequent cancer has been the subject of much study. It seems clear that individuals who were exposed to mustard daily for long periods (eg, workers in mustard production plants) have a slightly higher incidence of cancer of the airways, primarily the upper airways.[89–91] According to two separate reports,[92,93] the association of one or two exposures on the battlefield with subsequent cancer is not clear; in a third report,[94] the relation between mustard exposure and subsequent cancer is equivocal. Interested readers may consult Watson and associates' 1989 review[95] of the mustard exposure–cancer incidence relation.

In 1991, the National Academy of Science appointed a committee to survey the health effects of mustard and Lewisite.[94] Veterans of World War II, who, as subjects in test programs, had been exposed to mustard and Lewisite, were presenting at Veterans Administration hospitals with complaints of illnesses that they believed were associated with these test programs. The committee was requested to survey the literature to assess the strength of association between these chemical agents and the development of specific diseases. The committee reported finding a causal relationship between exposure and various cancers and chronic diseases of the respiratory system; cancer and certain other problems of the skin; certain chronic eye conditions; psychological disorders; and sexual dysfunction. They found insufficient evidence for a causal relationship between exposure and gastrointestinal diseases, hematological diseases, neurological diseases, and cardiovascular diseases (except those resulting from infection following exposure). Some of these conclusions were not well supported. For example, there were no cases of skin cancer reported, and the alleged psychological disorders were from the trauma of exposure, not from the agent (see Chapter 8, Long-Term Health Effects of Nerve Agents and Mustard).

LEWISITE

Lewisite (ß-chlorovinyldichloroarsine) is an arsenical vesicant but of

only secondary importance in the vesicant group of agents. It was synthesized[96] in the early 20th century and has seen little or no battlefield use. Lewisite is similar to mustard in that it damages the skin, eyes, and airways; however, it differs from mustard because its clinical effects appear within seconds of exposure. An antidote, British anti-Lewisite (BAL), can ameliorate the effects of Lewisite if used soon after exposure. Lewisite has some advantages over mustard but also some disadvantages.



L

Military Use

A research team headed by U.S. Army Captain W. L. Lewis is generally credited with the synthesis of Lewisite in 1918,[96–98] although German scientists had studied this material earlier.[1,58] Large quantities were manufactured by the United States for use in Europe; however, World War I ended while the shipment was at sea and the vessel was sunk.[1,98]

There has been no verified use of Lewisite on a battlefield, although Japan may have used it against China between 1937 and 1944.[93] Currently, this vesicant is probably in the chemical warfare stockpile of several countries. Lewisite is sometimes mixed with mustard to lower the freezing point of mustard; Russia has this mixture.[99]

Properties

Pure Lewisite is an oily, colorless liquid, and impure Lewisite is amber to black. It has a characteristic odor of geraniums. Lewisite is much more volatile and persistent in colder climates than mustard. Lewisite remains fluid at lower temperatures, which makes it perfect for winter dispersal. Lewisite hydrolyzes rapidly, and, on a humid day, maintaining a biologically active concentration of vapor may be difficult.[100]

Toxicity

The toxicity of Lewisite vapor is very similar to that of mustard vapor; the LCt_{50} (the concentration • time that is lethal to 50% of the exposed population) by inhalation is estimated to be about 1,500 mg•min/m^3, and the LCt_{50} for eye and airway damage are about 150 and 500 mg•min/m^3, respectively. Vesication is caused by 14 µg of liquid, and the LD_{50} of liquid on the skin is about 30 mg/kg[100] (or probably higher[98]). Blister fluid from a Lewisite-caused blister is nonirritating,[58,98] but it does contain 0.8 to 1.3

mg/mL of arsenic.

Biochemical Mechanisms of Injury

Lewisite shares many biochemical mechanisms of injury with the other arsenical compounds. It inhibits many enzymes: in particular, those with thiol groups, such as pyruvic oxidase, alcohol dehydrogenase, succinic oxidase, hexokinase, and succinic dehydrogenase (Figure 7-13). As is true with mustard, the exact mechanism by which Lewisite damages cells has not been completely defined. Inactivation of carbohydrate metabolism, primarily because of inhibition of the pyruvate dehydrogenase complex, is thought to be a key factor.[98]

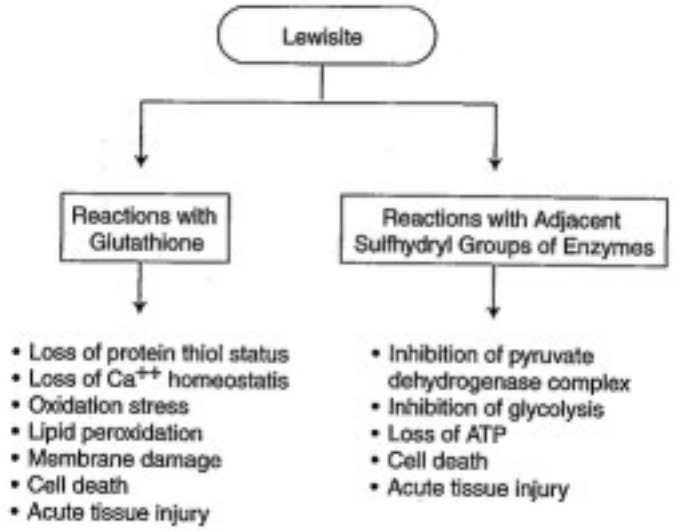


Fig. 7-13. The putative mechanisms by which Lewisite causes tissue damage. Adapted from US Army Medical Research Institute of Chemical Defense. A global picture of battlefield vesicants, I: A comparison of properties and effects. *Med Chem Def*. 1992;5(1):6.

Clinical Effects

Lewisite damages skin, eyes, and airways by direct contact and has systemic effects after absorption. Unlike mustard, it does not produce immunosuppression. Data on human exposure are few. Lewisite was applied to human skin in a few studies[58,101–103]; however, most information on its clinical effects is based on animal studies.

Skin

Lewisite liquid or vapor produces pain or irritation within seconds to minutes after contact. Pain caused by a Lewisite lesion is much less severe than that caused by mustard lesions, and it diminishes after blisters form.[58]

Erythema is evident within 15 to 30 minutes after exposure to liquid Lewisite, and blisters start within several hours; these times are somewhat longer after vapor exposure. Lewisite is absorbed by the skin within 3 to 5 minutes (compared with 20–30 min for an equal amount of mustard) and

spreads over a wider area than the same amount of mustard. The Lewisite blister begins as a small blister in the center of the erythematous area and expands to include the entire inflamed area, whereas vesication from mustard begins as a “string of pearls” at the periphery of the lesion, small blisters that eventually merge.[58] Other differences between the lesions produced by these two chemical agents are

- the inflammatory reaction from Lewisite generally occurs much faster,
- the lesions from Lewisite heal much faster,
- secondary infection is less common after Lewisite exposure, and
- subsequent pigmentation is likewise less common.[58]

See Goldman and Dacre[104] for a further review of Lewisite and its toxicology.

Eyes

A person is less likely to receive severe eye injury from Lewisite vapor than from mustard vapor because the immediate irritation and pain caused by Lewisite will produce blepharospasm, effectively preventing further exposure. A small droplet of Lewisite (0.001 mL) can cause perforation and loss of an eye.[105]

In tests performed on rabbits,[105] Lewisite caused almost immediate edema of the lids, conjunctiva, and cornea (which was maximal after the lid edema had subsided) and early and severe involvement of the iris and ciliary body, followed by gradual depigmentation and shrinkage of the iris stroma. Miosis appeared early. In this same study, miosis was not noted after mustard exposure. No long-term effects of Lewisite were noted, such as the delayed keratitis seen after mustard.

Airways

Lewisite vapor is extremely irritating to the nose and lower airways, causing individuals exposed to it to seek immediate protection, thus limiting further exposure. The airway lesion of Lewisite is very similar to the lesion caused by mustard exposure except that the Lewisite vapor is extremely irritating to the mucous membranes. In large amounts, Lewisite causes pulmonary edema.

After exposure to Lewisite, dogs exhibited massive nasal secretions, lacrimation, retching, vomiting, and labored respiration. These symptoms worsened until death finally occurred. On autopsy, the lungs were

edematous, and a pseudomembrane often extended from the nostrils to the bronchi. Tracheal and bronchial mucosa was destroyed and the submucosa was congested and edematous. Bronchopneumonia was commonly mixed with edema.[60]

Other Effects

"Lewisite shock" is seen after exposure to large amounts of Lewisite. This condition is the result of protein and plasma leakage from the capillaries and subsequent hemoconcentration and hypotension.

A small amount of Lewisite on the skin will cause local edema because of the effects of this agent on local capillaries. With a large amount of Lewisite, the pulmonary capillaries are also affected (because they are more sensitive to Lewisite than other capillaries or because absorbed Lewisite reaches the lungs before it reaches the systemic circulation); there is edema at the site of exposure and pulmonary edema. With even larger amounts of Lewisite, all capillaries are affected, and proteins and plasma leak from the circulation into the periphery. Even after small amounts of Lewisite, the fluid loss can be sufficient to cause diminution of renal function and hypotension.[104]

Arsines are known to cause hemolytic anemia, but there is little mention of this in reports on Lewisite exposure. A "true or hemolytic anemia" was noted with Lewisite shock.[104]

Diagnosis

Lewisite exposure can be distinguished from mustard exposure by the history of pain on contact with the agent. Phosgene oxime also causes pain on contact, but phosgene oxime does not produce a liquid-filled blister. If a single individual has an isolated blister, other plant or animal causes of vesication should be sought.

Laboratory Tests

There is no specific laboratory test for Lewisite. Urinary arsenic excretion might be helpful in identifying possible exposure to Lewisite, however.

Patient Management

Medical personnel should follow the same principles for managing Lewisite skin, eye, and airway lesions that they follow for managing mustard lesions. A specific antidote, BAL (dimercaprol), will prevent or greatly decrease the severity of skin and eye lesions if applied topically

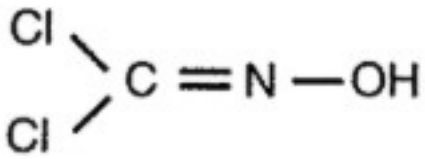
within minutes after the exposure and decontamination (however, preparations of BAL for use in the eyes and on the skin are no longer available). Given intramuscularly, BAL will reduce the severity of systemic effects. BAL binds to the arsenic of Lewisite more strongly than do tissue enzymes, thereby displacing Lewisite from the cellular receptor sites.[98,104]

BAL reduced the mortality in dogs when it was given within 100 minutes after they had inhaled a lethal amount of Lewisite.[106] Burns of the eyes from Lewisite can be prevented if BAL is applied within 2 to 5 minutes of exposure[104]; when it was applied within an hour after exposure, BAL prevented vesication in humans.[107] BAL has some unpleasant side effects, including hypertension and tachycardia; the user should read the package insert.

Long-Term Effects

There are no data on human exposure from which to predict the long-term effects from Lewisite. There is no substantial evidence to suggest that Lewisite is carcinogenic, teratogenic, or mutagenic.[104] The committee appointed by the National Academy of Science reported[94] a causal relationship between Lewisite exposure and chronic respiratory diseases, and also that acute, severe injuries to the eye from Lewisite will persist.

PHOSGENE OXIME



CX

Phosgene oxime (CX) is not a true vesicant because it does not produce vesicles. Instead, phosgene oxime is an urticant or nettle agent: it causes erythema, wheals, and urticaria. Its lesions have been compared with those caused by nettle stings. Because it causes extensive tissue damage, phosgene oxime has been called a corrosive agent. Phosgene oxime is not known to have been used on a battlefield, and there is very little information regarding its effects on humans. This compound must be distinguished from phosgene (CG), which exerts its effects on the alveolar–capillary membrane.

Military Use

German scientists first synthesized phosgene oxime in 1929,[108] and Russia as well as Germany had developed it before World War II. Both countries may have had weapons that contained this agent.[109] The United

States also had studied phosgene oxime before World War II but rejected it as a possible chemical agent because of its biological effects—or lack thereof—and its instability.[109] The apparent lack of biological effects was later found to be due to the low concentrations (1%–2%) used in the pre–World War II studies. Later studies indicated that concentrations below 8% cause no or inconsistent effects.[109,110]

Phosgene oxime is of military interest because

- it penetrates garments and rubber much more quickly than do other chemical agents, and
- it produces a rapid onset of severe and prolonged effects.

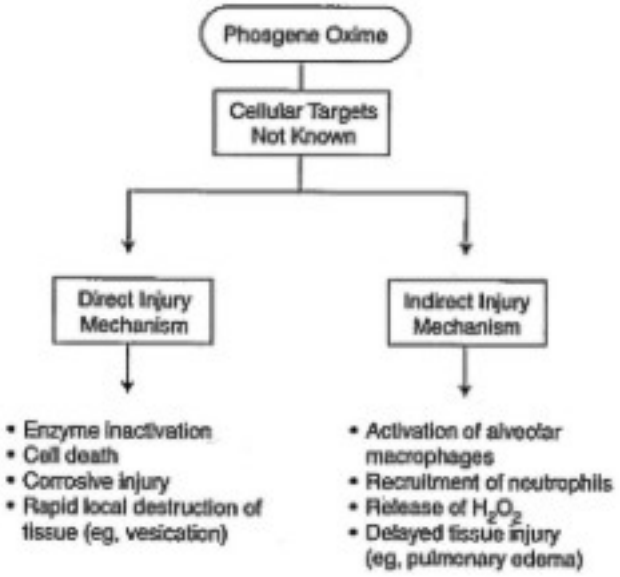
When mixed with another chemical agent (eg, VX), the rapid skin damage caused by phosgene oxime will render the skin more susceptible to the second agent. Also, if an unmasked soldier were exposed to phosgene oxime before donning his mask, the pain caused by phosgene oxime will prompt him to unmask again.

Properties

Pure phosgene oxime (dichloroformoxime) is a colorless, crystalline solid; the munitions grade compound is a yellowish-brown liquid. Its melting point is 35°C to 40°C (95°F–104°F). The solid material will produce enough vapor to cause symptoms.[100]

Biochemical Mechanisms of Injury

Phosgene oxime is the least well studied of the chemical agents discussed in this volume, and its mechanism of action is unknown. It might produce biological damage because of the necrotizing effects of the chlorine, because of the direct effect of the oxime, or because of the carbonyl group (Figure 7-14). The skin lesions, in particular, are similar to those caused by a



strong acid. This agent seems to cause its greatest systemic effects in the first capillary bed it encounters. For example, cutaneous application or intravenous injection of phosgene oxime causes pulmonary edema, while injection into the portal vein produces hepatic necrosis but not pulmonary edema.[110]

Fig. 7-14. The putative mechanisms by which phosgene oxime causes tissue damage. Adapted from US Army Medical Research Institute of Chemical Defense. A global picture of battlefield vesicants, I: A comparison of properties and effects. *Med Chem Def*. 1992;5(1):6.

Clinical Effects

Phosgene oxime affects the skin, the eyes, and the lungs. The effects are almost instantaneous, and it causes more severe tissue damage than other vesicants. A characteristic of phosgene oxime is the immediate pain or irritation it produces on the skin, in the eyes, and in the airways. No other chemical agent produces such an immediately painful onset that is followed by rapid tissue necrosis.

Skin

Pain occurs immediately on contact with the liquid or solid form of this agent. Approximately 5 to 20 seconds after solutions containing 8% to 70% phosgene oxime were applied, pain and blanching occurred at the application site. Following the initial exposure, the site became grayish with a border of erythema. Within 5 to 30 minutes after the exposure, edema formed around the edges of the tissue; the tissue later became necrotic. During the next 30 minutes, a wheal formed but disappeared overnight. The edema regressed over the following 24 hours and the original blanched area became pigmented. A dark eschar formed over the following 7 days; this gradually healed from below by granulation. The lesion extended into the underlying panniculus and muscle and was surrounded by an inflammatory reaction. In some subjects, healing was incomplete 4 to 6 months after exposure.[109] In both animal and human subjects, the skin had completely absorbed the phosgene oxime within seconds—by the time pallor appeared.[110]

Eyes

The eye lesions from phosgene oxime are similar to those caused by Lewisite; these lesions result in immediate pain, conjunctivitis, and keratitis.[109–111] An exact description of these effects, however, is not available.

Airways

The main lesion of phosgene oxime in the lungs is pulmonary edema. This effect occurs after either inhalation or systemic absorption of the agent. The pulmonary edema may be accompanied by necrotizing bronchiolitis and thrombosis of pulmonary venules. A large amount of phosgene oxime on the skin may produce pulmonary edema after a several-hour delay; pulmonary thromboses are prominent.[110]

Patient Management

There is no antidote for phosgene oxime, nor is there a recommended therapeutic regimen. Medical personnel should treat necrotic areas of the skin the same way other necrotic lesions are treated—by keeping them clean and avoiding infection. The eye lesions require the same care as one would supply for damage from a corrosive substance. The pulmonary lesion, noncardiac pulmonary edema, should be managed as suggested in Chapter 9, Toxic Inhalational Injury.

Decontamination, or self-aid, must be accomplished immediately after contact because the agent is absorbed from the skin within seconds.

SUMMARY

The military has considered vesicants to be major chemical warfare agents since 1917. Mustard, however, is the only vesicant known to have been used on the battlefield. Mustard and Lewisite, in much smaller amounts, are known to be in the stockpiles of other countries.

Mustard was used on a large scale in World War I, causing a great number of casualties; it was also used during the Iran–Iraq War. Data from the Iran–Iraq War are scanty; however, data from World War I indicate that more than 95% of mustard casualties survived but most required lengthy hospitalizations. If mustard is ever used again, military medical personnel must be prepared to accept and care for large numbers of casualties, who will require long-term hospitalization.

ACKNOWLEDGMENT

The authors thank John P. Petrali, Ph.D., U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland, for allowing us to use the previously unpublished photographs shown in Figure 7-6.

REFERENCES

1. Prentiss AM. *Chemicals in War: A Treatise on Chemical War*. New York, NY: McGraw-Hill; 1937.
2. Heller CE. *Chemical Warfare in World War I: The American Experience, 1917–1918*. Fort Leavenworth, Kan: US Army Command and General Staff College, Combat Studies Institute; 1984. Leavenworth Papers No. 10.
3. Medema J. Mustard gas: The science of H. *NBC Defense Technol Int*. 1986;1:66–71.
4. Jacobson LO, Spurr C, Barron ESG, Smith T, Lushbaugh C, Dick GF. Nitrogen mustard therapy. *JAMA*. 1946;32:263–271.
5. Gilman A. The initial clinical trial of nitrogen mustard. *Am J Surg*. 1963;105:574–578.
6. Goodman LS, Wintrobe MM, Dameshek W, Goodman MJ, Gilman A, McLennan MT. Nitrogen mustard therapy. *JAMA*. 1946;132:126–132.
7. Rhoads CP. Nitrogen mustards in the treatment of neoplastic disease: Official statement. *JAMA*. 1946;131:656–658.
8. Fries AA, West CJ. Dichloroethylsulfide: Mustard gas. In: Fries AA, West CJ, eds. *Chemical Warfar*e. New York, NY: McGraw Hill; 1921: 150–179.
9. Graef I, Karnofsky DA, Jager VB, Krichesky B, Smith HW. The clinical and pathologic effects of nitrogen and sulfur mustards in laboratory animals. *Am J Pathol*. 1948;24:1–47.
10. Gilchrist HL. Statistical consideration of gas casualties, I: Gas casualties. In: Weed FW, ed. *Medical Aspects of Gas Warfare*. Vol 14. In: *The Medical Department of the United States Army in the World War*. Washington, DC: Government Printing Office; 1926: 273–279.
11. Balali-Mood M, Farhoodi M, Panjvani FK. Report of three fatal cases of war gas poisoning. In: *Proceedings of the 2nd World Congress on New Compounds in Biological and Chemical Warfare*. Ghent, Belgium: International Association of Forensic Toxicologists; 1993. Abstract.
12. Alexander SF. Medical report of the Bari Harbor mustard casualties. *Military*

Surg. 1947;101:1–17.

13. Infield G. *Disaster at Bari*. New York, NY: Bantam; 1988.

14. Deeter DP, Gaydos JC, eds. *Occupational Health: The Soldier and the Industrial Base.* Part 3, Vol 2. In: Zajtchuk R, Bellamy RF, eds. *Textbook of Military Medicine*. Washington, DC: US Department of the Army, Office of The Surgeon General, and Borden Institute; 1993.

15. Carus WS. *Chemical Weapons in the Middle East*. Washington, DC: The Washington Institute for Near East Policy; 1988. Research Memorandum 9.

16. Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg*. 1989;3S:1–61.

17. Aasted A, Darre MD, Wulf HC. Mustard gas: Clinical, toxicological, and mutagenic aspects based on modern experience. *Ann Plast Surg*. 1987;19:330–333.

18. Aasted A, Wulf HC, Darre E, Niebuhr E. Fishermen exposed to mustard gas: Clinical experiences and risk evaluation. *Ugeskr Laeger*. 1985;147:2213–2216.

19. Jorgensen BS, Olesen B, Berntsen O. Mustard gas accidents on Bornholm. *Ugeskr Laeger*. 1985;147:2251–2254.

20. Wulf HC, Aasted A, Darre E, Neibuhr E. Sister chromatid exchanges in fishermen exposed to leaking mustard gas shells. *Lancet*. 1985;1:690–691.

21. Hobbs FB. A fatal case of mustard gas poisoning. *Br Med J*. 1944;2:306–307.

22. Heully F, Gruninger M. Collective intoxication caused by the explosion of a mustard gas shell. *Ann Med Legal*. 1956;36:195–204.

23. Jakubowski EM, Sidell FR, Evans RA, et al. Accidental human sulfur mustard exposure: Verification and quantification by monitoring thiodiglycol levels. *J Anal Toxicol*. 1997. In press.

24. Aitken RS. Effects of accidental exposure to mustard-gas vapor. *Lancet*. 1943;245:602–603.

25. Ruhl CM, Park DJ, Danisa O, et al. A serious skin sulfur mustard burn from artillery shell. *J Emerg Med*. 1994;12(2):159–166.

26. Chemical Research and Development Engineering Command. *Persistency Times of Chemical Agents on CARC Painted Vehicles and Sand.* Aberdeen Proving Ground, Md; 1990. CRDEC SMCCR-OPA.

27. Blewett WK. *Defense Against Mustard: A P2NBC2 Review and Analysis.* Aberdeen Proving Ground, Md: Physical Protection Directorate; 1992. Chemical Research and Development Engineering Command Technical Report 3270.

28. Mann I, Pullinger BD. A study of mustard-gas lesions of the eyes of rabbits and

men. *Am J Ophthalmol*. 1944;26:1253–1277.

29. Papirmeister B, Feister AJ, Robinson SI, Ford RD. *Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological Implications.* Boca Raton, Fla: CRC Press; 1991.

30. Marshall EK Jr, Lynch V, Smith HW. On dichlorethylsulphide (mustard gas), II: Variations in susceptibility of the skin to dichlorethylsulphide. *J Pharm Exp Therap*. 1919;12:291–301.

31. Smith WJ, Dunn MA. Medical defense against blistering chemical warfare agents. *Arch Dermatol*. 1991;127: 1207–1213.

32. Somani SM, Babu SR. Toxicodynamics of sulfur mustard. *Int J Clin Pharm Therap Toxicol*. 1989;9:419–435.

33. Anslow WP, Houck CR. Systemic pharmacology and pathology of sulfur and nitrogen mustards. In: *Chemical Warfare Agents, and Related Chemical Problems*. Parts 3–6. Washington, DC: Office of Scientific Research and Development, National Defense Research Committee, Div 9; 1946: 440–478.

34. Papirmeister B, Gross CL, Meier HL, Petrali JP, Johnson JB. Molecular basis for mustard-induced vesication. *Fund Appl Toxicol*. 1985;5:S134–S149.

35. Gross CL, Meier HL, Papirmeister B, Brinkley FB, Johnson JB. Sulfur mustard lowers nicotinamide dinucleotide concentrations in human skin grafted to athymic nude mice. *Toxicol Appl Pharmacol*. 1985;81:85–90.

36. Meier HL, Gross CL, Papirmeister B. 2,2'-dichlorodiethyl sulfide (sulfur mustard) decreases NAD^+ levels in human leukocytes. *Toxicol Lett*. 1987;39:109–122.

37. Mol MAE, van de Ruit AMBC, Kluivers AW. NAD^+ levels and glucose uptake of cultured human epidermal cells exposed to sulfur mustard. *Toxicol Appl Pharmacol.* 1989;98:159–165.

38. Dixon M, Needham DM. Biochemical research on chemical warfare agents. *Nature*. 1946;158:432–438.

39. Schnyder J, Bagglionoi M. Induction of plasminogen activator secretion in macrophages by electrochemical stimulation of the hexose monophosphate shunt by methylene blue. *Proc Natl Acad Sci USA*. 1980;77:414–417.

40. Papirmeister B, Gross CL, Petrali JP, Hixson CL. Pathology produced by sulfur mustard in human skin grafts on athymic nude mice, I: Gross and light microscopic changes. *J Toxicol Cutan Ocular Toxicol*. 1984;3:371–391.

41. Smith WJ, Gross CL, Chan P, Meier HL. The use of human epidermal keratinocytes in culture as a model for studying sulfur mustard toxicity. *Cell Biol Toxicol*. 1990;6:285–291.

42. Yourick JJ, Clark CR, Mitcheltree LW. Niacinamide pretreatment reduces

microvesicle formation in hairless guinea pigs cutaneously exposed to sulfur mustard. *Fund Appl Toxicol*. 1991;17:533–542.

43. Petrali JP, Oglesby SB, Meier HL. Ultrastructural correlates of the protection afforded by niacinamide against sulfur mustard-induced cytotoxicology of human lymphocytes in vitro. *Ultrastructural Pathol*. 1990;14:253–262.

44. Martens ME, Smith WJ. Mechanisms of sulfur mustard induced metabolic injury. *Proceedings of the 1993 Medical Chemical Defense Bioscience Review.* May 1993: 257–363. Defense Technical Information Center A275667.

45. Cowan FM, Broomfield CA, Smith WJ. Effect of sulfur mustard exposure on protease activity in human peripheral blood lymphocytes. *Cell Biol Toxicol*. 1991;7:239–248.

46. Cowan FM, Broomfield CA, Smith WJ. Inhibition of sulfur mustard-increased protease activity by niacinamide, N-acetyl cysteine or dexamethasone. *Cell Biol Toxicol*. 1992;8:129–138.

47. Smith WJ, Cowan FM, Broomfield CA. Increased proteolytic activity in human epithelial cells following exposure to sulfur mustard. *FASEB J*. 1991;5:A828.

48. Gentilhomme E, Neveux Y, Hua A, Thiriot C, Faure M, Thivolet J. Action of bis(betachloroethyl)sulphide (BCES) on human epidermis reconstituted in culture: Morphological alterations and biochemical depletion of glutathione. *Toxicology in Vitro*. 1992;6:139–147.

49. Orrenius S, Nicotera P. On the role of calcium in chemical toxicity. *Acta Toxicol*. 1987;11:S11–S19.

50. Ministry of Defence. A literature review upon the toxicology, mechanism of action, and treatment of sulphur and nitrogen mustard poisoning. United Kingdom: Ministry of Defence; n.d. Unpublished report.

51. Ray R, Legere RH, Broomfield CA, Petrali JP. Mechanism of action of alkylating agents: Membrane effects. In: *Proceedings of the 1991 Medical Defense Bioscience Review.* Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1991: 139–142. AD B158588.

52. Miccadei S, Kyle ME, Gilfor D, Farber JL. Toxic consequences of the abrupt depletion of glutathione in cultured rat hepatocytes. *Arch Biochem Biophys*. 1988;265:311–320.

53. Paulet G. Metabolisme cellulaire et action cutanée du sulfure d’ethyle dichlore (yperite): Role devolu au potentiel d’oxydation cellulaire [in French]. *CR Séances Soc Bio*. 1952;146:925–928.

54. Elsayed NM, Omaye ST, Klain GJ, Inase JL, Wheeler CW, Korte DW. Response of mouse brain to a single subcutaneous injection of the monofunctional sulfur mustard, butyl 2-chloroethyl sulfide (BCS). *Toxicology*. 1989;58:11–20.

55. Karnofsky DA, Graef I, Smith HW. Studies on the mechanism of action of the nitrogen and sulfur mustards in vivo. *Am J Pathol*. 1948;24:275–291.

56. Davison C, Rozman RS, Smith PK. Metabolism of bis-beta-chloroethyl sulfide (sulfur mustard gas). *Biochem Pharmacol*. 1961;7:65–74.

57. Zhang B, Wu Y. Toxicokinetics of sulfur mustard. *Chinese J Pharm Toxicol*. 1987;1:188–194.

58. Buscher H; Conway N, trans. *Green and Yellow Cross*. Cincinnati, Ohio: Kettering Laboratory of Applied Physiology, University of Cincinnati; 1944.

59. Keeler JR. Lieutenant Colonel, US Army Nurse Corps. Personal communication, 1990.

60. Vedder EB. Vesicants. In: Vedder EB, ed. *The Medical Aspects of Chemical Warfare*. Baltimore, Md: Williams & Wilkins; 1925: 125–166.

61. Gilchrist HL. *A Comparative Study of WWI Casualties From Gas and Other Weapons*. Edgewood Arsenal, Md: US Chemical Warfare School; 1928: 1–51.

62. Balali-Mood M, Navaeian A. Clinical and paraclinical findings in 233 patients with sulfur mustard poisoning. In: *Proceedings of the 2nd World Congress on New Compounds in Biological and Chemical Warfare*. Ghent, Belgium; 1986: 464–473.

63. Momeni A, Enshaeih S, Meghdadi M, Amindjavaheri M. Skin manifestations of mustard gas. *Arch Dermatol*. 1992;128:775–780.

64. Renshaw B. Mechanisms in production of cutaneous injuries by sulfur and nitrogen mustards. In: *Chemical Warfare Agents, and Related Chemical Problems*. Parts 3–6. Washington, DC: Office of Scientific Research and Development, National Defense Research Committee, Div 9; 1946: 478–520.

65. Warthin AS. Pathologic action of mustard gas (dichlorethylsulphide). In: Weed FW, ed. Medical Aspects of Gas Warfare. Vol. 14. In: *The Medical Department of the United States Army in the World War*. Washington, DC: Government Printing Office; 1926: 512–661.

66. Henriques FC Jr, Moritz AR, Breyfogle HS, Paterson LA. The mechanism of cutaneous injury by mustard gas: An experimental study using mustard prepared with radioactive sulfur. In: *Chemical Warfare Agents, and Related Chemical Problems*. Parts 3–6. Washington, DC: Office of Scientific Research and Development, National Defense Research Committee, Div 9; 1946.

67. Ginzler AM, Davis MIJ. *The Pathology of Mustard Burns of Human Skin.* Edgewood Arsenal, Md: US Army Medical Research Laboratory: 1943.

68. Petrali JP, Oglesby SB, Mills KR. Ultrastructural correlates of sulfur mustard toxicity. *J Toxicol Cutan Ocular Toxicol*. 1990;9:193–204.

69. Reed CI. The minimum concentration of dichlorethylsulphide (mustard gas)

effective for the eyes of man. *J Pharm Exp Therap*. 1920;15:77–80.

70. Geeraets WJ, Abedi S, Blank RV. Acute corneal injury by mustard gas. *South Med J*. 1977;70:348–350.

71. Warthin AS, Weller CV. *The Medical Aspects of Mustard Gas Poisoning*. St. Louis, Mo: C. V. Mosby; 1919.

72. Maumenee AE, Scholz RO. The histopathology of the ocular lesions produced by the sulfur and nitrogen mustards. *Bull Johns Hopkins Hosp*. 1948;82:121–147.

73. Atkinson WS. Delayed keratitis due to mustard gas (dichlorodiethyl sulfide) burns. *Arch Ophthalmol*. 1948;40: 291–301.

74. Gilchrist HL. Symptoms and treatment. In: Weed FW, ed. *Medical Aspects of Gas Warfare*. Vol 14. In: *The Medical Department of the United States Army in the World War*. Washington, DC: Government Printing Office; 1926: 250–272.

75. Pappenheimer AM. Pathological action of war gases. In: Weed, FW, ed. *Medical Aspects of Gas Warfare*. Vol 14. In: *The Medical Department of the United States Army in the World War*. Washington, DC: Government Printing Office; 1926: 87–249.

76. Winternitz MC, Finney WP Jr. The pathology of mustard poisoning. In: *Collected Studies on the Pathology of War Gas Poisoning*. New Haven, Conn: Yale University Press; 1920: 99–114.

77. Freitag L, Firusian N, Stamatis G, Greschuchna D. The role of bronchoscopy in pulmonary complications due to mustard gas inhalation. *Chest*. 1991;100:1436–1441.

78. Houck CR, Crawford B, Bannon JH, Smith HW. Studies on the mechanism of death in dogs after systemic intoxication by the intravenous injection of methyl-bis(beta-chloroethyl)amine or tris(beta-chloroethyl)amine. *J Pharm Exp Therap*. 1947;90:277–292.

79. Sohrabpour H. Clinical manifestations of chemical agents on Iranian combatants during the Iran–Iraq conflict. In: Heyndrickx A, ed. *Proceedings of the 1st World Congress on New Compounds in Biological and Chemical Warfare: Toxicological Evaluation.* Ghent, Belgium; 1984: 291–297.

80. Marshall EK Jr. Physiological action of dichlorethyl sulphide (mustard gas). In: Weed FW, ed. *Medical Aspects of Gas Warfare*. Vol 14. In: *The Medical Department of the United States Army in the World War*. Washington, DC: Government Printing Office; 1926: 369–406.

81. Philips FS. Recent contributions to the pharmacology of bis(2-haloethyl) amines and sulfides. *Pharmacol Rev*. 1950;2:281–323.

82. Wils ERJ, Hulst AG, de John AL, Verweij A, Boter HL. Analysis of thiodiglycol in urine of victims of an alleged attack with mustard gas. *J Anal Toxicol.*

1985;9:254–257.

83. Wils ERJ, Hulst AG, van Laar J. Analysis of thiodiglycol in urine of victims of an alleged attack with mustard gas, II. *J Anal Toxicol*. 1988;12:15–19.

84. US Department of the Army. *Assay Techniques for Detection of Exposure to Sulfur Mustard, Cholinesterase Inhibitors, Sarin, Soman, GF, and Cyanide.* Washington, DC: Headquarters, DA; May 1996. Technical Bulletin Medical 296.

85. Vojvodic V, Milosavljevic Z, Boskovic B, Bojanic N. The protective effect of different drugs in rats poisoned by sulfur and nitrogen mustards. *Fund Appl Toxicol*. 1985;5:S160–S168.

86. Weger N. Therapy in cases of poisoning with mustard gas (yellow cross). *Deutsches Arzteblatt*. 1975;23: 1749–1750.

87. Fasth A, Sorbo B. Protective effect of thiosulfate and metabolic thiosulfate precursors against toxicity of nitrogen mustard (HN 2). *Biochem Pharmacol*. 1973;22:1337–1351.

88. Morgenstern P, Koss FR, Alexander WW. Residual mustard gas bronchitis: Effects of prolonged exposure to low concentrations of mustard gas. *Ann Intern Med*. 1947;26:27–40.

89. Tokuoka S, Hayashi Y, Inai K, et al. Early cancer and related lesions in the bronchial epithelium in former workers of a mustard gas factory. *Acta Pathol Jpn*. 1986;36:533–542.

90. Wada S, Miyanishi M, Nishimoto Y, Kambe S, Miller RW. Mustard gas as a cause of respiratory neoplasia in man. *Lancet*. 1968;1161–1163.

91. Manning KP, Skegg DCG, Stell PM, Doll R. Cancer of the larynx and other occupational hazards of mustard gas workers. *Clin Otolaryngol*. 1981;6:165–170.

92. Norman JE. Lung cancer mortality in World War I veterans with mustard-gas injury: 1919–1965. *J Natl Cancer Inst*. 1975;54:311–317.

93. Beebe GW. Lung cancer in World War I veterans: Possible relation to mustard-gas injury and 1918 influenza epidemic. *J Natl Cancer Inst*. 1960;25:1231–1252.

94. Pechura CM, Rall DP, eds. *Veterans at Risk*. Washington, DC: National Academy Press; 1993.

95. Watson AP, Jones TD, Griffin GD. Sulfur mustard as a carcinogen: Application of relative potency analysis to the chemical warfare agents H, HD, and HT. *Regul Toxicol Pharmacol*. 1989;10:1–25.

96. Lewis WL, Stiegler HW. The beta-chlorovinyl-arsines and their derivatives. *Am Chem Soc*. 1925;47:2546–2555.

97. Harris R, Paxman J. *A Higher Form of Killing*. New York, NY: Hill and Wang;

1982.

98. Trammel GL. Toxicodynamics of organoarsenic chemical warfare agents. In: Somani SM, ed. *Chemical Warfare Agents*. San Diego, Calif: Academic Press; 1992: 255–270.

99. Madsen J. Major, Medical Corps, US Army. Personal communication, 1995.

100. US Department of Defense. *Potential Military Chemical/Biological Agents and Compounds.* Washington, DC: Headquarters, Departments of the Army, Navy, and Air Force; 1990. Field Manual 3-9, Air Force Regulation 355-7, NAVFAC P-467.

101. Rovida G. Lewisite, III: Action on the human skin. *Sperimentale*. 1929;83:115–120.

102. Wardell EL. *Lewisite (M-1): Summary of Physiologic and Toxicologic Data*. Edgewood Arsenal, Md: Chemical Warfare Service; 1940. Edgewood Arsenal Technical Report 285.

103. Dailey LE, Clark JW, Stolp BN, Conner JC Jr. *A Controlled Laboratory Experiment to Compare Lesions Resulting From Application of Mustard, Lewisite and Nitrogen Mustards to the Skin of the Forearms of Humans.* Washington, DC: Naval Research Laboratory; 1941. NRL Report P-2364.

104. Goldman M, Dacre JC. Lewisite: Its chemistry, toxicology, and biological effects. *Rev Environ Contam Toxicol*. 1989;110:75–115.

105. Mann I, Pirie A, Pullinger BD. A study of Lewisite lesions of the eyes of rabbits. *Am J Ophthalmol*. 1946;29:1215–1227.

106. Harrison HE, Ordway HK, Durlacher SH, Albrink WS, Bunting H. Poisoning from inhalation of the vapors of Lewisite and phenyldichlorarsine: Its pathology in the dog and treatment with 2,3-dimercaptopropanol (BAL). *J Pharm Exp Therap*. 1946;87:76–80.

107. Peters RA, Stocken LA, Thompson RHS. British anti-Lewisite (BAL). *Nature*. 1945;156:616–619.

108. Prandtl W, Sennewald K. Trichloronitrosomethane, dichloroformoxime (phosgene oxime) and some of their derivatives. *Berichte*. 1929;62B:1754–1768.

109. Joffe MH, Barry MC, Marzulli FN. *Effects of Aqueous Solutions of Cutaneously Applied Phosgene Oxime on Humans*. Army Chemical Center, Md; 1954. Medical Laboratories Research Report 288.

110. McAdams AJ Jr, Joffe MH. *A Toxico-pathologic Study of Phosgene Oxime*. Army Chemical Center, Md; 1955. Medical Laboratories Research Report 381.

111. Augerson WS, Cadigan FC Jr, Goyer MM, Sivak A. *Chemical Casualty Treatment Protocol*. Cambridge, Mass: Arthur D. Little, Inc; 1987: Chap 3: 3-

1–3-10.

Chapter 8

LONGTERM HEALTH EFFECTS OF NERVE AGENTS AND MUSTARD

FREDERICK R. SIDELL, M.D.*; AND CHARLES G. HURST, M.D.†

INTRODUCTION
- Nerve Agents
- Mustard

NERVE AGENTS
- Polyneuropathy
- Muscle Necrosis
- Intermediate Syndrome
- Neuropsychiatric Effects
- Electroencephalographic Abnormalities
- Toxicological Studies on Nerve Agents

MUSTARD
- Carcinogenesis
- Chronic Pulmonary Disease
- Chronic Eye Disease
- Scarring of Epithelial Surfaces
- Central Nervous System
- Mutagenesis, Teratogenesis, and Reproductive Toxicity

SUMMARY

* *Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014*

† *Colonel, Medical Corps, U.S. Army; currently, Special Assistant for Medical Programs, Office of the Deputy Assistant Secretary of Defense, Counterproliferation and Chemical/Biological Matters, Room 3E808, 3050 Defense Pentagon, Washington, D.C. 20301-3050; formerly, Commander, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425*

INTRODUCTION

Chemical warfare agents were used extensively in World War I (the United States had approximately 70,000 chemical casualties[1]) and have been employed or allegedly employed in a dozen or so conflicts since.[2] The most recent large-scale use of these weapons was by Iraq in its war with Iran in the late 1980s. During that conflict, Iraq used nerve agents and the vesicant mustard[3] and maintained a stockpile of, and a manufacturing capability for, these two agents after the war. Before the coalition forces liberated Kuwait early in 1991 during the Persian Gulf War, Iraq was expected to use these agents when attacked. No reports of the use of chemical weapons during that conflict were made, however, despite the watchfulness of a vigilant press corps, who expected such use; and personnel of the military medical departments, who were trained to report, investigate, and care for chemical casualties.[4,5] One U.S. soldier developed skin blisters 8 hours after exploring an underground bunker.[4] His clinical findings and mass spectroscopy readings (performed by a chemical detection team) from his clothing and the bunker supported a diagnosis of accidental mustard exposure, which was mild and resulted in no loss of duty time. The exposure was not confirmed by later testing of clothing samples, from which trace amounts of the agent may have dissipated.

Although the acute effects of the nerve agents and of mustard agent are well known,[6,7] the longterm effects after a single exposure or multiple exposures are less well recognized. The nerve agents are the subject of Chapter 5, Nerve Agents, and mustard is a subject of Chapter 7, Vesicants, but this chapter focuses on the longterm effects. A synopsis of the nature and activity of the agents follows.

Nerve Agents

Nerve agents are esters of phosphonic acid and are extremely potent chemicals. Their military designations are GA (tabun), GB (sarin), GD (soman), GF, and VX. The agents GF and VX have no common names.

The toxic effects of nerve agents are due primarily to their inhibition of

acetylcholinesterase and the resulting accumulation of acetylcholine.[8] Other biological activities of these agents have been described, but the relation of these activities to clinical effects has not been recognized. For example, some nerve agents affect ionic channels,[9] and all affect structures other than acetylcholinesterase.[10]

Several milligrams of VX, the least volatile nerve agent, absorbed through the skin will cause clinical signs and symptoms.[11,12] A C*t* (the concentration [C] of agent vapor or aerosol in air, as mg/m^3, multiplied by the time [*t*] of exposure, in minutes) of 2 to 3 $mg \cdot min/m^3$ of sarin will produce miosis and rhinorrhea in man.[13] This C*t* can be attained with exposure to a concentration of 2 mg/m^3 for 1 minute or a concentration of 0.05 mg/m^3 for 40 minutes.

The initial signs of exposure to small quantities of agent vapor are miosis, rhinorrhea, and airway constriction.[7,14] Larger amounts will cause loss of consciousness, seizure activity,[14] cessation of respiration[15] and cardiac activity, and death, unless there is medical intervention. Effects occur within minutes of exposure,[14,15] and after a large exposure (C*t* of 10–200 $mg \cdot min/m^3$, depending on the agent[16]), death occurs in 10 to 15 minutes.

After exposure to a sublethal amount on the skin (1–3 mg), the onset time for clinical effects may be hours.[11,12] The initial effect is usually vomiting, which may be followed by muscular weakness. A lethal amount on the skin (10 mg of VX, the most toxic by percutaneous absorption[16]) will cause effects within several minutes[14] and death will occur shortly afterwards.

Treatment consists of the administration of atropine, a drug that blocks the effects of the excess acetylcholine at muscarinic acetylcholine receptor sites; and of 2-pyridine aldoxime methyl chloride (2-PAM Cl, also called pralidoxime chloride), an oxime that will remove the agent from acetylcholinesterase, thereby reactivating the enzyme after poisoning by some agents.[17] 2-PAM Cl, however, is ineffective against soman intoxication[14] because of soman's rapid *aging*. (Aging is the process by which one of the nerve agent's alkyl groups leaves the molecule. After dealkylation, an acetylcholinesterase-bound nerve agent molecule can no longer be removed from the enzyme by an oxime. The aging half-time of soman is about 2 min.) Ventilatory support is necessary when breathing has stopped or is inadequate,[14,15] and the anticonvulsant diazepam may need to be administered.

Mustard

Two well-known forms of mustard exist. Sulfur mustard (designated by the military as H or HD) was first synthesized in the early 1800s, has been used in warfare on several occasions, and is a major chemical warfare agent.[6] Nitrogen mustard is of more recent origin, has not been used in warfare, and is a cancer chemotherapeutic agent. Throughout this report, the word mustard will refer to sulfur mustard.

Mustard is best known as a skin vesicant, but in a series of Iranian patients exposed to mustard, 95% had airway effects, 92% had eye injuries, and 83% had skin lesions.[18] After absorption, mustard, an extremely potent alkylating agent, has the potential to damage all cells and all organs.[6] Absorption and systemic distribution of a significant amount of mustard damages the bone marrow (where it destroys the precursor cells, leading to pancytopenia).[6] Less commonly, clinical effects are seen in the gastrointestinal tract (usually as a terminal event)[19,20] and in the central nervous system (CNS) (with ill-defined symptoms such as lethargy and apathy).[18,21]

On the skin, a C*t* of 50 mg•min/m^3 or a droplet of 10 µg of mustard is adequate to produce vesication.[6] (One study[22] indicates that 8 of the 10 µg evaporate and 1 µg enters the systemic circulation, leaving 1 µg to produce the skin lesion.) Eye lesions can be produced by a C*t* of 10 mg•min/m^3.[23] Airway injury occurs at a C*t* of 100 mg•min/m^3 or higher.[6]

The mode of biological activity of mustard is less well defined than that of the nerve agents. The initial event is felt to be a reaction of mustard and deoxyribonucleic acid (DNA) with subsequent damage to the DNA. A series of intracellular events then occur, leading to cellular damage accompanied by inflammation and cellular death. Cellular damage begins within 1 to 2 minutes of contact of mustard to skin or mucous membranes.[6]

The onset of clinical effects following exposure to mustard occurs hours after the exposure.[6] The delay usually ranges from 2 to 24 hours and is inversely proportional to the amount of mustard and other factors.

No specific therapy for mustard exposure exists.[6] Decontamination within a minute or two will prevent or diminish the lesion, and later care consists of symptomatic management of the lesion.

NERVE AGENTS

Much information on both the short-term and the long-term effects of mustard in man comes from its battlefield use in World War I and the

Iran–Iraq War, and from experimental studies during the World War I and World War II periods.[24] In contrast, no data from the battlefield use of nerve agents are available. Information on the effects of nerve agents in man comes from the accidental exposure of hundreds of people who were mildly or moderately exposed while working with nerve agents and from handful of workers who had severe exposures. Investigational studies carried out in hundreds of people also provide information.

Information on the effects of organophosphorus insecticides is included so that medical officers can compare and contrast the two. Because nerve agents and insecticides are both organophosphates, people often tend to extrapolate from the biological effects of one of these types to the other, but in fact the differences between insecticides and nerve agents are great. The authors of some reports did not recognize the differences and grouped them together.[25,26]

Although the organophosphate insecticides are similar to nerve agents in inhibiting cholinesterase, they differ in other characteristics. For example, the cholinergic crisis caused by acute, severe intoxication with the insecticides is generally much longer than that caused by nerve agents (days to weeks for insecticides[27,28] vs hours for nerve agents[14,15]). Not only do the insecticides differ from nerve agents, they also differ among themselves in some of their biological effects; for example, some cause polyneuropathy, others do not.[29] Because of these differences, all of which have probably not been defined, the similarity between the effects of insecticides in man and the effects of nerve agents in man cannot be assumed. We repeat, insecticides are included in this review *only* so that the similarities can be noted and the differences contrasted. The reader should be careful not to confuse one with the other.

Polyneuropathy

Insecticides

Organophosphorus ester–induced delayed neurotoxicity (OPIDN) has been recognized as a clinical syndrome in humans and animals for over 50 years. After an exposure to certain organophosphates occurs, incoordination, ataxia, spasticity, and flaccid paralysis develop over the following 1 to 3 weeks; the paralysis begins distally in the lower limbs and eventually spreads to the upper limbs. Part or all of the lesion may be reversible, but in its most severe form it can cause lifetime quadriplegia. Structural changes begin at the distal, nonmyelinated portion of the nerve, followed by progressive demyelination associated with degeneration of more proximal nerve segments.[29]

This syndrome was initially associated with ingestion of triorthocresyl

phosphate (TOCP), not an insecticide. After organophosphate insecticides became available, the syndrome was seen after exposure to some, but not all, of them.[29]

The best animal model for studying the effects of exposure to organophosphates is the chicken.[29,30] Extensive studies have been performed to elucidate the mechanism of action that causes OPIDN and to screen new organophosphate insecticides for this effect.[29,30] The exact mechanism of action is still unknown, but much evidence suggests that the inhibition of neurotoxic esterase in nerve tissue is involved.[31] Administration of oximes and atropine has no effect on the production of this neurotoxicity.[32]

OPIDN is not seen with all insecticides.[29,30] Generally, insecticides that have been shown to cause polyneuropathy have been removed from the market; only those that have been demonstrated not to cause this effect in animal models are available.

Nerve Agents

Nerve agents have caused polyneuropathy in animals only at doses manyfold greater than the LD_{50} (the dose [D] that is lethal [L] to 50% of the exposed population)—doses that require massive pretreatment and therapy to ensure survival of the animals. Davies et al[33] produced polyneuropathy in chickens with sarin only at 60 or more times the LD_{50}. (The animals were protected with atropine and oxime to permit survival.) Neuropathy was not detected at 8 times the medial lethal dose of soman. Davies's group also detected no polyneuropathy at doses of VX of 45 µmol/kg.[34]

In another study,[35] polyneuropathy was found in hens after 30 to 60 times the LD_{50} for sarin was administered, but not at 38 times the LD_{50} for soman or 82 times the LD_{50} for tabun. VX was not examined in this study because its ability to inhibit neurotoxic esterase is negligible. At 120 times the acute LD_{50} in hens, soman and tabun caused polyneuropathy in some surviving animals.[36] GF is a stronger inhibitor of neurotoxic esterase in vitro than the other nerve agents.[37] However, GF, along with tabun, soman, and VX, did not cause polyneuropathy at very high doses.[38] This syndrome has not been noted in the handful of humans severely exposed to nerve agents or in the hundreds of humans with mild-to-moderate effects from nerve agents. Studies using smaller doses of tabun, sarin, and soman are described later in this chapter, in the section on toxicology.

Muscle Necrosis

Insecticides

Necrosis of rat skeletal muscle in the region of the motor endplate has been noted after administration of cholinesterase-inhibiting compounds in amounts sufficient to cause signs.[39] Swelling, eosinophilia, and loss of striations of myofibers can be observed by light microscopy in the motor endplate regions as early as 2 hours after administration of the organophosphate, and the lesion is fully developed in 12 to 24 hours. In affected fibers, the sarcolemma remains intact and is the focus of later repair of the fiber. Recovery begins in 2 days and is complete by 2 weeks. The lesion can be prevented or lessened by denervation or by administration of atropine and oxime within the first 2 hours; the lesion is more severe in muscles of high activity, such as the diaphragm, and in Type II muscle fibers of the “fast twitch” category.[39]

Muscle necrosis was seen in the diaphragm of a man who died after drinking parathion. No cholinesterase could be demonstrated in the myoneural junctions of any muscle, but necrosis was limited to the diaphragm. Each focus involved 1 to 4 sarcomeres of both types of myofibers and varied from acute swelling to vacuolar disintegration of the fibers. The nerve endings in the segmental necrotic zones were degenerated.[40]

Nerve Agents

The circumscribed muscular necrosis seen with insecticides has also been seen after sarin[41,42] and tabun[43] administration to experimental animals. Soman produced necrosis in one study,[44] but not in another.[43] On stimulation of the nerve, the muscle was unable to sustain a tetanic contraction at frequencies of 100 and 200 Hz.[43]

Intermediate Syndrome

Insecticides

A second type of delayed neurological manifestation of organophosphate insecticide poisoning is the “intermediate syndrome.” In a series of 200 consecutive cases of organophosphate insecticide poisoning, 36 patients developed a weakness of the proximal muscles of the limbs, cranial nerve weaknesses, bilateral pyramidal tract signs, and areflexia.[45] This disturbance began 12 to 84 hours after hospital admission. In most cases, the cholinergic crisis had resolved, and the 21 patients who survived recovered completely by 96 hours. The lesion was

unresponsive to large amounts of atropine; 2-PAM Cl was not available.

The authors of the report[45] divided the signs of organophosphate intoxication into two groups, which they called Type I and Type II. According to these authors, Type I signs were muscarinic in nature and were amenable to atropine therapy, and Type II signs were nicotinic in nature, appeared 12 to 48 hours after exposure, and were resistant to atropine therapy.

Ten additional cases were later described.[46] These patients received atropine (up to 40 mg every 24 h) and 2-PAM Cl (1 g every 12 h for 24 to 48 h) during the cholinergic-crisis phase. About 24 to 96 hours after poisoning, the 10 patients developed a syndrome that included palsies of cranial nerves III, IV, VI, VII, and X; weakness of the respiratory muscles (four patients required immediate intubation and assisted ventilation at the onset of the syndrome); weakness of the proximal limb muscles; and pyramidal tract signs. Recovery occurred in 5 to 18 days. Electromyography in limb muscles and nerve conduction were normal. Tetanic stimulation of the abductor pollicis brevis showed a marked fade with no posttetanic facilitation. The authors of this report[46] called this the "intermediate syndrome," meaning that it is intermediate between the acute cholinergic effects and the later, well-recognized delayed polyneuropathy. Consequently, the term intermediate syndrome, rather than Type II signs, has been adopted.

Two additional cases of this syndrome were reported several years later; both patients required ventilatory support during the paralytic phase.[47] In another series, 29 of 90 patients with organophosphate poisoning had the intermediate syndrome.[48] Tetanic fade with no posttetanic facilitation was maximal between days 4 and 6, and the response to electrical stimulation had returned to normal by 8 to 10 days. The author suggested that a neuromuscular junction defect was responsible for the lesion.

Other cases have since been reported[49–52] and in some, the weakness or paralysis lasted for days to weeks. One suggestion was that lack of early oxime therapy might contribute to the development of the syndrome,[53] but it has occurred with adequate amounts of oxime.[49,50,54] The cause of this neuromuscular dysfunction has not been elucidated, nor has an animal model been described. Intermediate syndrome may be related to the myopathy seen at the neuromuscular junction.

Nerve Agents

The intermediate syndrome, associated with insecticide poisoning, has not been described after administration of nerve agents to animals, nor has it been noted in the handful of individuals severely exposed to nerve

agents.

Neuropsychiatric Effects

Many neuropsychiatric problems have been associated with a single exposure or repeated exposures to insecticides and to nerve agents. Generally, these symptoms were studied shortly after the patients were exposed, and the duration of the problems was not noted. However, several studies examined the effects long after the acute insult. The effects include disturbances in memory, sleep, and vigilance; depression; anxiety and irritability; and problems with information processing.

Insecticides

In 1961, Gershon and Shaw[55] described 16 patients with psychiatric problems who had been exposed to pesticides repeatedly over a 1.5- to 10-year period. Five were schizophrenic, 7 were severely depressed, 1 was in a state of fugue, and all had impairment of memory and concentration. These conditions followed multiple symptomatic exposures to organophosphate insecticides, and the patients recovered within 6 to 12 months after the onset of their signs and symptoms. Because neuropsychiatric sequelae of organophosphate insecticides had not been widely recognized, the authors suggested that these sequelae might be more common than generally thought.

Gershon and Shaw's report was criticized[56,57] because no information on the exposure history was included; because few objective measures, either of mental status or of blood cholinesterase were used; and because the conditions reported had not been reported in much larger series of patients exposed to organophosphate insecticides. Later studies failed to find evidence of thought disorders after pesticide exposure,[58,59] although diisopropyl fluorophosphate (DFP) administration aggravated psychosis.[60] Less-severe neuropsychiatric manifestations of organophosphate insecticide exposure, occurring either acutely or as sequelae, have been subsequently reported.

Durham et al[61] examined 187 individuals who were routinely involved in pesticide work (eg, crop dusters) for mental alertness. His subjects were studied, using a complex reaction time, (*a*) at the time of maximal work with insecticides and (*b*) during “nonexposure” periods. Control subjects were studied at similar times. Both groups, subjects and controls, did better on the tests during nonexposure periods, and both groups scored poorer during the higher risk periods. The performance of the exposed subjects improved during and after convalescence. The authors emphasized repeatedly that mental effects were not seen in the absence of clinical signs of poisoning.

Problems with memory after insecticide exposure were reported by Gershon and Shaw[55] (the problems cleared in 6 to 12 months after the acute exposure) and by Metcalf and Holmes[59] (the patients were studied more than a year after exposure). In the latter study, testing was performed to corroborate the report of memory deficit. Other reports have mentioned memory problems, but they provide few data.

Anxiety, irritability, giddiness, tension, and restlessness persisting for months after exposure to insecticides were reported by Namba et al[62] and by Gershon and Shaw.[55] (Both studies emphasized that these effects occurred only in patients who had demonstrated symptoms of exposure.) Metcalf and Holmes[59] reported similar effects, but did not indicate their duration or the time after exposure that they occurred.

Depression has been reported[62] from insecticide exposure immediately following the acute symptomatic exposure, but it did not persist. More-prolonged (6 to 12 mo) depression has been reported[55] after insecticide exposure. In contrast, Levin et al[58] found no evidence of depression using a structured interview and a depression inventory in asymptomatic workers with histories of chronic exposure.

Sleep disturbances, such as excessive dreaming, nightmares, and insomnia, have been reported[59,62] after insecticide exposure and generally are of relatively short duration (days to weeks).

Psychomotor performance has been evaluated after exposure to insecticides. Rowntree et al[60] found that daily administration of an organophosphate compound caused slowness in thought and decreased performance speed. Metcalf and Holmes[59] noted slowed thinking and calculation in patients who had been exposed to insecticides more than a year previously.

Difficulties in concentration and vigilance have been reported after insecticide exposure.[55,59,61–63] Some studies indicate marginal decreases, and others lack objective data (eg, Gershon and Shaw[55]). In all, the impairment occurred after an episode in which the patient had

exhibited symptoms of exposure to the compound.

Tabershaw and Cooper[64] evaluated 87 patients who had been exposed to an organophosphate insecticide more than 3 years previously and who had had persistent complaints for over a 6-month period. The symptoms involved the visual, gastrointestinal, cardiorespiratory, and neuropsychiatric systems. In each instance, the complaint could be attributed to other problems; for example, several cases of visual blurring were due to presbyopia, a case of chronic abdominal pain was due to a peptic ulcer, and in one case, nervousness and tremors were due to chronic alcoholism.

In a more recent study, Rosenstock et al[65] examined 38 patients more than a year after their hospitalization for organophosphate insecticide exposure. Control subjects had also worked with organophosphate insecticides but had not had a symptomatic exposure. The poisoned group did significantly less well than the control group on tests assessing a wide variety of neuropsychological functions, including auditory attention, visual memory, visuomotor speed, sequencing and problem solving, and motor steadiness, reaction, and dexterity.

Nerve Agents

Bowers et al[11] reported that subjects had difficulty with memory for 24 hours after they were given VX, but had no evidence of major thought disorders. Other investigators[66] noted depression acutely after nerve agent exposure,[66] but the depression did not persist. Sleep disturbances were also short-lived.[11,66,67] After exposure to VX, subjects had decreased performance on an arithmetic test, decreased reading comprehension, and decreased ability to play chess.[11] In some instances these performance decrements occurred before other signs of intoxication or in the absence of other signs. Impaired concentration and vigilance have been reported after nerve agent exposure.[66] These effects can persist for several weeks after symptomatic exposure to nerve agents (personal observations, F.R.S.).

A report[67] of 297 cases of accidental exposure to nerve agent among manufacturing workers indicated that about 20% of the individuals had neuropsychiatric effects such as disturbed sleep, disturbance in mood, irritability, nervousness, disturbance in ability to think clearly, absentmindedness, fatigability, and lightheadedness. The duration of these effects was not indicated, but the report noted that supervisors and coworkers detected these effects when the casualties returned to work prematurely.

A single subject, a biochemist exposed to soman, was evaluated at 2 weeks, at 4 months, and at 6 months after exposure, using a psychiatric interview and a battery of psychological tests (this case is also discussed in Chapter 5, Nerve Agents).[14] The person had been severely exposed, requiring ventilatory support for about 30 minutes. On initial testing, he had high scores on the hypochondriasis and hysteria scales on the Minnesota Multiphasic Personality Inventory; these improved on later testing. On the initial testing he did poorly on a visual retention task, on word association, proverbs, and an ink blot test. While taking the tests, he used delaying tactics, had difficulty generating verbal associations, and failed the harder proverbs. Results on the later tests were much improved and indicated full use of his intellectual facilities.

In another case, a physician was severely exposed to sarin and required ventilatory support for longer than 3 hours. Although psychiatric and psychological studies were not performed, after recovery he returned to work with no apparent problems.[15]

While few data on the duration of these neuropsychiatric effects in people exist, evidence suggests that they are relatively short-lived (days, weeks). Because of the nature of the work, people employed in manufacturing, at depots, or in research and development facilities were relatively few in number, tended to remain in the same job for a long period, and were a closely knit group. Most were thoroughly familiar with the effects of nerve agents, and most knew their coworkers very well. If a worker did not seem “right,” his coworker or supervisor recognized it.[67] A medical facility dedicated to the treatment of nerve agent casualties and with a staff experienced in this type of injury was always available; workers were encouraged to use it, and supervisors were instructed to send employees who were not “normal” to the medical facility for evaluation.

One of the authors (F.R.S.) worked in such a medical facility for over a decade. While there was no routine, formal follow-up procedure, (eg, psychological testing of exposed casualties), informal follow-up visits for several weeks after the incident for eye examinations (miosis takes 3–6 wk to recede[14]) and to discuss general health problems were common. Significant lingering effects were very likely to be mentioned, therefore, and detected.

No formal follow-up program existed for subjects exposed to nerve agents experimentally, but again, these individuals were seen on a regular basis for several weeks after exposure. Since these people were the subjects of research study on the effects of nerve agents, lingering effects were very likely to be carefully sought and reported if found.

In summary, studies intended to examine the neuropsychiatric effects of organophosphate compounds vary in their adequacy, and in some

instances the results are contradictory. Most studies agree, however, that acute neuropsychiatric effects result from exposure to both insecticides and nerve agents. These effects include inability to concentrate, memory problems, sleep disturbances, anxiety, irritability, depression, and problems with information processing and psychomotor tasks. With pesticides, these effects do not occur in the absence of the conventional signs of poisoning.

The duration of these effects is less well studied. Some studies suggest that after exposure to insecticides, problems might persist for a year or longer, but supporting data are not always provided. The two reports of patients exposed to nerve agents and personal observation suggest that these effects are of shorter duration in this class of compounds.

Electroencephalographic Abnormalities

Insecticides and Other Organophosphates

Electroencephalographic abnormalities were reported in subjects given daily doses of diisopropyl fluorophosphate for 2 to 7 days.[68] These abnormalities consisted of faster frequencies, higher voltages, and occasional bursts of slow waves of high voltage at 3 to 6 Hz. Their severity was directly related to the degree of initial cholinesterase inhibition. The changes persisted for 3 to 4 weeks. Changes were noted in the electroencephalograms (EEGs) of 50 industrial and agricultural workers within 72 hours of accidental exposure to insecticides (both organophosphate and chlorinated hydrocarbons, on separate occasions), although the relationship to work history; blood cholinesterase; and exposure type, duration, and severity were not mentioned.[59]

Nerve Agents

In a patient severely intoxicated with sarin, an EEG (taken after the loss of consciousness but before the onset of convulsions) showed marked slowing, with bursts of high-voltage slow waves at 5 Hz in the temporofrontal leads. These abnormalities persisted for 6 days, after which no residual effects were noted.[66]

Because of the reports on insecticides and concern for employees working with or in the vicinity of nerve agents, the U.S. government sponsored a series of studies[69–72] on the long-term effects of sarin exposure as seen in EEG examinations. In the first study, monkeys were dosed with sarin in one of two dose schedules: (1) a single, large dose that produced convulsions or (2) a series of 10 weekly doses that caused no clinical effects. In the second study, workers who had had at least one

documented exposure to sarin (signs, cholinesterase depression) more than a year before the study were evaluated. Control subjects were co-workers who had no possibility of organophosphate exposure.

In the nonhuman primates, animals from both dose schedules had increases in high-frequency beta activity a year after exposure. Spectral analysis of the EEGs of the humans showed increased beta activity in the sarin-exposed population compared to the control population. Visual reading of the records suggested decreased amounts of alpha and increased amounts of slow delta and theta activity in the exposed group. Increased amounts of rapid-eye-movement sleep in the exposed group were also found. Individual records could not be categorized. The investigators noted that the relationship between these changes and alterations in brain function was not known.

Toxicological Studies on Nerve Agents

The effects of exposure to nerve agents on a chronic or subchronic basis were reported in two studies on animals. In a two-part, 90-day study[73,74] of subchronic exposure, rats were given one of three doses of tabun or soman 5 days per week by gavage. At the end of the study, no abnormalities were found on gross or histological examination of tissue. In a study[75] of chronic exposure to sarin, dogs received a C*t* of 10 mg•min/m^3 of sarin over a 6-month period. Some animals were dosed 5 days per week, and some were dosed 7 days per week. No tissue abnormalities that could be attributed to the agent were noted on gross or microscopic examination. Several of the male animals were bred after the exposure and the pups were normal.

No evidence of polyneuropathy was noted clinically or on microscopic examination in studies[76–79] in which tabun, sarin, and soman were given to hens in single or multiple doses. The doses were those maximally tolerated with the coadministration of atropine.

Sarin and soman were deemed not mutagenic after they were studied using the Ames *Salmonella*, mouse lymphoma, and Chinese hamster ovary cell systems.[80] Tabun was found to be weakly mutagenic in the mouse lymphoma cell test[81] and in the Chinese hamster ovary[82] and Ames bacterial systems.[83]

MUSTARD

Studies have established that the chemical agent mustard has long-term sequelae. Both Morgenstern et al[84] and Buscher[85] emphasize that chronic low-dose exposure over months to years in occupationally exposed workers leads to chronic bronchitis, bronchial asthma,

hoarseness, aphonia, and hypersensitivity to smoke, dust, and fumes. Such individuals typically show persistent disability, with increased susceptibility to respiratory tract infections and evidence of bronchitis and bronchiectasis.[6,84,85]

Laboratory animal studies[86–88] have found that mustard is mutagenic and carcinogenic, and thus it is not surprising that it is carcinogenic in man.[24]

In 1993, a study[24] sponsored by the Veterans Administration and conducted by the Institute of Medicine reported that a causal relationship exists between mustard exposure and the following conditions:

- chronic respiratory diseases (asthma, chronic bronchitis, emphysema, chronic obstructive pulmonary disease, chronic laryngitis),
- respiratory cancers (nasopharyngeal, laryngeal, and lung),
- pigmentation abnormalities of the skin,
- chronic skin ulceration and scar formation,
- skin cancer,
- chronic conjunctivitis,
- recurrent corneal ulcerative disease,
- delayed recurrent keratitis,
- leukemia (nitrogen mustard),
- bone marrow depression and (resulting) immunosuppression,
- psychological disorders (mood disorders, anxiety disorders, and traumatic stress dis-orders), and
- sexual dysfunction as a result of scrotal and penile scarring.

Although there may be laboratory evidence to suggest that all of these *might* occur, there are no data in humans to indicate that all *have* occurred. The study report recognized this by stating, “It is also possible that skin cancers did not occur in the studied populations…”[24(p218)] and “…underrepresented in human studies is information on chronic or delayed effects [on the bone marrow and immune system].”[24(p220)] The report also pointed out that the psychological disorders were from the stress of the exposure and not from the agent, and there seemed to be no data on sexual dysfunction. Moreover, it is not clear from the report whether the relationship between mustard exposure and these effects follows one or multiple exposures.

All human studies dealing with chronic mustard disease processes are retrospective and fraught with the problems inherent in retrospective studies. These problems include bias in the sampling populations; lack of

epidemiological controls for the effects of smoking, lifestyle, race, gender, age, or exposure to other chemicals; differential quality of available health care; and incorrect diagnosis.[6] These limitations make absolute interpretation of the studies difficult.

Carcinogenesis

Mustard is an alkylating agent similar to drugs that have been used in cancer chemotherapy, such as nitrogen mustard, Cytoxan (manufactured by Bristol-Myers Squibb Oncology Division, Princeton, N. J.), and methotrexate. Since DNA is one of mustard's most sensitive targets, it is not surprising that carcinogenesis and radiomimetic effects are seen.

In studies[88–90] conducted from 1949 through 1953 by W. E. Heston with mustard and strain-A mice (immunocompromised), the occurrence of pulmonary tumors was easily demonstrated. Studies conducted at Edgewood Arsenal, Maryland, examined the carcinogenic effects on rats in whole-body chamber exposures. Mustard readily produced skin malignancies in rats, but no excess tumors at other sites.[91] Subcutaneous injections totaling about 6 mg/kg of mustard produced sarcomas and other malignancies at injection sites in C3H, C3Hf, and strain-A mice, but did not produce an increase of malignancies at other sites.[90]

Human data on the carcinogenicity of mustard are from *(a)* battlefield exposures, *(b)* accidents, and *(c)* workers in chemical factories. Both British and American studies have investigated the increased incidence of pulmonary carcinoma arising from World War I battlefield exposure. All are difficult to interpret, owing to the lack of controls for age, chronic pulmonary disease, cigarette smoking, and other factors that might affect the outcome.[92–94]

In contrast to battlefield exposures, studies of factory workers involved in the production of mustard have shown a definite link between prolonged exposure to low doses of mustard and cancer.[6] Several studies[87,95–99] have provided evidence of an increased risk of respiratory tract cancers in factory workers. Easton et al[96] found a 45% increase in deaths due to lung cancer, a 170% increase in death from cancer of the larynx, and a 450% increase in deaths from cancer of the pharynx, compared with expected deaths in the general population. The risks for cancer of the pharynx and lung were significantly related to the duration of employment at the factory. For reasons analyzed more fully elsewhere,[100] the association between a single exposure to mustard and airway cancer is not as well established.

Japanese studies suggest a greater potential risk of cancer due to mustard than do the British studies. Easton et al[96] and Manning[87] suggest that the difference is related to the design of the Japanese studies and to

the lower industrial hygiene standards in Japan at the time of the studies.[6] The weight of the evidence—cellular, epidemiological, and toxicological—indicates a causal association between mustard exposure and the occurrence of excess respiratory cancer, skin cancer, and possibly leukemia. Inadequate exposure information limits accurate estimation of the cancer excesses that may be expected.[24]

Chronic Pulmonary Disease

Inhalation of mustard vapor primarily affects the laryngeal and tracheobronchial mucosa.[6] Evidence exists that suggests that mustard inhalation causes sustained respiratory difficulties even after the acute lesions have healed. Clinical follow-ups on 200 Iranian soldiers who were severely injured by mustard during the Iran–Iraq War indicate that about one third had experienced persistent respiratory effects 2 years after initial exposure. Reported problems included chronic bronchitis, asthma, rhinopharyngitis, tracheobronchitis, laryngitis, recurrent pneumonia, bronchiectasis, and in some cases, severe, unrelenting tracheobronchial stenosis.[101–105]

Of the British soldiers exposed to mustard in World War I, 12% were awarded disability compensation for respiratory disorders that were believed to be due to mustard exposures during combat.[106] Bronchitis was the major complaint; emphysema and asthma were also reported. However, epidemiological studies of the relationship between agent exposure and subsequent respiratory disability were severely limited for several reasons. Often, individuals had experienced multiple combined exposures to mustard and other chemical agents. Also, influenza and other respiratory ailments frequently made diagnosis of the mustard vapor injury difficult.[6] Finally, no epidemiological controls for smoking or for postexposure environmental and occupational histories were included in the studies.[107]

Wada et al[95] suggest a causal relationship between mustard exposure and subsequent bronchitis, tuberculosis, and pneumonia in factory workers involved in the production of mustard. Again Morgenstern et al[84] and Buscher[85] emphasize that chronic low-dose exposure over prolonged periods (presumably months to years) leads to lingering bronchitis, bronchial asthma, hoarseness, aphonia, and hypersensitivity to smoke, dust, and fumes. Such individuals typically show persistent disability, with increased susceptibility to respiratory tract infections and evidence of bronchitis and bronchiectasis.[6]

Little contemporary information regarding the pathogenesis of the respiratory lesions is available, and few data from people or animals exposed to nonlethal concentrations of mustard vapor exist. Even fewer studies investigate the histopathology of the recovery process in animals exposed to mustard.[24] However, two studies[19,108] conducted during World War I suggest that low-level exposure or survivable exposures in dogs and rabbits may produce scar tissue following small ulcerations in the trachea and larynx, causing contractions of these areas. The more severe respiratory tract lesions described in animals exposed to mustard vapor appear to be quite similar in type and location to those described in humans.[6]

Chronic Eye Disease

Individuals who sustain acute ocular injury due to high-dose mustard exposure may experience difficulties even after the initial effects of the injury have subsided.[109–112] Recurrent or persistent corneal ulceration can occur after latent periods of 10 to 25 years. This delayed keratopathy[111,113] may be accompanied by chronic conjunctivitis and corneal clouding. Anecdotal accounts suggest that low-dose exposure also causes increased sensitivity to later exposures to mustard,[114] although the existence of increased sensitivity is difficult to substantiate with available scientific evidence.[6] About 10% of those with eye injury in World War I had severely affected eyes, with both the cornea and the conjunctiva being involved. Members of this group developed the “delayed keratitis” noted above 8 to 25 years later.[110]

The 1993 Institute of Medicine study[24] of the effects of mustard and Lewisite exposure on the health of veterans concluded that acute, severe injury of the eye with mustard might result in recurrent corneal ulcerative disease for the remainder of the patient’s life, with a maximum incidence occurring 15 to 20 years after the injury. Based on extensive data, the study concluded that a causal relationship between severe exposure to mustard and the development of delayed recurrent keratitis exists.[109] The

study also found a causal relationship between exposure to mustard and the development of prolonged, intractable conjunctivitis.

Scarring of Epithelial Surfaces

Residual cutaneous lesions most often take the form of scars that result from uncontrolled fibroblastic activity and overgrowth of connective tissue during the process of wound repair. Even wounds that are well cared for on body sites and parts that are not easily immobilized, such as shoulders, knees, elbows, and male genitalia, often heal with severe residual scar formation. Pigmentation is often altered (either increased or decreased) at these sites, although the degree of alteration does not differ from that observed in injuries caused by burns and other forms of physical and chemical insult. In the absence of melanocyte destruction, hyperpigmentation predominates. If melanocytes are locally destroyed, and inward migration from destroyed adnexal structures does not occur, depigmentation predominates. Some previously injured sites have been described as being “sensitive” to subsequent mechanical injury. These sites may show recurrent blisters after mild injury.[24]

Skin cancers occurring at the site of old scar formation is an acknowledged biological phenomenon.[115,116] Cutaneous cancers resulting from acute mustard exposure usually localize in scars, whereas those caused by chronic exposure can occur on any exposed site.[117]

In a prospective study of delayed toxic effects from mustard exposure, Balali-Mood[104] followed a group of Iranian solders exposed to mustard gas during the Iran–Iraq War. After 2 years, 41% of the exposed victims were experiencing pigmentary disorders.

Renshaw[22] reported on the development of contact sensitivity in man following localized exposure to liquid mustard. Cutaneous sensitivity may be seen within 8 days following the first application, and a more pronounced effect is seen after 4 weeks. The incidence of hypersensitivity varies between 30% and 65% of exposed individuals. Sensitivity may be immediate hives or delayed dermatitis and appears to last a lifetime. Sensitivity may also take the form of flares of old, healed mustard injury sites after a fresh application of mustard to normal, unaffected skin.[22]

In its study of mustard and Lewisite effects,[24] the Institute of Medicine concluded that

- the evidence indicates a causal relation between acute, severe exposure to mustard agents and increased pigmentation and depigmentation in human skin;
- acute and severe exposure can lead to chronic skin ulceration, scar

formation, and the development of cutaneous cancer (but see the caveat in the previous discussion of this report's conclusions); and
- chronic exposure to minimally toxic and even subtoxic doses can lead to skin pigmentation abnormalities and cutaneous cancer.

Central Nervous System

Excitation of the CNS after mustard exposure, resulting in convulsions and followed by CNS depression, has been reported.[118] Convulsions and cardiac irregularities appear to occur only after extremely acute, high doses,[119] which are probably attainable only in laboratory settings.[6] Mustard casualties of the Iran–Iraq War did not display severe CNS or cardiac abnormalities.[101]

Acute neuropsychiatric symptoms, including severe depression and changes in mentation, are common after high-dose exposures to mustard agents. These symptoms are produced both directly by the chemical and secondarily to other physiological changes.[24] Follow-up of workers in German chemical warfare plants showed a high prevalence of various neurological disorders, including impaired concentration, diminished libido, and sensory hypersensitivity.[120] To what extent mustard agents were responsible is not clear because multiple exposures to other agents, including nerve agents, were known to have occurred.

Mutagenesis, Teratogenesis, and Reproductive Toxicity

Mustard causes cross-linking of DNA and is known to alkylate DNA at the O^6 position of guanine. Some authors[121,122] suggest that intrastrand DNA cross-links, rather than interstrand cross-links,[123,124] are the lesions primarily responsible for producing chromosomal aberrations. Mustard causes chromosomal breakage and induces sister chromatid exchanges in a wide variety of cells including mammalian cells.[125] The International Agency for Research on Cancer, Lyon, France (an agency of the World Health Organization), has classified mustard as a human carcinogen based on the findings of epidemiological studies. Taken together, these observations highlight the potential of this compound to induce genetic damage and become a long-term health hazard. They also suggest that mustard could be a reproductive toxin.[24]

The 1993 Institute of Medicine report[24] noted that the quality of human data on the reproductive toxicity of mustard is quite poor. Follow-up of the occupational or battlefield cohorts to determine the nature of any reproductive toxicity or teratogenic effects attributable to these exposures has been insufficient. The evidence suggests a causal relationship

between mustard exposure and reproductive toxicity in laboratory animals, but the database is far too small and unreliable to allow a clear understanding of human reproductive risk from exposure to mustard. Mustard can cause genetic alterations in the sperm of male rats after inhalation or gastric exposure, but rodent studies[126] showed that mustards are not detectable teratogens in animals. The human data are insufficient for reliable interpretation.[24]

SUMMARY

Available information implicates the nerve agents and mustard as the cause or probable cause of several long-term health effects.

Polyneuropathy, the major neuromuscular manifestation seen after exposure to organophosphate pesticides, has not been reported in humans after exposure to nerve agents. Studies suggest that these agents cause polyneuropathy in animals only at doses so high that survival is questionable even with massive pretreatment and therapy. The intermediate syndrome, a syndrome characterized by weakness of the proximal muscles of the limbs, weakness of the respiratory muscles, cranial nerve weaknesses, bilateral pyramidal tract signs, and areflexia, has not been reported in animals or humans after nerve agent exposure. Muscular necrosis, the neuromuscular effect that can be produced by nerve agent administration, occurs after high-dose exposure, is short-lived, reverses within weeks, and has not been reported in humans.

Other long-term consequences of exposure to organophosphate pesticides are neuropsychiatric effects and possible EEG changes. Both are documented as acute manifestations of nerve agent poisoning; mild neuropsychiatric changes occur after even low-dose nerve agent exposure. Several studies of people exposed to insecticides, in which the subjects were chosen because they had experienced one or more episodes of symptomatic poisoning (including cholinesterase inhibition), report neuropsychiatric changes a year or longer after the acute manifestation. The duration of the neuropsychiatric effects after nerve agent exposure is less well documented, but available information suggests that these effects persist for several weeks or possibly several months. Studies of EEG changes following organophosphate nerve agent exposure found differences between the exposed and control populations but suggested no relationship between their findings and alterations in brain function.

The many studies of English and Japanese mustard factory workers establish repeated symptomatic exposures to mustard over a period of years as a causal factor in an increased incidence of airway cancer. The association between a single exposure to mustard and airway cancer is

not as well established. The association between one-time mustard exposure and other chronic airway problems, such as chronic bronchitis, which is based on World War I data, seems more clearly established. In some cases, the long-term damage was probably a continuation of the original insult resulting from insufficient therapy in the preantibiotic era.

Several eye diseases, such as chronic conjunctivitis, appear after an acute, usually severe, insult to the eye. In particular, delayed keratitis has appeared more than 25 years after the acute, severe lesion. Similarly, skin scarring, pigment changes, and even cancer have either followed the initial wound as a continuation of the process (scarring) or later appeared at the site of the lesion.

The production of nonairway cancer by mustard has been demonstrated in animals, but scant evidence exists to implicate mustard as a causative factor in nonairway cancer in humans.

Mustard causes chromosomal breakage and induces sister chromatid exchanges in man and has been classed as a mutagen. No data that implicate mustard as a reproductive toxin in man seem to be available, despite the many thousands of people exposed to mustard in the past 80 years.

REFERENCES

1. Prentiss AM. *Chemicals in War: A Treatise on Chemical Warfare*. New York, NY: McGraw-Hill; 1937: 653.

2. Robinson JP. *The Problem of Chemical and Biological Warfare*. Vol 1. In: *The Rise of CB Weapons*. New York, NY: Humanities Press; 1971.

3. United Nations Security Council, *Report of Specialists Appointed by the Secretary General to Investigate Allegations by the Islamic Republic of Iran Concerning the Use of Chemical Weapons.* New York, NY: United Nations; 1986. *UN Report S/16433.*

4. Wade JV, Gum RM, Dunn MA. Medical chemical defense in Operations Desert Shield and Desert Storm. *J US Army Med Dept.* 1992;PB8-92-1/2:34–36.

5. Sidell FR. The medical management of chemical casualty course in CONUS and Europe during Desert Storm. *J US Army Med Dept.* 1992;PB 8-92-3/4:10–12.

6. Papirmeister B, Feister AJ, Robinson SI, Ford RD. *Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological Implications.* Boca Raton, Fla: CRC Press; 1991.

7. Sidell FR. Clinical considerations in nerve agent intoxication. In: Somani SM, ed. *Chemical Warfare Agents*. San Diego, Calif: Academic Press; 1992:

156–194.

8. Taylor P. Anticholinesterase agents. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. *The Pharmacological Basis of Therapeutics.* New York, NY: Pergamon Press; 1990: 131–149.

9. Albuquerque EX, Akaike A, Shaw KP, Rickett DL. The interaction of anticholinesterase agents with the acetylcholine receptor–ionic channel complex. *Fundam Appl Toxicol*. 1984;4:S27–S33.

10. O'Neill JJ. Non-cholinesterase effects of anticholinesterases. *Fundam Appl Toxicol.* 1981;1:154–169.

11. Bowers MB, Goodman E, Sim VM. Some behavioral changes in man following anticholinesterase administration. *J Nerv Ment Dis.* 1964;138:383–389.

12. Craig FN, Cummings EG, Sim VM. Environmental temperature and the percutaneous absorption of a cholinesterase inhibitor, VX. *J Invest Dermatol.* 1977;68:357–361.

13. Johns RJ. *The Effects of Low Concentrations of GB on the Human Eye.* Edgewood Arsenal, Md: Medical Research Laboratory; 1952. MRL Report 100.

14. Sidell FR. Soman and sarin: Clinical manifestations and treatment of accidental poisoning by organophosphates. *Clin Toxicol.* 1974;7:1–17.

15. Ward JR. Exposure to a nerve gas. In: Whittenberger JL, ed. *Artificial Respiration: Theory and Applications*. New York, NY: Harper & Row; 1962: 258–265.

16. Program Executive Officer–Program Manager of Chemical Demilitarization. *Chemical Stockpile Disposal Program Final Programmatic Environmental Impact Statement.* Aberdeen Proving Ground, Md: Program Executive Officer–Program Manager of Chemical Demilitarization; 1988: B-23–B-25.

17. Sidell FR, Groff WA. The reactivatibility of cholinesterase inhibited by VX and sarin in man. *Toxicol Appl Pharmacol.* 1974;27:241–252.

18. Balali-Mood M, Navaeian A. Clinical and paraclinical findings in 233 patients with sulfur mustard poisoning. In: Heyndrickx B, ed. *Proceedings of the 2nd World Congress on New Compounds in Biological and Chemical Warfare: Toxicological Evaluation, Industrial Chemical Disasters, Civil Protection and Treatment, 24–27 August 1986.* Ghent, Belgium, State University of Ghent; 1986: 464–473.

19. Warthin AS, Weller CV. The lesions of the respiratory and gastrointestinal tract produced by mustard gas (dichlorethyl sulphide). *J Lab Clin Med.* 1919;4:229–264.

20. Sohrabpour H. Clinical manifestations of chemical agents on Iranian combatants during the Iran–Iraq conflict. In: Heyndrickx A, ed. *Proceedings of the 1st World Congress on New Compounds in Biological and Chemical*

Warfare: Toxicological Evaluation, 21–23 May 1984. Ghent, Belgium: State University of Ghent; 1984: 291–297.

21. Vedder EB. *The Medical Aspects of Chemical Warfare.* Baltimore, Md: Williams & Wilkins; 1925: 125–166.

22. Renshaw B. Mechanisms in production of cutaneous injuries by sulfur and nitrogen mustards. In: *Chemical Warfare Agents and Related Chemical Problems.* Washington, DC: Office of Scientific Research and Development; 1946.

23. Reed CI. The minimum concentration of dichlorethylsulphide (mustard gas) effective for the eyes of man. *J Pharmacol Exp Ther.* 1920;15:77–80.

24. Pechura CM, Rall DP, eds. *Veterans at Risk: The Health Effects of Mustard Gas and Lewisite.* Washington, DC: Institute of Medicine, National Academy Press; 1993.

25. Boskovic B, Kusic R. Long-term effects of acute exposure to nerve gases upon human health. In: *Chemical Weapons: Destruction and Conversion.* New York, NY: Crane, Russak & Co; 1980: 113–116.

26. Fullerton CS, Ursano RJ. Behavioral and psychological responses to chemical and biological warfare. *Milit Med.* 1990;155:54–59.

27. Chew LS, Chee KT, Yeo JM, Jayaratnam FJ. Continuous atropine infusion in the management of organophosphorus insecticide poisoning. *Singapore Med J.* 1971;12:80–85.

28. LeBlanc FN, Benson BE, Gilg AD. A severe organophosphate poisoning requiring the use of an atropine drip. *Clin Toxicol.* 1986;24:69–76.

29. Metcalf RL. Historical perspective of organophosphorus ester-induced delayed neurotoxicity. In: Cranmer JM, Hixson EJ, eds. *Delayed Neurotoxicity.* Little Rock, Ark: Intox Press; 1984: 7–23.

30. Takade DY. Delayed neurotoxicity in perspective: Summary and objectives of the workshop. In: Cranmer JM, Hixson EJ, eds. *Delayed Neurotoxicity.* Little Rock, Ark: Intox Press; 1984: 2–6.

31. Johnson MK. Organophosphorus esters causing delayed neurotoxic effects. *Arch Toxicol.* 1975;34:259–288.

32. Davies DR, Holland P. Effect of oximes and atropine upon the development of delayed neurotoxic signs in chickens following poisoning by DFP and sarin. *Biochem Pharmacol.* 1972;21:3145–3151.

33. Davies DR, Holland P, Rumens MJ. The relationship between the chemical structure and neurotoxicity of alkyl organophosphorus compounds. *Brit J Pharmacol.* 1960;15:271–278.

34. Davies, et al. Cited in: Gordon JJ, Inns RH, Johnson MK, et al. The delayed

neuropathic effects of nerve agents and some other organophosphorus compounds. *Arch Toxicol.* 1983;52(3):71–81.

35. Gordon JJ, Inns RH, Johnson MK, et al. The delayed neuropathic effects of nerve agents and some other organophosphorus compounds. *Arch Toxicol.* 1983;52(3):71–81.

36. Willems JL, Nicaise M, De Bisschop HC. Delayed neuropathy by the organophosphorus nerve agents soman and tabun. *Arch Toxicol.* 1984;55:76–77.

37. Vranken MA, DeBisschop HC, Willems JL. “In vitro” inhibition of neurotoxic esterase by organophosphorus nerve agents. *Arch Int Pharmacodyn.* 1982;260:316–318.

38. Willems JL, Palate BM, Vranken MA, DeBisschop HC. Delayed neuropathy by organophosphorus nerve agents. In: *Proceedings of the International Symposium on Protection Against Chemical Warfare Agents.* Umea, Sweden: National Defence Research Institute; 1983.

39. Hayes WJ Jr. Organic phosphorus pesticides. In: *Pesticides Studied in Man.* Baltimore, Md: Williams & Wilkins; 1982: 294.

40. DeReuck J, Willems J. Acute parathion poisoning: Myopathic changes in the diaphragm. *J Neurol.* 1975;208:309–314.

41. Meshul CK, Boyne AF, Deshpande SS, Albuquerque EX. Comparison of the ultrastructural myopathy induced by anticholinesterase agents at the end plates of rat soleus and extensor muscles. *Exp Neurol.* 1985;89:96–114.

42. Kawabuchi M, Boyne AF, Deshpande SS, Albuquerque EX. The reversible carbamate (–) physostigmine reduced the size of synaptic end plate lesions induced by sarin, an irreversible organophosphate. *Toxicol Appl Pharmacol.* 1989;97:98–106.

43. Ariens AT, Meeter E, Wolthuis OL, van Benthem RMJ. Reversible necrosis at the end-plate region in striated muscles of the rat poisoned with cholinesterase inhibitors. *Experientia.* 1969;25:57–59.

44. Dettbarn W. Pesticide induced muscle necrosis: Mechanisms and prevention. *Fundam Appl Toxicol.* 1984;4:S18–S26.

45. Wadia RS, Sadagopan C, Amin RB, Sardesai HV. Neurological manifestations of organophosphorous insecticide poisoning. *J Neurol Neurosurg Psychiatry.* 1974;37:841–847.

46. Senanayake N, Karalliedde L. Neurotoxic effects of organophosphorus insecticides. *N Engl J Med.* 1987;316:761–763.

47. Karademir M, Erturk F, Kocak R. Two cases of organophosphate poisoning with development of intermediate syndrome. *Hum Exp Toxicol.* 1990;9:187–189.

48. Nadarajah B. Intermediate syndrome of organophosphorus insecticide poisoning: A neurophysiological study. *Neurology.* 1991;41(suppl 1):251.

49. DeBleecker J, Willems J, Neucker KVD, DeReuck J, Vogelaers D. Prolonged toxicity with intermediate syndrome after combined parathion and methyl parathion poisoning. *Clin Toxicol.* 1992;30:333–345.

50. DeBleecker J, Neucker KVD, Willems J. The intermediate syndrome in organophosphate poisoning: Presentation of a case and review of the literature. *Clin Toxicol.* 1992;30:321–329.

51. Perron R, Johnson BB. Insecticide poisoning. *N Engl J Med.* 1969;281:274–275.

52. Gadoth N, Fisher A. Late onset of neuromuscular block in organophosphorus poisoning. *Ann Intern Med.* 1978;88:654–655.

53. Benson B. Is the intermediate syndrome in organophosphate poisoning the result of insufficient oxime therapy? *Clin Toxicol.* 1992;30:347–349.

54. Haddad LM. Organophosphate poisoning—intermediate syndrome? *Clin Toxicol.* 1992;30:331–332.

55. Gershon S, Shaw FH. Psychiatric sequelae of chronic exposure to organophosphorus insecticides. *Lancet.* 1961;1:1371–1374.

56. Bidstrup PL. Psychiatric sequelae of chronic exposure to organophosphorus insecticides. *Lancet.* 1961;2:103. Letter.

57. Biskind MS. Psychiatric manifestations from insecticide exposure. *JAMA.* 1972;220:1248. Letter.

58. Levin HS, Rodnitzky RL, Mick DL. Anxiety associated with exposure to organophosphate compounds. *Arch Gen Psychiatry.* 1976;33:225–228.

59. Metcalf DR, Holmes JH. EEG, psychological, and neurological alterations in humans with organophosphorus exposure. *Ann N Y Acad Sci.* 1969;160:357–365.

60. Rowntree DW, Nevin S, Wilson A. The effects of diisopropylfluorophosphate in schizophrenic and manic depressive psychosis. *J Neurol Neurosurg Psychiatry.* 1950;13:47–62.

61. Durham WF, Wolfe HR, Quinby GE. Organophosphorus insecticides and mental alertness. *Arch Environ Health.* 1965;10:55–66.

62. Namba T, Nolte CT, Jackrel J, Grob D. Poisoning due to organophosphate insecticides. *Am J Med.* 1971;50:475.

63. Dille JR, Smith PW. Central nervous system effects of chronic exposure to organophosphate insecticides. *Aerospace Med.* 1964;35:475–478.

64. Tabershaw IR, Cooper WC. Sequelae of acute organic phosphate poisoning. *J Occup Med.* 1966;8:5–20.

65. Rosenstock L, Keifer M, Daniell WE, McConnell R, Claypoole K. Chronic central nervous system effects of acute organophosphate pesticide intoxication. *Lancet.* 1991;338:223–227.

66. Grob D. The manifestations and treatment of poisoning due to nerve gas and other organic phosphate anticholinesterase compounds. *Arch Intern Med.* 1956;98:221–239.

67. Gaon MD, Werne J. *Report of a Study of Mild Exposures to GB at Rocky Mountain Arsenal.* Rocky Mountain Arsenal, Colo: US Army Medical Department; n.d.

68. Grob D, Harvey AM, Langworthy OR, Lillienthal JL. The administration of di-isopropyl fluorophosphate (DFP) to man. *Bull Johns Hopkins Hosp.* 1947;31:257.

69. Duffy FH, Burchfiel JL. Long term effects of the organophosphate sarin on EEGs in monkeys and humans. *Neurotoxicol.* 1980;1:667–689.

70. Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. Long-term effects of an organophosphate upon the human electroencephalogram. *Toxicol Appl Pharmacol.* 1979;47:161–176.

71. Burchfiel JL, Duffy FH. Organophosphate neurotoxicity: Chronic effects of sarin on the electroencephalogram of monkey and man. *Neurobehav Toxicol Teratol.* 1982;4:767–778.

72. Burchfiel JL, Duffy FH, Sim V. Persistent effect of sarin and dieldrin upon the primate electroencephalogram. *Toxicol Appl Pharmacol.* 1976;35:365–379.

73. Bucci TJ, Parker RM, Crowell JA, Thurman JD, Gosnell PA. *Toxicity Studies on Agent GA (Phase II): 90 Day Subchronic Study of GA (Tabun) in CD Rats.* Jefferson, Ark: National Center for Toxicological Research; 1992.

74. Bucci TJ, Parker RM, Gosnell PA. *Toxicity Studies on Agents GB and GD (Phase II): 90-Day Subchronic Study of GD (Soman) in CD-Rats.* Jefferson, Ark: National Center for Toxicological Research; 1992.

75. Jacobson KH, Christensen MK, DeArmon IA, Oberst FW. Studies of chronic exposures of dogs to GB (isopropyl methylphosphono-fluoridate) vapor. *Arch Indust Health.* 1959;19:5–10.

76. Bucci TJ, Parker RM, Cosnell PA. *Toxicity Studies on Agents GB and GD (Phase II): Delayed Neuropathy Study of Sarin, Type I, in SPF White Leghorn Chickens.* Jefferson, Ark: National Center for Toxicological Research; 1992.

77. Bucci TJ, Parker RM, Gosnell PA. *Delayed Neuropathy Study of Sarin, Type II, in SPF White Leghorn Chickens.* Jefferson, Ark: National Center for Toxicological Research; 1992.

78. Henderson JD, Higgins RJ, Rosenblatt L, Wilson BW. *Toxicity Studies on Agent GA: Delayed Neurotoxicity—Acute and Repeated Exposures of GA (Tabun).* Davis, Calif: University of California Davis Lab for Energy; 1989.

79. Bucci TJ, Parker RM, Gosnell PA. *Toxicity Studies on Agents GB and GD.* Jefferson, Ark: National Center for Toxiological Research; 1992.

80. Goldman M, Klein AK, Kawakami TG, Rosenblatt LS. *Toxicity Studies on Agents GB and GD.* Davis, Calif: University of California Davis Laboratory for Energy; 1987.

81. Kawakami TG, Goldman M, Rosenblatt L, Wilson BW. *Toxicity Studies in Agent GA: Mutagenicity of Agent GA (Tabun) in the Mouse Lymphoma Assay.* Davis, Calif: University of California Davis Laboratory for Energy; 1989.

82. Nasr M, Cone N, Kawakami TG, Goldman M, Rosenblatt L. *Toxicity Studies on Agent GA: Mutagenicity of Agent GA (Tabun) in the In Vitro Cytogenetic Sister Chromatid Exchange Test Phase I.* Davis, Calif: University of California Davis Laboratory for Energy; 1988.

83. Goldman M, Nasr M, Cone N, Rosenblatt LS, Wilson BW. *Toxicity Studies on Agent GA: Mutagenicity of Tabun (GA) in the Ames Mutagenicity Assay.* Davis, Calif: University of California Davis Laboratory for Energy; 1989.

84. Morgenstern P, Koss FR, Alexander WW. Residual mustard gas bronchitis: Effects of prolonged exposure to low concentrations of mustard gas. *Ann Intern Med.* 1947;26:27–40.

85. Buscher H; Conway N, trans. G*reen and Yellow Cross.* Cincinnati, Oh: Kettering Laboratory of Applied Physiology, University of Cincinnati, Oh; 1944.

86. Prokes J, Svovoda V, Hynie I, Proksova M, Keel K. The influence of X-radiation and mustard gas on methionin-35-S incorporation in erythrocytes. *Neoplasma.* 1968;15:393–398.

87. Manning KP, Skegg DCG, Stell PM, Doll R. Cancer of the larynx and other occupational hazards of mustard gas workers. *Clin Otolaryngol.* 1981;6:165–170.

88. Heston WE. Induction of pulmonary tumors in strain A mice with methyl-bis (beta-chloroethyl)amine hydrochloride. *J Natl Cancer Inst.* 1949;10:125–130.

89. Heston WE. Carcinogenic action of the mustards. *J Natl Cancer Inst*. 1950;11:415–423.

90. Heston WE. Occurrence of tumors in mice injected subcutaneously with sulfur mustard and nitrogen mustard. *J Natl Cancer Inst.* 1953;14:131–140.

91. McNamara BP, Owens EJ, Christensen MK, Vocci FJ. *Toxicological Basis for Controlling Levels of Mustard in the Environment.* Aberdeen Proving Ground, Md: Biomedical Laboratory; 1975. EB-SP-74030.

92. Case RAM, Lea AJ. Mustard gas poisoning, chronic bronchitis, and lung cancer: An investigation into the possibility that poisoning by mustard gas in the 1914–1918 war might be a factor in the production of neoplasia.*Br J Prev Soc Med.* 1955;9:62–72.

93. Norman JR, Jr. Lung cancer mortality in World War I veterans with mustard-gas injury: 1919–1965. *J Natl Cancer Inst.* 1975;54:311–317.

94. Fletcher C, Peto R, Tinker C, Speizer FE. *The Natural History of Chronic Bronchitis and Emphysema.* Oxford, England: Oxford University Press; 1976.

95. Wada S, Miyanishi M, Nashimoto Y, Kambe S, Miller RW. Mustard gas as a cause of respiratory neoplasia in man. *Lancet.* 1968;1:1161–1163.

96. Easton DF, Peto J, Doll R. Cancers of the respiratory tract in mustard gas workers. *Br J Ind Med.* 1988;45:652–659.

97. Minoue R, Shizushiri S. Occupationally-related lung cancer—Cancer of the respiratory tract as sequentia from poison gas plants. *Jpn J Thorac Dis.* 1980;18:845–859.

98. Albro PW, Fishbein L. Gas chromatography of sulfur mustard and its analogs. *J Chromatogr.* 1970;46:202–203.

99. Yanagida J, Hozawa S, Ishioka S, et al. Somatic mutation in peripheral lymphocytes of former workers at the Okunojima poison gas factory. *Jpn J Cancer Res.* 1988;79:1276–1283.

100. Watson AP, Jones TD, Grinnin GD. Sulfur mustard as a carcinogen: Application of relative potency analysis to the chemical warfare agents H, HD, and HT. *Regul Toxicol Pharmacol.* 1989;10:1–25.

101. Willems JL. Clinical management of mustard gas casualties. *Ann Med Milit Belg.* 1989;3(suppl):1–61.

102. Urbanetti JS. Battlefield chemical inhalation injury. In: Loke J, ed. *Pathophysiology and Treatment of Inhalation Injuries.* New York, NY: Marcel Dekker; 1988.

103. Balali M. Clinical and laboratory findings in Iranian fighters with chemical gas poisoning. In: Heyndrickx B, ed. *Proceedings of the 1st World Congress on New Compounds in Biological and Chemical Warfare: Toxicological Evaluation, 21–23 May 1984.* Ghent, Belgium: State University of Ghent; 1984: 254–259.

104. Balali-Mood M. First report of delayed toxic effects of yperite poisoning in Iranian fighters. In: Heyndrickx B, ed. *Proceedings of the 2nd World Congress on New Compounds in Biological and Chemical Warfare: Toxicological Evaluation, Industrial Chemical Disasters, Civil Protection and Treatment, 24–27 August 1986.* Ghent, Belgium, State University of Ghent; 1986: 489–496.

105. Freitag L, Firusian N, Stamatis G, Greschuchna D. The role of bronchoscopy in pulmonary complications due to mustard gas inhalation. *Chest.* 1991;100:1436–1441.

106. Gilchrist HL. *A Comparative Study of World War Casualties From Gas and Other Weapons.* Washington, DC: Government Printing Office; 1928.

107. Beebe GW. Lung cancer in World War I veterans: Possible relation to mustard-gas injury and 1918 influenza epidemic. *J Natl Cancer Inst.* 1960;25:1231–1252.

108. Winternitz MC. Anatomical changes in the respiratory tract initiated by irritating gases. *Milit Surg.* 1919;44:476–493.

109. Rimm WR, Bahn CF. Vesicant injury to the eye. In: *Proceedings of the Vesicant Workshop.* Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1987.

110. Hughes WF Jr. Mustard gas injuries to the eyes. *Arch Ophthalmol.* 1942;27:582–601.

111. Blodi FC. Mustard gas keratopathy. *Int Ophthalmol Clin.* 1971;2:1–13.

112. Duke-Elder WS, MacFaul PA. Chemical injuries. In: Duke-Elder WS, MacFaul PA, eds. *System of Ophthalmology.* St. Louis, Mo: CV Mosby; 1994.

113. Duke-Elder WS, MacFaul PA. *System of Ophthalmology.* St. Louis, Mo: CV Mosby; 1972.

114. Otto CE. *A Preliminary Report on the Ocular Action of Dichlorethyl Sulfide (Mustard Gas) in Man as Seen at Edgewood Arsenal, Edgewood, Maryland.* Edgewood Arsenal, Md: Chemical Warfare Service; 1946. EAL 539.

115. Novick M, Gard DH, Hardy SB, Spira M. Burn scar carcinoma: A review and analysis of 46 cases. *J Trauma.* 1977;17:809–817.

116. Treves N, Pack GT. Development of cancer in burn scars: analysis and report of 34 cases. *Surg Gynecol Obstet.* 1930;51:749–782.

117. Inada S, Hiragun K, Seo K, Yamura T. Multiple Bowen's disease observed in former workers of a poison gas factory in Japan with special reference to mustard gas exposure. *J Dermatol.* 1978;5:49–60.

118. US Army, US Navy, and US Air Force. Vesicants (blister agents). Section I—Mustard and nitrogen mustard. In: *NATO Handbook on the Medical Aspects of NBC Defensive Operations.* Washington, DC: US Army, US Navy, US Air Force; 1973. AMedP-6.

119. Anslow WP, Houch CR. Systemic pharmacology and pathology of sulfur and nitrogen mustards. In: *Chemical Warfare Agents and Related Chemical Problems.* Washington, DC: Office of Scientific Research and Development; 1946.

120. Lohs K. *Delayed Toxic Effects of Chemical Warfare Agents.* Stockholm, Sweden: Almqvist & Wilksell; 1979. SIPRI monograph.

121. Lawley PD, Lethbridge JH, Edwards PA, Shooter KV. Inactivation of bacteriophage T7 by mono- and difunctional sulphur mustards in relation to crosslinking and depurination of bacteriophage DNA. *J Mol Biol.* 1969;39:181–198.

122. Flamm WG, Bernheim NJ, Fishbein L. On the existence of intrastrand crosslinks in DNA alkylated with sulfur mustard. *Biochim Biophys Acta.* 1970;224:657–659.

123. Fox M, Scott D. The genetic toxicology of nitrogen and sulphur mustard. *Mutat Res.* 1980;75:131–168.

124. Scott D, Fox M, Fox BW. The relationship between chromosomal aberrations, survival and DNA repair in tumor cell lines of differential sensitivity to X-rays and sulphur mustard. *Mutat Res.* 1974;22:207–221.

125. Wulf HC, Aasted A, Darre E, Neibuhr E. Sister chromatid exchanges in fishermen exposed to leaking mustard gas shells. *Lancet.* 1985;1:690–691.

126. Sasser LB, Miller RA, Kalkwarf DR, Buschbom RL, Cushing JA. *Toxicology Studies on Lewisite and Sulfur Mustard Agents: Two-Generation Reproduction Study of Sulfur Mustard (HD) in Rats.* Richland, Wash: Pacific Northwest Laboratory; 1989.

Chapter 9

TOXIC INHALATIONAL INJURY

JOHN S. URBANETTI, M.D., FRCP(C), FACP, FCCP *

INTRODUCTION

TOXIC INHALATIONAL INJURY
- Physical Aspects
- Clinical Effects
- Physiology
- Evaluation of Injury

GENERAL THERAPEUTIC CONSIDERATIONS
- Further Exposure
- Critical Care Concepts
- Clinical Abnormalities
- Steroid Therapy

EXERTION AND TOXIC INHALATIONAL INJURY
- Interactions of Pulmonary Toxic Inhalants and Exercise
- Therapy

HISTORICAL WAR GASES
- Chlorine
- Phosgene

SMOKES AND OTHER SUBSTANCES
- Zinc Oxide
- Phosphorus Smokes
- Sulfur Trioxide–Chlorosulfonic Acid
- Titanium Tetrachloride
- Nitrogen Oxides
- Organofluoride Polymers: Teflon and Perfluoroisobutylene

SUMMARY

Clinical Assistant Professor of Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

INTRODUCTION

Pulmonary toxic inhalants have been a military concern since the Age of Fire. Thucydides, in 423 BC, recorded the earliest belligerent use of a toxic inhalant. The Spartans used a burning mixture of pitch, naphtha, and sulfur to produce sulfur dioxide that was used in sieges of Athenian cities.[1] There is scant reference in the literature to further military use of toxic inhalants until World War I. In early 1914, both the French and Germans investigated various tear gases, which were later employed. By early 1915, the German war effort expanded its gas research to include inhaled toxicants. As a result, on 22 April 1915, at Ypres, Belgium, the Germans released about 150 tons of chlorine gas along a 7,000-m battlefront within a 10-minute period. Although the exact number of injuries and deaths are unknown, this “new form” of warfare produced a degree of demoralization theretofore unseen.[2] Although phosgene and chlorine have not been used militarily since 1918, vast amounts are produced annually for use in the industrial sector. The potential for accidental or deliberate exposure to a toxic inhalant exists, and military personnel should be prepared.

TOXIC INHALATIONAL INJURY

Physical Aspects

Airborne (and consequently inhaled) toxic material may be encountered in gaseous or particulate form (Exhibit 9-1). Airway distribution of toxic inhalants as a gas or vapor follows normal respiratory gas flow patterns. The central airways exchange gases with the environment as a result of the mechanical aspects of breathing. Each breath dilutes central airway gas with newly inhaled gas.

EXHIBIT 9-1
DEFINITIONS OF AIRBORNE TOXIC MATERIAL

Gas	The molecular form of a substance, in which molecules are dispersed widely enough to have little physical effect (attraction) on each other; therefore, there is no definite shape or volume to gas.
Vapor	A term used somewhat interchangeably with gas, vapor specifically refers to the gaseous state of a substance that at normal temperature and pressure would be liquid or solid (eg, mustard vapor or water vapor compared with oxygen gas). Vaporized substances often reliquefy and hence may have a combined inhalational and topical effect.
Mist	The particulate form of a liquid (ie, droplets) suspended in air, often as a result of an explosion or mechanical generation of particles (eg, by a spinning disk generator or sprayer). Particle size is a primary factor in determining the airborne persistence of a mist and the level of its deposition in the respiratory tract.

Fumes, Smokes, and Dusts	Solid particles of various sizes that are suspended in air. The particles may be formed by explosion or mechanical generation or as a by-product of chemical reaction or combustion. Fumes, smokes, and dusts may themselves be toxic or may carry, adsorbed to their surfaces, any of a variety of toxic gaseous substances. As these particles and surfaces collide, adsorbed gases may be liberated and produce local or even systemic toxic injury.
Aerosol	Particles, either liquid or solid, suspended in air. Mists, fumes, smokes, and dusts are all aerosols.

Gas moves by convection into the peripheral airways (airways of 2-mm diameter) and then by diffusion to the alveolar-capillary membranes. Consequently, there is a much slower dilution of gas at this level. Therefore, toxic inhalants reaching this level may have a more profound effect due to greater relative duration of exposure.

Particles (such as those present in mists, and in fumes, smokes, and dusts) present a more complex distribution pattern because the particle size affects its deposition at various levels of the airway. Such factors as sedimentation and impact rates also control particle deposition. Therefore, heavier particles may settle in the nasopharynx or upper airways, whereas lighter or smaller particles may reach more-peripheral airways. Once they have impacted, particles are susceptible to a variety of respiratory defense mechanisms. These mechanisms determine the efficiency with which particle removal progresses, thereby determining the particle's ultimate degree of adverse effects.

Clinical Effects

Toxic inhalants may cause damage in one or more of the following ways:

- Asphyxiation may result from a lack of oxygen (eg, as in closed-space fires) or by interference with oxygen transport (eg, by cyanide, which compromises oxygen uptake by preventing its transport to cellular metabolic sites).
- Topical damage to the respiratory tract may occur due to direct toxic inhalational injury to the airways or alveoli. Cellular damage with consequent airway obstruction, pulmonary interstitial damage, or alveolar-capillary damage ultimately compromises adequate oxygen–carbon dioxide exchange. Some substances are relatively more toxic to the central airways, whereas others are more toxic to the peripheral airways or alveoli.
- Systemic damage may occur as a result of systemic absorption of a toxicant through the respiratory membranes (eg, leukopenia following mustard inhalation) with consequent damage to other organ systems. Effects on other organ systems may be more obvious than the respiratory effects of exposure (as with mercury).
- Allergic response to an inhaled toxicant may result in a pulmonary or systemic reaction, which may be mediated by one or more of a variety of immunoglobulins. Secondary by-products of this reaction may cause cellular destruction or tissue swelling; consequently, oxygen–carbon dioxide exchange may be compromised, and there may be systemic inflammatory damage.

Physiology

The ultimate effect of a particular toxic inhalant on the respiratory system or the whole organism is the result of a complex interaction of multiple factors, including the intensity of

exposure, condition of exposed tissues, and intrinsic protective and reparative mechanisms.

Intensity of Exposure

The intensity of exposure variable is partially affected by the physical state and properties of the toxicant. Heavier-than-air gases are particularly affected by environmental conditions; for example, warm environments increase the vaporization of some substances (such as mustard), making inhalational toxicity more likely. Increased humidity increases particle size by hygroscopic effects. Increased particle size may decrease the respiratory exposure to a toxicant because larger particles may precipitate prior to inhalation, or they may be collected preferentially in the upper airways, which have better clearance mechanisms.

The intensity as well as the site of exposure is partially affected by the toxic inhalant's degree of water solubility: more soluble toxicants (such as chlorine) primarily affect the upper airways and the more central airways. Despite the relatively high rate of airflow in central airways, the more soluble toxic gases are almost entirely deposited there. The less-soluble gases tend to produce effects in the peripheral airways or alveoli. In peripheral airways, air motion is relatively slow, occurring primarily by molecular diffusion. Foreign substances (such as cigarette smoke and toxic gases) that have not been trapped at more-central sites tend to remain longer in the peripheral airways. These substances may induce a surprising degree of damage due to their prolonged effect.

The intensity of exposure is commonly measured by multiplying the concentration (C, in milligrams per cubic meter) of a substance by the time of exposure (*t*, in minutes); the product is known as the C*t*; the units of measurement as mg•min/m^3. Precise measurement of the toxicant's concentration at the site of topical effect is not possible. An inhaled breath of toxic inhalant is mixed into a greater volume of airway gas (containing oxygen, nitrogen, carbon dioxide, and water vapor). The distribution of that breath then depends on such variables as speed of inhalation, depth of inhalation, and even body position during inhalation. Additionally, the duration of exposure, particularly in a combat setting, may be exceptionally difficult to assess. Finally, little attention is paid to the additional variable of depth and frequency of respiration (minute ventilation). This variable is highly affected by the exercise state or metabolic rate of the soldier. Deeper and more frequent breathing during combat may expose the airways to a greater amount of toxic inhalants compared to the exposure of an individual at rest.

Because of difficulties in accurately measuring C*t*, the threshold limit value (TLV) of a substance is used more often; a thorough discussion of TLV can be found in another volume in the *Textbook of Military Medicine series, Occupational Health: The Soldier and the Industrial Base.*[3] Calculations are made of a maximum allowable exposure to a toxicant, typically expressed for a 15-minute or an 8-hour period. This calculation makes the development of environmental standards and alarm detection systems somewhat simpler. The TLV should not, however, be considered a definition of a safe level. The concept of safe level wrongly implies that exposure to toxicants below that level has no effect. Rather, the TLV should be considered an expedient method of defining the statistical risk of injury resulting from an exposure. As biological and medical testing techniques undergo revision, toxic inhalants are often found to have histological or physiological effects in experimental animals at levels well below the established TLV. Therefore, rather than defining a "safe" level of a toxicant, the TLV may simply describe a level at which there is no recognized biological effect.

Condition of Exposed Tissues

Preexisting airway damage (such as that caused by prior toxic inhalant exposure) may seriously compromise the respiratory system's normal protection and clearance mechanisms. Specifically, there may be depletion of critical enzyme systems. Cigarette smoking may severely compromise airway function with respect to both airway patency and clearance mechanisms. Hyperreactive airways (asthma in varying degrees) are seen in up to 15% of the

adult population. Toxic inhalant exposures may trigger bronchospasm in these individuals. This bronchospasm may delay the clearance of the toxicant, interfering further with gas transport. The development of an acute interstitial process (eg, phosgene-related pulmonary edema) may also trigger bronchospasm. Individuals with any of the following characteristics should be considered likely to develop bronchospasm as the result of a toxic inhalant exposure:

- prior history of asthma or hay fever (even as a child),
- prior history of eczema, or
- family history of asthma, hay fever, or eczema.

Individuals with hyperreactive airways will benefit from bronchodilator therapy and possibly from steroids after exposure to a toxic inhalant. This statement, however, does not constitute an endorsement for routine steroid use in all toxic inhalational injuries.

Evaluation of Injury

Individuals exposed to a toxic inhalant should be carefully examined by medical personnel who are well versed in military medicine in the area of pulmonary injury. Obtaining a medical history and examining an exposed individual require the examiner to have an extensive knowledge of military toxicology and a thorough background in the fundamental aspects of medical practice. Casualties with toxic inhalational injuries present with a history that is characteristically different from that of most injuries; the cause–effect relationship may be particularly difficult to assess. The possibility of delayed effects caused by toxic inhalants cannot be overemphasized. Evidence of physiological damage may not become apparent for 4 to 6 hours after a lethal exposure (eg, to mustard or phosgene). If such an exposure is suspected, the patient must be observed for at least 4 to 6 hours. Even if there is no obvious injury noted during the observation period, the patient must be carefully reassessed before being discharged from the medical system.

History

Collecting historical data from the casualty is a critical aspect of assessing and treating toxic inhalational injury. Careful questioning of an exposed individual will often greatly simplify the diagnosis and therapy of the injury.

- Environment. Were explosions observed? Was there obvious smoke? If so, what color was it? Was the smoke heavier than air? What were the weather conditions (temperature, rain, wind, daylight, fog)? Were pools of liquid or a thickened substance in evidence?
- Protective Posture. What was the level of mission-oriented protective posture (MOPP)? Was there face mask or suit damage? Did the face mask fit adequately? When was the filter changed? How well trained was the soldier in using the appropriate protective posture, in making clinical observations, and in choosing appropriate therapy? Were other factors present (eg, consumption of alcoholic beverages, exposure to other chemicals, psychiatric status)?
- Prior Exposure. Was there prior exposure to other chemical agents? Is the individual a cigarette smoker? (For how long? How recently? How many?)
- Pulmonary History. Is there a prior history of chest trauma, hay fever, asthma, pneumonia, tuberculosis, exposure to tuberculosis, recurrent bronchitis, chronic cough or sputum production, or shortness of breath on exertion?
- Cardiac and Endocrine History. Is there a history of cardiac or endocrine disorder?
- Acute Exposure History. What were the initial signs and symptoms?

- Eyes. Is there burning, itching, tearing, or pain? How long after exposure did symptoms occur: minutes, hours, days?
- Nose and sinuses. Was a gas odor detected? Is there rhinorrhea, epistaxis, or pain? How long after exposure did symptoms occur: minutes, hours, days?
- Mouth and throat. Is there pain, choking, or cough? How long after exposure did symptoms occur: minutes, hours, days?
- Pharynx and larynx. Are there swallowing difficulties, cough, stridor, hoarseness, or aphonia? How long after exposure did symptoms occur: minutes, hours, days?
- Trachea and mainstem bronchi. Is there coughing, wheezing, substernal burning, pain, or dyspnea? How long after exposure did symptoms occur: minutes, hours, days?
- Peripheral airways and parenchyma. Is there dyspnea or chest tightness? How long after exposure did symptoms occur: minutes, hours, days?
- Heart. Are there palpitations, angina, or syncope? How long after exposure did symptoms occur: minutes, hours, days?
- Central nervous system. Is there diffuse or focal neurological dysfunction?

Physical Examination

Physical examination may be particularly difficult in the event of combined toxic and conventional injuries; therefore, it is essential that medical personnel note the following conditions:

- Reliability. Is the casualty alert and oriented?
- Appearance. Is he anxious or tachypneic?
- Vital Signs. What are his weight, blood pressure, pulse, and temperature?
- Trauma. Is there a head injury? Are there burns in the region of the eyes, nose, or mouth?
- Skin. Are there signs of burns, erythema, sweating, or dryness?
- Eyes. Is there conjunctivitis, corneal burns or abrasion, miosis, or mydriasis?
- Nose. Is there erythema, rhinorrhea, or epistaxis?
- Oropharynx. Is there evidence of perioral burns or erythema?
- Neck. Is there hoarseness, stridor, or subcutaneous emphysema?
- Chest. Is there superficial chest wall trauma, tenderness, crepitation, dullness, or hyperresonance? Are crackles present? This measurement should be made by asking the patient to hold a forced expiration at residual volume for 30 seconds, then listening carefully at the lung bases for inspiratory crackles. Is wheezing present? This examination should be undertaken by listening for wheezes bilaterally in the chest both posteriorly and anteriorly under circumstances of forced expiration.

Laboratory Measurements

Sophisticated laboratory studies are of limited value in the immediate care of an exposed, injured individual. The following studies are of some predictive value in determining the severity of exposure and the likely outcome.

Chest Radiograph. The presence of hyperinflation suggests toxic injury of the smaller airways, which results in air being diffusely trapped in the alveoli (as occurs with phosgene exposure). The presence of “batwing” infiltrates suggests pulmonary edema secondary to toxic alveolar capillary membrane damage (as occurs with phosgene exposure). Atelectasis is often

seen with more-central toxic inhalant exposures (such as with chlorine exposure). As radiological changes may lag behind clinical changes by hours to days, the chest radiograph may be of limited value, particularly if normal.

Arterial Blood Gases. Hypoxia often results from exposure to toxicants, such as occurs following exposure to chlorine. Measurement of the partial pressure of oxygen (PO_2) is a sensitive but nonspecific tool in this setting; both the central and peripheral effects of toxic inhalant exposure may produce hypoxia. At 4 to 6 hours, normal arterial blood gas (ABG) values are a strong indication that a particular exposure has little likelihood of producing a lethal effect. Typically, carbon dioxide elevation is seen in individuals with underlying hyperreactive airways; in this circumstance, it is thought that bronchospasm is triggered by exposure to the toxic inhalant.

Pulmonary Function Tests. A variety of airway function measures and pulmonary parenchymal function measures can be performed in rear-echelon facilities. Initial and follow-up measurements of the flow-volume loop, lung volumes, and the lung diffusing capacity for carbon monoxide (DL_{CO}) are particularly useful in assessing and managing long-term effects of a toxic inhalant exposure. Although such laboratory studies are of minimal value in an acute-care setting, flow volume loop measures may document a previously unrecognized degree of airway obstruction. A degree of reversibility may also be demonstrated if bronchodilators are tested at the same time. Substantial airway obstruction may be present with little clinical evidence. In all cases of unexplained dyspnea, regardless of clinical findings, careful pulmonary function measurements should be undertaken. Ideally, these studies should be performed in an established pulmonary function laboratory and would include DL_{CO} and ABG measurements. These studies should also be performed during exertion if dyspnea on exertion is noted that cannot otherwise be explained by pulmonary function studies performed at rest.

GENERAL THERAPEUTIC CONSIDERATIONS

Further Exposure

A soldier's exposure to toxic inhalants is limited by removing him from the environment in which the toxicant is present. Careful decontamination serves to limit reexposure to the toxicant from body surfaces or clothing. Furthermore, decontamination reduces the risk of secondary exposure of other personnel.

Critical Care Concepts

Life-threatening pathophysiological processes arising from toxic inhalants usually develop in the upper airway, where laryngeal obstructions can cause death in a few minutes, and in the lower respiratory tract, where bronchospasm can cause death almost as rapidly. Severe bronchospasm may indicate exposure to a nerve agent. If there is any possibility that exposure to nerve agents has occurred, the use of one or more of the three Mark I Auto-Injector kits that are provided to all military members (see Chapter 5, Nerve Agents) should be considered an urgent part of initial therapy. Like the airway and breathing, cardiac function must likewise be assessed immediately.

Adequate control of the airway is important in all toxic inhalant exposures. Exposure to centrally absorbed toxic inhalants (such as chlorine) and to fires may be particularly dangerous, insofar as laryngeal or glottal edema may rapidly compromise upper airway patency. Evidence of perinasal or perioral inflammation indicates the need for more careful investigation of the oropharynx for erythema. Subsequent laryngoscopy or bronchoscopy may be of particular value and should be undertaken along with preparations for expedient intubation. The presence of stridor indicates the need for immediate airway control.

Primary failure of respiration after exposure to toxicants other than nerve agents suggests a severity of exposure that requires intensive medical support; at this point, a triage decision

may be needed. The presence of wheezing indicates severe bronchospasm, which requires immediate therapy. The presence of dyspnea necessitates careful observation of the patient for at least 4 to 6 hours, until severe, potentially lethal respiratory damage can be reasonably excluded.

Primary failure of cardiac function, like respiration, is a grave sign after exposure to toxicants other than nerve agents. Cardiac rate and rhythm abnormalities are often seen after toxicant exposure. These abnormalities are often transient and improve once the casualty is removed from the toxic environment and is provided with supplemental oxygen. Hypotension is a grave prognostic sign if not immediately reversed by fluid and volume resuscitation and intensive medical care.

Clinical Abnormalities

Clinical abnormalities that may lead to respiratory failure can also be observed after pulmonary toxic inhalational injury. These include hypoxia, hypercarbia, pulmonary edema, which are all signs of possible toxic inhalant exposure; and infection, which is a frequent complication, particularly in intubated patients.

Oxygen supplementation should be provided to maintain a PO_2 greater than 60 mm Hg. Very early application of positive end-expiratory pressure (PEEP) is important, with progression to intubation and positive pressure ventilation if PEEP fails to normalize the PO_2. Note that PEEP application may precipitate hypotension in individuals with marginally adequate intravascular volume (such as those with conventional trauma or pulmonary edema) or in individuals previously treated with drugs that have venodilating properties (such as morphine or diazepam). Particular attention to anemia is necessary if hypoxia is present.

An elevation of the partial pressure of carbon dioxide (PCO_2) greater than 45 mm Hg suggests that bronchospasm is the most likely cause of hypercarbia; therefore, bronchodilators should be used aggressively. If the patient has a prior history of clinical bronchospasm, steroids should be added immediately; steroids should also be considered if the patient has a history of hay fever or eczema and obvious bronchospasm with the current exposure. Occasionally, positive pressure ventilation may also be necessary. Interstitial lung water (early pulmonary edema) may trigger bronchospasm in individuals who are otherwise hyperreactive (such as those with “cardiac” asthma). Steroids are not primarily useful in this circumstance.

Pulmonary edema noted after a toxic inhalant exposure should be treated similarly to adult respiratory distress syndrome (ARDS) or “noncardiac” pulmonary edema. The early application of PEEP is desirable, possibly delaying or reducing the severity of pulmonary edema. Diuretics are of limited value; however, if diuretics are used, it is useful to monitor their effect by means of the pulmonary artery wedge pressure measurement because excessive diuretics may predispose the patient to hypotension if PEEP or positive-pressure ventilation is applied.

Toxic inhalant exposures typically cause acute fever, hypoxia, elevated polymorphonuclear white cell count, and radiologically detectable infiltrates. These changes should not be taken to indicate bacteriological infection during the first 3 to 4 days postexposure. Thus, routine or prophylactic use of antibiotics is not appropriate during this period.

Infectious bronchitis or pneumonitis commonly supervenes 3 or more days postexposure to a toxic inhalant, particularly in intubated patients. Close observation of secretions, along with daily surveillance bacterial culture, will permit the early identification and specific treatment of identified organisms.

Steroid Therapy

Systemic steroid therapy has been considered for use in certain toxic inhalational exposures. U.S. Army Field Manual 8-285, *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries,*[4] suggests a benefit in phosgene exposure, but human data supporting this approach are scanty. There is some support in the literature for steroid use in exposure to zinc/zinc oxide and oxides of nitrogen. However, there is no other strong

support in the literature for the treatment of other specific toxic inhalations with systemic steroids.

A significant percentage of the population has a degree of airway irritability or hypersensitivity, as exemplified by persons with asthma. These individuals are likely to display heightened sensitivity or even bronchospasm, nonspecifically after an inhalational exposure. The use of systemic steroids would be indicated in this population if their bronchospasm were not readily controlled with more routine bronchodilators. Systemic steroids may, if used in this setting, be required for prolonged periods, particularly if superinfection should supervene.

Inhaled steroids may be less effective than systemic steroids in circumstances of acute exposure—especially if later infected. Inhaled steroids appear most useful as an adjunct to the gradual reduction or weaning or both from a systemic steroid use.

EXERTION AND TOXIC INHALATIONAL INJURY

"The extra amount of oxygen needed in the exertion could not be supplied and the results showed at once.... [M]en who had been comfortable while lying became rapidly worse and sometimes died suddenly if they walked or sat about,"[5(p424)] wrote Herringham in his article describing gassed casualties in World War I.

There is scant, primarily anecdotal, information relating to the effects of exercise postexposure to toxic inhalants. During World War I, attempts to assess the effects of toxic inhalant exposure on exercise tolerance were severely constrained by a limited understanding of basic exercise physiology and by limited technological capacity. Some early observations in exercise-limited victims of gassing included observations of limited "depth of respiration" and tachycardia after "mildest exercise"[6(p3)]; and a heartbeat that "rises more than normal for given exercise...returns to normal more slowly after exercise [seen in 116 of 320 gassed patients]."[7(p10)]

As a result of the World War I experience, it was clear that cardiorespiratory damage resulting from toxic inhalant exposures could severely limit exercise capacity. Of primary concern, however, was whether exercise undertaken after a toxic inhalant exposure could, in some way, exacerbate the effects of that exposure and thus increase the morbidity or mortality of exposed individuals. This was a particularly practical concern in light of the military needs to return soldiers to active duty as soon as possible and to require soldiers to participate (insofar as they appeared able) in their own evacuation.

Toxic inhalant exposures may produce direct pulmonary effects, indirect cardiac effects, and other systemic effects (eg, central nervous system [CNS] effects of mercury inhalation). Severe damage to those systems will be readily apparent; however, identification of lesser damage may require increasingly sophisticated examination. Minor organ dysfunction is best identified during stress; that is, an organ system that is functioning near its maximum capacity is more likely to demonstrate physiological limitation than a system that is functioning under conditions of rest. The principle of organ stress as a method of functional assessment is well recognized. Both cardiologists and endocrinologists have devised stress testing methods that allow earlier and more sensitive demonstration of cardiac and endocrine limitations. Systems with small degrees of physiological limitation are much more likely to display such limitations during stress than at rest. Conversely, an organ system that is impaired may become so dysfunctional during stress that it exceeds its compensatory mechanisms (and those of other support systems) and fails, with resulting catastrophic consequences for the organism as a whole.

The normal human body is designed to function at a wide range of activity. Normal cardiopulmonary reserves permit a 25- to 30-fold increase in oxygen delivery to working muscles. Other compensatory mechanisms permit transient activities even beyond these limits. Ultimately, however, any activity requires adequate oxygen delivery and carbon dioxide extraction for cell function. A damaged cardiorespiratory system that is unable to accomplish normal oxygen–carbon dioxide exchange will first appear limited at the extreme of exertion.

Maximum exercise capacity will be restricted. With increasing damage to the cardiorespiratory system, exercise capacity becomes further limited until symptoms (eg, dyspnea or orthopnea) appear even at rest.

Although oxygen–carbon dioxide exchange may be adequate at rest (oxygen delivery of approximately 250 mL/min), the requirements of oxygen delivery during exercise (up to 5,000 mL/min) cannot be met if there is cardiorespiratory damage: with cardiorespiratory damage, cellular hypoxia results during exercise. Anaerobic mechanisms are transiently effective but are inadequate to provide energy for extended periods of time, and metabolic acidosis ensues. Ordinarily the metabolic acidosis (lactate-predominant) causes enough discomfort and discoordination of activity to limit the exertion. Lactate is then cleared from the circulation and excreted from the body as carbon dioxide. However, should respiratory limitation also be present, then excess carbon dioxide load is not readily exchanged. A respiratory acidosis is superimposed. Acidosis develops more rapidly than systemic buffer systems can accommodate it. The resulting hydrogen ion excess compromises myocardial contractility, increases pulmonary artery resistance, and causes peripheral venous dilation. Cardiac output then diminishes relative to metabolic needs.

Furthermore, a diminished intravascular blood volume—which is secondary to losses to the extravascular space, particularly to lung parenchyma—and increased intrathoracic pressure (air trapping secondary to bronchospasm) reduce venous return. This contributes to a further, severe compromise of cardiac output. Hypotension then exceeds other compensatory mechanisms (such as tachycardia), with further restriction of oxygen delivery to metabolizing tissue. Finally, more intense acidosis occurs and, ultimately, death supervenes.

Interactions of Pulmonary Toxic Inhalants and Exercise

Airway resistance may increase because of toxic inhalational injury, resulting in increased work of respiration. Air trapping secondary to increased airway resistance increases intrathoracic pressure. Increased work of respiration and decreased venous return result in exercise limitation. Ventilation–perfusion abnormalities of disordered airway function limit oxygen delivery and carbon dioxide clearance, which also compromises exercise tolerance.

Acute, short-term changes in interstitial function secondary to pulmonary edema, or long-term changes secondary to interstitial inflammation or fibrosis subsequently limit diffusing capacity, a particularly exercise-sensitive portion of the gas exchange system.

During exercise, the airways and the alveoli have greater exposure to a toxicant because of increased ventilation. Hyperventilation may itself induce mild bronchospasm, delaying toxicant clearance from the lung periphery. Already limited oxygen delivery may be critically compromised by exercise, resulting in systemic hypoxia (including cerebral, cardiac, renal, and hepatic systems).

With regard to interstitial mechanisms, increments of pulmonary arterial pressure with increased cardiac output may aggravate capillary leaks, increasing interstitial edema. Inflammatory cells may accumulate in the interstitium, creating local toxicity as they degenerate.

Therapy

After a toxic inhalational exposure, exercise may further compromise the patient. Hypoxia is a primary factor. Oxygen supplementation is necessary at rest and especially during exercise. Exercise may aggravate the effects of toxicant exposure and should be limited or restricted if possible.

Toxicant exposure may produce pulmonary abnormalities which result in dyspnea. Clinical or laboratory testing may fail to confirm this symptom in the early hours postexposure; hence examination of the patient must also take place at 4 to 6 hours postexposure, if no abnormalities are initially identified.

Dyspnea may be present long after the chest radiograph, physical examination, and resting values for ABG return to normal. Exercise evaluation of these individuals (with ABG readings as a

necessary part of that evaluation) must be undertaken to understand the exposed individual's complaints. Failure to make these measurements in a soldier complaining of exercise limitation displays a lack of understanding of both toxic inhalant exposures and basic exercise physiology.

HISTORICAL WAR GASES

A variety of chemical agents were used as toxic inhalants during World War I; of these, several are considered current threats. Some toxicants have current military relevance either because of their presence in stockpiles or because of their current or recent use in military operations in other countries. Other toxicants exist in large quantities as a result of their industrial use. Because of the military's preparedness in managing large-scale exposure to toxicants, such as poisonous gases, military assistance may be required in the event of a major accident involving toxic inhalants.

The following section discusses chlorine and phosgene, the two war gases that were used during World War I. Although they are not considered current threats, under the right conditions they could pose a threat to military personnel.

Chlorine

Chlorine is a dense, acrid, pungent, greenish-yellow gas that is easily recognized by both color and odor. Because of its density and tendency to settle in low-lying areas, this gas is hazardous in closed spaces. Because the gas has a characteristic odor and its odor threshold is well below acute TLVs, chlorine is said to have good warning properties. However, chronic exposures are thought to lead to a progressive degradation of the odor threshold. As a result, workers with frequent or long-term occupational exposures to chlorine are at greater risk of inhalational damage in later years.[8–10]

Clinical Effects of Exposure

An almost characteristic initial complaint of chlorine exposure is that of suffocation: the inability to get enough air.[11] Typically, low exposures produce a rapid-onset ocular irritation with nasal irritation, followed shortly by spasmodic coughing and a rapidly increasing choking sensation. Substernal tightness is noted early. Complaints are particularly evident in individuals who have a history of hyperreactive airways (asthma). Minimal to mild cyanosis may be evident during exertion, and complaints of exertional dyspnea are prominent. Deep inspiration produces a typical persistent, hacking cough.

Moderate chlorine exposures result in an immediate cough and a choking sensation. Severe substernal discomfort and a sense of suffocation develop early. Hoarseness or aphonia is often seen, and stridor may follow. Symptoms and signs of pulmonary edema may appear within 2 to 4 hours; radiological changes typically lag behind the clinical symptoms. There may be retching and vomiting, and the gastric contents often have a distinctive odor of chlorine. Figure 9-1 shows the chest radiograph of a chemical worker 2 hours postexposure to chlorine: note the diffuse pulmonary edema without significant cardiomegaly. This patient experienced severe resting dyspnea, diffuse crackles on auscultation, and had a PO_2 of 32 mm Hg breathing room air. Figure 9-2 is a section of lung biopsy taken from the same worker 6 weeks postexposure. The section shows no residual injury from the chlorine exposure. The patient's PO_2 was 80 mm Hg breathing room air.

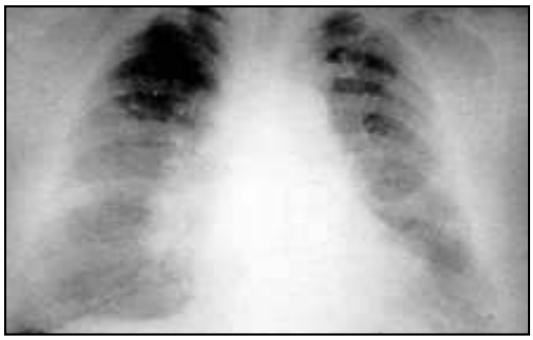


Fig. 9-1. The chest radiograph of a 36-year-old female chemical worker 2 hours postexposure to chlorine inhalant. She had severe resting dyspnea during the second hour, diffuse crackles/rhonchi on auscultation, and a P_{O_2} of 32 mm Hg breathing room air. The radiograph shows diffuse pulmonary edema without significant cardiomegaly.

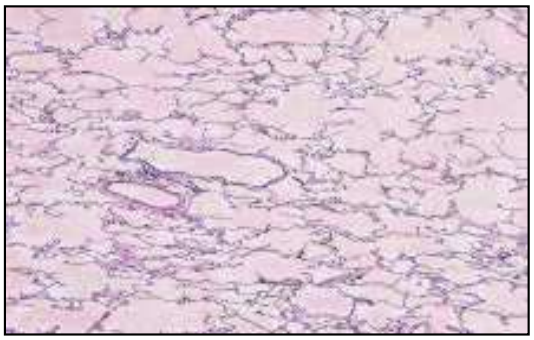


Fig. 9-2. A section from a lung biopsy (from the patient whose chest radiograph is seen in Figure 9-1) taken 6 weeks postexposure to chlorine. At that time, she had no clinical abnormalities and a P_{O_2} of 80 mm Hg breathing room air. The section shows normal lung tissues without evidence of interstitial fibrosis/inflammation. Hematoxylin and eosin stain; original magnification × 100.

Intense toxic inhalant exposures may cause pulmonary edema within 30 to 60 minutes. Secretions from both the nasopharynx and the tracheobronchial tree are copious, with quantities of up to 1 L/ h reported.[12] Severe dyspnea is so prominent that the patient may refuse to move. On physical examination, the chest may be hyperinflated. Mediastinal emphysema secondary to peripheral air trapping may dissect to the skin and present as subcutaneous emphysema. The sudden death that occurs with massive toxic inhalant exposure is thought to be secondary to laryngeal spasm.[13]

Therapy

There is no chemically specific prophylactic or postexposure therapy for chlorine inhalation; therefore, postexposure therapy is directed toward treating the observed physiological signs and symptoms. Most deaths occur within the first 24 hours and are caused by respiratory failure. Individuals who survive a single, acute exposure generally demonstrate little or no long-term pathological or physiological sequelae. Individuals with underlying cardiopulmonary disease or those who suffer complications (such as pneumonia) during therapy are at risk for developing chronic bronchitis or (rarely) a gradual and progressive bronchiolitis obliterans. Chronic bronchitis was thought to be common after World War I chlorine inhalant exposures. Current assessment of these gassed individuals suggests that their chronic or progressive illness is more likely to have resulted from a combination of inadequately treated complicating infections and cigarette smoking than from the destructive effects of a single, acute exposure.[14,15]

Secretions are typically copious but generally thin; mucolytics are not required. Careful attention to the appearance of secretions will assist in the early identification of bacterial superinfection, which may be associated with secretions that are other than clear or white.

Bacterial superinfection is commonly noted 3 to 5 days postexposure. Early, aggressive antibiotic therapy should be directed as specifically as possible against identified organisms. Careful, frequent Gram's stains and cultures of sputum are used to identify a predominant organism. Persistent fever, infiltrates, or elevated white blood cell count in the presence of thickened, colored secretions should prompt the institution of a broad-spectrum antibiotic (such

as ampicillin or a cephalosporin). The choice of antibiotic should be based on local experience with either community-acquired or nosocomial organisms. Antibiotics are not used prophylactically in this setting; such therapy would only serve to select a resistant bacterial population in the injured individual.

Bronchospasm is an early and prominent complication of chlorine exposure. Aggressive bronchodilator therapy (a combination of adrenergic agent and theophylline) is appropriate. Steroids are used if the patient has a history of hyperreactive airways. Bronchodilators are used at least until the antibiotics are discontinued and there is no further evidence of clinical response (eg, as indicated by laboratory testing). Steroid doses should be tapered as rapidly as clinical circumstances warrant after the first 3 to 4 days of (uncomplicated) recovery. Superinfection may complicate prolonged steroid therapy.

Hypoxia improves as the bronchospasm improves, and long-term oxygen supplementation is rarely required. If long-term oxygen supplementation is needed, a search for other causes of hypoxia should be undertaken. Early institution of positive airway pressure (such as using a PEEP mask) may be useful. Positive pressure ventilation may be necessary if PEEP is insufficient to maintain PO_2 greater than 60 mm Hg. Occasional reports of subcutaneous emphysema after chlorine exposure should not be considered a contraindication to using PEEP or positive pressure ventilation.[16,17]

Generally, the patient gradually recovers from his acute injury in 36 to 72 hours, depending on the degree of exposure. Delay in recovery may be the result of superinfection. Pleural effusions of up to 600 mL have been identified, generally in association with pulmonary edema. Areas of pneumonic consolidation may be evident on the chest radiograph.[16] Follow-up studies of acute toxic inhalant exposures have generally demonstrated that patients who had no acute complications developed no significant long-term effects.[15] Pulmonary function and respiratory symptoms in individuals with repetitive, long-term, or low-dose toxic inhalant exposures have been reviewed in a number of reports.[18] Although accurate records are difficult to maintain and, consequently, the data may be somewhat difficult to interpret, long-term or multiple, low-dose toxic inhalant exposures appear to produce no significant physiological defects when the results are corrected for smoking.[18]

Clinical Summary

Long-term complications from chlorine exposure are not found in those individuals who survive an acute exposure. There is little or no evidence that significant long-term respiratory compromise occurs, unless there has been a superimposed bronchitis or pneumonitis. The toxic effects of chlorine in the absence of superinfection are relatively short lived. Bronchospasm may require prolonged therapy, occasionally with steroids. There is little evidence for significant long-term pathophysiological abnormalities with either acute, severe chlorine exposure or repetitive low-dose, long-term exposures. A patient’s failure to demonstrate substantial recovery within 3 to 4 days should prompt an investigation for the possible presence of bacterial superinfection or other complicating features.

Phosgene

Phosgene (military designation, CG) appears at usual battlefield temperatures as a white cloud whose density is due, in part, to hydrolysis. The gas is heavier than air and at low concentrations has a characteristic odor of newly mown hay. At higher concentrations, a more acrid, pungent odor may be noted. An odor threshold of 1.5 ppm has been reported but does not apply to all observers. This odor threshold is inadequate to protect against toxic inhalant exposures to this substance. Furthermore, a relatively rapid nasal adaptation limits the usefulness of odor as a detection mechanism.[19] Synonyms for phosgene include carbonyl chloride, D-Stoff, and green cross.

Clinical Effects of Exposure

In the first 30 minutes following exposure to phosgene, low concentrations may produce mild cough, a sense of chest discomfort, and dyspnea. Exposure to moderate concentrations triggers lacrimation and the unique complaint that smoking tobacco produces an objectionable taste.[20] High concentrations may trigger a rapidly developing pulmonary edema with attendant severe cough, dyspnea, and frothy sputum. Onset of pulmonary edema within 2 to 6 hours is predictive of severe injury. High concentrations may produce a severe cough with laryngospasm that results in sudden death; this could possibly be due to phosgene hydrolysis, which releases free hydrochloric acid at the level of the larynx.[21]

In the first 12 hours after toxic inhalant exposure, depending on the intensity of exposure, a substernal tightness with moderate resting dyspnea and prominent exertional dyspnea become evident. These symptoms are often a prelude to the characteristic development of pulmonary edema. Initially small, then greater, amounts of thin airway secretions may appear. The delayed and insidious onset of severe pulmonary edema often has resulted in a casualty's being medically evaluated and discharged from the medical facility, only to return some hours later with severe and occasionally lethal pulmonary edema. The chest radiograph of a female chemical worker 2 hours postexposure to phosgene (Figure 9-3) shows bilateral perihilar, fluffy, and diffuse interstitial infiltrates. A section of the lung (Figure 9-4) from the same patient shows nonhemorrhagic pulmonary edema with few scattered inflammatory cells.

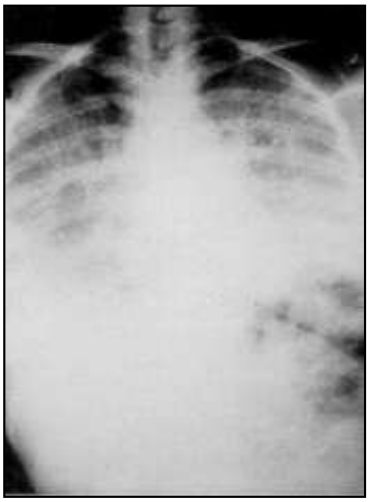


Fig. 9-3. The chest radiograph of a 42-year-old female chemical worker 2 hours postexposure to phosgene. Dyspnea progressed rapidly over the second hour; P_{O_2} was 40 mm Hg breathing room air. This radiograph shows bilateral perihilar, fluffy, and diffuse interstitial infiltrates. The patient died 6 hours postexposure.

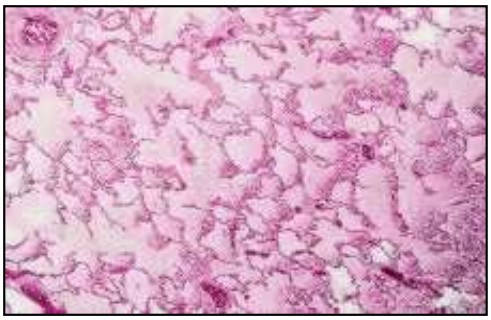


Fig. 9-4. A lung section of the patient whose chest radiograph is seen in Figure 9-3. This patient died 6 hours after exposure to phosgene; the biopsy section was taken at postmortem examination. The section shows nonhemorrhagic pulmonary edema with few scattered inflammatory cells. Hematoxylin and eosin stain; original magnification × 100.

An individual may remain relatively asymptomatic for up to 72 hours after inhalant exposure. During that time, dyspnea or pulmonary edema may be triggered by exertion (see the preceding section, Exertion and Toxic Inhalational Injury).

Therapy

Pulmonary edema is the most serious clinical aspect of phosgene exposure and begins with few, if any, clinical signs. Consequently, early diagnosis of pulmonary edema requires that careful attention be paid to the patient's symptoms of dyspnea or chest tightness. The presence of these symptoms in a setting of possible inhalant exposure requires expeditious auscultation, chest radiograph, and ABG measurements.

If abnormal, these measurements mandate close observation and support at the intensive care level.

If the measurements are normal, they all must be repeated 4 to 6 hours after the suspected exposure; only then can an individual be released to a lower medical priority status. Abnormality of any one of those measures, in the absence of other explanation, should prompt institution of therapy for noncardiac pulmonary edema. At the early stages of treatment, therapy should include positive airway pressure with early application of the PEEP mask. Later application of positive pressure ventilation through intubation may be required if the PEEP mask fails to maintain adequate arterial PO_2.

Figures 9-5 and 9-6 are the chest radiographs of a male chemical worker 2 hours and 7 hours, respectively, postexposure to phosgene. The chest radiograph taken 2 hours postexposure was normal. The patient presented with mild resting dyspnea, but otherwise his physical examination was normal. The same individual, 7 hours postexposure, presented with moderate resting dyspnea and a few crackles on auscultation. His chest radiograph showed mild interstitial edema. Figure 9-7 is a lung section from this patient, who died 4 years later from unrelated causes. These tissues are normal, and there is no evidence of interstitial fibrosis.

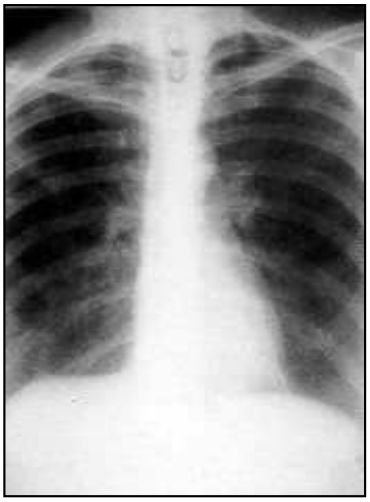


Fig. 9-5. The chest radiograph of a 40-year-old male chemical worker 2 hours postexposure to phosgene. The patient experienced mild resting dyspnea for the second hour; however, his physical examination was normal with a PO_2 of 88 mm Hg breathing room air. This radiograph is normal.

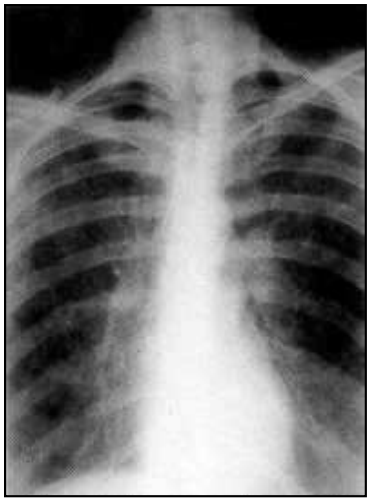


Fig. 9-6. The same patient seen in Figure 9-5, now 7 hours postexposure to phosgene. He had moderate resting dyspnea, a few crackles on auscultation, and a PO_2 of 64 mm Hg breathing room air. This radiograph shows mild interstitial edema.

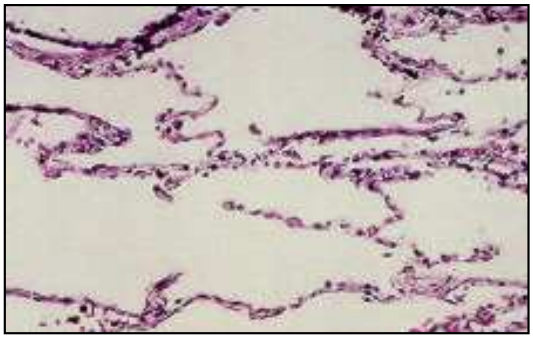


Fig. 9-7. A lung section from the patient whose chest radiographs are shown in Figures 9-5 and 9-6, who died 4 years later from causes unrelated to his exposure to phosgene. This section shows normal lung tissues without evidence of interstitial fibrosis or inflammation. Hematoxylin and eosin stain; original magnification × 400.

Diuretics may be of minor value in reducing capillary pressure and consequently decreasing the rate of fluid loss through a (presumptively) damaged alveolar–capillary membrane. It should be kept in mind that intravascular volume reduction (such as that induced by diuretics) may lead to serious hypotension if positive pressure ventilation is required. Steroids have not been found to be clinically useful in treating phosgene-induced pneumonitis. There has been some discussion in the literature concerning the use of hexamethamine tetramine. However, reliable comparative data supporting this therapeutic intervention are not available.[22–24]

Clinical Summary

The toxic effects of phosgene in the absence of superinfection or other complications are relatively short-lived. There is little evidence of significant long-term pathophysiological abnormalities with either acute, severe phosgene exposures or repetitive, low-dose, long-term exposures. A patient's failure to demonstrate substantial recovery within 3 to 4 days should prompt an investigation for the possible

presence of bacterial superinfection or other complicating features.[25]

SMOKES AND OTHER SUBSTANCES

Smokes and obscurants comprise a category of materials that are not used militarily as direct chemical agents. They may, however, produce toxic injury to the airways. The particulate nature of smokes may lead to a mechanical irritation of the upper and lower airways—therefore inducing bronchospasm in some hypersensitive individuals (eg, those with asthma). Certain smokes contain chemicals with a degree of tissue reactivity that results in damage to the airways. A discussion of obscurant smokes is followed by a discussion of certain explosion-related (oxides of nitrogen) or pyrolysis-related (perfluoroisobutylene) substances that are important in military practice.

Zinc Oxide

During World War I, the difficulties experienced by the Allies in using white phosphorus as an obscurant smoke led both the French and the U.S. Chemical Warfare Service to search for other smokes; zinc oxide (military designation, HC or HC smoke) is an outgrowth of that search. HC contains equal percentages of zinc oxide and hexachloroethane, with approximately 7% grained aluminum. The material is currently formulated for use in smoke pots, smoke grenades, and artillery rounds.

On combustion, the reaction products are zinc chloride and up to 10% chlorinated hydrocarbons such as phosgene, carbon tetrachloride, ethyl tetrachloride, hexachloroethane, and hexachlorobenzene. In addition to these products, hydrogen chloride, chlorine, and carbon monoxide are also produced. As the zinc chloride particles form, they hydrolyze in ambient water vapor to produce a dense white smoke. The toxicity of this chemical agent is generally attributed to the topical toxic effects of zinc chloride. However, carbon monoxide, phosgene, hexachloroethane, and other combustion products may also contribute to the observed respiratory effects, depending on the circumstances of the munitions ignition.[26]

Clinical Effects of Exposure

Since World War I, there have been numerous reports of accidental exposures to the combustion products of HC. Depending on the intensity of the exposure, a wide range of clinical effects occurs; exposures as brief as 1 minute may lead to death.

Low-dose toxic inhalant exposures are characterized by sensations of dyspnea without subsequent radiological, auscultatory, or blood gas abnormalities. These patients should be watched carefully for 4 to 6 hours postexposure (see the earlier discussion on phosgene); however, severe clinical sequelae are uncommon.

Moderate exposures to HC are characterized by severe dyspnea, which may show a relatively rapid clinical improvement during the first 4 to 6 hours. Because the chest radiograph is typically unremarkable at this time, the patient is often inappropriately discharged. These patients usually return to the medical facility within 24 to 36 hours complaining of rapidly increasing shortness of breath. By that time, the chest radiograph typically demonstrates dense infiltrative processes, which clear slowly with further care. Moderate to severe hypoxia may persist during the period of radiological abnormality.

After exposure, the chest radiograph is characteristically unremarkable within the first hours despite severe clinical symptoms, which include rapid respirations and severe dyspnea. An elevated temperature is often noted within the first 4 to 6 hours and may remain over the ensuing days. At 4 to 6 hours, the chest radiograph begins to show a dense infiltrative process that is thought to represent edema. However, bronchopneumonia may supervene in the following days; a long-standing, diffuse interstitial fibrosis may become evident, with only very gradual recovery.[27,28]

Figure 9-8 is the chest radiograph of a 60-year-old male 8 hours postexposure to HC; it

shows diffuse, dense, peripheral pulmonary infiltrates. The patient presented with moderately severe resting dyspnea and diffuse coarse crackles on auscultation. Taken 14 weeks postexposure, Figure 9-9 is a section from an open lung biopsy from the same patient. Diffuse interstitial fibrosis with inflammatory cells are evident. This patient presented with a persistent, moderate resting dyspnea. At this point, the patient had not been treated with steroids.

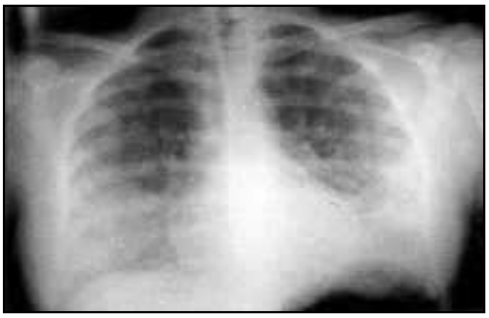


Fig. 9-8. The chest radiograph of a 60-year-old male sailor who inhaled zinc oxide (HC) in a closed space, taken 8 hours postexposure. He had moderately severe resting dyspnea during the seventh and eighth hours, diffuse coarse crackles on auscultation, and a P_{O_2} of 41 mm Hg breathing room air. The radiograph shows diffuse dense peripheral pulmonary infiltrates.

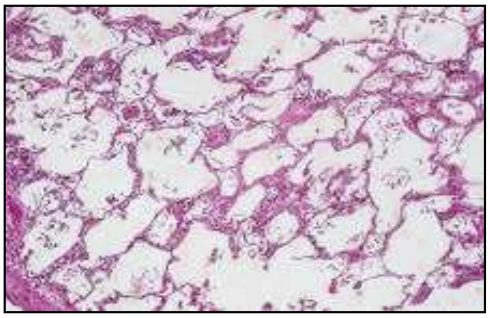


Fig. 9-9. A section from an open lung biopsy of the patient whose chest radiograph is shown in Figure 9-8, 14 weeks postexposure to zinc oxide (HC). He had persistent moderate dyspnea at rest and a P_{O_2} of 61 mm Hg breathing room air. Steroids had not been used. The section shows diffuse interstitial fibrosis with few inflammatory cells. Hematoxylin and eosin stain; original magnification x 400.

Exposures to very high doses of HC commonly result in sudden, early collapse and death, which are thought to be due to rapid-onset laryngeal edema and glottal spasm with consequent asphyxia. Exposures to high but nonlethal doses typically produce very early, severe hemorrhagic ulceration of the upper airway. Such exposures may also produce a relatively rapid onset of pulmonary edema.

Prolonged, severe exposures typically result in relatively rapid onset of severe dyspnea, tachypnea, sore throat, and hoarseness. A characteristic paroxysmal cough often produces bloody secretions. An acute tracheobronchitis may lead to death within hours.

Therapy

There is no chemically specific prophylactic or postexposure therapy for exposure to HC. Routine clinical support for specific complaints of acute tracheobronchitis and noncardiac pulmonary edema has been detailed previously (see the section titled General Therapeutic Considerations). Systemic steroid therapy is thought to be useful in treatment of the inflammatory fibrosis seen with this disorder.[29–32]

There are no reports of the clinical efficacy of chelating agents; however, the use of such agents as British anti-Lewisite (BAL) and calcium ethylenediaminetetraacetic acid (CaEDTA) has been suggested because of their capacity to reduce serum zinc levels. After acute exposure, however, long-term pulmonary function testing is indicated until the patient's condition is stable by that measurement. The continued presence of exertional dyspnea 2 to 3 months postexposure implies further development of interstitial fibrotic changes. Exercise testing would be of value to obtain a more accurate assessment of the degree of oxygen transport limitation. There are no data evaluating the use of long-term steroid therapy in the

treatment of interstitial disease secondary to HC inhalation.[29–32]

Clinical Summary

Although most patients with zinc chloride inhalational injuries progress to complete recovery, a smaller group of approximately 10% to 20% of exposed individuals may go on to develop fibrotic pulmonary changes. Both groups show early clinical recovery, making it difficult to distinguish patients who are likely to recover from those who are likely to develop more permanent changes. Early steroid use may modify the intensity of pulmonary reaction and the resulting fibrosis; therefore, steroids appear indicated in the acute therapy of zinc oxide exposure.

Phosphorus Smokes

Phosphorus occurs in three allotropic forms: white, red, and black. Of these, white phosphorus was used most often during World War II in military formulations for smoke screens, marker shells, incendiaries, hand grenades, smoke markers, colored flares, and tracer bullets.

White phosphorus is a very active chemical that will readily combine with oxygen in the air, even at room temperature. As oxidation occurs, white phosphorus becomes luminous and bursts into flames within minutes. Complete submersion in water is the only way to extinguish the flames. Vapors from white phosphorus are toxic; however, because these vapors quickly oxidize into phosphorus pentoxide and phosphoric acid, they are harmless to humans and animals at usual field concentrations.

Clinical Effects of Exposure

At room temperature, white phosphorus is somewhat volatile and may produce a toxic inhalational injury. Over a period of years, repetitive exposures can result in systemic poisoning.[33] At warmer temperatures, a slow oxidation to phosphorus trioxide (P_2O_3), which smells like garlic, can occur. At still higher temperatures (approximately 32°C [90°F]) and with adequate air exposure, dense white clouds of phosphorus pentoxide (P_2O_5) result from the combustion of phosphorus.

In moist air, the phosphorus pentoxide produces phosphoric acid. This acid, depending on concentration and duration of exposure, may produce a variety of topically irritative injuries. Irritation of the eyes and irritation of the mucous membranes are the most commonly seen injuries. These complaints remit spontaneously with the soldier’s removal from the exposure site. With intense exposures, a very explosive cough may occur, which renders gas mask adjustment difficult. There are no reported deaths resulting from exposure to phosphorus smokes.

Because of the toxicity associated with the manufacture of white phosphorus and because of its field risks, a gradual shift to red phosphorus (95% phosphorus in a 5% butyl rubber base) was undertaken after World War II. The British smoke grenade (L8-A1-3), which used red phosphorus, produced adequate field concentrations of smoke and functioned as an effective tank screen. Oxidation of red phosphorus produces a variety of phosphorus acids that, on exposure to water vapor, produce polyphosphoric acids. These acids may produce mild toxic injuries to the upper airways that result in a cough and irritation. There are no reported deaths resulting from exposure to red phosphorus smokes.

Therapy

Phosphorus smokes are generated by a variety of munitions. Some of these munitions (such as the MA25 155-mm round) may, on explosion, distribute particles of incompletely oxidized white phosphorus. Contact with these particles can cause local burns, and systemic

toxicity may occur if therapy is not administered. Therapy consists of topical use of a bicarbonate solution to neutralize phosphoric acids and mechanical removal and debridement of particles. A Wood's lamp in a darkened room may help to identify remaining luminescent particles.

Clinical Summary

The principal form of toxicity of absorbed elemental phosphorus, a destructive jaw process (phossy jaw), has been virtually eliminated by using *(a)* less white phosphorus in all industrial settings other than the military and *(b)* efficient adjunctive dental screening of exposed individuals for periods of up to 2 years postexposure. Careful dental screening consists of regular radiological assessment of the mandible for characteristic lesions, early and aggressive treatment of those lesions, and absolute prohibition of further phosphorus exposure.[33]

Sulfur Trioxide–Chlorosulfonic Acid

Sulfur trioxide–chlorosulfonic acid (military designation, FS smoke) consists of 50% (weight/weight) sulfur trioxide (SO_3) and 50% (weight/weight) chlorosulfonic acid [$SO_2(OH)Cl$]. FS smoke is typically dispersed by spray atomization. The sulfur trioxide evaporates from spray particles, reacts with moisture in the air, and forms sulfur acid, which condenses into droplets that produce a dense white cloud. The highly corrosive nature of FS smoke in the presence of moisture resulted in the army's abandoning its use.

Clinical Effects of Exposure

Contact with liquid FS smoke produces a typical mineral acid burn of exposed tissues. Exposure to the smoke—which constitutes exposure to sulfuric acids—produces irritation of the eyes, nose, and exposed skin and complaints of cough, substernal ache, and soreness. After a severe exposure to FS smoke, a casualty demonstrates profuse salivation and an explosive cough, which could render respirator adjustment difficult. The highly irritative nature of the substance acts as an adequate warning, however, and prompts an immediate evacuation from the smoke cloud.

Therapy

Severe exposures (sufficient to give rise to the rapid onset of symptoms) may require therapeutic intervention similar to that for chlorine exposure. Individuals with highly reactive airways are particularly at risk. In the event of triggered bronchospasm, these patients would benefit from aggressive bronchodilator use; consideration should also be given to the early use of steroids as well as positive-pressure ventilation.[4,29,34]

Titanium Tetrachloride

Titanium tetrachloride (military designation, FM smoke) is a corrosive substance typically dispersed by spray or explosive munitions. A dense, white smoke results from the decomposition of FM smoke into hydrochloric acid, titanium oxychloride, and titanium dioxide. Because titanium tetrachloride is extremely irritating and corrosive in both liquid and smoke formulations, FM smoke is not commonly used.

Exposure to the liquid may create burns similar to those of mineral acids on conjunctiva or skin. Exposure to the dense, white smoke in sufficient concentration may produce conjunctivitis or cough. There are no reports of exposure-related human deaths, nor are data available regarding pulmonary function assessment in extensively exposed individuals. Individuals with hyperreactive airways would be expected to develop bronchospasm with exposure to this substance.

Aggressive bronchodilator therapy and, possibly, steroid therapy should be considered.[4]

Nitrogen Oxides

The oxides of nitrogen (military designation, NO_x), particularly nitrogen dioxide, are components of photochemical smog. Their concentrations in smog are generally thought to be low enough to be of no significant clinical concern for the normal, healthy individual.[35]

There are four forms of nitrogen oxide. The first is nitrous oxide (N_2O), which is commonly used as an anesthetic; however, in the absence of oxygen, it acts as an asphyxiant. The second is nitric oxide (NO), which rapidly decomposes to nitrogen dioxide in the presence of moisture and air. Nitrogen dioxide exists in two forms: NO_2 and N_2O_4. At room temperatures, nitrogen dioxide is a reddish brown vapor consisting of approximately 70% N_2O_4 and 30% NO_2. Injuries from this toxic inhalant are seen most often in nonmilitary settings of silage storage.[36,37]

In a military or industrial setting, nitrogen dioxide occurs in the presence of electric arcs or other high-temperature welding or burning processes, and particularly where nitrate-based explosives are used in enclosed environments (such as tanks and ships). Diesel engine exhaust also contains substantial quantities of nitrogen dioxide.[36–39]

Clinical Effects of Exposure

At NO_x concentrations of 0.5 ppm or less, individuals with preexisting airways disease show no clinical effects postexposure. At 0.5 ppm to approximately 1.5 ppm, individuals with asthma may note minor airway irritation. Concentrations of 1.5 ppm may produce changes in pulmonary function measurements in normal individuals, including airway narrowing, reduction of diffusing capacity of the lung, and widening of the alveolar–arterial difference in the partial pressure of oxygen ($P_{AO_2} – P_{aO_2}$) gradient.

This level of toxic exposure may occur with little initial discomfort. The soldier may ignore the resultant mild coughing or choking. Because of rapid symptomatic accommodation to this cough, continued exposure may occur. A lethal exposure can occur within 0.5 hour, with the soldier only experiencing a minimal sense of discomfort.

The clinical response to NO_x exposure is essentially triphasic. In phase 1, symptoms appear more or less quickly, depending on the intensity of exposure. With a low dose, initial eye irritation, throat tightness, chest tightness, cough, and mild nausea may appear. Once the casualty is removed from the source of exposure, these symptoms disappear spontaneously over the next 24 hours. However, at 24 to 36 hours postexposure, a particularly severe respiratory symptom complex may appear suddenly; exertion seems to be a prominent precipitating factor. There may be severe cough, dyspnea, and rapid onset of pulmonary edema. If the patient survives this stage, spontaneous remission occurs within 48 to 72 hours postexposure. More intense exposures produce a relatively rapid onset of acute bronchiolitis with severe cough, dyspnea, and weakness, without the above-mentioned latent period. Again, spontaneous remission occurs at approximately 3 to 4 days postexposure.[40]

Phase 2 is a relatively asymptomatic period lasting approximately 2 to 5 weeks. There may be a mild residual cough with malaise and perhaps minimal shortness of breath, and there could be a sense of weakness that may progress. The chest radiograph, however, typically is clear.

In phase 3, symptoms may recur 3 to 6 weeks after the initial exposure. Severe cough, fever, chills, dyspnea, and cyanosis may develop. Crackles are identified on physical examination of the lung. The polymorphonuclear white blood cell count is elevated, and the partial pressure of carbon dioxide (P_{CO_2}) may be elevated as well.[41] The chest radiograph demonstrates diffuse, scattered, fluffy nodules of various sizes, which may become confluent progressively, with a butterfly pulmonary edema pattern and a prominent acinar component. At this point, pathological study demonstrates classic bronchiolitis fibrosa obliterans, which may

clear spontaneously or may progress to severe, occasionally lethal respiratory failure. The fluffy nodular changes noted in the chest radiograph typically show no clinical improvement. Pulmonary function testing may show long-term persistence of airways obstruction.[37,42,43]

Therapy

There is no chemically specific prophylactic or postexposure therapy for NO_x inhalational injury. Current therapy consists of intervention directed at specific symptoms (see the earlier discussion titled General Therapeutic Considerations). Pneumonitis appears to complicate the initial pulmonary edema relatively early; nevertheless, the use of prophylactic antibiotics is not indicated.

The use of steroids early in phase 3 has been reported; and although controlled studies are not available, these reports strongly suggest the value of steroid therapy in modifying the bronchiolitis obliterans that develops in the third stage.[42] Despite therapy, persistent limitation of airway function is typically seen, as demonstrated by long-term abnormalities in pulmonary function.[44]

Clinical Summary

Exposure to NO_x may produce a variety of delayed and severe pulmonary sequelae that are not readily predicted from early clinical or laboratory data. Careful, long-term observation appears to be important. Steroid therapy may be a useful therapeutic tool.

Organofluoride Polymers: Teflon and Perfluoroisobutylene

Teflon (polytetrafluoroethylene, manufactured by Du Pont Polymers, Wilmington, Del.) and other similar highly polymerized organofluoride polymers (eg, perfluoroethylpropylene) enjoy wide-spread use in a variety of industrial and commercial settings. Because of their desirable chemical and physical properties (eg, lubricity, high dielectric constant, chemical inertness), organofluoride polymers are considered important and are used extensively in military vehicles such as tanks and aircraft. The occurrence of closed-space fires in such settings has led to toxicity studies of the resultant by-products created from incinerated organofluorines. Inhalation of a mixture of pyrolysis by-products of these substances produces a constellation of symptoms termed polymer fume fever.

A primary high-temperature pyrolysis by-product of these substances is perfluoroisobutylene (PFIB). Inhalation of this material may produce a “permeability” or “noncardiac” type of toxic pulmonary edema very much like that produced by phosgene.

General Description of Polymer Fume Fever

Teflon pyrolysis occurring at temperatures of approximately 450°C produces a mixture of particulate and gaseous by-products; in 1951, Harris[45] was the first to describe polymer fume fever, the clinical syndrome that resulted from inhaling this mixture. Within 1 to 2 hours postexposure, a syndrome known as polymer fume fever appears. Often mistaken for influenza, polymer fume fever causes malaise, chills, fever to 104°F (40°C), sore throat, sweating, and chest tightness. Once the patient is removed from the site of exposure, the symptoms spontaneously and gradually disappear over 24 to 48 hours without any specific treatment. The patient typically has no long-term complaints or sequelae.[46] Much of this symptomatology may be due to a millipore-filtrable particulate. Animal studies have shown an alleviation of symptoms with filtration of the pyrolyzed materials[47] and restoration of symptoms with parenteral injection of the filtered material.[48]

Polymer fume fever may be induced by smoking Teflon-contaminated cigarettes (the burning tip temperature reaches 884°C[46]). Smoking contaminated cigarettes can also induce the signs and symptoms of pulmonary edema. Onset of pulmonary edema is typically rapid

(2–4 h), mild in degree (rarely requiring oxygen supplementation as part of treatment, and rapidly remits (clears within 48 h).[49]

As Teflon is pyrolyzed at a higher temperatures, more variety and higher concentrations of organofluoride by-products are noted.[50] Among these, PFIB is found to be the most toxic (Exhibit 9-2), with an LCt_{50} (ie, the vapor or aerosol exposure [concentration • time] that is lethal to 50% of the exposed population) of 1,500 mg•min/m^3 (in comparison, the LCt_{50} of phosgene is 3,000 mg•min/m^3).[51]

EXHIBIT 9-2
PROPERTIES OF PERFLUOROISOBUTYLENE (PFIB)

Chemical formula:	$(CF_3)_2C{=}CF_2$
Molecular weight:	200
Boiling point at 1 atm:	7.0° C
Gas density (dry air, at 15° C and 760 mm Hg = 1.22 g/L):	8.2 g/L
Colorless	
Odorless	
Very slightly soluble	

Data source: Smith W, Gardner RJ, Kennedy GL. Short-term inhalation toxicity of perfluoroisobutylene. *Drug and Chemical Toxicology*. 1982;5:295–303.

Clinical Effects of Exposure to Perfluoroisobutylene

Inhalation of PFIB is followed by a very rapid toxic effect on the pulmonary tissues (see the section below titled Clinical Pathology), with microscopic changes of perivascular edema evident within 5 minutes.[52] Exposure to a very high concentration of this colorless, odorless gas may produce early conjunctivitis[53]; in animal studies, it has produced sudden death ("lightning death" reported by Karpov, which occurred within 1 min).[54] No such sudden human deaths have been reported.

Less-intense exposures to PFIB are followed by a clinically asymptomatic period (clinical latent period) of a variable duration. During this period, normal biological compensatory mechanisms control the developing pulmonary edema until those compensatory mechanisms are overwhelmed. The length of this interval depends on the intensity and duration of exposure and on the presence or absence of postexposure exercise. Recent animal studies of PFIB inhalation strongly support World War I anecdotal human observations (with phosgene) that the resulting toxic pulmonary edema appears earlier and with more intensity when the casualty is required to exercise postexposure. It may be that increased pulmonary blood flow increases the permeability of the already damaged alveolar-capillary membrane.[55]

The clinical latent period for PFIB injury is 1 to 4 hours (compared with 1–24 h for phosgene). Subsequent symptoms of pulmonary edema progressively appear: initially, dyspnea on exertion; then orthopnea; and later dyspnea at rest. Radiological signs and clinical findings of pulmonary edema progress for up to 12 hours, then gradually clear with complete recovery by 72 hours. Typically, there are no long-term sequelae[49,56–59]; however, two deaths have been reported.[53,60] The death reported by Auclair et al[53] occurred after very severe pulmonary edema (requiring intubation), hypotension, and ultimately, Gram-negative superinfection.

Four reports on the long-term effects of PFIB exposure are available. Brubaker[56] reported a decrement in DL_{CO} 2 months after a single exposure. Capodaglio et al[61] reported that 3 of 4 exposed individuals showed pulmonary function test (PFT) abnormalities 6 weeks to 6 months postexposure. Paulet and Bernard[62] reported four workers with abnormal PFTs and DL_{CO}s during the 4 months following exposure. Williams et al[63] reported an individual with pulmonary interstitial fibrosis after multiple episodes of polymer fume fever.

Clinical Pathology of PFIB Inhalation

PFIB primarily affects the pulmonary system. Although animal studies occasionally report disseminated intravascular coagulation and other organ involvement, these effects only occur with substantial pulmonary injury, suggesting that systemic hypoxia is a major factor.[64] No human studies report organ involvement other than the respiratory system.

Pathological data on acute human exposure to PFIB are not available; however, pathological data on animals show both histological and ultramicroscopic changes occurring within 5 minutes of exposure.[52] Interstitial edema with alveolar fibrin deposition progresses rapidly over the next 24 hours, then gradually subsides until the patient is fully recovered. At 72 hours, a type II pneumocyte hyperplasia is seen (interpreted as consistent with known reparative processes). Although some long-term animal pathological changes have been reported,[54] most animal studies do not identify such long-term changes.

Human long-term pathological data are available in only one reported case: a 50-year-old female experienced approximately 40 episodes of polymer fume fever—typically occurring from smoking contaminated cigarettes. Eighteen months after her last episode, progressive exercise dyspnea was noted. A cardiopulmonary physical examination, chest radiograph, and ABG were all normal. Pulmonary function testing supported a provisional diagnosis of alveolar capillary block syndrome (decreased D_{LCO}, increased exercise $P_{AO_2} - Pa_{O_2}$ gradient, and minimal airway disease). Death occurred from an unrelated cause. The autopsy provided histological evidence of moderate interstitial fibrosis with minimal chronic inflammatory cell infiltrate.[63] Only two human deaths from pyrolysis products of polymerized organofluorides have been reported. [53,60]

Therapy

There is no recognized prophylactic therapy for human PFIB exposure. Animal studies suggest that increasing pulmonary concentrations of oxygen free-radical scavengers containing thiol groups may be of value; *N*-acetyl cysteine has been found effective.[65,66] No postexposure medical or chemical therapy that effectively impedes or reverses the effects of this toxic inhalational injury is known.

Specific therapy for the observed “noncardiac” pulmonary edema symptoms has been derived from clinical experience with ARDS. Pulmonary edema responds clinically to application of positive airway pressure. PEEP/CPAP (continuous positive airway pressure) masks are of initial value. Intubation may be required.

Oxygen supplementation is provided for evident hypoxia or cyanosis. Expeditious fluid replacement is mandatory when hypotension is present. Combined systemic hypotension and hypoxia may damage other organ systems. Bacterial superinfection is sufficiently common to warrant careful surveillance cultures. There is no literature support, however, for use of routine prophylactic antibiotics.

Steroid therapy for PFIB exposure has been reported in only two instances—both for the same worker.[49] The fact that recovery may be spontaneous and rapid makes it difficult to decide whether steroid use improves recovery.

Laboratory studies clearly support the observation that rest subsequent to exposure is useful. Because the intensity of injury and the duration of the clinically latent period are both affected by exercise, bed rest and litter transport are preferred.

SUMMARY

During World War I, the number and types of pulmonary toxicants available to the military increased substantially. At least 14 different respiratory agents were used, as well as obscurants (smokes), harassing agents (chloracetone), and vesicants (mustard) that could cause pulmonary injury. Today, only a handful of these toxicants still exist in stockpiles around the world, but several, such as chlorine and phosgene, are currently produced in large

quantities for industrial purposes. Whether produced for military or industrial uses, these chemical agents pose a very real threat to military personnel.

Toxic inhalational injury poses a 2-fold problem for military personnel:

1. No specific therapy exists for impeding or reversing toxic inhalant exposures.
2. Toxic inhalational injury can cause large numbers of casualties that can significantly burden medical facilities.

The pathophysiological processes that develop in the upper and lower respiratory tract can greatly incapacitate a casualty or result in death within minutes of exposure. It is therefore imperative that adequate control of the casualty's airways be maintained. Medical personnel should look for hypoxia, hypercarbia, and pulmonary edema, all of which are signs of possible toxic inhalant exposure. Infectious bronchitis or pneumonitis (particularly in intubated patients) is a frequent complication. The importance of chronic health problems that occur postexposure to toxic inhalants is a contentious subject because of the nebulous signs and symptoms that mimic degenerative diseases, such as emphysema, common to the general population.

REFERENCES

1. Thucydides; Crawley, trans. *The Complete Writings of Thucydides: The Peloponnesian War.* New York, NY: Random House; 1951. Unabridged.
2. Haber LF. *The Poisonous Cloud.* Oxford, England: Clarendon Press; 1986.
3. Weyandt TB, Ridgeley CD Jr. Carbon monoxide. In: Deeter DP, Gaydos JC. *Occupational Health: The Soldier and the Industrial Base.* Part 3, Vol 2. In: Zajtchuk R, Jenkins DJ, Bellamy RF, eds. *Textbook of Military Medicine.* Washington, DC: US Department of the Army, Office of The Surgeon General, and Borden Institute; 1993: 419.
4. US Department of the Army. Smokes. In: *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries.* Washington, DC: DA; 1990: 8-1–8-8. Field Manual 8-285.
5. Herringham WP. Gas poisoning. Lancet. 1920;1:423–424.
6. Haldane JS. The reflex restriction of respiration after gas poisoning. In: *Reports of the Chemical Warfare Committee, Medical Research Committee.* London, England: Chemical Warfare Department, Army Medical Service; 1918: 3–4.
7. Hunt GH. Changes observed in the heart and circulation and the general after-effects of irritant gas poisoning. In: *Reports of the Chemical Warfare Committee, Medical Research Committee.* London, England: Chemical Warfare Department, Army Medical Service; 1918: 10.
8. Patil LRS, Smith RG, Worwald AJ, Mooney TF. The health of diaphragm cell workers exposed to chlorine. *Am Ind Hyg Assoc J.* 1970;31:678–686.
9. Beck H. *Experimentelle Ermittlung von Gerüchsschwelleneiniger wichtige Reizgare (Chlor, Schwefeldioxyd, Ozon, Nitrose) und Ersheimüngen bei Einwirkung geringer Konzentrationen auf deu Meuschen.* Würzburg, Germany: University of Würzburg; 1959: 1–69. PhD dissertation.
10. Laciak M, Sipa K. The importance of the sense of smelling, in workers of some branches of the chemical industry. *Medycyna Pracy.* 1958;9:85–90.
11. Berghoff S. The more common gasses: Their effect on the respiratory tract. *Arch Int Med.* 1919;24:678–684.
12. Bunting H. Clinical findings in acute chlorine poisoning. In: *Respiratory Tract.* Vol 2. In: *Fasciculus on Chemical Warfare Medicine.* Washington, DC: Committee on Treatment of Gas Casualties, National Research Council; 1945: 51–60.

13. Gilchrist HL, Matz RB. The residual effects of warfare gases: The use of phosgene gas, with report of cases. *Med Bull Veterans Admin.* 1933;10:1–37.

14. Hoveid P. The chlorine accident in Mjondalen (Norway) 26 January 1940: An after investigation. *Nord Hyg Tidskr.* 1956;37:59–66.

15. Weill HR, Schwarz GM, Ziskind M. Late evaluation of pulmonary function after acute exposure to chlorine gas. *Am Rev Respir Dis.* 1969;99:374–379.

16. Bunting H. The pathology of chlorine poisoning. In: *Respiratory Tract.* Vol 2. In: *Fasciculus on Chemical Warfare Medicine.* Washington, DC: Committee on Treatment of Gas Casualties, National Research Council; 1945: 24–36.

17. The acute lung irritant gases. In: *History of the Great War.* Vol 2. London, England: H. M. Statistical Office; 1923: 621.

18. Chester EH, Gillespie DG, Krause FD. The prevalence of chronic obstructive pulmonary disease in chlorine gas workers. *Am Rev Respir Dis.* 1969;99:365–373.

19. Bunting H. Changes in the oxygen saturation of the blood in phosgene poisoning. In: *Respiratory Tract.* Vol 2. In: *Fasciculus on Chemical Warfare Medicine.* Washington, DC: Committee on Treatment of Gas Casualties, National Research Council. 1945: 484–491.

20. Underhill FP. *The Lethal War Gases: Physiology and Experimental Treatment.* New Haven, Conn: Yale University Press; 1920.

21. Gilchrist HL. *A Comparative Study of WWI Casualties from Gas and Other Weapons.* Edgewood Arsenal, Edgewood, Md: US Chemical Warfare School; 1928: 1–51.

22. Cucinell SA. Review of the toxicity of long-term phosgene exposure. *Arch Environ Health.* 1974;28:272–275.

23. Galdston M, Leutscher JA Jr, Longcope WT, Ballich NL. A study of the residual effects of phosgene poisoning in human subjects, I: After acute exposure. *J Clin Invest.* 1947;26:145–168.

24. Brand P. *Effect of a Local Inhalation Dexamethasone Isonicotinate Therapy on Toxic Pulmonary Edemas Caused by Phosgene Poisoning.* Würzburg, Germany: University of Würzburg; 1971. Dissertation.

25. Diller WF. Medical phosgene problems and their possible solution. *J Occup Med.* 1978;20:189–193.

26. Wang Y, Lee LKH, Poh S. Phosgene poisoning from a smoke grenade. *Eur J Respir Dis.* 1987;70:126–128.

27. Pare CMB, Sandler M. Smoke-bomb pneumonitis: Description of a case. *J R Army Med Corps.* 1954;100:320–324.

28. Evans EH. Casualties following exposure to zinc chloride smoke. *Lancet.* 1945;2:368–370.

29. Cullumbine H. The toxicity of screening smokes. *J R Army Med Corps.* 1957;103:119–122.

30. Hjortso E, Qvist J, Bud MI, et al. ARDS after accidental inhalation of zinc chloride smoke. *Intensive Care Med.* 1988;14:17–24.

31. Johnson FA, Stonehill RB. Chemical pneumonitis from inhalation of zinc chloride. *Dis Chest.* 1961;40:619–624.

32. Milliken JA, Waugh D, Kadish ME. Acute interstitial pulmonary fibrosis caused by a smoke bomb. *Can Med Assoc J.* 1963;88:36–39.

33. Hunter D. *The Diseases of Occupations.* London, England: Hodder and Stoughton; 1978.

34. Cameron GR. Toxicity of chlorsulphonic acid-sulphur trioxide mixture smoke clouds. *J Pathol Bacteriol.* 1954;68:197–204.

35. Ozone in fog. *Lancet.* 1975;2:1077. Editorial.

36. Scott EG, Hunt WB Jr. Silo-filler's disease. *Chest.* 1973;63:701–706.

37. Ramirez RJ, Dowell AR. Silo-filler's disease: Nitrogen dioxide–induced lung injury. *Ann Intern Med.* 1961;74:569–576.

38. LaFleche LR, Boivin C, Leonard C. Nitrogen dioxide—A respiratory irritant. *Can Med Assoc J.* 1961;84:1438–1440.

39. Delaney LT Jr, Schmidt HW, Stroebel CF. Silo-filler's disease. *Mayo Clin Proc.* 1956;31:189–194.

40. Ramirez RJ. The first death from nitrogen dioxide fumes. *JAMA.* 1974;229:1181–1182.

41. Lowry T, Schuman LM. Silo-filler's disease. *JAMA.* 1956;162:153–155.

42. Becklake MR, Goldman HI, Bosman AR, Freed CC. The long-term effects of exposure to nitrous fumes. *Am Rev Tuberc Pulm Dis.* 1957;76:398–412.

43. Jones GR, Proudfoot AT, Hall JT. Pulmonary effects of acute exposure to nitrous fumes. *Thorax.* 1973;28:61–65.

44. Leib RMP, Davis WN, Brown T, McQuiggan N. Chronic pulmonary insufficiency secondary to silo-filler's disease. *Am J Med.* 1958;24:471–478.

45. Harris DK. Polymer-fume fever. *Lancet.* 1951;2:1008–1011.

46. Williams N, Smith FK. Polymer-fume fever, an elusive diagnosis. *JAMA.* 1972;219:1587–1589.

47. Smith W, Gardner RJ, Kennedy GL. Short-term inhalation toxicity of perfluoroisobutylene. *Drug and Chemical Toxicology.* 1982;5:295–303.

48. Cavagna G, Finulli M, Vigliani EC. Studio sperimentale sulla patogensi della febre da inalazione di fumi di Teflon (politetrafluoroetilene) [in Italian]. *Med Lav.* 1961;52:251–261.

49. Lewis CE, Kerby GR. An epidemic of polymer-fume fever. *JAMA.* 1965;191:103–106.

50. Waritz RS, Kwon BK. The inhalation toxicity of pyrolysis products of polytetrafluoroethylene heated below 500 degrees Centigrade. *Am Indust Hygiene Assoc J.* 1968;19–26.

51. Clayton JW. Toxicology of the fluoroalkenes: Review and research needs. *Environ Health Perspect.* 1977;21:255–267.

52. Nold JB, Petrali JP, Wall HG, Moore DH. Progressive pulmonary pathology of two organofluorine compounds in rats. *Inhalation Toxicol.* 1991;3:123–137.

53. Auclair F, Baudot P, Beiler D, Limasset JC. Accidents benins et mortels dus aux "traitements" du polytetrafluoroethylène en milieu industriel: Observations cliniques et mesures physico-chimiques des atmosphères polluees. *Toxicological European Reasearch.* 1983;5(1):43–48.

54. Karpov BD. Establishment of upper and lower toxicity parameters of perfluroisobutylene toxicity. *Tr Lenig Sanit-Gig Med Inst.* 1977;111:30–33.

55. Moore DH. Effects of exercise on pulmonary injury induced by two organohalides in rats. In: *Proceedings of the Workshop on Acute Lung Injury and Pulmonary Edema, 4–5 May 1989.* Aberdeen Proving Ground, Md: Medical Research Institute of Chemical Defense; 1993: 12–19.

56. Brubaker RE. Pulmonary problems associated with the use of polytetrafluoroethylene. *J Occup Med.* 1977;19:693–695.

57. Evans EA. Pulmonary edema after inhalation of fumes from polytetrafluoroethylene (PTFE). *J Occup Med.* 1973;15:599–601.

58. Nuttall CJB, Kelly MJ, Smith CBS, Whiteside CCKJ. Inflight toxic reaction resulting from fluorocarbon resin pyrolysis. *Aerospace Med*. 1964;35:676–683.

59. Robbins JJ, Ware RL. Pulmonary edema from Teflon fumes. *N Engl J Med.* 1964;271:360–361.

60. Makulova ID. Clinical picture of acute poisoning with perflurosebutylene. *Gig Tr Prof Zabol.* 1965;9:20–23.

61. Capodaglio E, Monarca G, Divito VG. Respiratory syndrome by intermediate volatile products of polytetrafluoroethylene. *Rass Med Insusdr.* 1961;30:124–139.

62. Paulet G, Bernard JP. Heavy products appearing during the fabrication of polytetrafluorethylene: Toxicity-physiopathologic action; therapy. *Biol-Med (Paris).* 1968;57:247–301.

63. Williams N, Atkinson GW, Patchefsky AS. Polymer-fume fever: Not so benign. *J Occup Med.* 1974;16:519–522.

64. Zook BC, Malek DE, Kenney RA. Pathological findings in rats following inhalation of combustion products of polytetrafluoroethylene (PTFE). *Toxicology.* 1983;26:25–26.

65. Bridgeman MME, Marsden M, MacNee W, Flenley DC, Ryle AP. Cysteine and glutathione concentrations in plasma and bronchoalveolar lavage fluid after treatment with N-acetylcysteine. *Thorax.* 1991;46:39–42.

66. Lailey AF, Hill L, Lawston IW, Stanton D, Upshall DG. Protection by cysteine esters against chemically induced pulmonary edema. *Biochem Pharmacol.* 1991;42:S47–S54.

Chapter 10

CYANIDE POISONING

STEVEN I. BASKIN, PHARM.D., PH.D., FCP, FACC, DABT, FATS*; AND THOMAS G. BREWER, M.D., FACP†

INTRODUCTION

HISTORY AND USE
- Military Uses
- Nonmilitary Uses

BIOCHEMICAL BASIS FOR POISONING
- Cyanide Pharmacokinetics and Pharmacodynamics
- Toxicity

CLINICAL PRESENTATION AND MANAGEMENT OF CASUALTIES
- Laboratory Findings
- Principles of Therapy

SPECIFIC ANTIDOTES
- Drugs Used in the United States
- Other Therapeutic Drugs
- Investigational Drugs

SUMMARY

Pharmacology Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425
†Colonel, Medical Corps, U.S. Army; formerly, Experimental Therapeutics Division, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100; currently, Commander, U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, APO Area Pacific 96546, 315/6 Rajvithi Road, Bangkok 10400, Thailand

INTRODUCTION

Cyanide, long considered a toxic, deadly substance, has been used as a poison for thousands of years. It was not highly successful as a chemical warfare agent in World War I, possibly because of the way it was delivered. The effects of a high dose of cyanide are quick, and death occurs within minutes. Antidotes are effective if administered in time.

Cyanide is ubiquitous. It is present in some foods, in the products of combustion of synthetic materials, and is widely used in industry. Much of the cyanide used is in the form of salts, such as sodium, potassium, or calcium cyanide. The cyanides of military interest are the volatile liquids hydrocyanic acid (hydrogen cyanide, HCN; North American Treaty Organization [NATO] designation: AC) and cyanogen chloride (NATO designation: CK) (Table 10-1).

TABLE 10-1
CHEMICAL, PHYSICAL, ENVIRONMENTAL, AND BIOLOGICAL PROPERTIES OF CYANIDES

Properties	Hydrogen Cyanide (AC)	Cyanogen Chloride (CK)
Chemical and Physical		
Boiling Point	25.7°C	12.9°C
Vapor Pressure	740 mm Hg	1,000 mg Hg
Density:		
Vapor	0.99 at 20°C	2.1
Liquid	0.68 g/mL at 25°C	1.18 g/mL at 20°C
Solid		Crystal: 0.93 g/mL at -40°C
Volatility	1.1×10^6 mg/m^3 at 25°C	2.6×10^6 mg/m^3 at 12.9°C
Appearance and Odor	Gas: Odor of bitter almonds or peach kernels	Colorless gas or liquid
Solubility:		
In Water	Complete at 25°C	6.9 g/100 mL at 20°C
In Other Solvents	Completely miscible in almost all organic solvents	Most organic solvents (mixtures are unstable)
Environmental and Biological		
Detection	ICAD; M254A1 kit	M256A1 kit
Persistency:		
In Soil	<1 h	Nonpersistent
On Materiel	Low	Nonpersistent
Skin Decontamination	Water; soap and water	Water; soap and water
Biologically Effective	Amount:	
Vapor (mg•min/m^3)	LCt_{50}: 2,500–5,000 (time-dependent)	LCt_{50}: 11,000
Liquid (mg/kg)	LD_{50} (skin): 100	–

ICAD: individual chemical agent detector
LCt_{50}: the vapor or aerosol exposure [concentration • time] that is lethal to 50% of the exposed population
LD_{50}: the dose that is lethal to 50% of the exposed population

HISTORY AND USE OF CYANIDE

Although substances containing cyanide had been used for centuries as poisons, it was not until 1782 that cyanide itself was identified. It was first isolated by the Swedish chemist Scheele, who later may have died from cyanide poisoning in a laboratory accident.

Military Uses

Since the days of ancient Rome, cyanide and the derivatives of this highly toxic substance have been used as weapons.[1] Nero used cherry laurel water, which contains cyanide as its chief toxic component, to poison members of his family and others who displeased him. Napoleon III proposed the use of cyanides to enhance the effectiveness of his soldiers' bayonets during the Franco–Prussian War.

During World War I, in late 1915 and early 1916, the French were the only proponents for using cyanide and its derivative, hydrocyanic acid. This was made by distilling a concentrated solution of potassium cyanide with dilute sulfuric acid. Its use, however, proved to produce less than its desired effect. A highly volatile gas and lighter than air, hydrocyanic acid persisted for only a few minutes in the open air; this made it difficult to disperse a lethal concentration (also, the munitions used had a small payload). The effects of cyanide were not cumulative. In addition, the Germans, on learning of its use, equipped their troops with a mask that was capable of filtering out the gas. These combined factors made hydrocyanic acid less effective as a weapon.

About September 1916, the French tried another cyanide-based poison, cyanogen chloride, which is heavier and less volatile than hydrocyanic acid and which had a cumulative effect on its victims. Cyanogen chloride was produced by chlorinating a saturated solution of potassium cyanide at 0°C (32°F). Its toxicity was similar to that of hydrocyanic acid, but cyanogen chloride was more effective at low concentrations (it irritated the eyes and lungs). Cyanogen chloride also had a delayed toxic effect similar to such lung irritants as chlorine and phosgene. At high concentrations, cyanogen chloride is capable of killing by rapidly paralyzing the respiratory system's nerve center.[2]

At about the same time that the French launched cyanogen chloride, the Austrians introduced their own poisonous gas, which was derived from potassium cyanide and bromine. The resulting cyanogen bromide was highly volatile, yet it had only a quarter of the volatility of hydrocyanic acid and was less toxic. Cyanogen bromide had a strong irritating effect on the conjunctiva and on the mucous membranes of the respiratory system; however, because it corroded metals and was unstable in storage (gradually polymerizing into a toxicologically inert substance), the Austrians abandoned its use.[2]

During World War II, the Nazis employed hydrocyanic acid adsorbed onto a dispersible pharmaceutical base (Zyklon B) to exterminate millions of civilians and enemy soldiers in the death camps.[3,4] Zyklon B was a fumigant and rodenticide. One of its uses in the United States and other countries was to rid ships of rodents.

In the late 1980s, reports indicated that cyanide-like agents may have been used against the inhabitants of the Syrian city of Hama[5] and the inhabitants of the Kurdish city of Halabja, Iraq,[6] and possibly in Shahabad, Iran, during the Iran–Iraq War.[7] Based on this recent history, acute cyanide poisoning continues to constitute a threat for U.S. soldiers in future conventional or nonconventional conflicts.

Nonmilitary Uses

There has been little use of cyanide by the military; most of the information on cyanide poisoning has been from civilian experience in poisoning, fires, and industrial accidents. Most people probably contact cyanide in some form almost every day. Hundreds of thousands of

tons of cyanide are manufactured annually in this country. Cyanide is used in many chemical syntheses, electroplating, plastics processing, gold and silver extraction, tanning, metallurgy, and as a fumigant. Cyanides are in some foods; are pyrolysis products of many substances; and have gained notoriety for their use in executions, homicides, and suicides.

Cyanide poisoning has been reported[8] from eating chokecherries, bitter almonds, and apricot pits. In addition, cyanide is found in lima beans and cassava beans and roots. Cassava is a staple in certain countries and is blamed for the high incidence of tropical ataxic neuropathy in those areas.

Combustion of synthetic products that contain carbon and nitrogen, such as plastics and synthetic fibers, releases cyanide. Cigarette smoke contains cyanide; the nonsmoker averages 0.06 µg/mL of cyanide in blood, whereas the smoker has 0.17 µg/mL.[9] The effects of cyanide and of carbon monoxide, also formed in fires, are additive because they both contribute to tissue hypoxia by different mechanisms. The two gases are major causes of combustion-related fatalities.[10] In residential fires, cyanide poisoning may be more significant than has previously been appreciated. The short half-life of cyanide in blood contributes to the low concentrations of cyanide found in fire victims when blood is drawn after the victims reach the hospital.

From April 1988 through April 1989, a team of French investigators[11] collected samples—on the scene—from 109 victims of residential fires in and around Paris, France. The data they gathered were compared with data from a control group (N = 114) of individuals whose injuries were not caused by fire.

Blood cyanide concentrations were much higher in the fire victims than in the control group (Table 10-2), and victims who died had significantly higher levels (> 5-fold) than victims who survived. Contrary to what previous researchers have concluded, the results from this study "suggest that cyanide poisoning may prevail over carbon monoxide poisoning as the cause of death in some fire victims."[11(p1765)] Therefore, military medical officers need to be aware that victims of smoke inhalation from fires may be suffering the effects of cyanide poisoning, and might benefit from early antidotal cyanide therapy.

TABLE 10-2
BLOOD CYANIDE LEVELS IN VICTIMS OF SMOKE INHALATION

Total Subjects	Blood Cyanide Levels (Mean ± SD)
Fire Victims* (N = 109)	
Survivors (n = 66)	21.6 ± 36.4 µmol/L (P < .001)
Fatalities (n = 43)	116.4 ± 89.6 µmol/L (P < .001)
Controls (N = 114)	5.0 ± 5.5 µmol/L

* Of the 66 fire victims who survived, 9 had blood cyanide levels above 40 µmol/L and 3 had levels above 100 µmol/L. Of the 43 fire victims who died, 32 had blood cyanide levels above 40 µmol/L and 20 had levels above 100 µmol/L.
Data source: Baud FJ, Barriot P, Toffis V, et al. Elevated blood cyanide concentrations in victims of smoke inhalation. *N Engl J Med*. 1991;325(25):Fig 1:1763.

In addition, cyanide is used by governments, terrorists, corporations, and individuals to achieve various economic, beneficial, humanitarian, or harmful ends:

- Cyanide is the agent used in "gas chambers," in which a cyanide salt is dropped into an acid to produce HCN. (These chemicals—an acid and a cyanide salt—were found in several subway restrooms in Tokyo, Japan, in the weeks following the release of nerve agents in Tokyo in March 1995.)[12]

- It was illicitly placed in bottles of Tylenol (acetaminophen, manufactured by McNeill Consumer Products Co., Fort Washington, Pa.) in the Chicago area in 1982, killing seven people.[13]
- In 1978 near Port Kaituma, Guyana, the followers of the Reverend Jim Jones drank a grape-flavored drink laced with cyanide, and more than 900 children and adult members of the People's Temple committed mass suicide.[14]
- Furthermore, cyanide is a metabolic product of ingested Laetrile (an alleged cancer chemotherapeutic compound not available in this country) and may have been responsible for the deaths of some patients who took this substance.

Chronic ingestion of cyanide in the form of organic cyanogens is a public health problem in areas such as East Africa and Southeast Asia. Several common plants contain cyanogenic glycosides[15,16]; ingestion coupled with improper processing of such cyanogenic glycosides can result in death. Consumption of foodstuffs such as cassava result in tropical neuropathies in Africa[17] and in other diseases, such as tropical thyroid disease and tobacco amblyopia.

BIOCHEMICAL BASIS FOR POISONING

Although cyanide is known to bind and inactivate several enzymes, it is thought to exert its ultimate lethal effect of histotoxic anoxia by binding to the active site of cytochrome oxidase (Figure 10-1), thereby stopping aerobic cell metabolism[18,19] after an initial effect on excitable tissue. The binding to the cytochrome oxidase can occur over minutes. A more rapid effect appears to occur on neuronal transmission. No antagonists are known for the latter reaction, although it appears to be feasible to develop competitive antagonists for cyanide at a cytochrome oxidase–binding site.[20]

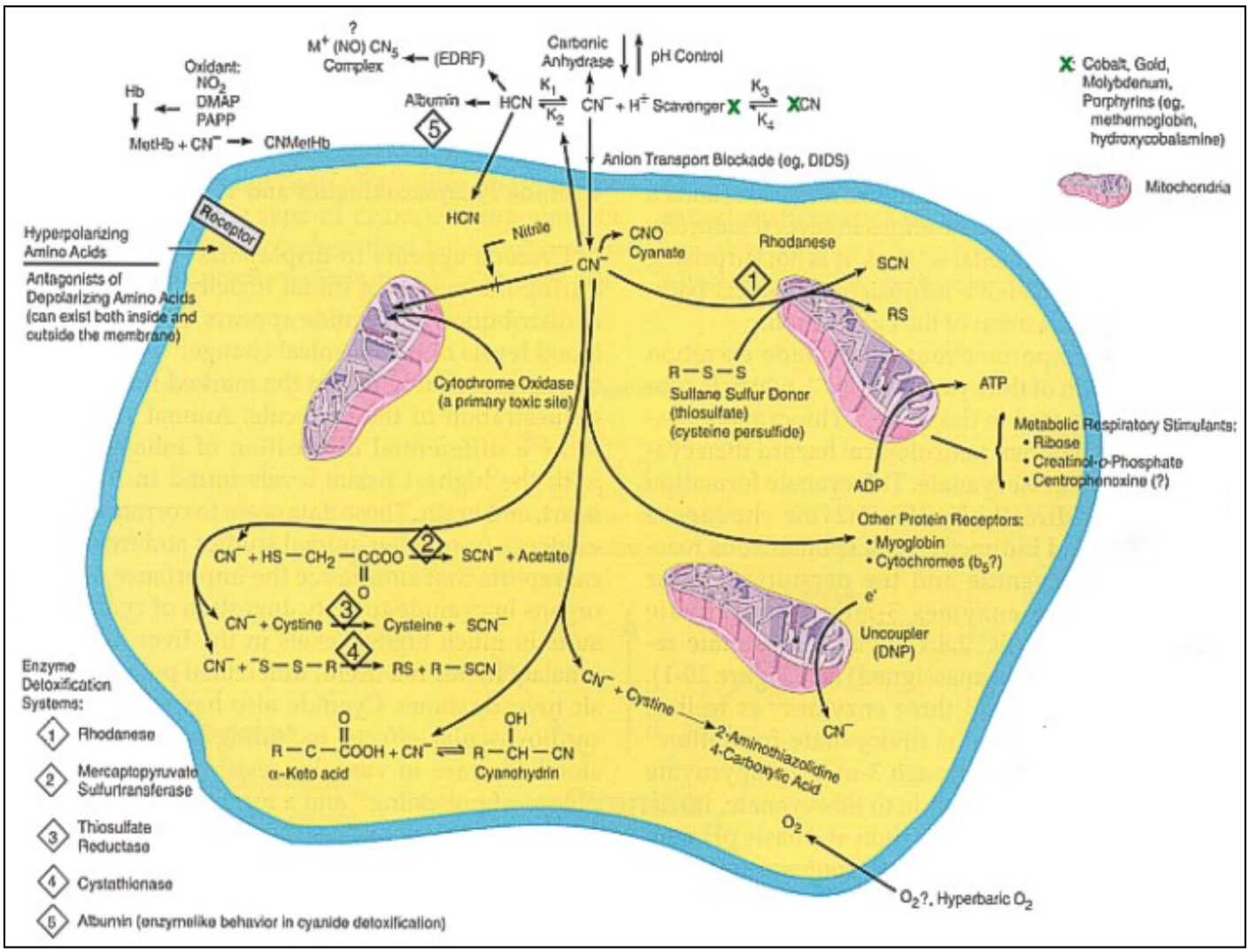


Fig. 10-1. The possible detoxification reactions for cyanide are shown for this hypothetical cell.

Cyanide can be removed by several processes before it can enter the cell. Perhaps of greatest importance is the formation of cyanomethemoglobin (CNMetHb), which is produced when the cyanide ion (CN^-) reacts with MetHb. Methemoglobin is formed when hemoglobin (Hb) reacts with a variety of oxidants (eg, nitrite, dimethylaminopheno [DMAP], and *p*–aminopropiophenone [PAPP]). Cyanide may complex with endothelial-derived relaxing factor (EDRF, which is thought to be nitric oxide). Cyanide can interfere with the action of carbonic anhydrase and lower pH, thereby decreasing the concentration of CN^- in the extracellular space. Heavy metals (eg, gold, molybdenum, or cobalt salts) or organic compounds (eg, hydroxocobalamin) may scavenge CN^-, effectively removing it from the milieu of the cell. Finally, albumin can exhibit enzymelike behavior and use bound elemental sulfur[1] to detoxify cyanide. It is also theoretically possible to prevent entrance of cyanide ions into the cell by blocking transport mechanisms with substances such as DIDS.

At lease four intracellular enzymes may be involved for cyanide detoxification. The generalized reactions of rhodanese, mercaptopyruvate sulfurtransferase, thiosulfate reductase, and cystathionase are shown within the cell.

Several broad classes of reactions may serve to ameliorate cyanide toxicity. They include but are not limited to the following. Metabolic respiratory stimulants may cross into the cell and stimulate adenosine triphosphate (ATP) production through a scavenger-ATP pathway (ie, ribose) or substrate augmentation (ie, creatinol-*o*-phosphate) or a free radical mechanism (ie, centrophenoxine). Hyperbaric oxygen or perhaps oxygen itself can reduce cyanide toxicity by competing with cyanide at some site (such as cytochrome oxidase in the mitochondria, which is thought to be a primary site for cyanide poisoning). Substances that act as hyperpolarizing agents or that antagonize depolarizing amino acids at ion channel receptors may ameliorate the convulsions from cyanide. Other possible reactions are formation of cyanohydrin with α-keto acids, competitive blockade by other nitriles, and reaction with other sites such as myoglobin, cytochrome b_5, or other electron transport system (ETS) compounds (eg, dinitropheno [DNP]).

(1) Source for this statement: Lieske CN, Clark CR, Zoeffel LD, et al. Temperature effects in cyanolysis using elemental sulfur. *J Appl Toxicol.* 1996;16:171–175.

DIDS: 4,4'-diisothiocyano-2,2'-disulfonic stilbene

Cyanide is readily diffusible through epithelium. This property contributes to its lethal toxicity after inhalation of hydrogen cyanide (HCN) gas (the usual route of military exposure), ingestion of cyanide salts or cyanogens, or percutaneous absorption of cyanide from high-concentration solutions. Because cyanides are present at low concentrations in several naturally occurring environmental sources, it is not surprising that most animals have intrinsic biochemical pathways for detoxification of the cyanide ion.

The most important route of cyanide excretion is by formation of thiocyanate (SCN^-), which is subsequently excreted in the urine.[17] Thiocyanate possesses a less inherent toxicological hazard than cyanide, cyanate, or isocyanate. Thiocyanate formation is catalyzed directly by the enzyme rhodanese (EC 2.8.1.1) and indirectly via a spontaneous reaction between cyanide and the persulfide sulfur products of the enzymes 3-mercaptopyruvate sulfurtransferase (EC 2.8.1.2)[21] and thiosulfate reductase (EC number unassigned) (see Figure 10-1). The mechanisms of all three enzymes[22] as well as the pharmacokinetics of thiocyanate formation[23] have been studied. Although 3-mercaptopyruvate functions to convert cyanide to this cyanate, its instability and sulf-auto-oxidation at a basic pH may mask this effect.[24] The enzymatic routes are efficient but have an insufficient capacity for detoxification in acute poisoning because of lack of sulfur donors. The mitochondrial sulfurtransferase reactions are exploited by the administration of sodium thiosulfate (used in therapy and discussed later in this chapter) in the treatment of acute poisonings. It is still not known with any certainty, however, what specific endogenous sulfur sources participate in the formation of thiocyanate from cyanide.[25]

A minor route of metabolism is the oxidation of cyanide to cyanate (CNO^-), which occurs via enzymatic and nonenzymatic pathways. The interaction of cystine and cyanide to form 2-amino thiazoline 4-carboxylic acid and its tautomer accounts for approximately 20% of cyanide metabolism. This increases with toxic doses of cyanide. However, the protection conferred by forming cyanate derivatives is limited because of the cell's inability to utilize oxygen during cyanide intoxication.

Combined, these metabolic routes detoxify 0.017 mg of cyanide per kilogram of body weight per minute in the average human. Cyanide is one of the few chemical agents that does

not follow Haber's law, which states that the C*t* (the product of concentration and time) necessary to cause a given biological effect is constant over a range of concentrations and times. For this reason, the LCt_{50} (the vapor or aerosol exposure that is lethal to 50% of the exposed population) for a short exposure to a high concentration is different from a long exposure to a low concentration.

Cyanide Pharmacokinetics and Pharmacodynamics

Cyanide appears to display first-order kinetics during the period of initial toxicity.[23] The volume of distribution for cyanide appears to change as the blood levels of the chemical change,[26] but these alterations probably reflect the marked intracellular sequestration of the molecule. Animal studies[27,28] show a differential disposition of inhaled HCN, with the highest tissue levels found in the lung, heart, and brain. These data seem to corroborate the evidence from other animal studies and from clinical reports that emphasize the importance of these organs in cyanide toxicity. Ingestion of cyanide results in much higher levels in the liver than does inhalation; this is a useful differential point in forensic investigations. Cyanide also has wide-ranging cardiovascular effects, including a poorly understood increase in vascular resistance in the early phases of poisoning[29] and a marked increase in cerebral blood flow in dogs.[30]

Data from rodent studies suggest that a single, acute administration of a cyanide salt leads either to death or to complete recovery. However, data from HCN inhalational studies in dogs, rabbits, monkeys, and humans suggests that death may be delayed for up to 8 days.[31,32] The neurological sequelae of cyanide intoxication may be delayed for up to a year.[1] These delayed changes in regional sensitivities of the brain are thought to be due to hypoxic stress and are analogous to those seen following sublethal carbon monoxide poisoning.

Toxicity

Although they are generally considered to be very toxic substances, when compared with other lethal chemical warfare agents, cyanides are among the least toxic. The LCt_{50} for hydrogen cyanide (hydrocyanic acid) is generally stated to be 2,500–5,000 mg•min/m^3; for cyanogen chloride, about 11,000 mg•min/m^3. (Comparable values for the nerve agents are 10–200 mg•min/m^3; for sulfur mustard, 1,500 mg•min/m^3; and for phosgene, 3,000 mg•min/m^3.)

The estimated intravenous dose that is lethal to 50% of the exposed population (LD_{50}) of hydrogen cyanide for man is 1.0 mg/kg, and the estimated LD_{50} for liquid on the skin is about 100 mg/kg.

CLINICAL PRESENTATION AND MANAGEMENT

The effects from cyanide poisoning are those of progressive histotoxic tissue hypoxia (Figure 10-2). The symptoms, signs, and physical findings are directly related to the dose of cyanide, the route of exposure, and the type of cyanide compound. In addition to the effects described below, cyanogen chloride also produces irritation of the eyes and mucous membranes similar to that produced by riot control agents.

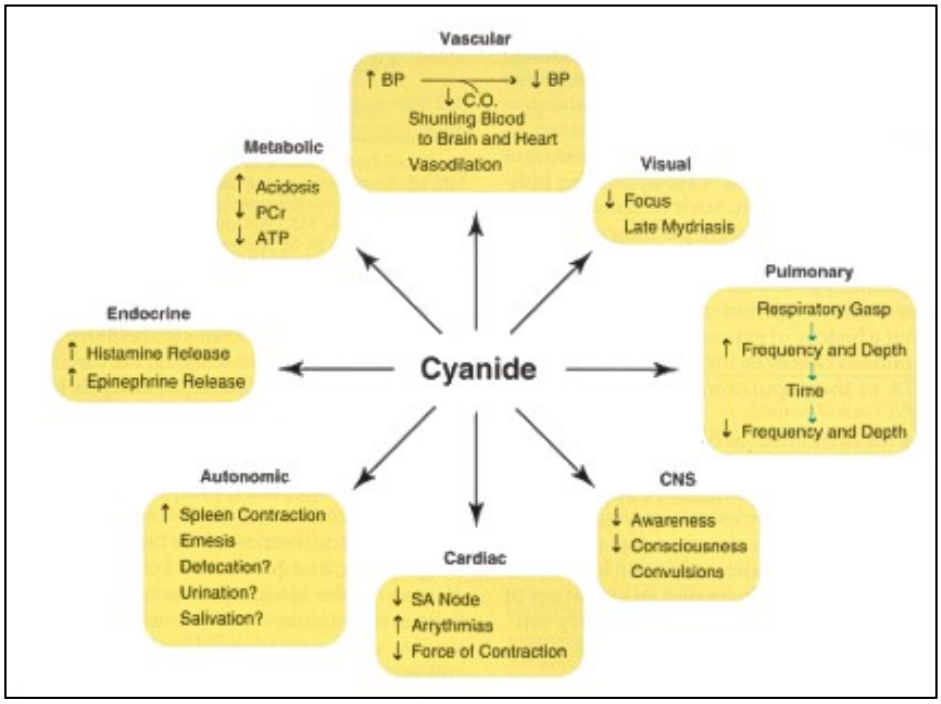


Fig. 10-2. Cyanide can affect many functions in the body, including the vascular, visual, pulmonary, central nervous, cardiac, autonomic, endocrine, and metabolic systems. The toxicodynamic effects can vary depending on the dose, route and speed of administration, chemical form of the cyanide, and other factors including the gender, age, weight, stress level, and general physical condition of the recipient. Proceeding clockwise from the top of the diagram: ***Vascular*** effects for cyanide can include an initial transient increase, followed by a decrease, in cardiac output. Blood pressure falls as the cardiac inotropic effect decreases and as vasodilation occurs. ***Visual*** effects can include a decrease in the capacity to focus, with late-onset mydriasis secondary to hypoxia. One of the first ***pulmonary*** effects from cyanide is a respiratory gasp, which is caused by stimulation of chemoreceptor bodies near the aortic bifurcation. Hyperventilation follows this response. Over time (the response is dose-dependent, but seconds to minutes), the frequency and depth of breathing diminish. ***Central nervous system*** effects initially manifest as decreased awareness and increased release of enkephalins followed by loss of consciousness and convulsions. ***Cardiac*** effects after cyanide exposure are an increase in heart rate, then a decrease; both are accompanied by arrhythmias and negative inotropy. Cyanide produces a number of ***autonomic nervous system*** effects, based on the route and dose of the agent. Cyanide can also produce multiple ***endocrine*** effects including epinephrine and histamine release, and ***metabolic*** actions that decrease energy production by the inhibition of the use of cytochrome oxidase.
PCr: phosphocreatine
ATP: adenosine triphosphate
C.O.: cardiac output

On a military battlefield, casualties will be from exposure to cyanide gas; this can be fatal within minutes after exposure to high concentrations. An initial hyperpnea (15 sec after exposure), due to the effect of cyanide on the chemoreceptor bodies, is closely followed by a loss of consciousness (30 sec after exposure). This progresses to apnea (3–5 min after exposure), cessation of cardiac activity (5–8 min after exposure), and death.

After exposure to lower concentrations, or exposure to lethal amounts via the oral or percutaneous routes, the effects are slower to develop. For example, after ingestion of a lethal dose of a cyanide salt, the casualty might have 15 to 30 minutes of survival time during which an antidote could be administered.

Prominent early signs and symptoms of cyanide poisoning include a transient hyperpnea, headache, dyspnea, and findings of general central nervous system (CNS) excitement, including anxiety, personality changes, and agitation progressing to seizures.[33] Diaphoresis, flushing, weakness, and vertigo may also be present. Late-appearing indications of CNS depression, such as coma and dilated, unresponsive pupils, are prominent signs of cyanide

intoxication.[33–35] These signs are not specific for cyanide poisoning, which makes the distinction from other types of poisoning very difficult without a history of exposure. The telltale odor of bitter almonds cannot be used as a guide because 40% to 60% of the population is unable to detect the odor.[36]

Because the toxic effect of cyanide is to block tissue uptake and utilization of oxygen, the casualty is transiently flushed and may have other, related signs of poor tissue oxygen extraction. For example, funduscopic examination shows an equally bright red color for retinal arteries and veins because of poor oxygen extraction. Increased oxygenation of venous blood is also responsible for a “cherry-red” skin color, but this sign may not always be present.

Laboratory Findings

Relevant laboratory findings include an early decreased arteriovenous difference in the partial pressure of oxygen (PO_2) with progressive lactic acidosis.

Timely measurements of blood and urine concentrations for suspected intoxicants are useful in guiding clinical therapy, especially when there is toxicity associated with the treatment agents. Unfortunately, analysis of cyanide in biological fluids is a difficult task for a variety of reasons.[37] Also, measurements of blood cyanide concentrations are almost never available during the treatment phase. Blood concentrations of cyanide and associated clinical effects are shown in Table 10-3.

TABLE 10-3
BLOOD CONCENTRATIONS OF CYANIDE AND ASSOCIATED CLINICAL EFFECTS

Cyanide Concentration (µg/mL)	Signs and Symptoms
0.2–0.5	None
0.5–1.0	Flushing, tachycardia
1.0–2.5	Obtunded
2.5–3.0	Coma
≥ 3.0	Death

Reprinted from Rumack BH. Cyanide poisoning. In: Newball HH, ed. Respiratory care of chemical casualties. In: *Proceedings of the Symposium on Respiratory Care of Chemical Casualties*. Fort Detrick, Frederick, Md: US Army Medical Research and Development Command; 28–30 November 1983: 186.

Documentation of blood cyanide levels is useful in confirming the clinical diagnosis and in subsequent follow-up investigations. The red blood cells contain most of the cyanide in the blood, so an assay of whole blood is necessary. Furthermore, cyanide levels tend to fall in stored samples because of the compound’s short half-life, and this process can only partially be limited by optimal storage conditions. Therefore, the time of sampling and the conditions of storage are very important factors to consider and record.

Cyanide concentrations in tissue, such as liver, lung, spleen, and heart, may be more accurate indicators of the blood cyanide intoxication levels. Estimates of tissue levels are necessary adjunctive studies in forensic cases.[38] For details on collecting and analyzing blood and tissue for cyanide, see *Assay Techniques for Detection of Exposure to Sulfur Mustard, Cholinesterase Inhibitors, Sarin, Soman, GF, and Cyanide,* Technical Bulletin Medical 296.[39]

Principles of Therapy

An understanding of the toxicology and pathophysiology of cyanide poisoning leads directly to the principles of therapy: eliminate further exposure, and institute supportive and specific antidotal therapy. (The specific antidotes are discussed separately in the section that follows.) In addition to the difficulties of diagnosing cyanide poisoning, each of the specific antidotal therapies discussed in this chapter has its own inherent toxicity. This knowledge should temper

their use in the typical field medical treatment facility and other emergency setting.

Elimination of Further Exposure

The first principle of therapy is the obvious one: eliminate any potential source of continuing cyanide poisoning:

- Remove the patient from an environment containing cyanide.
- Remove all contaminated clothing; rinse skin with soap and water or water alone if there is liquid on the skin.
- Gavage and administer activated charcoal if cyanide was ingested.

Following these steps can significantly decrease the amount of poison that has to be eliminated from the body.[40]

Supportive Therapy

Possibly the most important elements of therapy are general supportive actions, which, by themselves, can effect the recovery of most casualties without further risk from specific antidotal therapy.[41] They are probably the only indicated therapies for casualties of cyanide poisoning who arrive conscious at the emergency medical treatment station.

As early as 1840, Blake[42] found that the lethal effects of cyanide are neutralized by mechanical resuscitation. The use of hyperbaric oxygen in cyanide intoxication is still controversial. Supplemental oxygen with or without assisted ventilation clearly augments the effect of specific antidotes in animal studies; however, despite encouraging reports,[43,44] there is inconclusive evidence of further benefit from the use of hyperbaric oxygen.

Lactic acidosis resulting from anaerobic metabolism should be treated by intravenous administration of sodium bicarbonate, and seizures should be controlled by the administration of anticonvulsants such as diazepam.[45] Because correction of deficiencies in tissue perfusion and oxygenation is the ultimate goal of supportive therapy and is also important for the success of specific antidotal therapy, it is critically important to maintain an effective cardiac rhythm; this can be accomplished with cardiopulmonary resuscitation, if necessary, in the early stages of treatment.

Specific Antidotal Therapy

Casualties with advanced toxicity from a large amount of cyanide may require specific antidotal therapy in addition to the vigorous supportive therapy outlined above. The recommended agents or components of specific antidotal therapies for cyanide poisoning vary according to country and medical custom (Table 10-4). This diversity seems to be based, in part, on where the drugs were initially developed and used. The aims of the recommended therapies are generally similar, however, in that one drug is given for immediate relief from the histotoxic

TABLE 10-4
CYANIDE ANTIDOTAL COMPOUNDS IN USE

Antidote (Route of Administration)	Country
Sodium nitrite and sodium thiosulfate (IV)	United States,[1] Yugoslavia [2]
4-Dimethylaminophenol (IV or IM) and sodium thiosulfate (IV)	Germany [3, 4]
Dicobalt edetate (IV)	United States,[5] Netherlands [6]
Hydroxocobalamin (IV)	United States,[7, 8] France [9]

effect of cyanide complexed with cytochrome oxidase.

In most of the regimens discussed below, the action of displacing cyanide from cytochrome oxidase is accomplished by the formation of methemoglobin. Methemoglobin removes cyanide from the extracellular fluid space and, by so doing, displaces cyanide from the intracellular fluid. The second component of most regimens is sodium thiosulfate, which is given as a specific antidote to augment the systemic clearance of cyanide via thiocyanate formation by sulfur transferases.

IM: intramuscular; IV: intravenous
Sources: (1) Chen KK, Rose CL, Clowes GHA. Methylene blue, nitrites and sodium thiosulfate against cyanide poisoning. *Proc Soc Exp Biol Med*. 1933;31:250–251. (2) Binenfeld Z. Antidote therapy in cases of poisoning by some heavy metals and cyanides and its risks. *Farm Glas*. 1971;27:1–6. (3) Bright JE, Marrs TC. A model for the induction of moderate levels of methaemoglobinaemia in man using 4-methylaminophenol. *Arch Toxicol.* 1982;50:57–64. (4) Kiese M. *Methemoglobinemia: A Comprehensive Treatise*. Cleveland, Ohio: CRC Press; 1974. (5) Marrs TC, Swanston DW, Bright JE. 4-Dimethylaminophenol and dicobalt edetate (Kelocyanor) in the treatment of experimental cyanide poisoning. *Hum Toxicol*. 1985;4:591–600. (6) Nagler J, Provoost RA, Parizel G. Hydrogen cyanide poisoning: Treatment with cobalt EDTA. *J Occup Med.* 1978;20:414–416. (7) Cottrell JE, Casthely P, Brodie JD, Patel K, Klein A, Turndorf H. Prevention of nitroprusside-induced cyanide toxicity with hydroxocobalamin. *JAMA*. 1978;298:809–811. (8) Mushett CW, Kelley KL, Boxer GE, Rickards JC. Antidotal efficacy of vitamin B_{12a} (hydroxocobalamin) in experimental cyanide poisoning. *Proc Soc Exp Biol Med*. 1952;81:234–237. (9) Yacoub J, Faure J, Morena H, Vincent M, Faure H. Acute cyanide poisoning: Current data on the metabolism of cyanide and treatment with vitamin B_{12a}. *Eur J Toxicol*. 1974;7:22–29.

SPECIFIC ANTIDOTES

The clinical use of most antidotes is based on animal experiments[46] and on extrapolations made from a small number of clinical cases. Comparing results from animal studies has limitations because of the differences in experimental design from one study to another as well as marked interspecies differences in cyanide and drug metabolism. Moreover, the studies were not designed to resemble the usual emergency medical or battlefield scenario. The disparity of antidotes for cyanide is due to the following factors[47,48]:

- the small number of patients;
- the fact that most cyanide victims understandably receive several treatment agents;
- the lack of readily available, adequate analysis of blood and tissue concentrations; and
- the limited comparison studies that are available in animal models.

Antidotes are usually unnecessary if the casualty is conscious.

Drugs Used in the United States

Nitrites

Amyl nitrite and sodium nitrite, with or without sodium thiosulfate, are used as antidotes for cyanide.[49] The antidotal action of amyl nitrite was first noted as early as 1888.[50]

The oxidized form of heme iron (Fe^{3+}) in methemoglobin has a higher binding affinity for cyanide than does cytochrome oxidase. The preferential binding of cyanide to methemoglobin to form cyanomethemoglobin frees cytochrome oxidase to resume its role in aerobic metabolism.[51] In the United States and other countries, sodium nitrite has been used as the methemoglobin-inducing drug of choice.

In addition to methemoglobin formation, both sodium and amyl nitrite cause significant vasodilation, which warrants careful monitoring of blood pressure. Marked vasodilation with orthostatic hypotension, dizziness, and headache, in addition to the unpredictable levels of methemoglobin formed,[52] limit the utility of amyl nitrite in an upright casualty. Therefore, if a casualty is conscious and able to stand, he should not receive any nitrite. These factors, together with other concerns, have caused amyl nitrite to be removed from the cyanide antidote kit in the U.S. Army formulary for field units.

Sodium nitrite is available in the Lilly Cyanide Antidote Kit (manufactured by Eli Lilly and Company, Indianapolis, Ind.) in 10-mL ampules containing 300 mg for intravenous administration. (The kit also contains amyl nitrite encased in glass “pearls,” which are meant to be broken so the drug can be inhaled.) The solution of sodium nitrite (30 mg/mL) should be given to an adult intravenously over 5 to 15 minutes, with careful monitoring of blood pressure. A single dose is sufficient to raise the methemoglobin level to 20% in an adult,[53] and a second dose, up to half as large as the initial one, can be given. Methemoglobin levels should be monitored if possible and kept below 35% to 40%, the range that is associated with oxygen-carrying deficits caused by methemoglobin itself.[54]

Because most automated clinical analyzers do not detect cyanomethemoglobin, the residual normal hemoglobin capable of oxygen transport can be overestimated by measuring total hemoglobin only. Methemoglobin-inducing substances should not be given to fire victims, even if cyanide intoxication is suspected, because neither methemoglobin nor carboxyhemoglobin (formed by carbon monoxide) transport oxygen. Alternative therapy in this situation consists of administering oxygen, thiosulfate, and other standard supportive measures.

In children, sodium nitrite can cause lethal methemoglobin levels if the dose is too high. The recommended dose for children is 0.33 mL of the 10% solution per kilogram of body weight.[34,55]

Some data indicate that nitrites exert their action by a mechanism other than methemoglobin formation. It has been suggested that the protective effect is due to the vasodilating effect of nitrite.[52] Several α-adrenergic antagonists (eg, chlorpromazine, promethazine, promazine, and phenoxybenzamine) that cause vasodilation also antagonize cyanide toxicity.[56,57] Further information is needed to determine the mechanism or mechanisms by which chlorpromazine and phenoxybenzamine reverse cyanide intoxication.[57]

Several alternative methemoglobin-forming drugs that have different pharmacological properties from sodium nitrite have been investigated and used in other countries (see Table 10-4).

Other Methemoglobin-Forming Drugs

4-Dimethylaminophenol (4-DMAP), *p*-amino-propiophenone (PAPP), *p*-aminoheptanoylphenone (PAHP), and *p*-aminooctanoylphenone (PAOP) are also methemoglobin-forming compounds that have protective effects against cyanide. 4-DMAP was proposed as a fast-acting antidote with low toxicity.[58,59] This German-developed compound is used in the German military and by the civilian population. In humans, intravenous injection of 4-DMAP (3 mg/kg) can produce a level of 15% methemoglobin within 1 minute.[60] In dogs, a dose of 4-DMAP that produces a 30% level of methemoglobin will save animals that have received 2 to 3 LD_{50} of cyanide.[61]

The disadvantages of 4-DMAP are (1) the appearance of necrosis in the area of injection after intramuscular administration and (2) the possibility that extremely high levels of methemoglobin may occasionally result. Increases in pain, fever, and muscle enzymes also occur after intramuscular administration of the drug. 4-DMAP has been reported to produce positive results in the Ames test, which suggests that the compound may be a mutagen. PAHP appears to be the safest phenone of the series.[62]

Thiosulfate and Other Sulfur Donors

Thiosulfate has been used primarily with sodium nitrite in a fixed antidotal regimen. The standard dose of sodium thiosulfate, which is supplied in the Lilly Cyanide Antidote Kit in 50-mL ampules, is 50 mL of the 250 mg/mL (12.5 g), given intravenously. A second treatment with half of the initial dose may be given. The pediatric dose is 1.65 mL per kilogram of body weight.[55]

The utility of thiosulfate is limited because of its short biological half-life and its small volume of distribution.[23] This combination of pharmacological properties, in addition to the suggestion that the presence of cyanide increases the intracellular availability of the thiosulfate,[63] indicates that this compound probably cannot be used as a prophylactic but only as an antidote. Compounds containing the more-lipophilic sulfane sulfur (R—S—S$^-$) or compounds that can be actively transported into the cells may be more beneficial as cyanide antagonists,[64–66] but none are commercially available.

Other Therapeutic Drugs

Cobalt Salts

Cobalt salts have been shown to be an effective means for binding cyanide in vitro and in vivo.[67,68] Kelocyanor, the cobalt salt of ethylenediamine-tetraacetic acid (EDTA), which is commercially available in Europe but not in the United States, is administered intravenously.[69] In comparison studies against nitrite and hyposulfite, the cobalt chelate was thought to be superior[31]; however, in other studies the nitrite-thiosulfate combination was found to be superior.[70]

The drawback of cobalt compounds is their rather severe toxicity. Cardiac effects such as angina pectoris and ventricular arrhythmias, edema around the eyes, vomiting, and death have been observed.[71] A clinical caveat is that severe toxicity from cobalt can be seen even after initial recovery from acute cyanide poisoning.

Hydroxocobalamin

Hydroxocobalamin (vitamin B_{12a}) is an effective antagonist of cyanide intoxication that binds cyanide directly without forming methemoglobin. It is useful under certain conditions such as in fire victims who may already have a decreased concentration of functioning hemoglobin.[72] It has a low toxicity even at high doses.[73] In addition, in vitro cyanide studies[74] have shown a greater affinity for hydroxocobalamin than for cytochrome oxidase. Sodium thiosulfate can improve the protection provided by hydroxocobalamin alone.[46]

There are several disadvantages in the clinical use of this drug. In rare instances, urticaria can result from hydroxocobalamin administration. More importantly, hydroxocobalamin has a relatively short shelf life because it decomposes in light. It may cause tachyphylaxis, which may limit its usefulness.[75] The cost of the drug is high, in part because so much is needed on a molar basis. At 1,346 grams per mole of hydroxocobalamin, a dose of at least 4 g is needed to neutralize a lethal amount of cyanide.

Investigational Drugs

Prophylactic Drugs

PAPP and PAOP are also aminophenol derivatives. PAPP may not be active against cyanide intoxication; its metabolite, *p*-hydroxylamino-propiophenone, may be the active compound. This could account, in part, for the fact that cyanide toxicity *itself* changes the pharmacokinetics of the antidote.[76] PAPP reduces cyanide levels within red blood cells,[77] and its effect is greatly enhanced in the presence of sodium thiosulfate.[78] PAPP, PAHP, and PAOP all have pharmacokinetics that may allow for prophylactic administration.[62,79] Prophylactic methemoglobin formation could present its own problems, however, for victims of smoke inhalation or in other settings where high levels of carbon monoxide occur.[33,80]

A number of 8-aminoquinoline analogs of primaquine have also been studied as potential prophylactic drugs because they create elevated methemoglobin levels of long duration. Only preliminary information is available regarding the methemoglobin- forming potential of these

drugs in humans.[62,81,82]

Cyanohydrin-Forming Drugs

Aldehydes and carbonyl-related compounds (such as pyruvate, α-ketoglutaric acid, glyoxal, and reducing sugars) form cyanohydrins from cyanide. This action may be a mechanism for improving the protection provided by nitrites.[83] Steinberg and Thomas[84] found that glyoxal trimer may be the most effective compound of the group that they examined. The relatively short half-lives of these reversible reactions and the drug dose required[85] may limit the clinical utility of this class of compounds.[24]

SUMMARY

Cyanide, an ancient compound, is often associated with murders and assassinations. Because of the high amount needed to cause death and the inefficient weapons in which it was used, cyanide was not an effective chemical weapon in World War I; however, it was possibly used by Iraq against the Kurds in the Iran–Iraq War during the late 1980s.

Cyanide causes intracellular hypoxia by inhibiting the intracellular electron transport mechanism, the cytochrome enzymes. After inhalation of a large amount of cyanide—as either hydrocyanic acid or cyanogen chloride—the onset of effects is within seconds, symptoms are few, physical findings are scanty, and death occurs within minutes.

The antidotes used in the United States, sodium nitrite and sodium thiosulfate, are quite effective if given before cessation of cardiac activity.

ACKNOWLEDGMENT

The authors thank Michael P. Whitmer and Eric W. Nealley, U.S. Army Medical Research Institute of Chemical Defense, and Adam S. Szczepaniak, Jr., Wood Technical Library, Aberdeen Proving Ground, Maryland, for their excellent technical assistance.

REFERENCES

1. Sykes AH. Early studies on the toxicology of cyanide. In: Vennesland B, Conn EE, Knowles CJ, Westley J, Wissing F, eds. *Cyanide in Biology.* New York, NY: Academic Press; 1981: 1–9.
2. Prentiss AM. *Chemicals in War: A Treatise on Chemical War.* New York, NY: McGraw-Hill; 1937: 171–175.
3. Robinson JP. The problem of chemical and biological warfare. Vol 1. In: Robinson JP, ed. *The Rise of CB Weapons: A Study of the Historical, Technical, Military, Legal and Political Aspects of CBW, and Possible Disarmament Measures.* New York, NY: Humanities Press; 1971: 155–156.
4. Baskin SI. Zyklon. In: La Cleur W, ed. *Encyclopedia of the Holocaust.* New Haven, Conn: Yale University Press; 1998: in press.
5. Lang JS, Mullin D, Fenyvesi C, Rosenberg R, Barnes J. Is the “protector of lions” losing his touch? *US News & World Report.* November 1986;10:29.
6. Heylin M, ed. US decries apparent chemical arms attack. *Chem Eng News.* 1988;66:23.
7. Medical expert reports use of chemical weapons in Iran–Iraq War. *UN Chronicle.* 1985;22:24–26.
8. Hall AH, Rumack BH, Schaffer MI, Linden CH. Clinical toxicology of cyanide: North American clinical experiences. In: Ballantyne B, Marrs TC, eds. *Clinical and Experimental Toxicology of Cyanides.* Bristol, England: Wright; 1987: Chap 12: 313–314.

9. Clark CJ, Campbell D, Reid WH. Blood carboxyhaemoglobin and cyanide levels in fire survivors. *Lancet.* 1981;i:1332–1335. Quoted in: Hall AH, Rumack BH, Schaffer MI, Linden CH. Clinical toxicology of cyanide: North American clinical experiences. In: Ballantyne B, Marrs TC, eds. *Clinical and Experimental Toxicology of Cyanides.* Bristol, England: Wright; 1987; Chap 12: 321–333.

10. Anderson RA, Harland WA. Fire deaths in the Glasgow area, III: The role of hydrogen cyanide. *Med Sci Law.* 1982;22:35–40.

11. Baud FJ, Barriot P, Toffis V, et al. Elevated blood cyanide concentrations in victims of smoke inhalation. *N Engl J Med.* 1991;325(25):1761–1766.

12. Sidell FR. Chemical Casualty Consultant, Bel Air, Md. Personal communication, August 1996.

13. Wolnick KA, Fricke FL, Bonnin E, Gaston CM, Satzger RD. The Tylenol tampering incident—Tracing the source. *Anal Chem.* 1984;56(3):466A–470A, 474A.

14. Thompson RL, Manders WW, Cowan RW. Postmortem findings of the victims of the Jonestown tragedy. *J Forensic Sci.* 1987;32(2):433–443.

15. Hibbs CM. Cyanide and nitrate toxicoses of cattle. *Vet Hum Toxicol.* 1979;21:401–403.

16. Conn EE. Introduction. In: Evered D, Harnett S, eds. *Cyanide Compounds in Biology.* London, England: Wiley; 1988: 1–2. CIBA Foundation Symposium 140.

17. Vennesland B, Castric PA, Conn EE, Solomonson LP, Volini M, Westley J. Cyanide metabolism. *Fed Proc.* 1982;41:2639–2648.

18. Warburg O. Inhibition of the action of prussic acid in living cells. *Hoppe-Seyler's Z Physiol Chem.* 1911;76:331–346.

19. Keilin D. Cytochrome and respiratory enzymes. *Proc R Soc Lond B Biol Sci.* 1929;104:206–251.

20. Alexander K, Baskin SI. The inhibition of cytochrome oxidase by diaminomalenitrile. *Biochem Biophys Acta.* 1987;912:41–47.

21. Jarabak R, Westley J. Steady-state kinetics of 3-mercaptopyruvate sulfurtransferase from bovine kidney. *Arch Biochem Biophys.* 1978;185:458–465.

22. Westley J. Cyanide and sulfane sulfur. In: Vennesland B, Conn EE, Knowles CJ, Westley J, Wissing F, eds. *Cyanide in Biology.* New York, NY: Academic Press; 1981: 61–76.

23. Sylvester DM, Hayton WL, Morgan RL, Way JL. Effects of thiosulfate on cyanide pharmacokinetics in dogs. *Toxicol Appl Pharmacol.* 1983;69:265–271.

24. Porter DW, Baskin SI. The effect of three α-keto acids on 3-mercaptopyruvate sulfurtransferase activity. *J Biochem Toxicol.* 1996;11:45–50.

25. Sorbo B. Enzymatic conversion of cyanide to thiocyanate. In: *Proceedings of the 1st International Pharmacology Meeting.* Vol 6. Stockholm, Sweden; 1962: 121–136.

26. Marrs TC. Antidotal treatment of acute cyanide poisoning. *Adverse Drug React Acute Poisoning Rev.* 1988;4:179–206.

27. Ballantyne B, Bright J, Swanston DW, Williams P. Toxicity and distribution of free cyanide given intramuscularly. *Med Sci Law.* 1972;12:209–219.

28. Ballantyne B. Toxicology of cyanides. In: Ballantyne B, Marrs TC, eds. *Clinical and Experimental Toxicology of Cyanides.* Bristol, England: Wright; 1987: 41–126.

29. Baskin SI, Wilkerson G, Alexander K, Blitstein AG. Cardiac effects of cyanide. In: Ballantyne B, Marrs TC, eds. *Clinical and Experimental Toxicology of Cyanides.* Bristol, England: Wright; 1987: 138–155.

30. Pitt BR, Radford EP, Gurtner GH, Traystman RJ. Interaction of carbon monoxide and cyanide on cerebral circulation and metabolism. *Arch Environ Health.* 1979;34:354–359.

31. Paulet G, Chary R, Bocquet P. The comparative value of sodium nitrite and cobalt chelates in the

treatment of cyanide intoxication in non-anesthetized animals. *Arch Int Pharmacodyn.* 1969;127:104–117.

32. Haymaker W, Ginzler AM, Ferguson RL. Residual neuropathological effects of cyanide poisoning in dogs: A study of the central nervous system of 23 dogs exposed to cyanide compounds. *Mil Surg.* 1952;3:231–246.

33. Hall AH, Rumack BH. Clinical toxicology of cyanide. *Ann Emerg Med.* 1986;15:1067–1074.

34. Egekeze JO, Oehme FW. Cyanides and their toxicity: A literature review. *Vet Q.* 1980;2:104–114.

35. Izraeli S, Israeli A, Danon Y. Pharmacological treatment of cyanide poisoning. *Harefuah.* 1988;114:338–342.

36. Kirk RL, Stenhaus NS. Ability to smell solutions of KCN. *Nature.* 1953;171:698–699.

37. Groff WA Sr, Stemler FW, Kaminskis A, Froehlich HL, Johnson RP. Plasma free cyanide and blood total cyanide: A rapid completely automated microdistillation assay. *Clin Toxicol.* 1985;23:133–163.

38. Sunshine I, Finkle B. The necessity for tissue studies in fatal cyanide poisoning. *Int Archiv Gewerbepathol Gewerbehyg.* 1964;20:558–561.

39. Department of the US Army. *Assay Techniques for Detection of Exposure to Sulfur Mustard, Cholinesterase Inhibitors, Sarin, Soman, GF, and Cyanide.* Washington, DC: Headquarters, DA; 22 May 1996. Technical Bulletin Medical 296.

40. Lambert RJ, Kindler BL, Schaeffer DJ. The efficacy of superactivated charcoal in treating rats exposed to a lethal oral dose of potassium cyanide. *Ann Emerg Med.* 1988;17:595–598.

41. Graham DL, Laman D, Theodore J, Robin ED. Acute cyanide poisoning complicated by lactic acidosis and pulmonary edema. *Arch Intern Med.* 1977;137:1051–1055.

42. Blake J. Observations and experiments on the mode in which various poisonous agents act on the animal body. *Edinb Med Surg.* 1840;53:35–49.

43. Taitelman UZ. Oxygen. In: Meredith TJ, Jacobsen D, Haines JA, Berger J-C, van Heijst ANP, eds. *Antidotes for Poisoning by Cyanide.* Vol 2. In: *International Program on Chemical Safety/Commission of the European Communities Evaluation of Antidotes Series.* Geneva, Switzerland: World Health Organization and Commission of the European Communities; 1993: 27–40. Publication EUR 14280 EN.

44. Takano T, Miyazaki Y, Nashimoto I. Effect of hyperbaric oxygen on cyanide intoxication: In situ changes in intracellular oxidation reduction. *Undersea Biomed Res.* 1980;7:191–197.

45. Brivet F, Delfraissy JF, Bertrand P, Dormont J. Acute cyanide poisoning: Recovery with non-specific supportive therapy. *Intensive Care Med.* 1983;9:33–35.

46. Way JL, Sylvester D, Morgan RL, et al. Recent perspectives on the toxicodynamic basis of cyanide antagonism. *Fundam Appl Toxicol.* 1984;4:S231–S239.

47. Tadic V. Terapija akutnog trovanja cijanidima [in Serbo-Croatian]. *Vojnosanit Pregl.* 1981;38:117–119.

48. Meredith TJ, Jacobsen D, Haines JA, Berger J-C, van Heijst ANP, eds. *Antidotes for Poisoning by Cyanide.* Vol 2. In: *International Program on Chemical Safety/Commission of the European Communities Evaluation of Antidotes Series.* Geneva, Switzerland: World Health Organization and Commission of the European Communities: 1993. Publication EUR 14280 EN.

49. Potter AL. The successful treatment of two recent cases of cyanide poisoning. *Br J Ind Med.* 1950;7:125–130.

50. Pedigo LG. Antagonism between amyl nitrite and prussic acid. *Trans Med Soc Virginia.* 1888;19:124–131.

51. Groff WA Sr, Kaminskis A, Cucinell SA. Simultaneous determination of methemoglobin and total hemoglobin by a continuous-flow method. *Clin Chem.* 1974;20:1116–1120.

52. Way JL. Cyanide intoxication and its mechanism of antagonism. *Annu Rev Pharmacol Toxicol.* 1984;24:451–481.

53. Chen KK, Rose CL. Nitrite and thiosulfate therapy in cyanide poisoning. *JAMA.* 1952;149:113–119.

54. Bunn HF. Disorders of hemoglobin. In: Braunwald E, Wilson JD, Martin JB, Fauci AS. *Harrison's Principles of Internal Medicine.* 11th ed. New York, NY: McGraw-Hill; 1987: 1518–1527.

55. Berlin CM. The treatment of cyanide poisoning in children. *Pediatrics.* 1970;6:793–796.

56. Guth PS, Sprites MA. Antagonism of cyanide intoxication by chlorpromazine. *Fed Proc.* 1958;17:374.

57. Pettersen JC, Cohen SD. Antagonism of cyanide poisoning by chlorpromazine and sodium thiosulfate. *Toxicol Appl Pharmacol.* 1985;81:265–273.

58. Weger NP. Treatment of cyanide poisoning with 4-DMAP—Experimental and clinical overview. *Fundam Appl Toxicol.* 1983;3:387–396.

59. Vick JA, Froehlich H. Treatment of cyanide poisoning. *J Toxicol Clin Exp.* 1988;25:125–138.

60. Kiese M, Weger N. Formation of ferrihaemoglobin with aminophenols in the human for the treatment of cyanide poisoning. *Eur J Pharmacol.* 1969;7:97–105.

61. Lorcher W, Weger N. Optimal concentration of ferrihemoglobin for the treatment of cyanide poisoning. *Naunyn-Schmiedeberg's Arch Exp Pathol Pharmakol.* 1971;270(suppl):R88.

62. Rockwood GA, Baskin SI, Romano JA Jr, et al. *Effects of* p-*Aminopropiophenone (PAPP),* p-*Aminoheptanoylphenone (PAHP), and* p-*Aminooctanoylphenone (PAOP) Exposure on Methemoglobin, Sulfhemoglobin, Oxyhemoglobin, Oxygen Content, Reduced Hemoglobin, Oxygen Saturation, Carboxyhemoglobin, and Oxygen Capacity in Mice.* Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; April 1996. USAMRICD-TR-95-06.

63. Binenfeld Z. Antidote therapy in cases of poisoning by some heavy metals and cyanides and its risks. *Farm Glas.* 1971;27:1–6.

64. Frankenberg L, Sorbo B. Effect of cyanide antidotes on the metabolic conversion of cyanide to thiocyanate. *Arch Toxicol.* 1975;33:81–89.

65. Baskin SI, Legere RH, Kirby SD, et al. Biochemical and pharmacological approaches to the development of cyanide antidotes. In: *Proceedings of NATO Research Study Group 3, Panel VIII/RSG-3.* Part 1. Aberdeen Proving Ground, Edgewood, Md: US Army Medical Research Institute of Chemical Defense; 1988: 539–544.

66. Rockwood GA, Porter DW, Baskin SI, Romano JA Jr. An in vitro assessment of cyanide (CN) detoxifying sulfur compounds. *Toxicologist.* 1993;13:249.

67. Evans CL. Cobalt compounds as antidotes for hydrocyanic acid. *Br J Pharmacol.* 1964;23:455–475.

68. Hillman B, Bardhan KD, Bain JTB. 1974. The use of dicobalt edetate (Kelocyanor) in cyanide poisoning. *Postgrad Med J.* 1974;50:171–174.

69. Marrs TC. Dicobalt edetate (Kelocyanor). In: Meredith TJ, Jacobsen D, Haines JA, Berger J-C, van Heijst ANP, eds. *Antidotes for Poisoning by Cyanide.* Vol 2. In: *International Program on Chemical Safety/Commission of the European Communities Evaluation of Antidotes Series.* Geneva, Switzerland: World Health Organization and Commission of the European Communities; 1993: 79–94. Publication EUR 14280 EN.

70. Rose CL, Worth RM, Kikuchi K, Chen KK. Cobalt salts in acute cyanide poisoning. *Proc Soc Exp Biol Med.* 1965;120:780–783.

71. Reynolds JEF, Prasad AB, eds. Dicobalt edetate (1033-p). In: *Martindale: The Extra Pharmacopoeia.* 28th ed. London, England: Pharmaceutical Press; 1982: 382.

72. Rose CL, Worth RM, Chen KK. Hydroxocobalamine and acute cyanide poisoning in dogs. *Life Sci.* 1965;4:1785–1789.

73. Vesey CL, Cole PV, Linnell JC, Wilson J. Some metabolic effects of sodium nitroprusside in man. *Br Med J.* 1974;2:140–142.

74. Lopes LCV, Campello AP. Effect of hydroxocobalamin on the inhibition of cytochrome *c* oxidase by cyanide, II: In isolated cytochrome *c* oxidase. *Res Commun Chem Pathol Pharmacol.* 1976;14:177–191.

75. Posner MA, Rodkey FL, Tobey RE. Nitroprusside-induced cyanide poisoning: Antidotal effect of hydroxocobalamin. *Anesthesiology.* 1976;44:330–335.

76. Liu L, Huang R. Pharmacokinetics and pharmacodynamics of *p*-aminopropiophenone in rabbits. *Zhonggua Yaoli Xuebao.* 1988;9:178–181.

77. Marrs TC, Bright JE. Effect on blood and plasma cyanide levels and on methaemoglobin levels of cyanide administered with or without previous protection using PAPP. *Hum Toxicol.* 1987;6:139–145.

78. Rose CL, Welles JS, Fink RD, Chen KK. The antidotal action of *p*-aminopropiophenone with or without sodium thiosulfate in cyanide poisoning. *J Pharmacol Exp Ther.* 1947;89:109–114.

79. Rockwood GA, Romano JA Jr, Scharf BA, Baskin SI. The effects of *p*-aminopropiophenone (PAPP) and *p*-aminooctoylphenone (PAOP) against sodium cyanide (CN) challenge and on righting and motor activity in mice. *Toxicologist.* 1992;12:271.

80. Hall AH, Rumack BH. Management of cyanide poisoning. *Ann Emerg Med.* 1988;17:108–109.

81. Steinhaus RK, Baskin SI, Clark JH, Kirby SD. Formation of methemoglobin and metmyoglobin using 8-aminoquinoline derivatives or sodium nitrite and subsequent reaction with cyanide. *J Appl Toxicol.* 1990;10(5):345–351.

82. Levine BC, Wheeler CW, Tomlinson MJ. Acute and subchronic oral toxicity of the anticyanide drug WR242511 tartrate. *The Toxicologist.* 1996;16:106. Abstract.

83. Moore SJ, Norris JC, Ho IK. The efficacy of α-ketoglutaric acid in the antagonism of cyanide intoxication. *Toxicol Appl Pharmacol.* 1986;82:40–44.

84. Steinberg GM, Thomas NC. *Aldehyde Treatment in Cyanide Poisoning in Mice.* Alexandria, Va: Defense Technical Information Center; 1974. Report AD-779540.

85. Johnson WD. *Effects of Methemoglobin Versus Potassium Cyanide Intoxication.* Fort Detrick, Frederick, Md: US Army Research and Development Command; 1987. USAMRDC Report 7583-14: 1–75.

Chapter 11

INCAPACITATING AGENTS

JAMES S. KETCHUM, M.D., ABPN*; AND FREDERICK R. SIDELL, M.D.†

INTRODUCTION

USE OF INCAPACITATING AGENTS
- Historical Precedents
- Contemporary Use

POSSIBLE APPROACHES TO INCAPACITATION
- Nonchemical Agents
- Chemical and Biological Warfare Agents
- Psychochemical Agents

THE ANTICHOLINERGICS AS CANDIDATE INCAPACITATING AGENTS
- General Characteristics of Anticholinergics
- The Most Likely Candidate: BZ or a Related Glycolate
- Clinical Pharmacology of BZ
- Anticholinergic Delirium Produced by BZ

DIAGNOSIS OF INCAPACITATING AGENT SYNDROMES

MEDICAL MANAGEMENT
- BZ and Other Anticholinergics
- LSD, Other Indoles, and Phenethylamine Derivatives
- Opioids

SUMMARY

* *Colonel, Medical Corps, U.S. Army (Ret); Assistant Clinical Professor, Department of Psychiatry, University of California at Los Angeles, Los Angeles, California 90024*
† *Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014*

INTRODUCTION

As defined in *The American Heritage Dictionary of the English Language,* to “incapacitate” means “to deprive of strength or ability.” The word is not synonymous with paralysis, confusion, or any other specific affliction. It is a general term, implying neither global inability to act nor any particular type of disability. For example, blurred near vision might be incapacitating for a computer programmer or air traffic controller but probably would not be incapacitating for a laborer or a football player. Consequently, when the word incapacitating is used, we should ask, “incapacitating for what activity?”

Used in a military context, incapacitation is understood to mean inability to perform one’s military mission. Since missions vary, we could theoretically consider a particular agent to be incapacitating if it disrupts aspects of performance vital to a particular mission. Impaired hearing might incapacitate a translator, a severe tremor might incapacitate a sniper, and so forth. In this chapter, however, incapacitation means the inability to perform *any* military task effectively and implies that the condition was achieved via the deliberate use of a *nonlethal* weapon.

Finding a suitable nonlethal weapon to substitute for a lethal one poses formidable problems. Consider the criteria—military, medical, and budgetary—that should govern the selection of an ideal incapacitating agent: effectiveness, relative lack of toxicity, persistence, logistical feasibility, treatability, predictability, manageability of casualties, and expense (Exhibit 11-1). No proposed incapacitating agent meets all of these demanding requirements, but any compound worthy of standardization (ie, production without further modification) needs to fulfill most of them.

EXHIBIT 11-1
CRITERIA FOR THE IDEAL INCAPACITATING AGENT

1. Effectiveness	The agent must be able to severely impair or completely disrupt an enemy's ability to fight.
2. Relative lack of toxicity	When used as intended, the agent must produce few deaths or permanent injuries.
3. Persistence	Effects must be temporary: preferably minutes to hours; at most, a few days.
4. Logistical feasibility	The compound must be potent, chemically stable, and capable of being incorporated into practical munitions.
5. Treatability	Effects brought about by the agent should be fully or partially reversible by relatively simple medical treatment. Even without treatment, ill effects should not cause permanent disability.
6. Predictability	The behaviors produced by the agent must be relatively predictable and unlikely to bring about disastrous secondary consequences to civilians or other noncombatants. Nor should the agent increase the likelihood of the senseless activation of weapons of mass destruction by affected individuals.
7. Manageability of casualties	Once captured, incapacitated individuals should be controllable. Deaths and injuries due to an interaction between the altered mental state and natural dangers in the immediate environment must be prevented.
8. Expense	The cost of production must be affordable within the context of the overall military budget.

Source: Departments of the Army, Navy, and Air Force, and Commandant, Marine Corps. *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries*. Washington, DC: HQ: DA, DN, DAF, Commandant, MC; 22 Dec 95: 3–1. Field Manual 8-285, NAVMED P-5041, Air Force Joint Manual 44-149, Fleet Marine Force Manual 11-11.

Further considerations would come into play before such an agent could be used. For example, methods and equipment must be designed to manufacture, store, and transport the agent. Troops in the field would need training to operate what might prove to be quite complex delivery systems. Medical personnel would need to learn how best to treat casualties, working within the confines of the battlefield.

USE OF INCAPACITATING AGENTS

Historical Precedents

Few references to the historical use of drugs for military purposes appear in contemporary publications. Scholars interested in chemical warfare, therefore, may easily assume that this concept is of recent origin, arising out of the technological revolution of the late 20th century. Actually, a substantial literature describing a variety of tactical efforts to incapacitate enemy forces by intoxicating them with mind-altering chemicals is available. The fact that such material is rarely cited in current publications, both lay and professional, probably has several explanations. The exponential expansion of pharmaceutical discovery, for example, has shifted attention from those few drugs that were the mainstay of medical treatment before the turn of the century. In addition, in the wake of an explosion of new information and the exponential proliferation of journals and textbooks, the older literature has been eclipsed and is rarely included in current indexes and computerized databases.

In 1961, Goodman[1] carried out a systematic perusal of 100 years of four leading American and British medical journals *(Journal of the American Medical Association, Boston Medical and Surgical Journal* [continued as *New England Journal of Medicine], Lancet,* and *British Medical Journal)* and a more limited survey of several respected German medical periodicals *(Fuhner-Wielands Sammulung von Vergiftungsfallen* [continued as *Archiv fur Toxikologie]* 1930–1961, and *Deutsche Zeitschrift fur die Gersamte Gerichtliche Medizin* 1922–1939). Goodman uncovered an astonishing variety of reports of the deliberate pharmacological induction, particularly by atropine and related drugs, of “behavioral toxicity” (a term introduced by Brady in 1956). Most of these were individual cases of poisoning for

nefarious purposes, but many can be considered instances of the early use of drugs as “weapons of mass destruction” (a late–20th-century term that seemingly does not ethically distinguish nonlethal incapacitating chemical agents from lethal chemical agents, or from biological and nuclear weapons).

A few excerpts from Goodman’s lengthy historical review are worthy of inclusion here, if only to show that incapacitating agents are by no means a new approach to military conflict:

> According to Sextus Julius Frontinus, Maharbal, an officer in Hannibal’s army about 200 BC,…sent by the Carthaginians against the rebellious Africans, knowing that the tribe was passionately fond of wine, mixed a large quantity of wine with mandragora, which in potency is something between a poison and a soporific. Then, after an insignificant skirmish, he deliberately withdrew. At dead of night, leaving in the camp some of his baggage and all the drugged wine, he feigned flight. When the barbarians captured the camp and in frenzy of delight greedily drank the drugged wine, Maharbal returned, and either took them prisoners or slaughtered them while they lay stretched out as if dead.[2(p139)]
>
> . . . .
>
> Another example of the use of atropinic plants for military purposes occurred during the reign of Duncan, the 84th King of Scotland (AD 1034–1040), who used wine dosed with “sleepy nightshade” against the troops of Sweno, King of Norway. After a battle near Culross, Duncan sent messengers to Sweno to negotiate surrender, and during the discussions supplied the Norwegians with provisions. As expected, this was looked on as a sign of weakness. The Scottish forces under Bancho entered Sweno’s camp while the invaders were intoxicated and rapidly vanquished them.[3,4(pp537–538),5]
>
> . . . .
>
> During his assault in 1672 on the city of Groningen, the Bishop of Muenster tried to use grenades and projectiles containing belladonna against the defender. Unfortunately, capricious winds often blew the smoke back, creating effects opposite to those intended. As a result of this and other incidents in which chemicals were used in battle, a treaty was signed in 1675

between the French and the Germans, outlawing further use of chemical warfare.[4(p563)]

. . . .

In 1813 the inhabitants of an area being invaded by French troops received fortuitous help from local flora. A company of starving French soldiers was rendered helpless when they impulsively consumed wild berries containing belladonna alkaloids. Gaultier, the company surgeon, vividly recorded the generalized confusion and self-destruction that took place as delirious soldiers wandered half-clothed through the bog. Others, in response to their hallucinations, cried out “Aux armes!” and threw themselves into blazing campfires.[6]

. . . .

Later in the same century a peaceful railway surveying expedition under Lieutenant-Colonel Paul Flatters was proceeding to the Sudan from Algeria, through the territory of the Touareg. These Barbers, who, unlike other North African, veil the men and not the women, are a raider people who did not completely surrender to French authorities until 1943. They called themselves “the Blue Men” and “the People of the Veil”; the other inhabitants, however, called them “the Abandoned of God.”[7,8]

Flatters, ignoring a warning letter from the Touareg marched into an ambush on 16 February 1881, losing approximately the entire “assault group,” which constituted half of his entire force. As his sixty-one remaining personnel tried to march to a French Outpost, they were trailed by approximately two hundred Touareg. On 8 March 1881, their supplies having been observed to be low, they were approached by three men who claimed to be members of another tribe. They were sold three bundles of dried dates, which were thrown into their camp the following day, and consumed in varying quantities by the troops. Shortly thereafter they exhibited the signs of solanaceous intoxication.[9]

Five men disappeared in the first minutes of confusion, and 31 of the remainder were so sick that they were unable to look after themselves. In the evening some attempted to crawl away into the desert. The other Frenchmen had been tied

> down by the senior indigenous soldier to prevent injury. The next morning, somewhat improved, "…they set off, half mad, bent double under excruciating pain, their legs crumbling away under them, their voices shrill, their words unintelligible." On the second day after the poisoning they reached an oasis, where a force of Touareg awaited them. By this time, however, the survivors were able to function as an effective fighting force and repulsed the attack. After finding water, and resorting to cannibalism to sustain life, 12 of the original soldiers reported to a French outpost 20 days after their initial "chemical warfare attack" by an "incapacitating agent" (later identified as *Hyoscyamus falezlez*).[9]
>
> . . . .
>
> Ironically, the first recorded 20th century use of solanaceae in a military situation occurred in Hanoi, French Indo-China (later known as North Vietnam) on 27 June 1908. On that day, two hundred French soldiers were poisoned by datura in their evening meal. One of the intoxicated soldiers saw ants on his bed, a second fled to a tree to escape from an hallucinated tiger and a third took aim at birds in the sky. The delirious troops were soon discovered and all recovered after medical attention. Two indigenous non-commissioned officers and an artilleryman were later convicted by courts-martial of plotting with ex-river pirates who had been influenced by "Chinese reformer agitators."[10,11]

Goodman's scholarly review[1] provides other examples, including the alleged use of cauldrons of burning hemp (containing tetrahydrocannabinol) or smoke from opium (morphine alkaloids) dating as far back as the first century BC; however, the foregoing examples should suffice to correct the notion that incapacitating chemical weapons are a modern invention.

Contemporary Use

Although warfare is tantamount to death and destruction, the international community, particularly in the latter half of the 20th century, has repeatedly tried to find ways to make war more “humane.” Remorse and indignation were widely expressed following the use during World War I of such repugnant weapons as chlorine, mustard, and phosgene, which killed or injured hundreds of thousands of soldiers in the European trenches. One consequence of this outcry was an international ban on chemical weapons adopted by the Geneva Convention in 1925. The United States, although not a signatory to this document until 1975, strongly supported its purpose.

Chemical weapons were apparently not used during World War II, although the German military was later found to have developed and stockpiled several different lethal anticholinesterase nerve agents. Fortunately they were not used, perhaps because of the swift and overwhelming invasion by Allied forces. Following the end of the war in 1945, research and development of nerve agents (GA [tabun], GB [sarin], and later GD [soman] and VX) continued in a number of countries, including the United States. Other than in a few isolated instances, however, (such as the Iraqi use against Iran in the 1980s) these agents have not been used in warfare. They remain in the United States arsenal, presumably as defensive weapons of last resort.

What was thought to be a novel concept—using chemicals to produce temporary disability—began to attract increasing interest as the acceptance of lethal agents declined. (As Goodman reported, of course, attempts to use various drugs and potions for this purpose are nothing new.) As credible military weapons, however, drugs did not receive serious consideration until the 1950s, when scientific psychopharmacology first came of age. At about this time, a number of research laboratories began to explore this possibility. During the next two decades, the feasibility of chemical incapacitation was systematically studied by nations on both sides of the Iron Curtain. The following, also from Goodman’s review, might be considered an example of “Cold War chemical warfare”[1]:

> A double agent revealed that in Munich, in 1959, salt shakers in a cafeteria serving 1,248 employees of Radio Free Europe were dosed with atropine. Chemical analysis of the contents of two shakers showed the presence of 2.36 per cent by weight of atropine.[12,13] (One gram of this concoction would thus contain almost 24 mg of atropine, more than enough to produce severe delirium.) Fortunately, the attempt was aborted.

In the United States, substantial resources were allocated for this study, reaching a peak in the mid-1960s. After extensive clinical study, a single incapacitating agent (BZ) was chosen for standardization by the U.S. Army Chemical Corps. By 1966, munitions capable of delivering BZ had been stockpiled and stored in military depots. In recent years, however, these have been dismantled and the contents destroyed. At the present time, incapacitating munitions are no longer in our armamentarium.

POSSIBLE APPROACHES TO INCAPACITATION

Virtually every imaginable chemical technique for producing military incapacitation has been tried at some time. Between 1953 and 1973, at the predecessor laboratories to what is now the U.S. Army Medical Research Institute of Chemical Defense, many of these were discussed and, when deemed feasible, systematically tested. Chemicals whose predominant effects were in the central nervous system were of primary interest and received the most intensive study. But other substances capable of disrupting military performance were also investigated, including some biological toxins. Nor were chemical agents and toxins the only possibilities considered; other candidates included noise, microwaves, light, and foul odors.

Nonchemical Agents

Physical disturbances (including loud noises, microwaves, and high-intensity light) and substances capable of causing intense sensory stimulation are among the nonchemical methods explored to impair performance.

Noise

Devices to produce loud or unpleasant sounds were built and, in some cases, were tested fairly extensively. Various sound patterns, including white noise and high frequencies, were tried. Although the sounds produced were annoying, they were relatively ineffective unless used at intensities that were either impractical to deliver over a large area or too likely to cause permanent hearing impairment.

It is interesting to note that the blare of trumpets has been used in battle throughout history to demoralize or confuse enemy forces. In addition, journalists reported that irritating rock-and-roll music was played during Operation Just Cause, the 1989 mission to Panama that resulted in the capture of Panamanian President Manuel Noriega. Similar loud music was played by the U.S. Treasury Department’s Alcohol, Firearms, and Tobacco team for the same purpose during the siege of the Branch Davidians in

1993 at Waco, Texas.

Microwave Bombardment

Microwave effects on the central nervous system were investigated (in laboratory animals) beginning in the late 1960s. Consideration was given to the possibility of using a microwave generator to produce military incapacitation. Concern about long-term adverse effects, however, caused this idea to be abandoned.

High-Intensity Photostimulation

Light sources of high intensity, adjusted to oscillate at frequencies that theoretically might impair visual perception and concentration were also studied for a short period, but this approach was ultimately judged to be impractical and probably unsafe (ie, it could cause retinal damage).

Olfactory Assault

Even the concept of olfactory incapacitating agents was briefly explored. Various obnoxious odors, including those produced by skatole derivatives, were found to be highly aversive, possibly sufficiently to impair performance. However, the relative ease of protecting against such odors (eg, wearing masks) and the probability that a highly motivated enemy would not be appreciably deterred by aversive odors alone caused this line of investigation (which was never very popular with the research team) to be abandoned.

Chemical and Biological Warfare Agents

A variety of chemicals—most of them known before 1950 and usually grouped with the chemical warfare agents—produce incapacitation through physiological rather than psychological effects. This category is included in this historical review of incapacitating agents only for the sake of completeness; each agent is discussed in detail in a separate chapter in this textbook.

Likewise, a number of noninfectious biological agents (eg, the staphylococcal enterotoxins) can cause severe malaise and other systemic symptoms, which would severely impair the ability to fight. The same is true of sublethal doses of organophosphates (cholinesterase inhibitors) such as VX or GB. None of these systemic agents are acceptable for use as incapacitating agents because of their unacceptable safety margins (ie, the ratio of the lethal to the incapacitating dose).

Vesicants

The vesicants, which include such substances as mustard, produce painful burns of the skin and respiratory tract. These chemical warfare agents were used extensively during World War I, with disastrous consequences. They have been used by some Third World nations, however, in the recent past. Similarly, phosgene and chlorine are generally not used. Although all vesicants can certainly incapacitate, they also often produce death and extreme suffering. (For further discussion, see Chapter 7, Vesicants.)

Irritants and Nausea-Producing Agents

Irritant agents, including lacrimators (such as CN, the original tear gas, and CS, its more potent successor), are generally fairly effective and safe when properly used. Their drawbacks are twofold: (1) their duration of action is relatively brief (adaptation to the chemical insult usually occurs after less than 30 min of continuous exposure) and (2) highly motivated individuals can fight through (ie, ignore) their effects. Thus, agents of this type would be relatively ineffective against dispersed, well-trained troops.

Nausea-producing agents (eg, DM) may have substantial effectiveness but they can be toxic. Highly potent relatives of apomorphine (a well-known emetic) are known but they have rather low safety margins. (For further discussion of CN, CS, and DM, see Chapter 12, Riot Control Agents.)

Psychochemical Agents

Virtually all drugs whose most prominent effects are psychological or behavioral (sometimes referred to as psychochemicals) can be classified into four fairly discrete categories: stimulants, depressants, pyschedelics, and deliriants. These drugs all can cross the blood–brain barrier with ease, and they exert their most dramatic effects on the central nervous system. Their interference with higher functions (as opposed to basic vegetative functions, which are primarily under brainstem control) are of greatest relevance to the goal of producing military incapacitation. The higher functions of the brain (attention, orientation, perception, memory, motivation, conceptual thinking, planning, and judgment) are more easily disrupted than are the more robust systems that regulate the physiological functions that are essential to life. Thus, it is possible to disable intelligent behavior at doses much lower than those that might have a direct lethal effect.

Stimulants

Stimulants include amphetamines, cocaine, caffeine, nicotine, and

epileptogenic substances such as strychnine and metrazole. Some phenethylamines (among which the amphetamines are perhaps the best known) have additional effects that can best be classified as “psychedelic” and will be discussed below. None of the conventional stimulants appears to have sufficient potency to be usable as an airborne incapacitating agent, and low doses could even prove counterproductive, since moderate stimulation might easily lead to a soldier’s more energetic and aggressive performance. Amphetamines, in fact, have successfully been employed to offset fatigue and enhance cognitive function.

Depressants

A large variety of compounds come under the heading of depressants, but none holds much promise as a practical incapacitating agent. Barbiturates generally require doses of several hundred milligrams to produce heavy sedation. For example, 200 mg of secobarbital was found to produce a decline of only about 20% in a 25-trial, time-reproduction task[14]; other studies have produced similar results. Morphine and other opioids could be incapacitating, and very potent analogs (eg, etorphine) have been synthesized,[15] but the lethal dose is only 10-to 20-fold greater than the incapacitating dose.

The more potent antipsychotic (neuroleptic) major tranquilizers such as haloperidol and other butyrophenones often produce relatively little sedation, although they reduce hyperactivity. Their tendency to produce extrapyramidal symptoms such as acute dystonia is an additional liability, and their potency, although considerable, generally would not satisfy logistical constraints. The minor tranquilizers would be virtually useless because of their relatively mild effects and limited potency.

Psychedelics

The psychedelic group includes D-lysergic acid diethylamide (LSD-25) as its most well known member. LSD was, in fact, a drug of great interest to the military (as well as the whole nation) for many years starting in the early 1950s. Systematic testing of LSD as a possible incapacitating agent was done mostly between 1959 and 1965. Although highly potent (capable of producing complete incapacitation after oral doses of approximately 2.5 µg/kg), LSD tends to produce unpredictable (although well-coordinated) behavior. Affected individuals usually cannot carry out a series of instructions or concentrate on a complex task, but might be capable of isolated, impulsive actions such as firing a weapon accurately enough to be dangerous. Studies[16] conducted in simulated military settings demonstrated conclusively that even well-trained units become totally disorganized following total oral doses of less than 200 µg. Phenothiazines and

benzodiazepines provide partial antidote effects, but there is no known complete antagonist.

The term psychedelic means “mind-manifesting” and refers to the alleged opening up and expansion of awareness that early clinicians attributed to LSD. This led to its popularity for many years as a psychotherapeutic agent (or as an adjunct to therapy). The flood of ideas and images released by LSD accounts for its disorganizing effects; for example, soldiers under the influence of LSD would find it impossible to carry out an assigned task because of distracting, sometimes amusing, thoughts.

When administered by the aerosol route, using a particle size of approximately 5 µm, the ID_{50} (ie, the dose that incapacitates 50% of the exposed population; this dose is retained in the lungs and is available for absorption) was estimated to be 5.6 µg/kg, approximately twice the ID_{50} by the parenteral route.[17]

LSD analogs are numerous, but none exceed it in potency. Several other naturally occurring psychedelics (structurally related to LSD in that they contain an indole ring) are known. Psilocybin, ibogaine, and harmine are examples, but none of these shares the potency of LSD. Studies in several species of animals have shown that the lethal dose is at least 1,000-fold greater than the incapacitating dose. On the other hand, a single, intramuscular dose of 297 mg (about 100 µg/kg) caused sudden death in an elephant tested in the Lincoln Park Zoo in Oklahoma City, Oklahoma.[18] In humans, doses above 10 µg/kg have occasionally produced grand mal seizures,[19] although in recreational users, ingestion of as much as 50 µg/kg without serious consequences has also been observed.[20]

Although substituted indoles were apparently the only known synthetic psychedelic drugs prior to 1955, compounds with psychedelic properties have since been produced by making appropriate additions to the phenethylamine skeleton (the nuclear constituent of dopamine and norepinephrine). They include 3,4-methylenedioxymethylamphetamine (MDMA), the drug popularly known as “ecstasy.” This and a number of related synthesized compounds[21] share many of the properties of LSD in that they induce an alteration of consciousness with startling perceptual changes that range from frightening to fascinating, depending on the user, his mental set, and the setting in which the drugs are taken. Some are very potent, but the same caveats that apply to LSD apply to their use in a military situation.

Phencyclidine (PCP) and the related compound ketamine have a mixture of clinical effects that reflects their complex pharmacology. Although acting on a specific subset of serotonin-type (HT-2) receptors in the forebrain,[22] PCP also has affinity for cholinergic, opiate, and dopamine receptors. This may explain PCP’s ability to produce subjective changes ranging from

euphoria to terror, as well as the apparent analgesia and “superman” effects described by police officers who have tried to arrest individuals under its influence. It was concluded quite early that PCP was not a suitable drug for military use, after its effects were observed in a few volunteers.

Deliriants

The fourth major group of psychoactive drugs can usefully be described as the deliriants. This category includes compounds that in small doses may produce clinically useful effects but in doses a few times larger produce delirium. Delirium is an incapacitating syndrome, involving confusion, hallucinosis, disorganized speech and behavior, as well as other features that will be described in greater detail below. Many drugs (and a number of metabolic alterations caused by disease states) can produce delirium. In their classic 1935 monograph, Wolff and Curran[23] enumerated more than 100 distinct etiologies of delirium. Many other drugs, some of them recently synthesized, could be added to that list. From this large number of possibilities, chemical compounds in a single subgroup—the “anticholinergics”—are regarded as most likely to be used as military incapacitating agents.

THE ANTICHOLINERGICS AS CANDIDATE INCAPACITATING AGENTS

“Anticholinergics” is the term generally used to refer to drugs that block the muscarinic effects of acetylcholine, in either the peripheral or the central nervous system. The best known are atropine and scopolamine, which are derived from solanaceous plants such as Jimson weed. Of course, many drugs in large overdose can produce delirium: tricyclic antidepressants, antihistamines, barbiturates, and phencyclidine are a few examples. But the anticholinergic BZ and a few structurally close synthetic relatives are the only ones known to be capable of producing delirium at very low dosage with a high safety margin—apart from scopolamine, which is one third as potent as BZ. A detailed discussion of BZ, and the nature of the delirium it produces, follows.

General Characteristics of Anticholinergics

The term anticholinergic as used in the context of this discussion refers more specifically to compounds that selectively block the brain’s muscarinic receptor (now known to consist of several sub-types). Atropine (hyoscyamine) and scopolamine (hyoscine) are the most familiar medicinal anticholinergics. Historically, they were obtained from of the botanical family Solanaceae, which includes Jimson (or loco) weed, mandrake root, henbane, belladonna, and nightshade. Atropine and scopolamine are esters

of tropic acid and contain a tertiary nitrogen moiety. This gives them the ability to cross the blood–brain barrier and block central muscarinic cholinergic receptors by competitive inhibition with acetylcholine, the natural neurotransmitter at these sites.

With regard to central activity, scopolamine is about 7-fold more potent than atropine but is shorter acting. An injection of as little as 1.5 mg of scopolamine hydrobromide is sufficient to cause the average 70-kg soldier to become delirious (and thus incapacitated) for 2 to 4 hours. Ten to twelve milligrams of atropine sulfate produces a similar effect, lasting 4 to 8 hours. In the peripheral cholinergic nervous system, both drugs produce parasympathetic blockade, causing tachycardia, elevation of blood pressure, hyperthermia (through blockade of sweat production), decrease in salivation, and reduction of gastrointestinal and urinary tract functions.

Impairment of near vision, attributable to a mixture of central and peripheral actions, is due to loss of accommodation (owing to ciliary muscle paralysis) and reduced depth of field (owing to pupillary enlargement). Alteration of skeletal muscle reflexes and tonus is related to central influences on the Renshaw interneurons in the spinal cord. Interaction between peripheral and central effects of anticholinergic drugs at different times following administration can cause biphasic changes in such parameters as heart rate and peripheral spinal reflexes. For example, heart rate may be slowed initially due to brainstem influences, after which vagal blockade tends to predominate, causing tachycardia. Similarly, knee and ankle reflexes may be exaggerated at first, but later are reduced. The pharmacokinetics that govern speed of distribution to the various drug compartments probably explain these biphasic phenomena. Although these variations in effects may seem to be academic distinctions, medical officers need to be aware of them when attempting to diagnose anticholinergic drug intoxication (which is discussed later in this chapter).

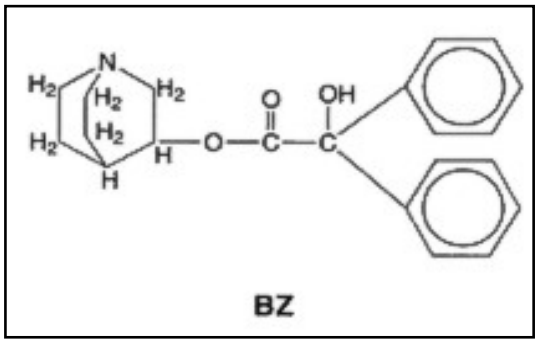
The above general characterization of anticholinergic drug effects produced by the familiar medications atropine and scopolamine also applies quite well to a number of synthetic anticholinergic compounds. Many of these compounds are more potent than atropine or scopolamine. In some cases, their duration of action is quite short and in other cases much longer than either atropine or scopolamine; furthermore, a few display even greater preferential central activity than scopolamine. Some synthetic agents[24] can cause delirium with little or no change in heart rate or other of the signs of peripheral muscarinic blockade.

The degree of preferential affinity for various muscarinic cholinergic receptors in brain, heart, and smooth muscle probably explains the relative lethality of the various anticholinergics.[25] For example, estimated potency of five different anticholinergics in man based on heart rate is closely correlated with their relative lethality in the rat,[26] suggesting that death at

high doses is probably due to cardiotoxicity. Previously, central toxicity was considered to be the cause of death from atropine. For humans, the dose that is lethal to 50% of the exposed population (LD_{50}) has been estimated to be about 100 mg.[27] However, using data from numerous reports of lethality and survival from high doses of atropine after reviewing more than 1,000 cases, Goodman calculated, using probit analysis,[28] the LD_{50} to be 453 mg (95% confidence level: 335–612 mg).

Combining these two analyses allows us to estimate the safety margin of various anticholinergics in humans. For BZ and atropine, the ID_{50} is approximately 40-fold lower than the LD_{50}; for scopolamine and other more centrally potent anticholinergics, the ID_{50} is approximately 100-fold lower than the LD_{50}. Computer models of dose distribution (using “Monte Carlo” statistical techniques) could perhaps use these dose ratios to help predict the probable number of lethalities (eg, if 50% of the target population were to be incapacitated by a particular agent, using a particular delivery system in a particular field situation). Clearly, however, this calculation would not be highly reliable.

The Most Likely Candidate: BZ or a Related Glycolate



BZ was first experimentally studied for therapy of gastrointestinal diseases. However, reports were received of confusion and hallucinations, suggesting that even small excesses of dosage were likely to cause problems. BZ was quickly withdrawn from commercial study and turned over to the U.S. Army as a drug of possible interest as an incapacitating agent.

Structurally, BZ is 3-quinuclidinyl benzilate, today known to neuropharmacologists as QNB, a research standard for measuring central antimuscarinic activity.[29,30] The code name BZ is probably derived from benzilate, a molecular member of the larger chemical family known as glycolates (glycolic acid esters). BZ is a stable (environmentally persistent), crystalline solid, which makes it suitable for dissemination by heat-producing (thermal) munitions.

Clinical Pharmacology of BZ

BZ's clinical profile closely resembles that of atropine, differing significantly only in duration of action and potency. Whereas the ID_{50} of atropine (as free base) is approximately 140 µg/kg (8–14 mg per soldier), the ID_{50} for BZ is only 6.2 µg/kg (roughly 0.5 mg per soldier).[31] Although BZ is roughly 25-fold more potent centrally than atropine, BZ is only 3-fold more potent than scopolamine. This distinction is often not appreciated by nonpharmacologists, and BZ erroneously gained the reputation as an exceptionally potent and dangerous drug—not only in the popular press but also in scientific publications. As a result, regulations were issued by the U.S. Army Chemical Corps prohibiting the transport of even a few milligrams of BZ (or any related agent of similar potency) aboard a commercial airline!

Unlike the much-shorter-acting scopolamine, BZ's effectiveness by the oral route of administration is about 80% that of the intravenous or intramuscular routes (which are virtually identical). By inhalation, if disseminated at an optimal particle size (diameter about 1.0 µm), BZ is approximately 40% to 50% as effective as it is by injection. When applied to the skin dissolved in propylene glycol (a common vehicle for transdermal administration), apparent absorption is only 5% to 10% and the effects are delayed approximately 24 hours. (This is surprising since historical treatises suggest that belladonna drugs are readily absorbed from poultices.[31])

MED_{50} is defined as the dose that is minimally effective for mild cognitive impairment in 50% of the exposed population; it produces mild impairment (25% decrease) in Number Facility (NF) performance.[32] The MED_{50} for BZ is approximately 2.5 µg/kg.[33] Recovery from this dose occurs within 24 hours. As stated above, the ID_{50} for BZ is 6.2 µg/kg and is defined as a persistent drop in performance on the NF to below 10% of baseline.

The effects of BZ by any route are slow in onset and long in duration. Performance decline is usually barely measurable at 1 hour, reaches a peak at about 8 hours, and subsides gradually over the next 48 to 72 hours. The duration of incapacitation (defined as the period during which NF performance remains below 25% of baseline) is approximately 24 hours at the ID_{50}. Doubling the dose produces incapacitation within 1 hour and prolongs recovery by approximately 48 hours. The effects of higher doses in humans are not known but would presumably occur even more rapidly and last considerably longer. In a field situation, wide variations in dosage would occur and mathematical models have been used to calculate the results under various conditions, but these can only give approximate predictions.

Anticholinergic Delirium Produced by BZ

As mentioned earlier, delirium is a nonspecific syndrome. Prior to the

systematic study of anticholinergic delirium, however, correlation of the clinical features of delirium with performance of cognitive and other tasks under controlled conditions had not been accomplished. In the following discussion, aspects of delirium produced by anticholinergic agents will be described in relation to associated impairment in performance of various scoreable tasks of military relevance.

Following the administration of BZ at the MED_{50}, delirium appears in its mildest form, represented by a drowsy state, with occasional lapses of attention and slight difficulty following complex instructions. Moderate delirium (following doses of about 4 µg/kg) generally is manifested by somnolence or mild stupor, indistinct speech, poor coordination, and a generalized slowing in thought process, with some confusion and perplexity.

Individuals receiving the ID_{50} or higher almost always develop the full syndrome of delirium. There is surprisingly little variation among individuals when anticholinergics are given. Perhaps this is because these drugs operate more directly on the “hardware” of the brain—neuronal systems where “all-or-none” activity is more characteristic. Drugs such as LSD or the amphetamines, for example, act on serotonin or dopamine systems. These are modulatory rather than discrete in their actions; that is, their effects may vary in accordance with the prevailing mood, arousal, and motivational state of the subject.

When delirium is present in its full-blown state, the individual seems to be in a “waking dream,” often staring and muttering, sometimes shouting, as simple items in the environment are variably perceived as elaborate structures, animals, or people. These hallucinations may arise from some trivial aspect of the surroundings, such as a strip of molding, a pillow, or an irregular spot on the floor. A total lack of insight generally surrounds these misperceptions.

Another striking characteristic of delirium is its fluctuation from moment to moment, with occasional lucid intervals and appropriate responses. An individual might answer “Shakespeare” when asked who wrote *Hamlet*, but when asked the same question 5 minutes later, might get down on the floor and attempt to remove an imaginary manhole cover, or become absorbed in a miniature World Series game being played out before his eyes.

“Phantom” behaviors, such as plucking or picking at the air or at garments, is characteristic (whence the old term “woolgathering”). This “carphologia,” as it was known in the 19th century, can be comical at times. When two individuals are both delirious they may play off of each other’s imaginings. A subject was once observed to mumble, “Gotta cigarette?” and when his companion held out an invisible pack, he followed with, “S’okay, don’t wanna take your last one.”

Recovery from drug-induced delirium is gradual, with a duration presumably determined by the pharmacokinetic persistence of the

causative agent. The more spectacular and florid hallucinations are gradually replaced by more modest distortions in perception. (Instead of large animals, mice and insects are described by the subject.) Awareness gradually returns and with it comes the subject's partial insight that his mental faculties are not what they should be. Ironically, paranoid tendencies often emerge at this stage, as the individual senses that something is amiss but cannot carry out the reality testing required to rule out malevolent manipulation of the environment by others. A period of restorative sleep generally precedes the return to normal cognitive function.

DIAGNOSIS OF INCAPACITATING AGENT SYNDROMES

There is little likelihood that incapacitating agents other than anticholinergics would be used on the battlefield. They are the only type known to be at all practical, as discussed earlier. Furthermore, most potential enemies on today's horizon seem to have little inclination to use weapons of great subtlety or ones that are designed to minimize lethality. There seems little probability that reversible incapacitating agents would even exist in their arsenals.

Nonetheless, in view of the elusive maladies reported following Agent Orange exposure in the Vietnam War and the so-called "Persian Gulf War syndrome," it is important that medical officers be able to recognize chemical intoxication if it should occur, lest impaired performance be inappropriately attributed to stress, lack of motivation, or psychiatric illness. Fortunately, the effects of most anticholinergic agents are usually easily recognized. Medical students were long taught the old medical adage "dry as a bone, red as a beet, hot as a hare, and mad as a hatter" as a means of remembering belladonna poisoning. The most useful diagnostic features of anticholinergics, indoles, cannabinoids, and anxiety reactions, all which can be confused with the signs of incapacitating agents, are summarized in Table 11-1.

TABLE 11-1
DIFFERENTIAL DIAGNOSIS FOR INCAPACITATING AGENTS

Sign or Symptom	Possible Etiology
Restlessness, dizziness, giddiness, failure to obey orders, confusion, erratic behavior, stumbling or staggering, vomiting	Anticholinergics, indoles, cannabinoids, anxiety reaction, other intoxications (such as alcohol, bromides, lead, barbiturates)
Dryness of mouth, tachycardia at rest, elevated temperature, flushed face, blurred vision, pupillary dilation, slurred or nonsensical speech, hallucinatory behavior, disrobing, mumbling, picking behavior, stupor, coma	Anticholinergics
Inappropriate smiling or laughing, irrational fear, distractibility, difficulty expressing self, perceptual distortions, labile increases in pupil size, heart rate, and blood pressure, stomach cramps and vomiting	Indoles (may mimic schizophrenic psychosis in some respects)
Euphoria, relaxation, day-dreaming, unconcerned attitude, easy laughter, hypotension and dizziness on sudden standing	Cannabinoids

Tremor, clinging or pleading, crying, clear answers, decrease in disturbance with reassurance, history of nervousness or immaturity, phobias, bodily disturbances such as blindness and paralysis	Anxiety reaction

Adapted from Departments of the Army, Navy, and Air Force, and Commandant, Marine Corps. *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries*. Washington, DC: HQ: DA, DN, DAF, Commandant, MC; 22 Dec 95: 3–1. Field Manual 8-285, NAVMED P-5041, Air Force Joint Manual 44-149, Fleet Marine Force Manual 11-11.

Depending on dosage and time elapsed following exposure, certain features may be more apparent. As mentioned earlier, peripheral symptoms of tachycardia, dryness of skin and mucous membranes, and moderately elevated blood pressure may be the most conspicuous signs during the first few hours. Incoordination, confusion, and slurred speech also appear early. Soon thereafter, mydriasis, stupor, and even coma may develop. If casualties are not examined until many hours have elapsed, peripheral cholinergic blockade may have largely subsided. Bizarre behavior (eg, groping, undressing, mumbling) with failure to respond to commands or conversation may then be the most conspicuous feature.

As recovery begins, apparent normality may be punctuated by sudden paranoid attempts to escape, or fleeting but vivid hallucinations may impinge on seemingly intact mentation. This is a time when panic, with its well-known dangers, may suddenly develop; vigilance (including restraint, if necessary) may be medically needed.

As noted earlier, the synthetic glycolate anticholinergics (those that are known so far) vary tremendously in their potency and duration of action. Signs and symptoms may last as little as 2 hours or as long as several weeks. Also, unfortunately, some glycolates produce few or no peripheral antimuscarinic features at the low end of the incapacitating dose range. Even the pupils may not be greatly enlarged. Such agents may produce signs that are difficult to distinguish from naturally occurring psychoses, unless the observer is well versed in the distinctly different pattern of cognitive changes that are pathognomonic of delirium. The occurrence of behaviors such as phantom drinking or smoking, picking or groping behavior, nonsensical speech, random disrobing, and the inability to follow simple instructions should greatly assist in making the differential diagnosis in such cases.

Detecting the covert use of other agents (those not suitable for large-scale dissemination) requires some knowledge of the effects of LSD and other psychedelics. Since LSD is a stimulant and usually prevents sleep, medical officers should not expect to see drowsiness or sedation. Staring, enigmatic smiling, and unusual preoccupation with ordinary objects are not uncommon. Responses to commands may be superficially normal, but laughter may supervene, or insubordinate and oppositional behavior. There are no practical diagnostic tests (although a sensitive fluorometric method for quantitative detection of LSD is known,[34] and blood samples could be useful in making a definitive diagnosis at a later time).

Marijuana intoxication is common in areas where the drug is indigenous, and the presence of reddened conjunctivae, along with the insouciance and relaxed joviality that marijuana produces, should make the diagnosis obvious. There is little likelihood that purified THC (tetrahydracannabinol, the active component of cannabis) would be used in a general military setting. Testing of blood and urine could be used if there is a need for definitive proof of its presence, but such tests are not always feasible or available.

With regard to covert use of incapacitating agents, the differential diagnostic problem is no different from that encountered in emergency rooms, and standard textbooks and manuals provide adequate guidelines. The possibility that secret research might produce some highly potent, unfamiliar variant of a known psychoactive drug cannot, of course, be ruled out, and blood analysis would seem the only way to determine its presence and chemical structure. However, medical officers in the field will be unlikely to encounter casualties produced by totally unfamiliar chemical substances.

MEDICAL MANAGEMENT

BZ and Other Anticholinergics

Prior to the mid 1960s, standard pharmacological textbooks taught that no antidote was available for the reversal of delirium caused by belladonnoid drugs. In fact, however, such an antidote had been reported by Kleinwachter[35] in the German literature in 1864. He noted that a lump of sugar saturated with extract of the Calabar bean (a natural source of physostigmine) proved efficacious in restoring lucid mental function to a prisoner who had become disoriented after drinking tincture of belladonna, thinking it was alcohol. Apparently this serendipitous finding was overlooked by early 20th-century clinical pharmacologists.

Nevertheless, reports[36,37] of reversal by physostigmine of coma (produced by injected doses of atropine as high as 212 mg, as therapy for certain psychiatric disorders) reappeared during the 1950s. Once again, this

useful finding apparently received little attention from mainstream clinicians. The fact that physostigmine had been largely replaced in clinical practice by neostigmine and pyridostigmine, neither of which has much central antimuscarinic activity, may account for this curious oversight.

In 1963, the usefulness of physostigmine as an antidote was once again reestablished during investigations of BZ's effects in volunteers.[31] In 1967, U.S. Army physicians published the first double-blind controlled study demonstrating the effectiveness of physostigmine in reversing scopolamine delirium.[38] Later, they reconfirmed this finding in studies[39] of atropine and Ditran (a mixture of two synthetic belladonnoid glycolates, no longer available, that had enjoyed brief popularity as a treatment for depression[40]). In the course of other military medical studies, the drug was also noted to reverse the delirium produced by a variety of synthetic anticholinergic glycolates.[24,31,41–43] Similar findings were soon reported in civilian studies.[44] Deliria produced by overdose with other drugs possessing anticholinergic side effects, such as tricyclic antidepressants[45] and antihistamines (personal observation, J.S.K.), were also noted to be treatable with physostigmine.

When given by the intravenous route, a dose of 30 μg/kg of physostigmine was found in further military studies[46] to be quite effective in the reversal of a variety of anticholinergics, although 45 μg/kg was the initial dose needed to reverse more-severe effects of delirium. It is interesting that physostigmine has also been used and reported to be effective for morphine-induced respiratory depression; alcohol withdrawal; and the effects of heroin, diazepam, ketamine, and fentanyl. In these instances, its activity may be due to a general direct cholinergic arousal effect, rather than to the inhibition of cholinesterase.[47] Case reports have come from the director of the Rocky Mountain Poison Control Center, near Denver, Colorado,[48] and the use of physostigmine as an antidote was reviewed by the director of the Munich, Germany, Poison Control Center.[49]

Although doses of as little as 2 to 3 mg of physostigmine alone may cause nausea and other signs of cholinergic excess (eg, salivation, intestinal cramping, and diarrhea), an intramuscular dose of 4 mg is generally well tolerated without any side effects when given as an antagonist to belladonnoid intoxication. In more than 100 subjects treated by one of the authors (J.S.K.), the only side effects were transient fasciculations in the platysmal area in one subject, and transient nausea and vomiting in a few others. If physostigmine should be given in error when no anticholinergic is present, its effects can be reversed by intravenous or intramuscular administration of 1 to 2 mg of atropine. Rapid intravenous use of physostigmine is not recommended because, in some individuals, the bolus effect on cardiovascular receptors may cause cardiac arrhythmias or even cardiac arrest. Most of these untoward outcomes, however, have

occurred in patients who were in poor general health or suffering from heart disease.

When in doubt as to diagnosis, an intramuscular test dose of 1 to 2 mg, repeated after 20 minutes if no effects are noted, is recommended. Once the diagnosis is established by a definite improvement in mental status, improvement can be sustained by repeating the treatment at intervals of 1 to 4 hours. Dosage and frequency should be dictated by clinical judgment. For example, after 30 to 60 minutes, heart rate will rise as the effects of physostigmine wane, and intellectual acuity will fade. Mental status can easily be estimated by having the subject do serial subtraction by 7 or 3; or by asking him to repeat a sentence containing a name, location, occupation, and employer; or by asking him simple questions regarding time, place, and person.

A solution of physostigmine is best administered orally, mixed with fruit juice to mask its bitter taste. Administered parenterally, only two thirds as much drug would be required to produce the same effects. A publication[50] distributed within the U.S. Army Medical Department contains detailed directions on how to dilute the parenteral preparation for oral use. In a combat zone, the oral route may be the only practical method to treat large number of casualties, relying on technicians (or, if necessary, other soldiers who are unaffected) to give measured amounts at specified intervals. With skillful titering, performance close to preexposure levels can be maintained. If the situation requires only that the soldier be comfortable and manageable, it is better to use a more conservative approach (eg, 1–2 mg by intramuscular injection or 2–4 mg administered orally every 2 h).

Once a suitable level of reversal is achieved (after 3 to 4 doses), delirium caused by BZ or other long-acting glycolates will usually relapse to its untreated severity in about 6 hours. (Shorter-acting belladonnoids such as atropine and scopolamine may be cleared from the brain by that time and thus further treatment may not be required.) The value of physostigmine in restoring and maintaining normal performance in a soldier given an incapacitating dose of BZ is illustrated in Figure 11-1.[51]

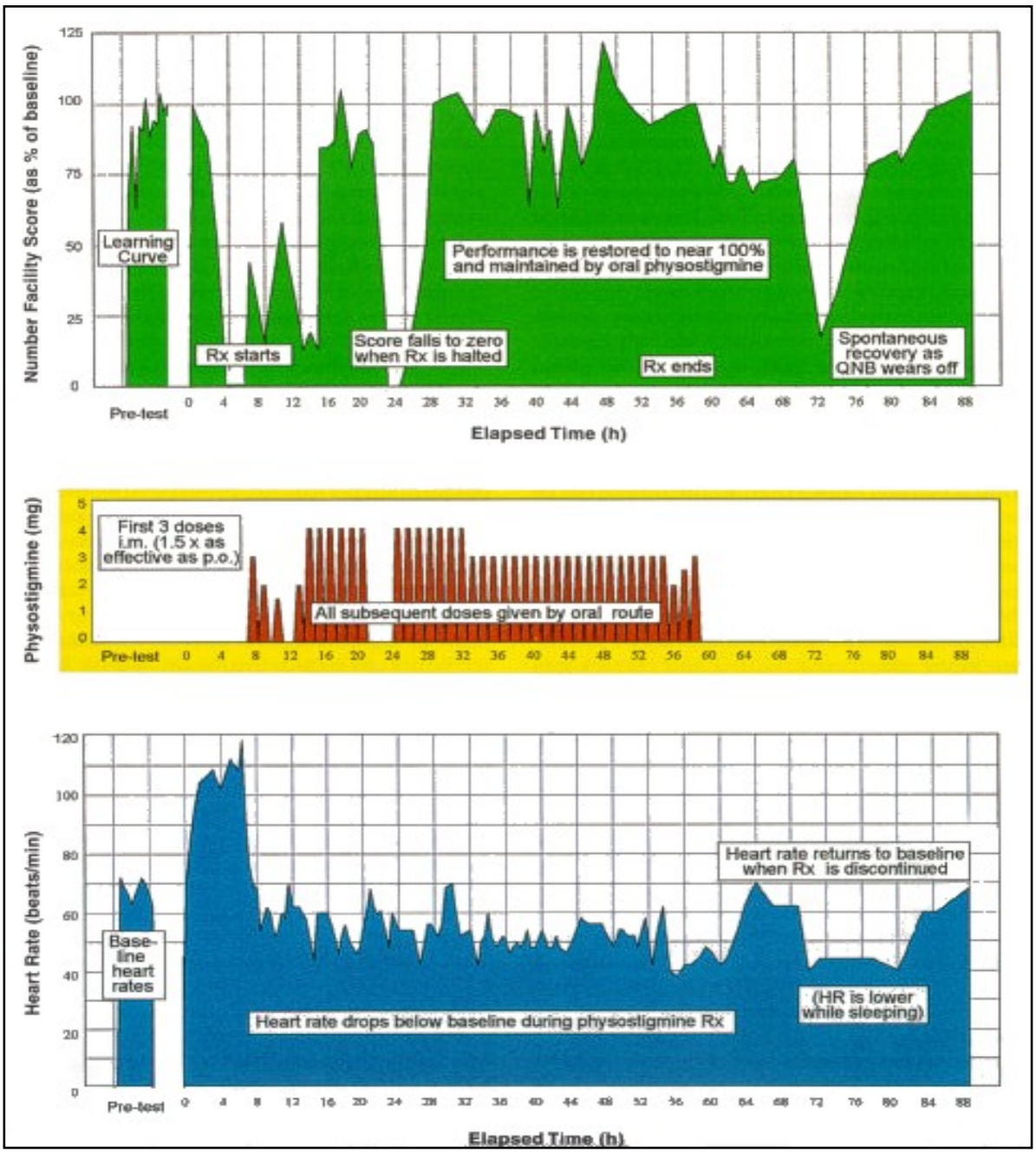

Number Facility Score (as % of baseline)
Learning Curve
Rx starts
Score falls to zero when Rx is halted
Performance is restored to near 100% and maintained by oral physostigmine
Rx ends
Spontaneous recovery as QNB wears off
Pre-test
Elapsed Time (h)
Physostigmine (mg)
First 3 doses i.m. (1.5 x as effective as p.o.)
All subsequent doses given by oral route
Pre-test
Heart Rate (beats/min)
Base-line heart rates
Heart rate returns to baseline when Rx is discontinued
Heart rate drops below baseline during physostigmine Rx
(HR is lower while sleeping)
Pre-test
Elapsed Time (h)

Fig. 11-1. The effectiveness of physostigmine in the treatment of 3-quinuclidinyl benzilate (QNB) intoxication. The three graphs above are the summary record of Number Facility performance, top, and heart rate (HR), bottom, in a single volunteer after the administration of QNB (the Rx), center. An aerosol dose (particle size < 1.0 μm) of approximately 7.5 μg/kg (120% of the incapacitating dose) was administered via the inhalational route. Treatment with physostigmine was begun 6 hours after aerosol exposure (peak impairment occurs at 6–10 h). Without treatment, recovery from an incapacitating dose of QNB is gradual, requiring 72 to 96 hours. The effectiveness of physostigmine is evident. Adapted from Ketchum JS, Tharp B, Crowell E, Sawhill D, Vancil M. *The Human Assessment of BZ Disseminated Under Field Conditions.* Edgewood Arsenal, Md; 1967. Edgewood Arsenal Technical Report 4140.

In another study, a single individual was given 6.4 mg of BZ intramuscularly and treated with a placebo; he was totally unable to perform simple arithmetic (the NF test) for approximately 48 hours. On a second occasion 14 days later, he was given the same dose of BZ and treated with a total of more than 200 mg of physostigmine over a 72-hour period without apparent ill effect. Not only could he perform at levels close to his baseline, but he generally felt normal and could eat, play pool, and read without difficulty as long as treatment was continued.[31]

For reasons that are not fully understood, physostigmine is relatively ineffective if given during the first 4 to 6 hours following the onset of BZ effects. Physostigmine is likewise ineffective if given earlier than 45 minutes following administration of the shorter-acting scopolamine.[38] Also, use of the antagonist does seem to not shorten the duration of the underlying intoxication; in fact, if treatment is not maintained, recovery from intoxication may be slightly prolonged.[31] Unknown pharmacokinetic or metabolic factors may underlie these curious phenomena. The practical implication, in any case, is that the treatment team should not be discouraged if early administration of physostigmine fails to bring about immediate, dramatic improvement.

Whether or not physostigmine is a fielded drug and therefore available to medical officers in deployable hospitals, ancillary supportive measures for such signs and symptoms of delirium as disorientation, hyperthermia, decreased salivation, and self-inflicted injuries to the skin are important, as is evacuation to an appropriate hospital (Exhibit 11-2).

EXHIBIT 11-2
ANCILLARY SUPPORTIVE MEASURES FOR THE TREATMENT OF DELIRIUM

1. Control and containment are of primary concern since delirium can easily lead to accidents and inadvertent injury to others. Comatose or stuporous casualties may emerge from immobility into a stage of persistent crawling or attempted climbing (primitive behaviors sometimes called “progresso ostinato” [obstinate progression] in 19th-century descriptions of delirium). It is better to tether or otherwise loosely restrain individuals who are disoriented than to let them move about freely without close supervision.

2. The danger of hyperthermia must be considered if the environment is warmer than 75°F. Death from relatively low doses of anticholinergics has occurred due to impairment of sweating. Wet cloth is effective to reduce body temperature, and the casualty should be placed in the shade, if available.

3. Dryness of the mouth and parching of the lips should be managed with moist swabs and small amounts of vaseline or unguents. Hard candy may induce sufficient salivation to keep the tongue moist. Fluids should be given sparingly and food withheld until the individual is obviously capable of normal chewing and swallowing.

4. Significant skin abrasions can be caused by persistent repetitive movements, especially against rough surfaces. The use of wrappings or gloves may be useful. A tendency to remove clothing is common, and reflects a general regression to simple habitual behaviors. If the environment is harsh, the casualty’s clothing may have to be secured so it cannot be removed.

5. Evacuation from the field to more adequate medical facilities is desirable in most cases. If not possible, separation of affected individuals into small groups (eg, in tents) is preferable to large aggregations, where a few confused and hyperactive individuals can lead to an escalating problem of crowd control.

As pharmacologists know well, physostigmine is an acetyl (or “true”) cholinesterase inhibitor and, as discussed above, is an effective antagonist to cholinergic blocking compounds. A simplistic view of this phenomenon is that the excess acetylcholine created by the inhibition of the enzyme might antagonize (overcome) the effects of the blocker. The nerve agent VX, also an acetylcholinesterase inhibitor, has also been used successfully in the reversal of BZ.[52] Pseudocholinesterase inhibitors, such as diisopropyl fluorophosphate (DFP), are less effective.[39] Tetrahydroaminoacridine (THA, also called tacrine), currently approved for use in senile dementia, Alzheimer’s type, is also primarily a pseudocholinesterase inhibitor. In four subjects, 200 mg of oral THA was administered as an antagonist against BZ and proved moderately effective.[31] It was noted, however, to cause temporary changes in hepatic function tests and therefore was abandoned.

Physostigmine is probably not as highly regarded as it was during the 1970s and 1980s, when it was given to patients with a variety of intoxications, often without a rational indication. On the other hand, physostigmine is probably not as dangerous as current thinking and textbooks would indicate. It has predictable effects, and there are specific indications for its use. A drug with such potent vagal blocking activity probably should not, as mentioned earlier, be given rapidly by the intravenous route (risking a sudden bolus effect on cardiovascular receptors), since it is almost equally effective when given intramuscularly. The brief delay in absorption will rarely make a significant difference.

If administered by the intravenous route, a dose of 30 μg/kg (about 2 mg in a 70- to 75-kg person) is quite effective in reducing peripheral effects such as tachycardia and hypertension; higher doses (at least 45 μg/kg) are usually needed to significantly reverse the more-severe central cognitive effects.[46] Again, this route of administration should be used with great caution in patients who are already receiving other drugs that predispose to convulsions or cardiac arrhythmias.[53] If in doubt, medical officers might consider trying a small (≤1 mg) test dose before administering fully effective quantities. This amount will not generally completely reverse the central nervous system signs but may give detectable indications that the antidote is effective. Furthermore, a dose this small will not adversely affect the average adult who has not been exposed to an anticholinergic compound.

LSD, Other Indoles, and Phenethylamine Derivatives

In the unlikely scenario where psychedelic compounds such as LSD or one of the more potent phenethylamine analogs are encountered on the battlefield, depressant drugs are useful, even if suboptimal, antidotes. For many years, chlorpromazine (Thorazine, manufactured by SmithKline Beecham Pharmaceuticals, Philadelphia, Pa.) was the preferred treatment

of “bad trips” caused by LSD. Only moderate (although dose-related) improvement in performance was demonstrated in our laboratories when a 50- to 100-μg/kg dose of Thorazine was administered orally, 60 minutes following intravenous administration of 2 μg/kg of LSD.[54] More recently, the short-acting benzodiazepines, such as lorazepam (Ativan, manufactured by Wyeth-Ayerst Laboratories, Philadelphia, Pa.), have been widely used to good effect. Surprisingly, a leading clinical psychopharmacologist[55] recommended barbiturates to one of the authors (J.S.K.) during a recorded interview, on the grounds that LSD was a highly effective antagonist to barbiturates in laboratory studies. To our knowledge, this suggestion has not systematically been tested in humans.

Opioids

The treatment of opioid overdose is well established. Naloxone (Narcan, manufactured by Du Pont Multi-Source Products, Garden City, N. Y.) in doses of 0.4 to 1.0 mg has been recommended,[56] and is the standard treatment in most emergency rooms. Naloxone can be given subcutaneously or, if the soldier appears to be deeply comatose with severely depressed respirations, by the intravenous route. Repeated injections at intervals as short as 30 to 60 minutes are usually required in the presence of a large overdose to prevent relapse into coma with a possibly fatal outcome.

SUMMARY

Incapacitating agents, capable of temporarily preventing military personnel from performing their duties (without permanent injury), have a long and colorful history. For a variety of reasons, they have not generally been used in overt warfare in the 20th century. Preference for conventional lethal weapons by most aggressors, and the many uncertainties applying to their use by friendly nations, have led to their elimination from the United States’s arsenal. However, in the attempt to find an incapacitating agent that would meet the numerous constraints imposed by practical and political concerns, many studies were conducted, especially by the U.S. Army, during the 1960s. Although an ideal incapacitating agent was never found, much was learned from the search.

Of all known psychochemical options, anticholinergics appear to be the most feasible for military use. 3-Quinuclidinyl benzilate (BZ) or a related potent glycolate seem to be the most likely candidates among the many that have been studied. Following an absorbed dose of less than 1 mg, BZ produces an acute brain syndrome, best described as delirium, that lasts 2 to 3 days.

Reversal of the effects of BZ by physostigmine and other

anticholinesterase agents has been clearly demonstrated to be both effective and safe when properly used in otherwise healthy individuals. The benefits and methods of use of physostigmine were brought to the attention of modern medicine by U.S. Army medical officers, who conducted these studies.

Incapacitation produced by less likely candidates such as LSD and other indole derivatives, psychedelic phenethylamines, and potent opioids is theoretically possible, but it is unlikely that any of these compounds would be employed militarily. Covert use, which is logistically easier to accomplish and has fewer constraints, opens a broader spectrum of possibilities. This, however, is a concept that involves considerations that generally extend beyond the scope of chemical warfare.

REFERENCES

1. Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

2. Frontinus SJ; Bennet CH, trans. *The Strategems.* London, England: William Heinemann; 1925. Quoted in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

3. Buchanan G; Watkins J, trans. *The History of Scotland.* London, England: Henry Fisher, Son, and P. Jackson; 1831. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

4. Lewin L. *Die Gifte in der Weltgeschichte*. Berlin, Germany: Julius Springer; 1920: 537–538. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

5. Mitchel TD. *Materia Medica and Therapeutics.* 2nd ed. Philadelphia, Pa: JB Lippincott; 1857: 233. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

6. Gaultier M, trans. Narrative of the poisoning of one hundred and eighty persons by the berries of belladonna. *Medical and Physical Journal* (London). 1814;32:390–393. Cited in: Goodman E. *The Descriptive Toxicology of Atropine*. Edgewood Arsenal, Md. Unpublished manuscript, 1961.

7. Leder R. *Sahara.* Garden City, NY: Hanover House; 1954: pp 151 ff. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

8. Skolle J. *Azalei.* New York, NY: Harper and Brother; 1956: pp 22 ff. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

9. Cornewin C. *Des Plantes Veneneuses.* Paris, France: Librarie de Firmin-Didot et

Cit; 1893: 473. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

10. Lewin L. *Gifte und Vergiftungen.* Berlin, Germany: Georg Stilke; 1929: 809. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

11. *The Times.* London, England: 3 July 1908:8; 9 July 1908:7. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

12. *Newsweek.* 28 Dec 1959;54(26):27. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

13. *US News and World Report.* 28 Dec 1959;47(26):10. Cited in: Goodman E. *The Descriptive Toxicology of Atropine.* Edgewood Arsenal, Md. Unpublished manuscript, 1961.

14. Ketchum JS. Effects of secobarbital on time estimation performance. Edgewood Arsenal, Md; 1962. Unpublished study.

15. Simon EJ, Hiller JM, Edelman I. Stereospecific binding of the potent narcotic analgesic (3H) etorphine to rat-brain homogenate. *Proc Natl Acad Sci USA.* 1973;70:1947–1949.

16. Sim VM. *Clinical Investigation of EA 1729.* Edgewood Arsenal, Md: Chemical Research and Development Laboratory; 1961. CRDL Technical Report 3074.

17. Ketchum JS, Aghajanian GK, Bing O. *The Human Assessment of EA 1729 and EA 3528 by the Inhalation Route.* Edgewood Arsenal, Md: Chemical Research and Development Laboratory; 1964. CRDL Technical Report 3226.

18. West LJ, Pierce CM, Thomas WD. Lysergic acid diethylamide: Its effects on a male Asiatic elephant. *Science.* 1962;138(3545):1100–1103.

19. Buckman J. Senior Medical Officer, Marlborough Day Hospital, St. John's Wood, London, England. Personal communication, July 1965.

20. Leib G. Captain, Medical Corps, US Army. Clinical Research Department, Medical Research Laboratory, Edgewood Arsenal, Md. Personal communication, 1968.

21. Shulgin A, Shulgin A. *PIHKAL: A Chemical Love Story.* Berkeley, Calif: Transform Press; 1991.

22. Sircar R, Zukin SR. Characterization of specific sigma opiate/phencyclidine (PCP)-binding sites in the human brain. *Life Sci.* 1983;33(suppl 1):259–262.

23. Wolff HG, Curran D. Nature of delirium and allied states: Dysergastic reaction. *Arch Neurol Psychiatr.* 1935;33:1175–1215.

24. Ketchum JS, Kitzes D, Mershon M, et al. *The Human Assessment of EA 3443.* Edgewood Arsenal, Md: 1967. Edgewood Arsenal Technical Report 4066.

25. Waelbroeck M, Tasknoy M, Camus J, Christophe J. Binding kinetics of quinuclidinyl benzilate and methyl quinuclidinyl benzilate evantiomes at neuronal (M1), cardiac (M2), and pancreatic (M3) muscarinic receptors. *Mol Pharmacol.* 1991;40:413–420.

26. Ketchum JS. Incapacitating compounds. In: *Proceedings of the 1st Meeting of the Quadripartite Standing Working Group on Chemical Warfare.* Edgewood Arsenal, Md: Chemical Research and Development Laboratory; 1965.

27. Innes IR, Nickerson M. Drugs inhibiting the action of acetylcholine on structures innervated by postganglionic parasympathetic nerves (antimuscarinic or atropinic drugs). In: Goodman LS, Gilman A, eds. *The Pharmacological Basis of Therapeutics.* 3rd ed. New York, NY: Macmillan; 1965: 521–545.

28. Bliss CI. *The Statistics of Bioassay.* New York, NY: Academic Press; 1952.

29. Yamamura HI, Kuhar MJ, Snyder SH. In vivo identification of muscarinic cholinergic receptor binding in rat brain. *Brain Res.* 1974;80:170–176.

30. Yamamura HI, Snyder SH. Muscarinic cholinergic receptor binding in the longitudinal muscle of the guinea pig ileum with [3H] quinuclidinyl benzilate. *Mol Pharmacol.* 1974;10:861–867.

31. Ketchum JS. *The Human Assessment of BZ.* Edgewood Arsenal, Md: Chemical Research and Development Laboratory; 1963. Technical Memorandum 20-29.

32. Moran LJ, Mefferd RB. Repetitive psychometric measures. *Psychol Rep.* 1959;3:209–275.

33. Kitzes DL, Vancil ME. *Estimate of Minimal Effective Doses of BZ by the Intramuscular Route in Man.* Edgewood Arsenal, Md: Chemical Research and Development Laboratory; 1965. Technical Memorandum 2-30.

34. Aghajanian GK, Bing OH. Persistence of lysergic acid diethylamide in the plasma of human subjects. *Clin Pharmacol Ther.* 1964;5:611–614.

35. Kleinwachter I. Observations concerning the effectiveness of extract of Calabar against atropine poisoning. *Berl klin Wschr.* 1864;1:369–377.

36. Forrer GR, Miller JJ. Atropine coma: A somatic therapy in psychiatry. *Am J Psychiatry.* 1958;115:455–458.

37. Forrer GR. Atropine toxicity in the treatment of schizophrenia. *Journal of the Michigan State Medical Society.* 1950;49:184–185.

38. Crowell EB, Ketchum JS. The treatment of scopolamine induced delirium with physostigmine. *Clin Pharmacol Ther.* 1967;8:409–414.

39. Ketchum JS, Sidell FR, Crowell EB, Aghajanian GK, Hayes AH. Atropine, scopolamine, and Ditran: Comparative pharmacology and antagonists in man. *Psychopharmacologia.* 1973;28:121–145.

40. Bell C, Gershon S, Carroll B, Holan G. Behavioural antagonism to a new

psychotomimetic: JB-329. *Arch Intern Pharmacodyn Ther.* 1964;147:9–25.

41. Kitzes DL, Ketchum JS, Weimer JT, Farrand RL. *The Human Assessment of EA 3580 by the Aerosol Route.* Edgewood Arsenal, Md; 1967. Edgewood Arsenal Technical Report 4041.

42. Ketchum JS, Kitzes D, Copelan H. *Effects of EA 3167 in Man.* Edgewood Arsenal, Md; 1973. Edgewood Arsenal Technical Report 4713.

43. McCarroll E, Markis J, Ketchum JS, Houff W, Sim VM. Effects on Performance of the Administration of EA 3834 to Members of a Small Infantry Element Under Simulated Combat Conditions. Edgewood Arsenal, Md; 1971. Edgewood Arsenal Technical Report 4633.

44. Duvoisin RC, Katz R. Reversal of central anticholinergic syndrome. *JAMA.* 1968;206:1963.

45. Heiser JF, Gillin JC. The reversal of anticholinergic drug-induced delirium and coma with physostigmine. *Amer J Psychiatry.* 1971;127:1050.

46. Sidell FR. *Use of Physostigmine by the Intravenous, Intramuscular, and Oral Routes in the Therapy of Anticholinergic Drug Intoxication.* Biomedical Laboratory, Edgewood Arsenal, Md; 1976. EB-TR-76-12.

47. Ghoneim MM. Antagonism of diazepam by physostigmine. *Anesthesiology.* 1980;54:372.

48. Rumack BH. Anticholinergic poisoning: Treatment with physostigmine. *Pediatrics.* 1973;52:449–451.

49. Daunderer M. Physostigmine salicylate as an antidote. *Int J Clin Pharmacol Ther Toxicol.* 1980;18:523–535.

50. Directorate of Medical Research. *Guide to the Management of BZ Casualties, I.* Edgewood Arsenal, Md; 1965.

51. Ketchum JS, Tharp B, Crowell E, Sawhill D, Vancil M. *The Human Assessment of BZ Disseminated Under Field Conditions.* Edgewood Arsenal, Md; 1967. Edgewood Arsenal Technical Report 4140.

52. Sidell FR, Aghajanian GK, Groff WA. The reversal of anticholinergic intoxication in man with the cholinesterase inhibitor VX. *Proc Soc Exp Biol Med.* 1973;144:725–730.

53. Lipka LJ, Lathera CM. Psychoactive agents, seizure production, and sudden death in epilepsy. *J Clin Pharmacol.* 1987;27:169–183.

54. Aghajanian GK, Bing O. 1963. Unpublished report. Cited in: Ketchum JS. Incapacitating compounds. In: *Proceedings of 1st Meeting of the Quadripartite Standing Working Group on Chemical Warfare.* Edgewood Arsenal, Md; 1965.

55. Freedman DX. Chairman and Professor of Psychiatry and Pharmacology, University of Chicago Medical School, Chicago, Ill. Personal communication,

1973.

56. Wikler A. Characteristics of opioid addiction. In: Jarvik ME, ed. *Psychopharmacology in the Practice of Medicine.* New York, NY: Appleton-Century-Crofts; 1977: 419–432.

Chapter 12

RIOT CONTROL AGENTS

FREDERICK R. SIDELL, M.D.*

INTRODUCTION

HISTORY

CS (*o*-CHLOROBENZYLIDENE MALONONITRILE)
- Physical Characteristics
- Clinical Effects

CN (1-CHLOROACETOPHENONE)
- Physical Characteristics
- Clinical Effects

SEVERE MEDICAL COMPLICATIONS FROM THE USE OF CS AND CN

OTHER RIOT CONTROL COMPOUNDS
- DM (Diphenylaminearsine)
- CR (Dibenz(*b,f*) -1:4-oxazepine)
- CA (Bromobenzylcyanide)

MEDICAL CARE
- Decontamination
- Skin
- Eye
- Respiratory Tract
- Cardiovascular System

FUTURE USE

SUMMARY

*Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense,

Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014

INTRODUCTION

Riot control agents are compounds that cause temporary incapacitation by irritation of the eyes (tearing and blepharospasm), causing them to close, and irritation of the upper respiratory tract. They are often called irritants, irritating agents, and harassing agents; the general public usually calls them tear gas. Like most of the other chemical agents discussed in this textbook, riot control agents are known by two-initial designators that are neither abbreviations nor acronyms of their chemical names but are most akin to code names. Hence an explanation of the derivations of the names is usually not attempted here.

Three types of riot control agents are recognized: lacrimators, which primarily cause lacrimation and eye irritation; sternutators, which mainly cause sneezing and irritation of the upper respiratory tract; and vomiting agents, which additionally cause vomiting. Because these compounds—CS, CN, DM, CR, and CA—have a number of characteristics in common, they are grouped together as riot control agents in this chapter. The small distinctions among them are noted in the discussion of each agent. Table 12-1 lists the chemical, physical, environmental, and biological properties of the three major agents: CS, CN, and DM. Characteristics common to all compounds in this category are

- a rapid time of onset of effects (seconds to several minutes),
- a relatively brief duration of effects (15–30 min) once the victim has escaped the contaminated atmosphere and has decontaminated (ie, removed the material from his clothing), and
- a high safety ratio (the ratio of the lethal dose [estimated] to the effective dose).

TABLE 12-1
CHEMICAL, PHYSICAL, ENVIRONMENTAL, AND BIOLOGICAL PROPERTIES OF CS, CN, AND DM

Properties	*o*-Chlorobenzylidene Malononitrile (CS)	1-Chloroacetophenone (CN)	Diphenylaminearsine (DM)
Chemical and Physical			
Boiling point	310°C	248°C	410°C with decomposition

Vapor pressure	0.00034 mm Hg at 20°C	0.0041 mm Hg at approx 20°C	4.5×10^{-11} mm Hg at 25°C
Density:			
Vapor	—	5.3*	—
Liquid	—	1.187 g/mL at approx 58°C	—
Solid	Bulk: 0.24–0.26 g/cm^3 Crystal: 1.04 g/cm^3	1.318 g/cm^3 at approx 20°C	Bulk: <1 g/cm^3 Crystal: 1.65 g/cm^3 at 20°C
Volatility	0.71 mg/m^3 at 25°C	34.3 mg/m^3 at approx 20°C	Not of practical significance
Appearance and odor	White crystalline powder with pungent odor (pepper)	Fragrant (like apple blossoms)	Yellow-green, odorless, crystalline substance
Solubility:			
In Water	Insoluble	Insoluble	0.0064 g/100 g at room temperature
In Other Solvents	Organic solvents: complete	Organic	Best: acetone, 13.03 g/100 g at 15°C
Environmental and Biological			
Detection	No detector	No detector	No detector
Persistency:			
In Soil	Varies	Short	Persistent
On Materiel	Varies	Short	Persistent
Biologically Effective Amount:			
Aerosol (mg• min/m^3)	LCt_{50}: 60,000 ICt_{50}: 3–5	LCt_{50}: 7,000–14,000 ICt_{50}: 20–40	LCt_{50}: 11,000–35,000 ICt_{50}: 22–150; nausea, vomiting: approx 370

*Compared with the density of air
LCt_{50}: the concentration • time (Ct) that is lethal to 50% of the population exposed
ICt_{50}: the Ct that incapacitates 50% of the population exposed

Riot control agents all produce effects by sensory irritation, causing extreme discomfort or pain in the organs affected. The eyes, nose, and respiratory tract are

the primary organs affected, although the skin is also often involved. The compounds produce temporary disability because the extreme eye irritation and blepharospasm cause the eyes to close temporarily, and the irritation of the airways causes coughing, shortness of breath, and sometimes retching or vomiting. One of these compounds, DM, is noted for also causing vomiting and malaise.

The United States does not recognize riot control agents as chemical warfare agents as defined in the Geneva Convention of 1925. The Geneva Gas Protocol of 1925 was ratified by the United States on 22 January 1975. At that time, the United States interpreted the protocol as prohibiting the first use of lethal chemicals, but not of nonlethal ones such as riot control agents or herbicides.

During the Vietnam War, before the protocol ratification, the United States had used the riot control agent CS (*o*-chlorobenzylidene malononitrile) extensively. On 8 April 1975, President Ford signed Executive Order 11850, which unilaterally renounced first use of riot control agents in armed conflict, with specified exceptions. These exceptions include first use for riot control in areas under direct U.S. military control (including control of rioting prisoners of war), use in rescue operations, use in situations in which civilians screen or mask attacks, and use in rear echelons to protect convoys from terrorists or similar groups. Presidential approval is required in advance for either first or retaliatory use of riot control agents in war.

Of all the compounds discussed in this book, riot control agents are perhaps the most scrutinized by the public. In civilian life, law enforcement agencies use riot control agents in civil disturbances, riots, or to avoid using deadly force. The military commonly uses them in training. The symptoms described below, therefore, will be familiar to most military personnel.

HISTORY

Irritant compounds were allegedly used by Marcus Fulvius against the Ambracians in the second century BC. The Byzantines apparently knew of the efficacy of using irritant substances to harass the enemy. Plutarch described a Roman general who used an irritant agent cloud in Spain to drive the enemy out of concealment in caves,[1] a use similar to that of the United States in Vietnam 2,000 years later.

Modern use probably began in the 1910–1914 period, when ethylbromoacetate was employed against criminals by French police. At the beginning of World War I, some of these former policemen, who were then in the French army, began to use some of these munitions on the battlefield with some degree of success. Although the German use of chlorine at Ypres, Belgium, on 22 April 1915 is generally heralded as the beginning of chemical warfare on the modern battlefield, irritating substances had already been in use for about a year. During World War I, approximately 30 different compounds were tried for their irritant effects, usually without much success.[2] As noted above, a riot control agent was widely used in the Vietnam War.

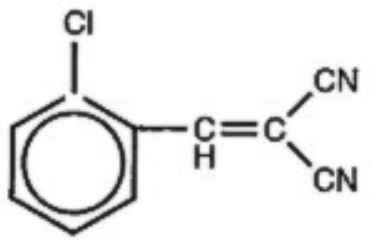
Riot control agents gained some notoriety when they were used in civil disturbances in Paris, France, in 1968; in Londonderry, Northern Ireland, in 1969;

in several protest demonstrations in the United States in the late 1960s; and in prison riots. More recently, riot control agents were used in an unsuccessful attempt to drive the Branch Davidians from their compound near Waco, Texas, in February 1993.

Probably the best known of these compounds is CN (1-chloroacetophenone); it has been used for many years and is commercially available in devices for self-protection under its proprietary name, Mace (the chemical, not the devices, is manufactured by General Ordnance Equipment Corp., Pittsburgh, Pa.). CS is the compound that is used by the military in most countries and almost exclusively by law enforcement agencies throughout the world.

CS (*o*-CHLOROBENZYLIDENE MALONONITRILE)

The riot control agent known as CS (*o*-chlorobenzylidene malononitrile) was first synthesized in 1928 by Corson and Stoughton (hence its code name). It replaced CN as the standard riot control or irritant agent in the U.S. Army in 1959. In the late 1950s, CS was also adopted by most U.S. law enforcement agencies and by the military and law enforcement agencies of other countries, because CS is more effective than CN (it causes effects at lower doses) and is less toxic (ie, its LCt_{50}, the vapor or aerosol exposure [concentration • time] that is lethal to 50% of the exposed population, is higher).



CS

Physical Characteristics

CS is a white, crystalline solid with a low vapor pressure. It is almost insoluble in water and only slightly soluble in ethyl alcohol and carbon tetrachloride. Because of these physical characteristics, decontaminating buildings, furniture, and other material after CS use in urban riots is difficult. Dissemination of CS can be by explosive dispersion of a powder or solution, by dispersion of the powder in a fine state, by spraying a solution, or by releasing as smoke from a pyrotechnic mixture.[3] The method of dissemination may influence the severity of the injury (see eye injury for CN). The Material Safety Data Sheet, which the manufacturer includes in each package, assigns it a flammability rating of 4 (on a scale of 0 to 4). The agent was a large contributor in the conflagration that burned the Branch Davidian compound and its inhabitants in Waco, Texas, in 1993.

CS tends to agglomerate when used and resists weathering poorly (losing its effectiveness). During the mid 1960s, hydrophobic formulations of CS, CS1 and CS2, were developed. The former is a micronized powder with 5% hydrophobic silica aerogel; the latter is a siliconized, microencapsulated form of CS1. CS1 and CS2 last for several weeks and are a persistent hazard during military operations. Because of their persistence, they have not been used for civil

disturbances.

Clinical Effects

Clinical effects common to all of these riot control agents are listed in Exhibit 12-1. In the eye, an initial burning feeling or irritation progresses to pain accompanied by blepharospasm, lacrimation, and conjunctival injection. The intense blepharospasm causes the eyes to close. Photophobia is often present and may linger for an hour. The mucous membranes of the mouth, including the tongue and palate, have a sensation of discomfort or burning, with excess salivation. Rhinorrhea is accompanied by pain inside the nose and perhaps around the external nares. When inhaled, these compounds cause a burning sensation or a feeling of tightness in the chest, with coughing, sneezing, and increased secretions. On unprotected skin, especially if the air is warm and moist (see skin effects of CS), these agents cause tingling or burning; within a few minutes, erythema may develop at the exposed sites.

EXHIBIT 12-1
CLINICAL EFFECTS OF RIOT CONTROL AGENTS

Eye
- Burning, irritation
- Conjunctival injection
- Tearing
- Blepharospasm
- Photophobia

Skin
- Burning
- Erythema

Gastrointestional Tract
- Gagging
- Retching
- Vomiting

Airways
- Sneezing
- Coughing
- Tightness in the chest
- Irritation
- Secretions

Nose
- Rhinorrhea
- Burning pain

Mouth
- Burning of mucous membranes
- Salivation

Tolerance to Exposure

Typically, effects appear within seconds of exposure to an aerosolized compound and worsen as long as one remains in the cloud. Most effects slowly dissipate, starting within a few minutes after one leaves the contaminated area. By 30 minutes, most effects have completely abated, although the usually mild erythema may persist for 1 to 2 hours. If one does not leave shortly after the onset of irritation, the effects might become more severe, with marked coughing, gagging, retching, and vomiting.

Most individuals note marked harassment at a concentration of 3 to 5 mg/m^3 and leave the area as soon as possible.[4] Tolerance develops, however, in those who have been in close contact with CS for a period of time, such as production or laboratory workers. Those who have developed tolerance can stay in their accustomed concentration of CS and the discomfort does not increase, and, in fact, may decrease. Those who work in a CS environment and get CS on their

clothing often become so accustomed to its effects that they wear the clothing out of the area without remembering, only to have others complain.

Tolerance was examined experimentally in an early study[5] in which men were placed in a concentration of 0.43 mg/m^3; the concentration was slowly increased to 2.0 mg/m^3 over 60 minutes. If the men were able to withstand the initial effects, they could remain at the higher concentration. During this time, some subjects played cards and two attempted to read.

In a similar study,[4] when four subjects were exposed to a low concentration that was increased to 6 mg/m^3 over 10 minutes, three subjects left before the time was up. In contrast, when the same subjects were exposed to the same low concentration that was slowly increased to 6 mg/m^3 over a 30-minute period, three remained for 30 more minutes (the fourth subject left after 2 min because of coughing, but voluntarily returned for the remainder of the period). Individuals did not develop tolerance to the compound after ten exposures of 1 to 13 mg/m^3 over a 2-week period.

Duration of tolerance was reduced in exercising individuals, presumably because of deeper breathing and deeper penetration of the particles into the lung, and chest symptoms were more pronounced than when the subjects were exposed while resting. An increase in tolerance was noted when the temperature was low (–18°C; 0°F); a slight decrease in tolerance occurred in a hot environment (36°C). Skin symptoms (such as a burning sensation) were more prominent at the hot temperature than at moderate (20°C–32°C) temperatures.[4]

One might expect that personality and mental set could determine tolerance to CS; a dedicated hijacker, for example, might be able to resist its effects. To test for a correlation between personality and tolerance to an irritant compound, a group of men were exposed to CS, then tested on the Minnesota Multiphasic Personality Inventory (MMPI).[6] Those individuals with less tolerance to CS were characterized by the MMPI by greater use of denial, repression, and somatic complaints than the more tolerant group. Furthermore, the more tolerant group had a higher mean general intelligence score (127 compared with 100 for the less tolerant group).

In a similar study,[7] subjects with high scores classified as abnormal on certain MMPI scales tolerated less CS than did subjects with normal scores. After the administration of diazepam, the tolerance to CS was significantly increased in the group with abnormal scores, but not in the group with normal scores. This result suggests that anxiety, which was reduced more by diazepam in the group with abnormal scores, plays a role in tolerance.

Respiratory Tract Effects

Inasmuch as CS is usually disseminated as an aerosol (powder or in solution), the most common route of absorption is by inhalation. In an LCt_{50} study,[8] four species (rat, rabbit, guinea pig, and mouse) were exposed to aerosolized CS powder for 5 to 60 minutes. The LCt_{50} values (based on mortality within 14 d) ranged from 50,010 mg•min/m^3 (in the mouse) to 88,480 mg•min/m^3 (in the rat). No animal died during exposure; most of those that died afterwards did so within 2 days. The lungs of those dying were congested and edematous, and many had hemorrhages. The trachea was congested with moderate amounts of mucus. On microscopical examination, moderate to marked congestion of alveolar capillaries and intrapulmonary veins, inter- and intra-alveolar hemorrhages, and excess secretions in the smaller airways were seen. Animals that died after 48 hours also had evidence of early bronchopneumonia. Those that survived for 14 days had normal lungs on gross and microscopic examination.

Pyrotechnically dispersed smoke from a CS grenade was used in a similar study design with the same four species.[9] At high concentrations and exposure times of 5 to 20 minutes, the LCt_{50} values (based on mortality within 14 days) ranged from 35,000 mg•min/m^3 (in the guinea pig) to 76,000 mg•min/m^3 (in the mouse). No animal died during exposure, and only two died within 12 hours of removal from the chamber. With concentrations ranging from 31.9 to 56.4 mg/m^3 and a 5-hour per day exposure for 1 to 7 days, the LCt_{50} values (14-day mortality) were from 25,000 mg•min/m^3 (rat) to 54,000 mg•min/m^3 (rabbit).

The lungs of animals that died before 14 days were edematous and congested, with areas of hemorrhage and excessive amounts of mucus in the trachea and bronchi. The alveolar capillaries and intrapulmonary veins were congested, with areas of alveolar hemorrhages and hemorrhagic atelectasis. A few had edema, but no inflammatory cell infiltration was noted. In addition, most animals had evidence of circulatory failure, with dilated right ventricles and enlarged livers, kidneys, and spleens.[9]

Animals that survived 14 days had no abnormalities on pathological examination. The investigators pointed out that the presence of pulmonary edema and hemorrhages in the absence of inflammatory cell infiltration suggests that the smoke caused direct injury to the pulmonary capillary endothelium and that the main cause of death was pulmonary damage. They also commented that, because of the agglomeration of the smoke particles and subsequent precipitation of the compound, concentrations as high as those used could not be maintained under operational conditions.[9]

Two hundred sixty-four rats and 250 hamsters were exposed to CS concentrations of 750, 480, or 150 mg/m^3 for 30, 60, or 120 minutes, respectively (the calculated Ct values were 22,500, 28,800, and 18,000 mg•min/m^3, respectively). Only one animal died in the first 6 hours after exposure; 33 died within 48 hours, and 31 of these were in the 480-mg/m^3 (60-min) group. Those dying within 48 hours had moderately severe congestion in the lungs, with

alveolar hemorrhage and edema in some. Acute tubular necrosis was present in some of the animals. In contrast, no deaths occurred (in 48 animals) within 48 hours in the 750-mg/m^3 group, and only two deaths (in 240 animals) occurred in the 150-mg/m^3 group. In these animals and in those sacrificed at 24 hours and onward, minimal abnormalities were found.[9]

In a continuation of this study,[9] rats were exposed to CS at 1,000 to 2,000 mg/m^3 for 5 minutes per day for 5 days. None of the rats died. Minimal pathological changes were found on sacrifice of the animals, but 5 of 56 had bronchopneumonia. A group of 50 rats was exposed to a concentration of 12 to 15 mg/m^3 for 80 minutes daily for 9 days.[9] Five rats died from bronchopneumonia and on sacrifice, 5 of the remaining 45 rats were found to have bronchopneumonia.

In a long-term study,[10] mice were exposed to 3 or 30 mg/m^3 of CS for 60 minutes per day for 55 exposures and then observed for 6 months longer. A daily exposure of 192 mg/m^3 for 60 minutes per day was stopped after three exposures because of deaths. Rats and mice were also exposed to these doses daily for 120 days; daily exposure at 236 mg/m^3 was stopped after 5 days. At the two low concentrations (3 and 30 mg/m^3), the number of deaths over the year of study did not exceed the number of deaths in control groups, which were exposed to air in the exposure chamber daily. After a year, mice receiving 30 mg/m^3 had a statistically significant increase in chronic laryngitis and tracheitis, but otherwise the pathological findings for these animals were not different from those of the control group. In particular, no relationship was found between specific tumors and the total dose of CS.

Dermatological Effects

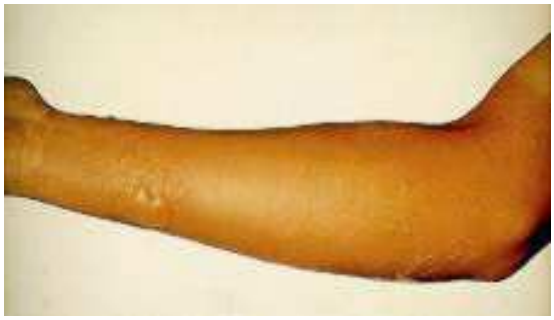
CS is a primary irritant to the skin. In addition, individuals may develop allergic contact dermatitis after an initial, uneventful exposure to it.

Typically, several minutes after an acute exposure to a low concentration of CS, a prickly feeling or burning is felt in exposed areas of skin. This sensation is more noticeable if the skin is wet or freshly abraded (eg, after shaving). The sensation may be accompanied or followed by erythema, which usually persists for an hour or less. Under certain circumstances—involving the amount of CS, the temperature, and the humidity—a more intense erythema may follow about 2 hours later. If the amount of CS, the temperature, and the humidity are all high, the erythema becomes even more severe, and edema and vesication appear hours later. The time course is the same as that for the skin damage after exposure to mustard.

To test the effects of CS on human skin, the arms of volunteers were exposed to high concentrations of CS thermally generated from an M7 grenade.[11] The exposure was at a temperature of 36°C and humidity of 100%; the average concentration was 300 mg/m^3, and exposure times ranged from 15 to 60 minutes. All subjects noted stinging about 5 minutes after onset of exposure. After being withdrawn from the apparatus, the arms were rinsed with cold, running water to remove the powder that clung to hairs; this procedure caused the stinging to increase. C*t* values of 4,440 and 9,480 mg•min/m^3 caused an immediate skin response: a patchy, vascular erythema, which subsided after 30 minutes with no further reaction.

C*t* values of 14,040 and 17,700 mg•min/m^3 caused a more severe initial dermal response, which required 3 hours to disappear. After 12 to 24 hours, a delayed reaction, consisting of first- and second-degree burns, appeared. Blistering occurred in four of the eight subjects (Figure 12-1). With treatment (discussed below), these lesions resolved in 10 to 14 days; by 6 weeks later, a small amount of post-inflammatory pigmentation remained.[11]

a



b

Fig. 12-1. (a) Erythema 25 hours after exposure to a high C*t* (the product of concentration of vapor or aerosol • time of exposure; in this instance, 14,040 mg•min/m^3) of CS at 97°F and 100% humidity. **(b)** The same skin lesions at 45 hours, with vesication. Reprinted from Hellreich A, Goldman RH, Bottiglieri NG, Weimer JT. *The Effects of Thermally-Generated CS Aerosols on Human Skin.* Edgewood Arsenal, Md: Medical Research Laboratories; 1967: 19. Technical Report 4075.

By means of a sleeve with removable patches, arms of volunteers in another study[12] were exposed to CS thermally generated from an M7 grenade. The patches were removed at appropriate times to give C*t* exposures of 1,550 to 33,120 mg•min/m^3 at tropical conditions (37°C; 98% relative humidity) or at one of three temperate conditions (14°C and 41% relative humidity; 20°C and 95% relative humidity; 22°C and 72% relative humidity). No subjects at 14°C or 22°C had the delayed erythema at C*t* values of up to 25,560 mg•min/m^3. At 20°C (95% relative humidity), all four subjects had minimal delayed erythema at C*t* values of 26,025 or 30,240 mg•min/m^3. In contrast, at the tropical conditions, the effective C*t* for producing delayed erythema was 3,500 mg•min/m^3.

The authors of the study pointed out that many variables make it difficult to predict which individuals might be more sensitive than others. Among these variables are skin pigmentation, eye color, complexion, and susceptibility to sunburn.[12]

Although the conditions of these studies were severe, serious skin reactions can occur under milder, more common conditions. First- and second-degree burns were produced in

a group of U.S. Army Chemical Corps officers on a field exercise.[13] Temperature and humidity were high, it had been raining heavily, and their uniforms were soaked through. The officers, who were wearing fatigues, ponchos, and M17 protective masks, were hit with a cloud of micropulverized CS1 from a disperser; soon afterwards, they noted burning of their unprotected skin. About 2 hours later, some of the men hosed off and some changed clothes, but most did neither. About 14 to 16 hours after exposure, blistering began, and all of the men who had not hosed off or changed clothes eventually developed vesication.

Firemen in Washington, D. C., were frequently exposed to CS during the riots of April 1968; in addition, they were exposed to CS as they entered buildings in which CS had been disseminated. The CS on floors or furniture was reaerosolized both by their movement and by the force of water from their hoses. They later developed erythema and edema of periorbital skin and other exposed areas.[14]

Earlier investigators reported vesication after CS patch testing.[4] They also mixed CS with sodium hypochlorite (household bleach) and found that in all subjects tested, the product caused a reaction that was much more severe than that produced by CS alone. For that reason, hypochlorite is not recommended for decontamination of CS on skin. (A hypochlorite is successfully used as a decontaminant for most other chemical agents.)

CS is a primary irritant and causes contact dermatitis, typically in workers in CS-manufacturing or -packing plants. A reaction is more common in warm weather and high humidity or in sweating subjects. The lesion begins some hours after exposure as an erythema, with burning and stinging; the area becomes edematous at about 24 hours, then vesicles or bullae may appear. Common sites are those of partial occlusion, such as the areas under the cuff or glove and under the shirt collar.

CS is also a sensitizer and can cause allergic contact dermatitis, which is the result of a delayed hypersensitivity reaction. An initial exposure may not cause a reaction, but a later exposure to even a small amount produces an often severe dermatitis, with erythema, edema, vesication, and, in severe instances, purpura and necrosis.

Differentiation of the two reactions—primary irritant dermatitis and allergic contact dermatitis—is often difficult clinically and usually requires patch testing.

Ophthalmological Effects

The eye is a sensitive target organ of riot control agents. In studies[14,15] on humans, CS (0.1% or 0.25% CS in water; 1.0% CS in trioctyl phosphate), when placed or sprayed into the eyes, caused inability to open the eyes for 10 to 135 seconds. A transient conjunctivitis but no corneal damage as assessed by slitlamp biomicroscopy resulted.

In another study,[16] subjects were exposed to CS2 (powder dispersal) or CS powder (thermally disseminated) at 0.1 to 6.7 mg•min/m^3 for 20 seconds to 10 minutes. Their visual acuity was tested at intervals during and after the exposure. Subjects who could keep their eyes open during the exposure to read the chart had minimally impaired visual acuity, and no appreciable change in acuity from

preexposure readings was found.

In an investigation of the ophthalmic toxicity of CS,[17] rabbit eyes were contaminated with CS in solution (0.5%–10% in polyethylene glycol), as a solid, and as a pyrotechnically generated smoke (15 minutes at 6,000 mg/m^3. The effects were most severe with the solution and least severe with the smoke. After exposure to the smoke, the eyes had a transient, slight excess of lacrimation and congestion of conjunctival vessels lasting 24 hours; the tissues were normal when examined 7 days later.

The solid (0.5–5.0 mg) caused lacrimation at all doses, blepharitis that increased with dose and lasted up to a week, and chemosis at 5 mg, which was mild and lasted 3 days. Minimal iritis and keratitis, of 24 hours' duration, were seen in two of five animals receiving 5 mg. At concentrations of 1% and higher, CS in solution caused conjunctivitis and iritis, chemosis, keratitis, and corneal vascularization; the lesion was more severe and lasted longer with the higher doses. Histological examination indicated patchy denudation of corneal epithelium and a neutrophilic infiltration of the cornea.[17]

Reports of severe eye injuries from riot control agents have involved the agent CN. They are discussed below in the CN section.

Gastrointestinal Tract Disturbances

A handful of instances in which an individual ate CS are known. In all but two cases, children were the victims. Typically, they were playing in an old impact area on a military installation and came across some shells containing a powdery substance, which they ate. One adult ingestion was an attempt at suicide by an otherwise healthy young man; the other was an individual who ate a CS pellet (820 mg) after a friend told him it was a vitamin pill.[18]

The oral LD_{50} (dose that is lethal to 50% of the exposed population) of CS was found to be 143 mg/kg in the female rabbit, the most sensitive of three species studied (the rat, about 1,300 mg/kg; the guinea pig, 212 mg/kg; and the male rabbit, 231 mg/kg).[8] The animals that died had multiple, extensive hemorrhagic erosions of the gastric mucosa, with perforation of the wall, and a few had increased peritoneal fluid. In those surviving for several days, intraabdominal adhesions were found. After male rats and female guinea pigs received 0.5 LD_{50} of CS by stomach tube, and male rabbits received 0.3 LD_{50} by this route, the incidence of wet or runny stools was no greater than that for the control vehicle, polyethylene glycol 300 (PEG300).[19] The investigators concluded that diarrhea is not an effect of ingested CS. They also suggested that rioters would not have diarrhea from CS exposure, since they would be unlikely to swallow this much, but that an intensely emotional experience such as being in a riot may itself be a cause of disturbed bowel function. In another study,[20] the oral LD_{50} varied widely in rats (178–358 mg/kg), depending on the solvent used. After death, moderate to severe gastroenteritis was noted on gross examination.

No deaths or severe complications in humans from ingestion of CS are known. The young man mentioned above who had attempted suicide by CS ingestion was given large amounts of what were described as “saline cathartics”

and over the next 24 hours had repeated episodes of severe abdominal cramps and diarrhea; whether these symptoms were due to the illness or the treatment is unknown. A surgical team examined the patient early and stood by during the acute phase. The patient recovered uneventfully. The adult who ate a CS pellet was given liquid antiacid and viscous lidocaine orally and droperidol intravenously. He vomited twice, had six voluminous watery bowel movements without blood, and otherwise recovered uneventfully. Blood cyanide was less than 1 µg/dL 18 hours after ingestion (see section on metabolism).[18]

Metabolic Effects

Both in vivo and, in water, in vitro, CS (*o*-chlorobenzylidene malononitrile) is hydrolyzed to 2-chlorobenzaldehyde and malononitrile. Malononitrile contains two cyanide moieties, and it is thought that at least one of these is liberated and attaches to sulfur via the enzyme rhodanese to form thiocyanate, which is excreted in the urine.

Some authors have suggested that cyanide contributes to mortality in CS-caused deaths.[21,22] In dogs given CS by the aerosol or intravenous routes, the plasma concentrations of thiocyanate increased over the following 24 to 48 hours, presumably because of transformation of the liberated cyanide to thiocyanate by combination with endogenous sulfur.[21] After CS was given intraperitoneally, the mortality was markedly decreased by the intravenous administration of thiosulfate, which may have provided additional sulfur for the transformation of cyanide to thiocyanate.[21] Also, after intravenous administration of CS or malononitrile, the signs and the times to death were similar (15–60 min), suggesting that both caused effects by the same mechanism.[22] In this report, the authors also noted the similarities of signs and times of death for these two compounds, compared with cyanide administered intravenously.

One author of the latter report, however, clearly notes in a later communication[23] that the mode and time of death differ depending on whether CS is administered by the intravenous route or by aerosol. As noted earlier in the discussion of respiratory effects for CS, animals exposed to far greater than the lethal C*t* do not die during exposure or immediately afterwards, but many die hours later, in contrast to the usually rapid death caused by cyanide. Moreover, the lung damage found on pathological examination is adequate to explain death.[8,9]

In addressing this issue, a British report[3] suggests that whereas cyanide might be a causative factor in the rapid deaths occurring after intravenous administration, it is not a factor in death after aerosol administration. If one were to absorb completely all the CS during a 1-minute exposure at 10 mg/m^3, and if both cyanides on the molecule were liberated—and evidence suggests that only one is liberated—the total amount of cyanide received would be equivalent to that received from two puffs of a cigarette.

Other Physiological Responses

When subjects were exposed to CS concentrations of 1 to 13 mg/m^3 daily for 10 days, their airway resistances, measured 2 to 4 minutes after the fourth and tenth exposures, were unchanged from the preexposure values.[4] Tidal volume, vital capacity, and peak flow in 36 subjects also were unchanged when they were measured immediately and 24 hours after exposure to CS.[5]

Heart rates of subjects were lower immediately after exposure compared with preexposure values.[4] Subjects entered a chamber of CS with masks on; immediately on removing their masks, their mean blood pressure increased by 20 mm Hg systolic and 11 mm Hg diastolic. After they had remained in the CS for 20 minutes, however, their blood pressures were comparable to the preexposure values.[4] The blood pressures of subjects drenched with dilute solutions of CS were transiently elevated to about 150/90 mm Hg.[24]

After daily exposures to CS for 10 days, seven subjects had no alterations in blood sodium, potassium, alkaline phosphatase, or bromsulfophthalein; one of the seven had an increase in thymol turbidity. No chest radiograph or urinary changes were seen.[4] In another study,[5] although significant changes were seen in some blood chemistries after exposure, all values were within the normal range.

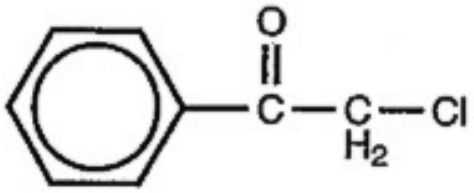
Pregnant rats and rabbits were exposed to CS aerosols at concentrations of 6, 20, or 60 mg/m^3 for 5 minutes on days 6 to 15 and 6 to 18 of gestation, respectively. In addition, rats were given CS (20 mg/kg) intraperitoneally on days 6, 8, 10, 12, and 14 of gestation. No embryolethality or teratogenicity was evident.[25]

CS and some of its metabolites were found not to have mutagenic effects in the Ames *Salmonella typhimurium* assay with microsome supplementation.[26] In addition, no mutagenic effects were found in assays for reverse mutations in *S typhimurium* after exposure to CS, in assays for sex-linked, recessive lethal mutations in sperm cells after *Drosophila* were fed CS, or in chromosomes of bone marrow erythrocytes of mice exposed to CS.[27] The authors of another study[28] of rats and *Salmonella* concluded that CS did not induce point mutations or carcinogenic processes mediated by DNA binding. However, CS did give a positive response in the forward mutation assay in mouse lymphoma cells.[29]

CN (1-CHLOROACETOPHENONE)

Physical Characteristics

Like CS, the riot control agent known as CN (1-chloroacetophenone) is a solid or powder and can be disseminated as a smoke generated from a grenade or other device, or in powder or liquid formulations. Under the trade name Mace, it is



CN

in most devices sold for self-
protection, although today it is commonly mixed with or is being replaced by capsaicin (pepper spray).

CN was first synthesized by Graebe in 1871 and was used in World War I. Before the late 1950s, it was the standard tear gas used by the military and law enforcement agencies.

The harassing concentration for CN is about 10 mg/m^3, compared with about 4 mg/m^3 for CS. It is more toxic than CS, and the human LCt_{50} (median lethal Ct) has been estimated to be 7,000 mg•min/m^3 for pure aerosol and 14,000 mg•min/m^3 for a commercial grenade.[30]

Clinical Effects

In general, the clinical effects caused by CN are the same as those caused by CS. The harassing dose is higher and CN is more toxic and more likely to cause serious effects, particularly in skin and eyes (see below). Most effects from exposures to a low concentration will disappear within 20 to 30 minutes.

Respiratory Tract Effects

In studies parallel to those described above for CS, CN was found to be 3- to 10-fold more toxic (lower LCt_{50}) than CS in rats, rabbits, guinea pigs, and mice.[8] In addition, the pathological findings in the lungs were more severe, with more edema; patchy acute inflammatory cell infiltration of the trachea, bronchi, and bronchioles; and more evidence of early bronchopneumonia.

Dermatological Effects

A textbook published in 1925 states that CN in field concentrations does not damage human skin; however, the powder might produce burning: “slight rubefaction, and sometimes small vesicles appear.”[31(p171)] Early cases of CN dermatitis—one of primary irritant dermatitis in a soldier and three in civilian employees who probably had allergic dermatitis from working around CN for years—were described several years later.[32]

A severe allergic reaction to CN developed after a 43-year-old military recruit went through the CN training chamber routine (ie, an individual spends 5 min in the chamber masked, then removes the mask and exits the chamber). Within 5 minutes after exiting, the patient complained of generalized itching, which became progressively worse over the following hours. Four hours after exiting, he had a diffuse and intense erythema over his entire body except his feet and the portion of his face covered by the mask. His temperature was 38.9°C (102°F) and rose to 39.4°C (103°F) the next day. By 48 hours after exposure, he had vesication and later developed severe subcutaneous edema that “strikingly altered the appearance of the face”[33(p1879)] and severe generalized itching. Over the next 4 days, the signs subsided, and desquamation, which was profuse at day 6, gradually decreased. The patient had developed itching during a tear gas

exercise 17 years previously but had not been exposed in the interim.[33]

A police officer received an initial exposure to CN and 5 years later, on repeated exposure, developed recurrent attacks of what was probably allergic contact dermatitis. The source of the repeated exposures was unrecognized until he realized that he had been using outdated CN bombs for eradication of rodents on his property.[34]

CN (0.5 mg), when left in place for 60 minutes, caused irritation and erythema on the skin of all humans tested in one study,[35] whereas CS caused no effects in amounts less than 20 mg. When the CN was moist, 0.5 mg caused vesication in most subjects, whereas vesication was not seen after exposure to 30 mg or less of CS.

In addition to being a more potent primary irritant on the skin than CS, CN is also a more potent skin sensitizer.[36] Several people developed allergic contact sensitivity to CN after patch testing.[37] Because of the high incidence of sensitization in test subjects, CN should be considered a potent allergic sensitizer, and those who are frequently exposed should be aware of the high likelihood of developing allergic dermatitis.[38]

Ophthalmological Effects

The irritation caused by CN in the eye signals avoidance and, by causing lacrimation and blepharospasm, initiates a defense mechanism. High concentrations of CN sprayed into the eyes from a distance have caused edema of the corneal epithelium and conjunctiva and many minute epithelial defects in the cornea.[39] Healing was rapid, however.

More lasting or permanent effects may occur when CN is released at close range (within a few meters), particularly if it is from a forceful blast from a cartridge, bomb, pistol, or spray. One study[40] based on case records from the files of the Armed Forces Institute of Pathology in Washington, D. C., reviewed eye injuries from tear gas; unfortunately, many of the histories were incomplete. In about half the cases, the injuries were self-inflicted and accidental; in the other half, the injuries were caused by a second person firing a weapon from close range with intent to injure the patient. In some instances, particles of agglomerated agent were driven into the eye tissues by the force of the blast; the authors of the study suggested that a chemical reaction caused damage over months or years. In other instances, the injury was probably caused by the blast or other foreign particles rather than by CN. The authors carefully pointed out that features of the weapon, such as the blast force, the propellant charge, the wadding, and the age of the cartridge (in older cartridges, the powder agglomerates and forms larger particles) should be considered in evaluating eye damage due to CN.

The author of another review[41] came to the same conclusion: the traumatic effect of the blast is a considerable factor, and one cannot always be sure that CN per se is the cause of permanent injury.

In a study[20] comparing the effects of CN and CS in the eyes of rabbits, CN at a concentration of 10% (wt/vol) caused iritis and conjunctivitis lasting longer than

7 days and corneal opacity lasting longer than 55 days. In contrast, CS, at the same concentration, caused moderate conjunctivitis but no iritis or corneal opacities; all eyes were normal at 7 days. Other evidence[30] indicates that when CN is applied directly to the eye in powder form or is sprayed at close range, a more severe reaction than that seen with CS may result.

Although permanent eye damage has been reported from the use of CN weapons at close range, separating the effects of the weapon from those of the compound is difficult. There is no evidence that CN at harassing or normal field concentrations causes permanent damage to the eye.

SEVERE MEDICAL COMPLICATIONS FROM THE USE OF CS AND CN

The indiscriminate use of large amounts of CN in confined spaces has caused injuries requiring medical attention and death. An incident of injury to an infant from CS has also been reported.

A 4-month-old infant was in a house into which police fired CS tear gas canisters for 2 to 3 hours to subdue a disturbed adult. Immediately on being removed from the house, the infant was taken to a hospital, where he was observed to have copious secretions of the nose and mouth and frequent sneezing and coughing. He required frequent suctioning to relieve upper airway obstruction. Physical examination was unremarkable except for the secretions, slight conjunctival injection, and rapid heart rate and respirations. On the second day, he had an episode of cyanosis, which cleared with suctioning. On examination, he was in respiratory distress with suprasternal retraction, wheezes, and rales bilaterally. The chest radiograph was clear. Antibiotics, high-dose steroids, and positive-pressure breathing were started. He slowly improved until the seventh hospital day, when his temperature rose to 40.4°C (104.4°F) and coughing increased. An infiltrate was noted on the chest radiograph. Physical findings were unremarkable except for coarse breath sounds throughout the lungs. He improved with further antibiotic and ventilatory therapy and was discharged on day 12, only to be readmitted on day 13 with an increasing cough and a progression of the infiltrate. With more antibiotics and other therapy, he gradually recovered and was discharged after 28 days in the hospital.[42]

In a prison incident, 44 inmates were in a cell block sprayed with CN; 28 inmates later sought medical attention, and 8 were hospitalized. All eight complained of malaise, lethargy, and anorexia. Five had pharyngitis, three of whom developed pseudomembranous exudates several days later. Three also developed tracheobronchitis with purulent sputum, but no infiltrates on chest radiograph. Four patients had facial burns, and three had bullae on the legs; the most severely affected had first- and second-degree burns over 25% of his body. One patient was admitted 5 days after the incident with a papulovesicular rash of his face, scalp, and trunk, which had appeared 2 days earlier. Ten prisoners were treated as outpatients for first- and second-degree burns, and six had localized papulovesicular rashes. Ten had conjunctivitis with edema of the conjunctiva, and in some the eyelids were closed by the swelling, but no patient had corneal injuries or permanent eye damage. The patients with

laryngotracheobronchitis were given bronchodilators, postural drainage, and positive-pressure exercises. Two were given short-term, high-dose steroids, but none received antibiotics. One required bronchodilator therapy 3 months later, but the others made prompt recoveries.[43]

The skin lesions were treated with debridement and applications of silver sulfadiazine and, in some cases, with topical steroids and antihistamines. Skin color was almost normal 3 months later. Topical steroids caused the conjunctival edema to begin to resolve in 48 hours. The only estimate of the amount of CN used was obtained from the prisoners, each of whom claimed to have been sprayed multiple times. Although the first- and third-floor windows were open, the exhaust system was off during the incident.[43]

In another prison incident, the windows and doors were closed and ventilation was off during what was described as a “prolonged gassing” of inmates confined to individual cells. It was later estimated that the incident lasted 110 minutes. Among the dispensers used were at least six thermal grenades of CN, fourteen 100-g projectiles of CN, and more than 500 mL of an 8% CS solution. Using only the amount in the CN projectiles, the authors of the report calculated that the prisoners were exposed to a Ct of 41,000 mg•min/m^3. The total number of prisoners exposed was not noted. Afterward, some had coughing with varying degrees of illness, and at least three received medical treatment (the authors carefully pointed out that they were unable to obtain details).[44]

A prisoner was found dead under his bunk[46] hours later. Other prisoners reported that he had had “red eyes,” had vomited “bloody” material, and had sought medical attention on several occasions. On autopsy, he was noted to have rigor mortis, cyanosis of the face and head, and no evidence of physical injury. His lungs had subpleural petechiae, hyperemia, mild edema, and patchy areas of consolidation; microscopic examination showed bronchopneumonia clustered around exudate-filled bronchioles. His larynx and tracheobronchial tree were lined with an exudative pseudomembrane; microscopic examination showed this was a fibrin-rich exudate containing polymorphonuclear leukocytes and their degenerating forms. There was no evidence of gastrointestinal hemorrhage; other organs had passive hyperemia.[44]

Another individual had an altercation with the police and locked himself into a room in his house. A single CN grenade (128 g) was thrown into the room (approximately 27 m^3), where the patient remained for 30 more minutes (128,000 mg • 30 min ÷ 27 m^3 provides an estimated Ct of about 142,500 mg•min/m^3, or an exposure 10-fold higher than the estimated lethal Ct_{50}).[45]

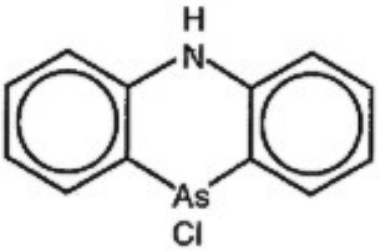
On admission to the hospital, his respirations were 24 per minute, his conjunctiva were suffused, his pupils were small and unreactive, mucoid discharge from his nose and mouth was abundant, his lungs were clear, and an occasional premature ventricular contraction was evident on the electrocardiogram. He remained “in a semicomatose condition for approximately 12 hours and then suddenly developed pulmonary edema and died.”[45(p375)] Relevant findings on autopsy included cyanosis, frothy fluid in the mouth and nose, acute necrosis of the mucosa of the respiratory tree with pseudomembrane

formation, desquamation of the lining of the bronchioles with edema and inflammation of the walls, and a protein-rich fluid in most of the alveolar spaces. Foci of early bronchopneumonia were present.[45]

Information on three other cases of death from CN, which the authors obtained from other medical examiners, are summarized in the same report.[45] Details were scanty, but the autopsy findings were similar; in each case, the individual was confined in a relatively small space. Exposure was for 10 minutes in one instance and for hours in the others (details of exposure were unknown).

OTHER RIOT CONTROL COMPOUNDS

DM (Diphenylaminearsine)



DM

The riot control agent known as DM (diphenylaminearsine) is one of a group of compounds that are known as vomiting agents. The others, which are of much less military importance, are the agents DA (diphenylchlorarsine) and DC (diphenylcyanoarsine). DM was first synthesized by the German chemist Wieland in 1915 and, independently, by the U.S. chemist Adams in 1918. DM is also known as adamsite.

DM is a yellow-green, odorless, crystalline substance that is not very volatile. It is insoluble in water and relatively insoluble in organic solvents. Its primary action is on the upper respiratory tract, causing irritation of the nasal mucosa and nasal sinuses, burning in the throat, tightness and pain in the chest, and uncontrollable coughing and sneezing. It also causes eye irritation and burning, however, with tearing, blepharospasm, and injected conjunctiva.

DM is more toxic than other riot control agents; the LCt_{50} for humans has been estimated to be 11,000 mg•min/m^3.[46] The amount that is intolerable for humans has been estimated by some to be 22 mg•min/m^3 and by others to be 150 mg•min/m^3.[46] The threshold for irritation in humans is about 1 mg/m^3, but men have tolerated Ct exposures of 100 to 150 mg•min/m^3.

Two characteristics make this class of compounds unique among the riot control agents. The first is that the effects do not appear immediately on exposure or seconds afterwards, but several minutes later. In the absence of symptoms, a soldier will not mask immediately; by the time he masks, he will have absorbed a significant amount. The effects may then cause him to unmask.

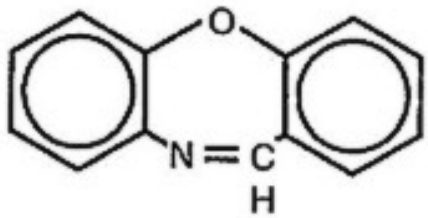
The second characteristic of these compounds is that there may be more prolonged systemic effects, such as headache, mental depression, chills, nausea, abdominal cramps, vomiting, and diarrhea, which last for several hours after exposure. DM and related compounds are known as vomiting agents, but

the incidence of vomiting and the amount of compound necessary to cause it are not known with certainty. In studies dating from 1922 to 1958,[46] humans were exposed to C*t*s ranging from 4.6 to 144 mg•min/m^3; nausea was noted in fewer than 10% of the subjects. Because of the lack of data, the C*t* necessary to cause nausea and vomiting has not been established,[46] but has been estimated to be about 370 mg•min/m^3.[24]

One death has been reported[46] from DM inhalation (the information on this fatality is incomplete). A DM generator was operated in a barrack, exposing 22 sleeping men. The estimated concentration was 1,130 to 2,260 mg/m^3, and the duration of exposure was estimated to be 5 minutes (by one source) or 30 minutes (by a second source). For a 5-minute exposure, the estimated C*t* would be 5,650 to 11,300 mg•min/m^3; for a 30-minute exposure, 33,900 to 67,800 mg•min/m^3. One individual died; the postmortem findings were severe airway and lung damage, similar to those seen after death from CN. Another source[47] reported severe pulmonary injury and death after accidental exposure to high concentrations of DM in confined spaces, but no details were given.

CR (Dibenz*(b, f)*-1:4-oxazepine)

The riot control agent known as CR (dibenz*(b,f)*-1: 4-oxazepine) is a relatively new compound, first synthesized in 1962 by Higginbottom and Suschitzkey. CR is more potent and less toxic than CS. Because of the low vapor pressure of CR solution, no respiratory tract effects are anticipated from its use. The LCt_{50} for animals exposed to grenade-generated smokes was found to be 167,500 mg•min/m^3. The estimated LCt_{50} for humans is probably higher than 100,000 mg•min/m^3.[24]



CR

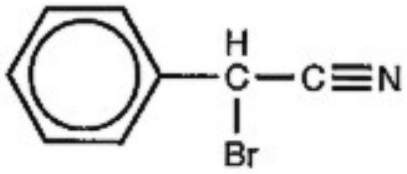
CR is sparingly soluble in water, and a cosolvent (PEG300 is frequently used) is necessary when it is dispersed in solution. Since CR does not degrade in water, it resists weathering and persists in the environment.

In humans, the effects caused by CR are qualitatively similar to those caused by CS, but there is an approximately 5-fold difference in potency. A splash of a solution in the range of 0.01% to 0.1% causes immediate eye pain, blepharospasm, and lacrimation, which persist for 15 to 30 minutes, and conjunctival injection and minimal edema of lid margins, which last for 3 to 6 hours. A solution splashed in the mouth causes burning of the tongue and palate and salivation for 5 to 10 minutes. If a splash enters the nose, it causes irritation and rhinorrhea. Skin exposure causes burning within a few minutes, which persists for 15 to 30 minutes, and an erythema lasting for 1 to 2 hours. A blood pressure increase may accompany the subjective discomfort; this is thought to be caused by the stress of the irritation, since the amount of CR that could be absorbed is much too small to cause a pharmacological effect.[24]

A transient erythema (1–2 h) occurs, but CR does not induce inflammatory cell infiltration, vesication, or contact sensitization, and it does not delay the healing of skin injuries.[24,48] The potential for eye damage is also significantly less than it is from CS or CN.[24] CR was neither teratogenic nor embryolethal in one study[49] when given as an aerosol or by gavage.

Compared with other riot control agents, CR is relatively new; no data from its use exist. Experimental studies indicate that its effects are similar to those of CS except that it causes almost no effects in the lower airways and lungs. It is much more potent than CS—a smaller concentration is needed to cause effects—and it appears to be much safer, as judged from the higher LCt_{50} and the lack of persistent skin and eye effects.

CA (Bromobenzylcyanide)



CA

The riot control agent known as CA (bromobenzylcyanide) was the last irritating agent introduced by the Allies in World War I, and it was the most potent. It corrodes iron and steel, is not chemically stable in storage, and is sensitive to heat, all characteristics that made it unsuitable for storage and use in artillery shells.[50]

CA irritates the eyes and causes lacrimation at concentrations of 0.15 and 0.3 mg/m^3; the LCt_{50} was estimated to be 27,000 mg•min/m^3.[50] More recent studies indicate that the estimated LCt_{50} for humans is 11,000 mg•min/m^3,[51] indicating that it is among the more toxic riot control agents. The health effects caused by CA are very similar to those caused by CS and CN.

CA is rarely used and is a relatively unimportant agent of this class. The compound is included here primarily because it is discussed in *Treatment of Chemical Agent Casualties and Conventional Military Chemical Casualties,*[52–54] field manuals published by the Department of Defense for use by the U.S. Army,

Navy, and Air Force.

MEDICAL CARE

The effects from riot control agents are usually self-limiting, and medical attention is usually not required. Exiting the contaminated area should bring some measure of relief in 15 to 30 minutes or sooner. In rare circumstances, complications may occur on the skin, in the eyes, or in the airways.

Decontamination

The use of water on the skin may result in transient worsening of the burning sensation. Soap and water may be more effective but may also cause a momentary increase in the symptoms. CS rapidly hydrolyzes in an alkaline solution; a solution containing 6% sodium bicarbonate, 3% sodium carbonate, and 1% benzalkonium chloride was found to bring prompt relief of symptoms and to hydrolyze the agent.[13] No form of hypochlorite should be used.

Skin

For dermatitis, a topical steroid preparation (eg, triamcinolone acetonide, fluocinolone acetonide, flurandrenolone, or betamethasone-17-valerate) is the principal therapeutic agent. Oozing lesions should be treated with wet dressings (moistened with fluids such as 1:40 Burow's solution). Appropriate antibiotics should be given for secondary infection, and oral antihistamines for itching.[13] Vesicating lesions have been successfully treated with compresses of a cold silver nitrate solution (1:1,000) for 1 hour, applied six times daily.[11] One person with severe lesions and marked discomfort was given a short course of an oral steroid. An antibiotic ointment was applied locally, but systemic antibiotics were not used.[11]

Eye

A local anesthetic might be applied once for severe pain, but continued use should be restricted. The eye should be thoroughly flushed to remove any particles of the agent. If the lesion is severe, the patient should be sent to an ophthalmologist.

Respiratory Tract

Usually, the cough, chest discomfort, and mild dyspnea are gone 30 minutes after exposure to clean air. However, both the animal data (detailed in the section on CS) and the clinical experience with the infant exposed to CS suggest that severe respiratory effects may not become manifest until 12 to 24 hours after exposure. An individual who has prolonged dyspnea or objective signs should be hospitalized under careful observation. Further care should be as described in Chapter 9, Toxic Inhalational Injury. Although people with chronic bronchitis have been exposed to riot control agents without untoward effects, any underlying lung

disease (eg, asthma, which affects one person in six in the general, or the military, population) might be exacerbated by exposure to CS.[3]

Cardiovascular System

Transient hypertension has been noted after exposure to riot control agents, primarily because of the anxiety or pain of exposure rather than a pharmacological effect of the compound. Whatever the cause, adverse effects may be seen in individuals with hypertension, cardiovascular disease, or an aneurysm.

FUTURE USE

More research is needed to illuminate the full health consequences of riot control agents, as one report[55] has suggested. Information gaps in this chapter indicate areas that might fruitfully be explored, although funding for such research is problematic. The limited resources of the military program in chemical defense are probably more wisely spent on investigating better defense against and medical care for victims of agents that cause more severe consequences and are more likely to be used on a battlefield. Law enforcement agencies generally have few funds for these purposes. Manufacturers probably do not have a large interest in this topic; it is unlikely that their profits from these compounds are large enough to support such an effort. Federal medical funding is generally concerned with more serious diseases affecting larger segments of the population.

Other concerns discussed in the report[55] were the “pattern of use” of these compounds. Are there circumstances in which the use of riot control agents can, or cannot, be condoned? The “pattern of use” might be difficult to regulate, particularly in the areas and under the circumstances in which the use of CS or CN has apparently been abused (eg, the West Bank and the Gaza Strip in the Middle East, and Seoul, South Korea). Public opinion and the Geneva Protocol did not dissuade Iraq from using several types of chemical weapons in the conflict with Iran, or prevent Libya from constructing a large manufacturing facility at Rabta, apparently for the manufacture of chemical weapons. Despite the concern about the loss of innocent lives and injury among innocent bystanders, there is serious doubt that a prohibition of the use of riot control agents would be effective.

While it is true that in some instances dialogue and negotiation should precede the use of riot control agents, one wonders how this suggestion might have been received by the desperate refugees. Although CS allegedly caused injury, the amount of injury was probably small compared to what might have been inflicted if CS had not been available and more extreme measures had been used. Possibly, the use of CS is sometimes the most benign solution in ugly and dangerous circumstances.

SUMMARY

Riot control agents are intended to harass or to cause temporary incapacitation. Their intended target might be the foe in an armed conflict—with the limitations outlined above—or rioters in a civil disturbance.

Much evidence suggests that riot control agents are safe if they are used as intended and if the response is as intended. When they are not used as intended, and the response is not as intended, however, there may be devastating consequences (eg, the deaths of the Branch Davidians at Waco, Tex.). Almost all of the reported adverse effects have resulted from indiscriminate use of weapons containing riot control agents or from resistance to the effects of the compounds, which increases the amount of exposure. Sometimes injury results from the effects of the delivery system of the weapon rather than from the compound; these two sources of injury should not be confused.

Indiscriminate or uncontrolled use of CS, or any riot control compound, is obviously not desired, nor is it necessary in circumstances in which a better, less drastic solution is possible. But the use of CS or CN might be more benign than the use of more deadly alternatives in desperate circumstances. As the data clearly suggest, CS is a relatively safe compound when used as intended.

REFERENCES

1. Robinson JP. *Problem of Chemical and Biological Warfare: A Study of Historical, Technical, Military, Legal, and Political Aspects of CBW.* Vol 1. The Rise of CB Weapons. New York, NY: SIPRI/Humanities Press; 1971.

2. Bestwick FW. Chemical agents used in riot control and warfare. *Hum Toxicol.* 1983;2:247–256.

3. Great Britain Home Office. *Report of the Enquiry into the Medical and Toxicological Aspects of CS (orthochlorobenzylidene malononitrile).* London: Her Majesty's Stationery Office. 1971. Cmnd. 4775.

4. Punte CL, Owens EJ, Gutentag PJ. Exposures to ortho-chlorobenzylidene malononitrile. *Arch Environ Health.* 1963;6:72–80.

5. Bestwick FW, Holland P, Kemp KH. Acute effects of exposure to orthochlorobenzylidene malononitrile (CS) and the development of tolerance. *Br J Ind Med.* 1972;29:298–306.

6. Klapper JA, McColloch MA, Merkey RP. *The Relationship of Personality to Tolerance of an Irritant Compound.* Edgewood Arsenal, Md: Medical Research Laboratories; 1971. Technical Report 4577.

7. Klapper JA, McColloch MA. *The Effect of Diazepam on Tolerance of a Mucous Membrane Irritant.* Edgewood Arsenal, Md: Medical Research Laboratories; 1971. Technical Report 4581.

8. Ballantyne B, Swanston DW. The comparative acute mammalian toxicity of 1-chloroacetophenone (CN) and 2-chlorobenzylidene malononitrile (CS). *Arch Toxicol*. 1978;40:75–95.

9. Ballantyne B, Callaway S. Inhalation toxicology and pathology of animals exposed to *o*-chlorobenzylidene malononitrile (CS). *Med Sci Law.* 1972;12:43–65.

10. Marrs TC, Colgrave HF, Cross NL, Gazzard MF, Brown RFR. A repeated dose study of the toxicity of inhaled 2-chlorobenzylidene malononitrile (CS) aerosol in three species of laboratory animal. *Arch Toxicol.* 1983;52:183–198.

11. Hellreich A, Goldman RH, Bottiglieri NG, Weimer JT. *The Effects of Thermally-Generated CS Aerosols on Human Skin.* Edgewood Arsenal, Md: Medical Research Laboratories; 1967. Technical Report 4075.

12. Hellreich A, Mershon MM, Weimer JT, Kysor KP, Bottiglieri NG. *An Evaluation of the Irritant Potential of CS Aerosols on Human Skin Under Tropical Climatic Conditions.* Edgewood Arsenal, Md: Medical Research Laboratories; 1969. Technical Report 4252.

13. Weigand DA. Cutaneous reaction to the riot control agent CS. *Milit Med.* 1969;134:437–440.

14. Rengstorff RH, Mershon MM. *CS in Trioctyl Phosphate: Effects on Human Eyes.* Edgewood Arsenal, Md: Medical Research Laboratories; 1969. Technical Report 4376.

15. Rengstorff RH, Mershon MM. *CS in Water: Effects on Human Eyes.* Edgewood Arsenal, Md: Medical Research Laboratories; 1969. Technical Report 4377.

16. Rengstorff RH. *The Effects of the Riot Control Agent CS on Visual Acuity.* Edgewood Arsenal, Md: Medical Research Laboratories; 1968. Technical Report 4246.

17. Ballantyne B, Gazzard MF, Swanston DW, Williams P. The ophthalmic toxicology of *o*-chlorobenzylidene malononitrile (CS). *Arch Toxicol.* 1974;32:149–168.

18. Pace S, MD. Emergency Department physician, Madigan Army Medical Center, Tacoma, Wash. Personal communication, 1990.

19. Ballantyne B, Beswick FW. On the possible relationship between diarrhoea and *o*-chlorobenzylidene malononitrile (CS). *Med Sci Law.* 1972;12:121–128.

20. Gaskins JR, Hehir RM, McCaulley DF, Ligon EW. Lacrimating agents (CS and CN) in rats and rabbits. *Arch Environ Health.* 1972;24:449–454.

21. Cucinell SA, Swentzel KC, Biskup R, et al. Biochemical interactions and metabolic fate of riot control agents. *Fed Proc.* 1971;30:86–91.

22. Jones GRN, Israel MS. Mechanism of toxicity of injected CS gas. *Nature.* 1970;228:1314–1316.

23. Jones GRN. Verdict on CS. *Br Med J.* 1971;Oct 16;4(780):170.

24. Ballantyne B. Riot control agents. In: Scott RB, Frazer J, eds. *Medical Annual.* Bristol, UK: Wright and Sons; 1977.

25. Upshall DG. Effects of *o*-chlorobenzylidene malononitrile (CS) and the stress of aerosol inhalation upon rat and rabbit embryonic development. *Toxicol Appl Pharmacol.* 1973;24:45–59.

26. Rietveld EC, Delbressine LPC, Waegemaekers THJM, Seutter-Berlage F. 2-Chlorobenzylmercapturic acid, a metabolite of the riot control agent 2-

chlorobenzylidene malononitrile (CS) in the rat. *Arch Toxicol.* 1983;54:139–144.

27. Wild D, Eckhardt K, Harnasch D, King, MT. Genotoxicity study of CS (*ortho*-chlorobenzylidene malononitrile) in *Salmonella, Drosophila,* and mice. *Arch Toxicol.* 1983;54:167–170.

28. Daniken A, Friederich U, Lutz WK, Schlatter C. Tests for mutagenicity in *Salmonella* and covalent binding to DNA and protein in the rat of the riot control agent *o*-chlorobenzylidene malononitrile (CS). *Arch Toxicol.* 1981;49:15–27.

29. McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Caspary WJ. Responses of the L51178Y tk+/tk–mouse lymphoma cell forward mutation assay. *Environ Mol Mutagen.* 1988;11:91–118.

30. McNamara BP, Vocci FJ, Owens EJ. *The Toxicology of CN.* Edgewood Arsenal: Md: Medical Research Laboratories; 1968. Technical Report 4207.

31. Vedder EB. *The Medical Aspects of Chemical Warfare.* Baltimore, Md: Williams & Wilkins; 1925: 171.

32. Kibler AL. *The After-Effects of Chloracetophenone.* Edgewood Arsenal, Md: Medical Research Laboratories; 1933. Technical Report 133.

33. Queen FB, Stander T. Allergic dermatitis following exposure to tear gas (chloroacetophenone). *JAMA.* 1941;117:1879.

34. Madden JF. Cutaneous hypersensitivity to tear gas (chloroacetophenone). *AMA Arch Dermatol Syphilol.* 1951;63:133.

35. Holland P, White RG. The cutaneous reactions produced by *o*-chlorobenzylidene malononitrile and 1-chloroacetophenone when applied directly to the skin of human subjects. *Br J Dermatol.* 1972;86:150–154.

36. Chung CW, Giles AL. Sensitization of guinea pigs to *alpha*-chloroacetophenone (CN) and *ortho*-chlorobenzylidene malononitrile (CS), tear gas chemicals. *J Immunol.* 1972;109:284–293.

37. Penneys NS, Israel RM, Indgin SM. Contact dermatitis due to 1-chloroacetophenone and chemical mace. *N Engl J Med.* 1969;281:413–415.

38. Penneys NS. Contact dermatitis due to chloracetophenone. *Fed Proc.* 1971;30:96–99.

39. Leopold IH, Lieberman TW. Chemical injuries of the cornea. *Fed Proc.* 1971;30:92–95.

40. Levine RA, Stahl CJ. Eye injury caused by tear-gas weapons. *Am J Ophthalmol.* 1968;65:497–508.

41. Rengstorff RH. Tear gas and riot control agents: A review of eye effects. *Optom Week.* 1969;60:25–28.

42. Park S, Giammona ST. Toxic effects of tear gas on an infant following prolonged exposure. *Am J Dis Child.* 1972;123:245–246.

43. Thorburn KM. Injuries after use of the lacrimatory agent chloroacetophenone in a confined space. *Arch Environ Health.* 1982;37:182–186.

44. Chapman AJ, White C. Death resulting from lacrimatory agents. *J Forensic Sci.*

1978;23:527–530.

45. Stein AA, Kirwan WE. Chloracetophenone (tear gas) poisoning: A clinico-pathologic report. *J Forensic Sci.* 1964;9:374–382.

46. Owens EJ, McNamara BP, Weimer JT, et al. *The Toxicology of DM.* Edgewood Arsenal, Md: Medical Research Laboratories; 1967. Technical Report 4108.

47. *Medical Manual of Defence Against Chemical Agents.* London, England: Ministry of Defence; 1987.

48. Holland P. The cutaneous reactions produced by dibenzoxazepine (CR). *Br J Dermatol.* 1974;90:657–659.

49. Upshall DG. The effects of dibenz (*b,f*)-1:4 oxazepine (CR) upon rat and rabbit embryonic development. *Toxicol Appl Pharmacol.* 1973;24:45–59.

50. Prentiss AM. *Chemicals in War.* New York, NY: McGraw-Hill; 1937.

51. Oberst FW, Crook JW, Swaim SF, et al. *Toxic Effects of High Concentrations of Bromobenzylnitrile (CA) Vapor in Various Animal Species.* Edgewood Arsenal, Md: Medical Research Laboratories; 1967. Technical Report 4078.

52. US Department of the Army. *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries.* Washington, DC: US Department of Defense; 1990. Field Manual 8-285.

53. US Department of the Navy. *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries.* Washington, DC: US Department of Defense; 1990. NAVMED P5041.

54. US Department of the Air Force. *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries.* Washington, DC: US Department of Defense; 1990. Air Force Manual 160-11.

55. Hu H, Fine J, Epstein P, Kelsey K. Tear gas—Harassing agent or toxic chemical weapon? *JAMA.* 1989;262:660–663.

Chapter 13

FIELD MANAGEMENT OF CHEMICAL CASUALTIES

FREDERICK R. SIDELL, M.D.*; RONALD R. BRESELL, M.S.†; ROBERT H. MOSEBAR, M.D.‡; K. MILLS MCNEILL, M.D., PH.D., FACPM§; AND ERNEST T. TAKAFUJI, M.D., M.P.H.¥

INTRODUCTION

HEALTH SERVICE SUPPORT ON THE BATTLEFIELD
- Echelon I: The Unit Level
- Echelon II: The Division Level
- Echelon III: The Corps Level
- Echelon IV: The Echelon Above Corps
- Echelon V: The Continental United States

MEDICAL SUPPORT IN A CHEMICAL ENVIRONMENT
- First Aid for a Chemical Casualty
- Casualty-Receiving Area

SUMMARY

* *Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical Casualty Consultant, 14 Brooks Road, Bel Air, Maryland 21014*

† *Major, Medical Service Corps, U.S. Army (Ret); Formerly, NBC Branch, Preventive Medicine Department, U.S. Army Medical Department Center and School, Fort Sam Houston, Texas 78234-6100; currently, Assistant Director, Safety, Radiation Safety Officer, University of Wisconsin, Madison, 30 North Murray Street, Madison, Wisconsin 53715*

‡ *Colonel, Medical Corps, U.S. Army (Ret); Chief of Clinical Consultants Division, Directorate of Combat and Doctrine Development, U.S. Army Medical Department Center and School, Fort Sam Houston, Texas 78234-6175*

§ *Colonel, Medical Corps, U.S. Army; Director, Preventive Medicine Services, Dwight David Eisenhower Army Medical Center, Fort Gordon, Georgia 30905-5650*

¥ *Colonel, Medical Corps, U.S. Army; Commander, Walter Reed Army Institute of Research, Washington, D. C. 20307-5010*

INTRODUCTION

In the event of an enemy attack with chemical agents, those in the military medical departments must consider first aid, treatment, evacuation, and decontamination procedures of contaminated casualties—some of whom may have injuries made by conventional weapons in addition to their chemical injuries. The ultimate objective in the management of all contaminated casualties is to provide the earliest and most effective treatment without compounding injuries or contaminating medical personnel and treatment facilities.

Although first aid will be initiated on the battlefield and continued during evacuation, these casualties must be moved to an uncontaminated environment as early as possible to permit the removal of protective gear and to allow initiation of optimal treatment in a “shirtsleeve” environment unencumbered by protective clothing or masks. Otherwise, medical personnel must don protective gear, which will significantly compromise their efficiency.

The basic threat to and management principles for chemical casualties have not changed since World War I. To survive and to accomplish the mission on the chemical battlefield, medical care providers must be able to respond quickly and effectively. Soldiers must be trained in first-aid procedures. Decontamination procedures must be practiced. And medical care providers must know how to perform their mission in a chemical environment. Continued, careful attention to each of these requirements will significantly reduce the chemical threat to our military personnel.

HEALTH SERVICE SUPPORT ON THE BATTLEFIELD

A brief review of pertinent Health Service Support (HSS) doctrine will provide a background for the discussion of chemical casualty care. The basic objectives of HSS are to

- reduce the incidence of disease and non-battle injury;
- provide treatment of acute illness, injury, and wounds; and
- return to duty as many soldiers as possible at each echelon or level.

These will be accomplished by emphasizing prevention, by providing far forward medical treatment including advanced trauma management (ATM) and by providing timely and efficient casualty evacuation. Determination of which casualties can be returned to duty will be made at the lowest possible level to preclude their being evacuated farther back than is necessary to provide appropriate care. HSS operations must conform to the tactical plan, allowing for rapid reinforcement or replacement of the forward echelon of medical support. It must provide for a continuum of care from the forward line of troops (FLOT) back to the continental United States (CONUS), emphasizing centralized control with flexibility of execution. Table 13-1 summarizes the various echelons of care and the treatment capabilities at each command level. Each higher echelon reflects an increase in medical capability while it retains the capabilities found in the lower echelons. Echelons I, II, and III are found in the combat zone; Echelon IV is in the communication zone; and Echelon V is in the Zone of the Interior (ZOI).

In the past, the terms “echelon,” “level of care,” and “level,” have sometimes been used in a confusing and seemingly indiscriminate manner. In an attempt to clarify this semantic imprecision, we use the definitions provided in the *American, British, Canadian, Australian Armies Medical Interoperability Handbook:*

> [T]he term *echelon* is used to describe the phased system of health care delivery in the Theater of Operations (such as far forward care is provided at Echelon I). The term level is used to describe the *level* of command (such as division, regiment, or corps).[1(p1-1)]

Echelon I: The Unit Level

Echelon I medical care, found at the unit level and all higher levels, consists of ATM, sick call, and evacuation capability provided by the medical platoon/section organic to combat maneuver battalions and to some combat support battalions. Major emphasis is placed on those measures necessary to resuscitate, stabilize, and prepare for the evacuation of the casualty to the next higher echelon of care. This care may be in the form of self-aid/buddy aid or it may be treatment provided by the combat lifesaver or the combat medic.

TABLE 13-1
TREATMENT EMPHASIS AT THE ECHELONS OF CARE

	CZ			COMMZ	ZOI
Treatment Emphasis	**Level I (Unit)**	**Level II (Division)**	**Level III(Corps)**	**Level IV (EAC)**	**Level V (CONUS)**
Emergency medical treatment, first aid, self-aid, buddy aid	Combat medic, combat lifesaver, all soldiers				
Emergency medical care (advanced trauma management)	Echelon I: Battalion/squadron aid stations		Echelon I: Aid station of the troop medical clinic	Echelon I: Aid station of the troop medical clinic	

Beginning resuscitation and emergency medical care (advanced trauma management)	Echelon II: Clearing station of the forward support medical company in the brigade support area Clearing stations of the medical battalion, or the medical company of the main support battalion in the division rear	Echelon II: Clearing stations of the medical companies of the area support medical battalion		Echelon II: Clearing stations of the medical companies of the area support medical battalion
Resuscitative surgery		Echelon III: Mobile army surgical hospitals,* combat surgical hospitals		Echelon III: Field hospitals
Definitive care			Echelon IV: General Hospitals	

Definitive and restorative care	Echelon V: Medical centers, medical department activities, federal hospitals

In the future, resuscitative surgery will be provided by forward surgical teams deployed at Level II
COMMZ: Communications Zone
CONUS: Continental United States
CZ: Combat Zone
EAC: Echelon Above Corps
ZOI: Zone of the Interior

The combat lifesaver is not a medic but is an ordinary soldier who has received, in addition to his primary military training, additional training beyond basic first aid and, when the situation permits, assists the combat medic by providing immediate care. The combat medic is the first individual in the HSS chain who makes medically substantiated decisions (including triage decisions) based on military occupation specialty (MOS) training. This individual is capable of providing emergency medical treatment.

The treatment squad or battalion aid station (BAS) is the other source of Echelon I care. Personnel here are trained and equipped to provide ATM as well as routine sick call.

Echelon II: The Division Level

Echelon II medical care, found at the division level and all higher levels, is provided at the clearing station by the treatment platoon of the medical company of the main support battalion and the forward support battalion of the Division Support Command (DISCOM). Here the casualty is evaluated to determine the priority for continued evacuation to the rear, or is treated and returned to duty.

Echelon II possesses an increased medical treatment capability plus emergency and sustaining dental care, radiology, laboratory, optometry, patient holding, preventive medicine, mental health, and medical supply capabilities. However, these capabilities do not exceed levels dictated by immediate necessity. Nondivisional units in the division sector receive medical support on an area basis from the nearest medical treatment facility (MTF). In the division, Echelons I and II medical care will not be bypassed, although this may occur in the corps area.

Echelon III: The Corps Level

Echelon III medical care, found at the corps level and higher, is at present provided in a Mobile Army Surgical Hospital (MASH), Combat Support Hospital (CSH), or Field Hospital (FH). A hospital presently being designed as part of the

Medical Reengineering Initiative (MRI) is planned to replace these hospitals in the near future. In addition, resuscitative surgical care will be provided by forward surgical teams at both Echelon III and the brigade division level. Echelon III facilities are staffed and equipped to provide care for all categories of casualties. Those whose injuries permit additional transportation without detriment to their conditions receive surgical care in a hospital farther to the rear.

Echelon IV: The Echelon Above Corps

Echelon IV medical care, found at the Echelon Above Corps (EAC) and higher, is presently provided in a General Hospital (GH). The MRI plans to replace the GH with a hospital of similar capability at the EAC. The GH or its replacement consists of general and specialized medical and surgical capability, including treatment that may be required to stabilize the casualties who require evacuation to CONUS.

Echelon V: The Continental United States

Echelon V medical care, provided by hospitals in the ZOI and CONUS, is the most comprehensive care available within the U.S. Army Medical Department (AMEDD) HSS system. Echelon V hospitals also provide all the types of medical care found at lower echelons.

MEDICAL SUPPORT IN A CHEMICAL ENVIRONMENT

Medical units, like their line counterparts, must be able to survive a chemical attack if they are to successfully perform their primary mission. Protective measures available to them fall into three categories: preattack, attack, and postattack.

Preattack measures include

- gaining knowledge of the characteristics of anticipated chemical agents and the effects of these agents on individuals and on unit operations;
- proper defensive planning (including use of individual protective equipment);
- a full understanding of self-aid, buddy aid, and medical pretreatment;
- casualty decontamination; and
- activation of collective protection and detection/monitoring equipment. An ability to implement protective measures including use of shelters, dispersal, and camouflage is essential, as is the accurate correlation of alert states with mission-oriented protective posture (MOPP) levels.

Attack measures during enemy use of one or more chemical agents include

- detection and monitoring for the continued presence of chemical agent,
- guidance to commanders on potential performance degradation,
- first aid measures,
- initial treatment and evacuation of casualties, and
- individual protection and collective protection, including chemically hardened shelters.

Postattack measures consist of

- monitoring and reporting of chemical contamination and effects;
- control of contamination (avoidance, limitation of spread, weathering/decay);
- damage assessment and control;
- monitoring for effects on command, control and communications elements;
- medical treatment, evacuation and/or quarantine of chemical casualties;
- use of casualty wraps;
- operation and supervision of casualty decontamination centers; and
- preparation for future attacks.

Several categories of casualties will require treatment depending on whether they sustain conventional wounds, chemical poisoning, or both. The number of chemical casualties is dependent on the level of unit training and discipline and the preparedness of the unit. Casualties' conditions may also be adversely affected because normal tasks such as driving an ambulance will take longer in protective equipment, thereby delaying the arrival of the casualty at the point of definitive medical care. For example, at MOPP 4 (ie, full protection), it is generally assumed that all operations will be performed at approximately 50% efficiency. Additionally, medical services must decontaminate liquid agent on individual protective equipment to the vapor-free state to permit entry into collective protection. This will further delay definitive treatment of the casualty, potentially aggravating the injuries.

Key objectives in the management of chemical casualties include

- minimizing chemical agent injuries,
- preventing aggravation of conventional injuries during first aid and decontamination procedures,
- controlling the spread of chemical contamination, and
- continuation of the primary medical mission.

The accomplishment of these objectives plus patient decontamination will require augmentation of the BAS and Forward Support Medical Company (FSMC) by 10 to 20 personnel from the supported unit.

Another important factor is heat stress. The wet bulb globe thermometer (WBGT) index determines the heat condition; this condition is assigned a number (1–5) or a corresponding color code (white, green, yellow, red, black) that can be displayed with flags or other devices. MOPP gear increases the ambient WBGT index by about 10°F; that is, 10° is added to the WBGT reading before the heat condition is designated. The recommended amount of water intake per hour for each heat condition and physical activity for each condition are shown in Table 13-2. Individuals wearing butyl rubber aprons on the decontamination line while at MOPP 4 may experience an even greater heat load. For someone at MOPP 4, a relatively comfortable WBGT of 82°F (heat category 2, green) would increase to a level of over 92°F (heat category 5, black), the most severe and debilitating level of heat

stress. Therefore, frequent rotation of personnel to reduce the occurrence of heat injuries is another factor to consider when determining total manpower requirements.

Specific medications and items of equipment to treat chemical casualties will be carried by units operating in an area of chemical threat. When collective protection systems are not available, casualties will be taken upwind 100 m or more to permit treatment to occur in a shirtsleeve environment. Chemical agent detection equipment, such as the chemical agent monitor (CAM), should be available to determine *(a)* if agent vapors have been absorbed on surfaces of the casualty's clothing or equipment before entering a treatment area, and *(b)* if decontamination procedures have been properly accomplished.

Medical facilities treating chemical casualties must divide their operations into two categories: contaminated (dirty) and uncontaminated (clean). Contaminated operations include triage, emergency treatment, and patient decontamination. Uncontaminated operations include treatment and final disposition. All activities conducted in the Casualty Decontamination Center (CDC) and not inside a collective protection shelter must be conducted at MOPP 4. Operational flexibility is essential. Therefore, the number and arrangement of functional areas will be adapted to both medical and tactical situations.

First Aid for a Chemical Casualty

The most important care for a chemical casualty is that provided within the first few minutes. This cannot be provided by medical personnel and must be done by each individual. This self-aid includes decontamination and the self-administration of the antidote kit if exposure was to a nerve agent.

After exposure to chemical agent vapor, the most important aspect of care is for the soldier to don his mask immediately to prevent further exposure. If the soldier is symptomatic from nerve agent exposure, he should immediately administer the contents of one MARK I kit to himself and notify his buddy of the exposure. For other agents (vesicants, cyanide, and pulmonary agents) there is no self-aid or first-aid therapy.

TABLE 13-2
HEAT CATEGORIES AND WORK/REST CYCLES

Criteria*			Controls: Physical Activity for Soldiers	
Heat Condition / Color Code	**WBGT Index (F°)**	**Water Intake (Qt/h)**	**Work/Rest† Cycles for Acclimatized‡ Soldiers**	**Unacclimatized Soldiers and Trainees**

1/White	78-81.9	At least 0.5	Continuous	Use discretion in planning heavy exercises. Suspend strenuous exercise during the first 3 wk of training. Training activities may be continued on a reduced scale after week 2 of training. Avoid activity in direct sunlight.
2/Green	82-84.9	At least 0.5	50 min work / 10 min rest	
3/Yellow	85-87.9	At least 1.0	45/15	
4/Red	88-89.9	At least 1.5	30/30	Curtail strenuous exercise for all personnel with < 12 wk of hot-weather training.
5/Black	≥ 90	>2	20/40	Physical training and strenuous exercise are suspended. Essential operational commitments not for training, where risk of heat casualties is warranted, are excluded from suspension. Enforce water intake to minimize expected heat injuries.

*Wearing mission-oriented protective posture (MOPP) gear or body armor adds 10°F to the wet bulb globe thermometer (WBGT) index.
† Rest: minimal physical activity, which should be accomplished in the shade if possible; however, any activity requiring only minimal physical activity can be performed during "rest" periods (training by lecture/demonstration, minor maintenance of vehicles/weapons, personal hygiene activities).
‡ Acclimatized: the soldier has worked in the given heat condition for 10–14 d.
Adapted from Department of the US Army. *Field Hygiene and Sanitation.* Washington, DC: HQ, DA; Nov 1988: 39. Field Manual 21-10.

After exposure to liquid agent, the most important aspect of care is to decontaminate (ie, remove the agent) as quickly as possible. Decontamination done within a minute or two after exposure to mustard will decrease skin damage; the severity of subsequent skin damage increases greatly each minute the agent is in contact with skin. Immediate decontamination of nerve agent droplets from the skin can prevent severe poisoning or death. Large amounts of cyanide must be present on skin to cause clinical effects, and pulmonary agents generally penetrate the skin

poorly or cause no effects by this route of exposure, so decontamination is of much less importance when exposure is to these agents.

A soldier who is symptomatic from a nerve agent should immediately take steps to prevent further exposure, and then he should self-administer the nerve agent antidote kit (the MARK I). Each member of the military is taught to administer one kit if he has minimal or moderate symptoms from a nerve agent. He administers one and waits 10 minutes. If he is no better after this time, he seeks out a buddy to evaluate him; if there has been no improvement, a second MARK I kit is given.[2]

For a casualty who is able to administer a MARK I kit and seek out a buddy, a total of two antidote kits usually will be sufficient. On the other hand, a soldier with effects so severe that he is unable to self-administer the antidotes must depend on buddy-aid. A soldier who is not walking or talking should be given three MARK I antidote kits and diazepam by his buddy as quickly as they can be administered. When the unit medic or combat lifesaver arrives, he can administer additional atropine and one or two more injectors of diazepam as conditions indicate.[2]

Casualties who improve significantly from one or two MARK I kits given for nerve agent symptoms will continue their mission on improvement. Those casualties who later develop symptoms after vesicant or pulmonary agent exposure will seek medical aid either at the unit aid station or at the BAS. Generally, they will decide to seek assistance before the effects become severe, and they will transport themselves to the medical site before they have received assistance from a combat lifesaver or medic.

Casualties with severe effects, those who require on-the-spot assistance from the unit lifesaver or medic, will generally be sent back to the aid post or BAS for further care. The decision by the unit lifesaver or medic to call for the litter team or ambulance team to evacuate that casualty is the first of many levels at which triage decisions are made on each casualty.

Ambulances that transport casualties from the FLOT to the first-echelon MTF are generally contaminated, or “dirty,” and the personnel on these vehicles are at MOPP 4.

Casualty-Receiving Area

Any MTF that receives contaminated casualties will have a casualty-receiving area, which consists of a dirty side and a clean side, separated by a “hotline” that must not be crossed by contaminated casualties, garments, or equipment. The area where casualties are received, on the dirty side of the line, is where initial triage is done, emergency medical care is provided, and the casualty is decontaminated (Figure 13-1).

The number and types of personnel in this area will be different at each echelon of care. For example, at a BAS, a single senior medic may staff both the triage and emergency treatment areas, but at a hospital, physicians’ assistants or physicians might be present at each area. Similarly, what is done with the casualty at each station will be different at each echelon. At the BAS or other first-echelon MTF, the goals are to return casualties with minor injuries to duty, and to stabilize casualties with more-severe injuries for evacuation to higher echelons of care. Of these, only two categories of casualties will be decontaminated. Those who have severe injuries will be decontaminated to enter the clean medical treatment area; those who can

return to duty may go through a MOPP-gear exchange process or go through decontamination to enter the clean area to don new protective gear, providing they have their own second set of gear.

Casualties with severe but stable injuries or others who must be evacuated without treatment will be sent directly from the triage area to the ambulance area to be evacuated dirty. At a higher-echelon MTF, such as a hospital, where more-complete care can be provided, all casualties will be decontaminated for entry into the clean treatment area.

Behind the contaminated receiving area and separated from it by a hotline is the clean treatment area.

A clearing company or a clearing company team will set up ambulance exchange points, which have a treatment squad to perform first aid and the capability to perform patient decontamination before further evacuation. As a rule, contaminated ambulances operate from the FLOT, transporting contaminated casualties back to the exchange point, while clean vehicles transport decontaminated casualties to Echelon III medical treatment facilities.

The Entry Point

The entry point is a clearly demarcated area into which all casualties arrive. Ambulances unload casualties at this point, and ambulatory casualties report to this point. The entry and exit roads must also be clearly marked. Organic (ie, intrinsic) staffing in this area may be minimal, and all casualties arriving at this area will be sent to the triage station.

The Triage Station

The triage officer sorts each casualty into one of the four triage categories: immediate, minimal, delayed, or expectant (Exhibit 13-1). At lower echelons of care, the triage officer may be a senior medic (who may also be the staff at the emergency treatment station); at higher echelons, he may be a physician's assistant, dentist, or physician.

As discussed in greater detail in Chapter 14, Triage of Chemical Casualties, the triage officer must know the natural history of the injuries he faces, including chemical injuries. He must also have knowledge of evacuation capabilities and the facilities at higher echelons of care as well as his own decontamination capabilities and assets for medical care.

The triage officer will send casualties (*a*) back to duty, (*b*) to the emergency treatment station, (*c*) to the decontamination area, or (*d*) to the dirty evacuation area.

The Emergency Treatment Station

In the most forward MTF, the emergency treatment station will likely be staffed by the same senior medic who functions as the triage officer. At higher echelons of care, a physician's assistant or physician might staff this station.

At the emergency treatment station, the casualty is provided emergency

lifesaving medical care and is stabilized for the 10- to 20-minute decontamination procedure that is necessary before he can enter the clean area of the MTF for more-elaborate treatment.

Fig. 13-1. A casualty receiving area, or chemical decontamination receiving area, is located in front of any medical treatment facility (MTF) that receives chemically contaminated casualties. Although the precise details will vary depending on the echelon of medical care and the resources available, any MTF at any echelon of care that might receive contaminated casualties will have a receiving area for contaminated casualties. This area, seen in the drawing, consists of the casualty arrival point, triage point, emergency medical treatment (EMT) station, decontamination area, and the hotline, which is set perpendicular to the wind direction. Only after a casualty has been decontaminated can he be taken across the hotline into the clean treatment area for more complete chemical casualty care. The pathway for nonambulatory casualties shown in this figure is shaded yellow; ambulatory casualties, further medical treatment, and the disposition of contaminated equipment and garments are not addressed in the following discussion.

(a) At the arrival point, the healthcare provider uses a chemical agent monitor (CAM) to assess the nature and magnitude of the casualty's chemical contamination.

(b) After the casualty has been triaged, treatment at the EMT station may involve emergency first aid for conventional injuries as well as the administration of chemical agent antidotes. Note that healthcare providers are at mission-oriented protective posture (MOPP) 4 and, in addition, are wearing butyl rubber aprons. At the litter decontamination station,

(c) the casualty's hood is decontaminated with 5% hypochlorite solution and

(d) is being cut away prior to removal;

(e) the outer garment is being excised prior to removal (not seen: the medical record card is inserted in a plastic bag and placed under the casualty's mask headstrap);

(f) the overboots are removed; and

(g) the battledress uniform is being excised prior to removal. After the casualty's underwear has been excised and removed, the casualty is monitored for additional contamination and

(h) the skin is spot decontaminated with 0.5% hypochlorite solution.

(i) At the shuffle pit, the still-masked, litter-borne casualty is transferred to the clean side of the hotline for further treatment at the MTF. (Not shown: the clean casualty is checked with the CAM before crossing the hot line.) The bag containing the casualty's medical record card, which has also been decontaminated with 5% hypochlorite solution, is transferred with the casualty. Total elapsed time for decontamination of a litter-borne casualty to this point is 10 to 20 minutes.

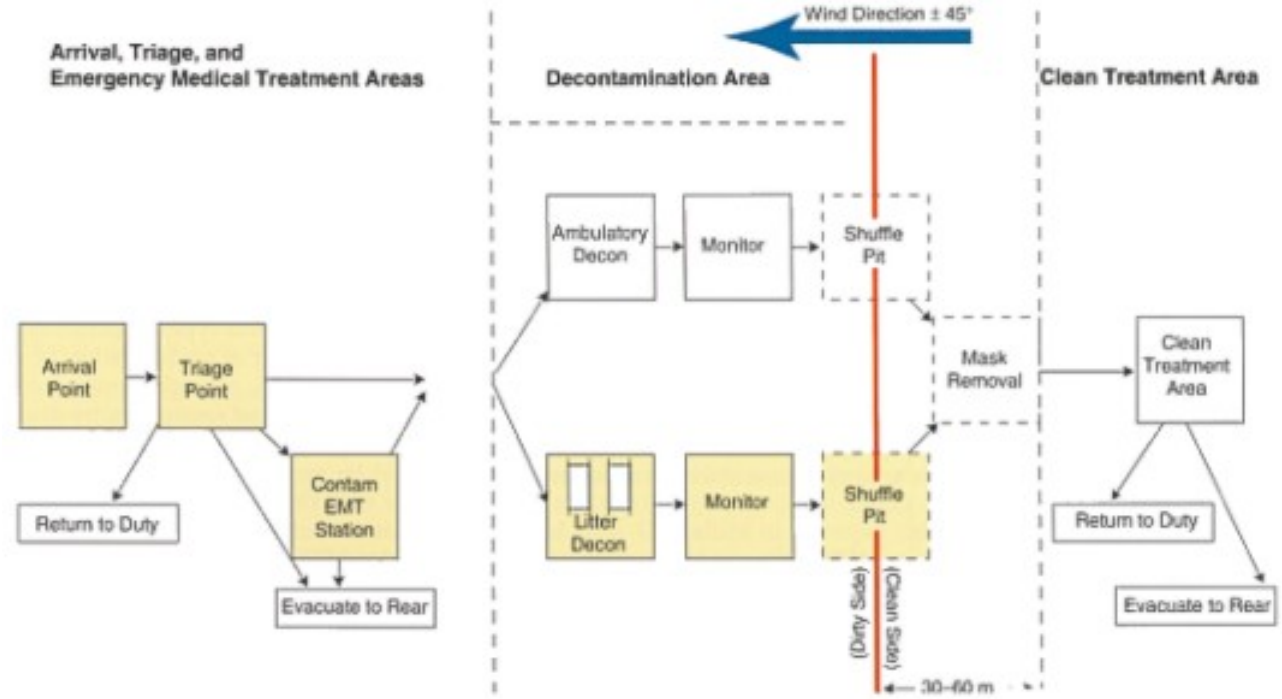


Diagram: Adapted from Combat Casualty Care Office. *Medical Management of Chemical Casualties Handbook.* Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1994: 194. Photographs: Reproduced electronically from *US Army Chemical Decontamination of Nonambulatory Casualties*. Fort Sam Houston, Tex: Health Sciences Media Division, US Army Medical Department Center and School; 1995. Training videorecording 710175; TVT 8-252.

EXHIBIT 13-1
TRIAGE CATEGORIES FOR CASUALTIES OF CHEMICAL WARFARE AGENTS

Immediate

A casualty in the immediate category needs to have a medical procedure performed within an hour or so to save his life. This may be something as simple as giving more atropine and additional diazepam, and inserting an airway for ventilation in a nerve agent casualty who is convulsing or who has just become apneic. In this instance, the triage officer would send the casualty to the emergency treatment station in the contaminated area. If the casualty needs more care and that care can be provided in his facility, the triage officer sends the patient for decontamination for entry into the clean (ie, not contaminated) medical treatment area of his facility. However, he might send the casualty to the contaminated emergency treatment area for stabilization before the 10- to 20-minute decontamination procedure. Rarely, he might send an immediate casualty for urgent evacuation to a facility at a higher echelon, but he would do this only if he were certain that the casualty could reach that echelon in a timely fashion and that that facility could provide the needed care on arrival.

In a higher-echelon medical facility, all immediate patients will be sent through decontamination for entry into the clean area. However, the casualty might require stabilization at the contaminated emergency treatment facility before entry into the lengthy decontamination process.

Minimal

A casualty in the minimal category is one who needs minor care and who is expected to return to duty within hours after that care is provided. In a noncontaminated environment, these casualties will generally not be evacuated.

In a contaminated environment, a minimal casualty might be one with a tear in his battledress overgarment through which he became wounded by chemical or conventional means (eg, a small tear through which he became exposed to mustard). At a lower-echelon medical treatment facility (MTF) the medical care can be provided, but a replacement for his torn garment cannot be provided by that facility. He can return to duty shortly, but needs new protective clothing; he can *(a)* go through ambulatory decontamination at that MTF and replace his protective gear with his own second set, *(b)* go through procedure for exchanging his mission-oriented protective posture gear if he has his own second set of protective gear, or *(c)* return to his unit for resupply. In the latter case, the triage officer might send the casualty to the contaminated evacuation area for evacuation in a dirty (ie, contaminated) vehicle. Or he might send him to be evacuated in a clean ambulance, in which case the casualty must go through decontamination.

Delayed

A casualty in the delayed category is one who has a serious injury, but who can wait for care. The delay will not change the ultimate outcome. Most vesicant casualties with skin lesions will be in this category. Generally, delayed casualties will not be sent to the emergency treatment area and will not be decontaminated at the lower-echelon facility. They will be evacuated in a dirty vehicle.

Expectant

A casualty in the expectant category is one who needs care that is beyond the capability of that MTF to provide. In addition, the needed care is required before the casualty can be evacuated to the MTF that can provide such care. Depending on his condition and the circumstances in the MTF at the time, the casualty will initially be set aside but will be decontaminated. As circumstances permit, he will be reexamined and possibly be retriaged to a higher category.

Casualties with minor wounds might be treated here if they can be returned to duty. However, most will need to have their protective garments replaced and will (1) go through decontamination, or (2) go through a MOPP exchange procedure if they have a second set of garments, or (3) be returned to their own units for resupply. These latter casualties may be evacuated in a dirty vehicle, or they might be decontaminated and sent in a clean vehicle.

Care at the emergency treatment station is limited. The care provider and the casualty are both in MOPP 4. The care provider can apply dressings, start intravenous fluids, and insert an endotracheal tube. In each case, the care provider must decontaminate the casualty's skin in the areas that he will touch, and he must ensure that his hands (gloves) are decontaminated. Although he will be able to insert an endotracheal tube, he may not have a ventilator, or if he has a mask-valve-bag device, he may not have the personnel to use it.

From the emergency treatment station the casualty will *(a)* return to duty, *(b)* go to the decontamination area, or *(c)* go to the ambulance area for evacuation in a dirty ambulance.

The Decontamination Area

At the first echelons of care the organic staff is small, and dedicated personnel are not available to decontaminate casualties. Personnel from the supported unit must be assigned to the MTF for this purpose, to allow all medical care providers to care for casualties. These augmentees should be identified early and be thoroughly trained for these tasks. To most effectively decontaminate a patient on a litter, three—or possibly two—people are needed. The actual size of the augmented decontamination staff needed, however, will be severalfold larger than this number because the personnel will need rest periods, the frequency and duration of which will depend on the ambient temperature.

A minority of casualties who are able to walk will be decontaminated at the BAS or other low echelon of care. Most walking casualties who require significant medical attention will have nonurgent injuries and can be evacuated to a higher echelon for needed care. Casualties in either of these categories, those who need significant care and those who can be returned to duty after MOPP replacement, can be evacuated in a dirty vehicle.

Casualties who need care at the first-echelon MTF or who need stabilization before evacuation will be decontaminated on a litter by the decontamination staff, who are supervised by a medic. The first stage in this process is the removal of the outer garment, followed by removal of the casualty's battledress uniform, gloves, protective boots, and boots. The field medical card (FMC) is placed in a sealed plastic bag and will remain with the casualty until the information is copied onto another FMC; personal effects will be bagged and tested later for contamination.

This is followed by decontamination of exposed areas of skin with 0.5% hypochlorite solution or the M291 (or M258A1) decontamination kits. The CAM or M8 paper may be used to test for contamination before and after decontamination. It is generally believed that removal of the protective clothing will remove 90% of contamination and removal of the uniform will remove 5% more, leaving only a small amount to be removed by skin decontamination.

The decontamination team then hands the nude, decontaminated casualty across the hotline to the medical staff. As a final step, far from the hotline the casualty's mask is removed. All contaminated material is placed in a dirty dump, which is located 100 m downwind and marked with the North Atlantic Treaty Organization "gas" marking.

During the decontamination process, the medic overseeing decontamination is responsible for splints, tourniquets, and bandages. Splints are soaked thoroughly with the decontaminating solution but are not removed. Tourniquets are removed after a new one is placed proximally over decontaminated skin. Bandages are removed and not replaced except as needed (ie, to control bleeding). Wounds that are neither truncal nor neurological are flushed with 0.5% hypochlorite; other wounds are thoroughly flushed with water or saline.

The decontamination process will usually take about 10 to 20 minutes; a well-trained team can decontaminate a casualty in slightly less than 10 minutes.

As the capability to provide medical care increases at higher echelons of care, a larger number of casualties will be decontaminated, and at hospitals, all contaminated casualties will go through this procedure.

The Clean Treatment Area

The capability to care for casualties increases greatly from the lowest echelon of care to the highest. The BAS will have a physician's assistant, a physician, or both, and several medics. The higher echelons, the hospitals, will have a full surgical staff including subspecialists, surgical facilities, and full support capabilities to provide all needed immediate care.

With limited resources available, the major tasks of the BAS are to provide lifesaving care and to prepare the casualty for evacuation. By necessity these must be short, simple procedures. After receiving care in a low-echelon MTF, the casualty is evacuated in a clean vehicle to a higher echelon for further care. If clean vehicles are not available, the casualty may be placed in a patient protective wrap and evacuated in a dirty vehicle (see Figure 16-42 in Chapter 16, Chemical Defense Equipment). At higher echelons, the treatment area will be located in a collective protection shelter; otherwise, this should be at least 100 m upwind from the receiving area.

SUMMARY

Field management of a contaminated casualty or of a casualty in a contaminated environment is cumbersome and manpower-intensive. In front of each medical care facility, from battalion aid station to field hospital, there must be a casualty-receiving station if casualties are contaminated, or if casualties are entering from a contaminated area. In this station, casualties are *(a)* triaged, *(b)* given the emergency care that can be provided with both casualty and medical care provider encapsulated in protective garments, *(c)* decontaminated, and then *(d)* taken into a noncontaminated—or clean—area for further care. At this stage or after the initial triage, the casualty may be evacuated to a higher-echelon facility, depending on the needs of the casualty and on the resources available. Initial triage is greatly hampered by the partial loss of the senses of sight and touch because of the

protective garments. Initial medical care in the contaminated area is rudimentary because of potential contamination on the casualty and because of the protective equipment. Decontamination of a casualty takes about 10 to 20 minutes.

REFERENCES

1. *American, British, Canadian, Australian Armies Medical Interoperability Handbook.* ABCA: March 1996. Initial draft.
2. Departments of the Army, the Navy, and the Air Force, and Commandant, Marine Corps. *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries.* Washington, DC: Headquarters, DA; DN; DAF; and Commandant, MC; 22 Dec 1995: Chap 2, Nerve Agents. Army FM 8-285, Navy NAVMED P-5041, Air Force AFJMAN 44-149, Marine Corps FMFM 11-11.

Chapter 14

TRIAGE OF CHEMICAL CASUALTIES

FREDERICK R. SIDELL, M.D.*

INTRODUCTION

TRIAGE OF CHEMICAL CASUALTIES

REVIEW OF CHEMICAL AGENT EFFECTS
- Nerve Agents
- Cyanide
- Vesicants
- Phosgene
- Incapacitating Agents

CATEGORIES FOR TRIAGE OF CHEMICAL CASUALTIES
- Immediate
- Delayed
- Minimal
- Expectant

CASUALTIES WITH COMBINED INJURIES
- Nerve Agents
- Mustard
- Phosgene
- Cyanide
- Incapacitating Agents

SUMMARY

*Formerly, Chief, Chemical Casualty Care Office, and Director, Medical Management of Chemical Casualties Course, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425; currently, Chemical

INTRODUCTION

The word triage comes from the French word *trier*, meaning to sort, to cull, or to select. Simply stated, triage is the process of sorting or prioritizing casualties when providing immediate and maximal care to each is impossible. Triage is practiced only when a mass casualty situation occurs and the needs of the casualties for care overwhelm the medical capabilities to provide that care. A triage situation need not involve large numbers of casualties: for example, when only one chest surgeon is available in a hospital to care for two auto accident victims with chest wounds needing immediate surgical intervention. More commonly, triage in peacetime is used in a large hospital after a disastrous accident with large numbers of casualties. On a battlefield, triage is required at a unit-level medical facility (such as the battalion aid station [BAS]), where medical personnel and capabilities are limited and the casualties are numerous. In addition to the sorting of casualties for care, the triage process on a battlefield also requires setting evacuation priorities; do not, however, confuse evacuation priorities with triage.

The intent of triage is to provide immediate help to those who need it; to delay care for those who have less threatening injuries; and to set aside, at least temporarily, both those who need care beyond the capabilities of the available medical assets (personnel, equipment, and facilities) and those who require such extensive care that the time and assets spent would delay or prevent care for those more likely to recover.

The latter concept, setting aside casualties who are in need, is unpopular among medical care providers, whose goal is to provide the ultimate care for each patient. It is understandable that the thought of setting aside a critically sick or injured patient is repugnant to someone who has not been in a mass casualty situation or who has given little thought to such situations. After all, in peacetime, every patient who enters the hospital emergency room receives the full attention of all personnel needed to provide optimal care. Barring a mass casualty situation, no need for triage exists under these circumstances and most medical care providers do not live with it or the thought of it.

In a mass casualty situation, whether in peacetime or on a battlefield, triage is carried out to provide immediate and appropriate care for casualties with treatable injuries, to delay care for those with less immediate needs, and to set aside those for whom care would be too time- or asset-consuming. It ensures the greatest care for the greatest number

and the maximal utilization of medical assets: personnel, supplies, and facilities.

It is essential that a triage officer know

- the natural course of a given injury,
- the medical resources on hand,
- the current and likely casualty flow, and
- the medical evacuation capabilities.

Commonly, the most experienced surgeon available performs triage. A surgeon is selected because physical injuries are involved in most triage situations, and surgeons have the most extensive training and experience in evaluating them. An experienced surgeon is desirable because he is most familiar with the natural course of the injury presented. Part of the triage process is the evaluation of the benefit that immediate assistance will provide. This evaluation is based, in part, on the natural course of the injury or disease. For example, dedicating medical assets to a casualty with an injury that will either heal or prove fatal no matter what immediate care is given would be of little benefit.

When working in a chemically contaminated environment, the triage officer is in protective gear and is not immediately available to assist with casualty care, which, ideally, is done within a collective protection area (a “shirtsleeve” environment). Examination of the casualty will not be as thorough as it might be in a clean (ie, not contaminated) environment, and very little care can be given a casualty in the emergency treatment section in the contaminated area. In chemically contaminated environments, therefore, in contrast to other triage situations, the most experienced surgeon is in the clean treatment area where he can provide maximum care. In these cases, the triage officer is a senior corpsman or someone else with medical training, such as a dentist.

In addition to knowing the natural course of the disease or injury, the triage officer also should be aware of the current medical assets, the current casualty population, the anticipated number and types of incoming casualties, the current status of the evacuation process, and the assets and casualty population at the evacuation site. Committing assets to the stabilization of a seriously injured casualty in anticipation of early evacuation and more definitive care would be pointless if evacuation could not be accomplished within the time needed for the casualty’s effective care, or if the assets at the evacuation site were already committed. The officer might also triage differently if, for example, he knew that the 10 casualties present were all that would need care in the next 24 hours or, on the other hand, that those 10 casualties were to be followed by 50 more within an hour.

Triage is not a static process but a dynamic one that occurs at every echelon of medical care, preferably several times. The first triage is done by the field medic or unit lifesaver when he encounters an injured soldier in the field. The medic first decides whether anything can be done for that soldier to save life or limb. If the answer is no, the medic moves on, perhaps after administering an analgesic. More commonly, the medic decides that care is indicated. Can the medic provide that care on the spot to return the soldier to duty quickly? Can the care wait until the battle is less intense or an ambulance arrives? Or must the care be given immediately if the casualty is to survive? In the latter case, the medic will do what is possible to return the casualty to the medical facility.

A casualty is triaged once more upon entry into a medical care facility and is triaged again and again within that facility as circumstances change. Those circumstances include the casualty's condition and the assets available. For example, a casualty set aside as expectant (see Triage Groups, below, for definitions of classification categories) because personnel are occupied with more salvageable casualties might be reclassified as immediate when those personnel become free. On the other hand, a casualty with a serious wound but in no immediate danger of loss of life might initially be classified as delayed, but if he suddenly developed unanticipated bleeding and if assets were available to care for him, he might be retriaged as immediate.

In an unfavorable tactical situation, another consideration may arise. Casualties with minor wounds, who otherwise may be classified minimal, might have highest priority for care to enable them to return to duty. The fighting strength thus preserved could save medical personnel and casualties from attack.

Even in the most sophisticated medical setting, a form of triage is usually performed, perhaps not always consciously by those doing it: separation of those casualties who will benefit from medical intervention from those who will not be helped by maximal care. However, in most circumstances in a large medical facility, care is administered anyway; for instance, an individual with a devastating head injury might receive life-support measures. The realization that in some settings assets cannot be spent in this manner is an integral part of triage.

TRIAGE OF CHEMICAL CASUALTIES

In the simplest form of triage, patients or casualties are separated into three groups. The first group is those for whom medical care cannot be provided because medical assets and time are not available to care for a wound or illness of the severity presented, and because the triage officer knows from experience that the casualty will die no matter what care is

given. Again, a casualty's classification might change as assets become available or when later reevaluation shows that the casualty's condition was not as serious as first anticipated. The second group consists of casualties who require immediate intervention to save life. In a conventional situation (ie, a noncontaminated environment), these casualties usually have injuries affecting the airway, breathing, or circulation—the "ABCs"—which can be treated effectively with the assets available within the time available. The third group consists of casualties who have injuries that place them in no immediate danger of loss of life. Casualties in this group might include someone with a minor injury who merely needs suturing and a bandage before being returned to duty, or someone who has an extensive injury necessitating long-term hospitalization, but who at present is stable.

The triage system commonly used by U.S. military medical departments and by civilian medical systems contains four categories: *immediate, delayed, minimal*, and *expectant* (Exhibit 14-1). Sometimes, as was done in the *NATO Emergency War Surgery Handbook,*[1] a fifth category, urgent, is added to denote a casualty for whom intervention must occur within minutes to save life. In Exhibit 14-1, this concept is included in the immediate category. Also, in some schemes, the term *chemical intermediate* is used for a casualty who requires that antidotes be given immediately to save life (as in nerve agent or cyanide poisoning). The triage categories used in this chapter do not make the distinction between chemical casualties and casualties whose injuries are caused by conventional weapons.

EXHIBIT 14-1
U.S. ARMY MEDICAL DEPARTMENT MASS CASUALTY TREATMENT PRIORITIES

Treatment priorities for mass casualties are as follows:

1. *Immediate:* casualties who require lifesaving care within a short time, when that care is available and of short duration. This care may be a procedure that can be done within minutes at an emergency treatment station by a corpsman (eg, relief of airway obstruction) or may be acute lifesaving surgery.

2. *Delayed:* casualties with severe injuries who are in need of major or prolonged surgery or other care and who will require hospitalization, but delay of this care will not

adversely affect the outcome of the injury. Fixation of a stable fracture is an example.

3. *Minimal:* casualties who have minor injuries, can be helped by nonphysician medical personnel, will not be evacuated, and will be able to return to duty shortly.

4. *Expectant:* casualties with severe life-threatening injuries who would not survive with optimal medical care, or casualties whose injuries are so severe that their chance of survival does not justify expenditure of resources.

Triage categories are based on the need for medical care, and they should not be confused with categories for evacuation to a higher-echelon medical treatment facility (MTF) for definitive care. The need for evacuation and, more importantly, the availability of evacuation assets will certainly influence the medical triage decision. For example, if a casualty at a BAS is urgently in need of short-term surgery to control bleeding and evacuation is not possible for several hours, his triage category might be expectant instead of immediate. The evacuation categories are *urgent* (life immediately threatened), *priority* (life or limb in serious jeopardy), and *routine.*

Because this is a textbook on the management of chemical casualties, triage of the conventionally wounded casualty is not discussed except in the context of *combined* casualties (ie, casualties whose wounds were caused by conventional weapons but who have also been exposed to a chemical agent; see Casualties With Combined Injuries, below). The distinction between the urgent and immediate groups has been ignored, as has the separation of the chemical immediate and immediate groups. Chemical casualties are discussed under the commonly used groups of immediate, minimal, delayed, and expectant.

At the first echelon of medical care, the chemical casualty is contaminated and both he and the triage officer are in protective clothing. Furthermore, the first medical care given to the casualty is in a contaminated area, on the “hot” or dirty side of the “hotline” at the emergency treatment station (see Figure 13-1 in Chapter 13, Field Management of Chemical Casualties). This is unlike the clean side of the hotline at any echelon of care where casualties are decontaminated before they enter, or unlike a hospital in peacetime where usually there is no contamination.

It must be remembered that triage refers to priority for medical or

surgical care, not priority for decontamination. *All* chemical casualties require decontamination. One might argue that a casualty exposed to vapor from a volatile agent, such as cyanide or phosgene, or from some of the volatile nerve agents does not need to be decontaminated. However, one can seldom be certain that in a situation in which vapor and liquid both exist, some liquid is not also present on the casualty.

It is extremely unlikely that immediate decontamination at the first echelon of medical care will change the fate of the chemical casualty or the outcome of the injury. Various estimates indicate that the casualty usually will not reach the first echelon of care for 15 to 60 minutes after the injury or onset of effects, except when the MTF is close to the battle line or is under attack and the injury occurs just outside. The casualty is unlikely to seek care until the injury becomes apparent, which is usually long after he becomes contaminated. For example, mustard, a vesicant, may be on the skin for many hours before a lesion becomes noticeable. Thus, it is likely that the agent has been completely absorbed or has evaporated from the skin by the time the casualty reaches the MTF. The small amount unabsorbed or the amount absorbed during a wait for decontamination is very unlikely to be significant.

The process of patient decontamination must be a factor in the judgment of the triage officer during triage. In a contaminated environment, emergency care is given by personnel in the highest level of mission-oriented protective posture (MOPP 4), whose capabilities are limited by their protective gear. After receiving emergency care, a casualty must go through the decontamination station before receiving more definitive care in a clean environment. Decontamination takes 10 to 20 minutes. No medical care is provided during this time or during the time spent waiting to begin the decontamination process. Therefore, before leaving the emergency care area, the patient must be stabilized to an extent that his condition will not deteriorate during this time. If stabilization cannot be achieved, the triage officer must consider this factor when making the triage judgment. A different type of decontamination—immediate spot-decontamination— must be performed at the triage or emergency treatment station in the dirty (ie, contaminated) area when there is a break in the clothing or a wound that is suspected to be the source of contamination.

Casualties from certain chemical agents, such as nerve agents, may be apneic or nearly apneic; one of the first interventions required is assisted ventilation. It is unlikely that the equipment and personnel needed to provide assistance will be available in the contaminated area. However, if a device for ventilatory assistance, such as a mask-valve-bag device, is available, should it be used in a contaminated area? If there is a brisk wind and if the medical facility is far upwind from the source of contamination,

there will be very little agent vapor in the air. One may choose the lesser of two undesirable circumstances and ventilate with air that is possibly minimally contaminated rather than let the casualty continue to be apneic. The apnea is certain to be fatal, whereas with further but minimal vapor inhalation, the casualty may possibly be assisted. The knowledge that a limited number of medical care providers are available in the contaminated area might affect the decision, however, because when care providers begin ventilation, they are committed to that process and cannot care for other casualties. However, a walking wounded (a casualty in the minimal category) can quickly be taught how to ventilate these casualties.

REVIEW OF CHEMICAL AGENT EFFECTS

Before discussing the triage groups and the types of chemical casualties that might be placed in each, a brief review of the type of casualty seen with each chemical agent is presented. Under the best of circumstances, a casualty probably will not reach a medical treatment area until at least 15 minutes after exposure (or after onset of effects, if onset immediately follows exposure). Moreover, a casualty will not seek medical attention until effects are apparent; an appreciable amount of time, therefore, may elapse before the casualty is seen.

Nerve Agents

In a unit-level MTF, nerve agent casualties might be classified as immediate, minimal, delayed, or expectant. In a full-care MTF, it would be unlikely to classify one as expectant.

If a nerve agent casualty is walking and talking, he can generally be treated and returned to duty within a short period (see Chapter 5, Nerve Agents, for a more complete discussion of nerve agent effects and treatment). In most cases he should not present himself at the triage point, but should self-administer his MARK I autoinjectors, which usually will be enough to reverse the respiratory effects of vapor exposure. If the casualty appears at the triage station, he should be classified as minimal because he can self-administer the antidote (or it can be given by a medic), evacuation is not anticipated, and he will return to duty shortly. If the casualty has received the contents of all three MARK I kits and continues to have dyspnea, if his dyspnea is increasing, or if he is beginning to have other systemic symptoms (such as nausea and vomiting, muscular twitching, or weakness), he should be classified as immediate. A source of continuing contamination, such as a break in protective clothing or a wound, should be sought and spot-decontaminated and irrigated. The progression of his illness can be stopped or reversed with a minimal

expenditure of time and effort in the emergency treatment area. More atropine should help considerably.

One additional consideration, which is contrary to the general advice about decontamination, must be remembered. It is quite possible that the condition of the casualty described above, who had a vapor exposure and administered the contents of his MARK I kits, continues to worsen because he also has had a liquid exposure, which is being absorbed through the skin. A break in his protective garb should be sought; if one is found, the skin under it should be quickly spot-decontaminated using whatever liquid is available (preferably bleach, but saline or water will help). If the casualty is conscious, has not convulsed, and is still breathing, prevention of further illness will ensure a quick return to duty. He will survive unless he continues to absorb agent.

At the other end of the spectrum, casualties who are seriously poisoned will usually not survive long enough to reach an MTF. There are exceptions. If the attack is near an MTF, casualties who are unconscious, apneic, and convulsing or postictal might be seen within minutes of exposure. Or, if the casualties have taken pyridostigmine, a nerve agent pretreatment, they might remain unconscious, convulsing, and with some impairment (but not cessation) of respiration for many minutes to hours. These patients, as well as those in a similar condition who have not used pyridostigmine, require immediate care. If they receive that care before circulation fails and convulsions have become prolonged (see Chapter 5, Nerve Agents), they eventually will recover and be able to return to duty.

Supporting this view is a report from the Tokyo subway terrorist incident of 1995. One hospital received two casualties who were apneic with no heartbeat. With vigorous resuscitation, cardiac activity was established in both. One resumed spontaneous respiration and walked out of the hospital several days later, and the other did not start breathing spontaneously and died days later. These anecdotes suggest that when circumstances permit, resuscitation should be attempted. In a contaminated area where resources, including personnel, are limited, the use of ventilatory support and closed chest cardiac compression must be balanced against other factors (see above), but the immediate administration of diazepam and additional atropine requires little effort and can be very rewarding in the casualty who still has apparent cardiopulmonary function.

Cyanide

Cyanide casualties present the triage officer with few problems. In general, a person exposed to a lethal amount of cyanide will die within 5 to 10 minutes and will not reach the MTF. Conversely, a person who does reach the MTF will not require therapy and will probably be in the minimal group, able to return to duty soon. If the exposure occurs near the treatment area, a severely exposed casualty might appear for treatment. He will be unconscious, convulsing or postictal, and apneic. If the circulation is still intact, the antidotes will restore the casualty to a reasonably functional status within a short period of time. The triage officer, however, must keep in mind that it takes 5 to 10 minutes to inject the two antidotes needed. In a unit-level MTF, a cyanide casualty might be immediate, minimal, or expectant; the last classification would apply if the antidote could not be administered or if the circulation had failed before the casualty reached medical care. In a full-care facility, the casualty might be classified as immediate or minimal.

Vesicants

Most casualties from mustard exposure will require evacuation to a facility where they can receive care for several days to months. The exceptions are those with small areas of erythema and those with only a few small, discrete blisters. Even these guidelines are not as clear-cut as they seem. If the casualty is seen early after exposure, erythema may be the only manifestation, but it may be the precursor of blister formation. Small, discrete blisters may appear innocuous, but on certain areas of the body they can be quite incapacitating, rendering the soldier unfit for duty (see Chapter 7, Vesicants, for a more complete discussion).

Mustard casualties, especially those with eye involvement, are often classified erroneously as immediate for purposes of decontamination. Little is to be gained by this. By the time the mustard lesion forms, the agent has been in contact with the skin, eye, or mucous membrane for a number of hours and the agent that will absorb into the skin or eye tissue has already been absorbed. Immediate decontamination at this time, rather than 30 to 60 minutes later, might prevent the last fraction of a percent of agent penetration, but this will rarely have a significant impact on the care of a casualty or the outcome. These casualties should be decontaminated only after those who require urgent medical care.

Casualties who have liquid mustard burns over 50% or more of body surface area or burns of lesser extent but with more than minimal pulmonary involvement pose a problem for the triage officer. An estimated LD_{50} (ie, the dose that is lethal to 50% of the exposed population) of liquid

mustard, 100 mg/kg, will cover 20% to 25% of body surface area. It is unlikely that a casualty will survive 2 LD_{50} because of the tissue damage from the radiomimetic effects of mustard. Two LD_{50} of liquid will cover about 50% of body surface area, and casualties with a burn this size or greater from liquid mustard should be considered expectant. They will require intensive care (which may include care in an aseptic environment because of leukopenia) for weeks to months, which can be provided only at the far-rear echelons or in the continental United States. Chances of survival are very low in the best of circumstances and are decreased by delays in evacuation. Furthermore, even in a major hospital, long-term care will require assets that might be used for more salvageable casualties. When such casualties are the only casualties, they will receive this care, but in a wartime situation, when beds and medical care are at a premium, medical care assets might best be used for more salvageable casualties elsewhere.

Under battlefield or other mass casualty conditions, casualties with conventional thermal burns covering greater than 70% of body surface area are usually put in the expectant group[1] when medical facilities are limited. This percentage is subject to downward modification (in increments of 10%) by other factors, including further restriction of healthcare availability, coexisting inhalational injury, and associated traumatic injury. There are differences between mustard burns and conventional burns. Conventional burns are likely to have a larger component of third-degree burns, whereas mustard burns are mostly second-degree. On the other hand, exposure to mustard causes problems not seen with conventional burns: hemopoietic suppression and the ensuing susceptibility to systemic infection, which is greater than that seen with conventional burns.

In general, mustard casualties will be classified delayed for both medical attention and decontamination. Exceptions are casualties with a very small lesion (< 5% of body surface area) in a nonsensitive area, who would be classified as minimal and returned to duty; those with large burn areas from liquid mustard (> 50% of body surface area) and those with more than minimal pulmonary involvement, who might be classified as expectant; and those with more than minimal pulmonary involvement, who might also be expectant. In a more favorable medical environment, every effort would be made to provide care for these casualties; at least those in the latter group would be classified as immediate.

In a unit-level MTF, a mustard casualty might be categorized as minimal, delayed, or expectant, but probably not immediate, because the care this casualty would require would not be available. Even if immediate evacuation is possible, the eventual cost in medical care for a casualty needing evacuation must be compared to the probable cost and outcome

of care for a casualty of another type. In a large medical facility where optimum care is available and the cost is negligible, a mustard casualty might be classified as minimal, delayed, or immediate.

Phosgene

The phosgene casualty also may present a dilemma to the triage officer. A casualty who is in marked distress, severely dyspneic, and coughing up frothy sputum might be saved if he entered a fully equipped and staffed hospital; at least, he would receive the full capabilities of that facility. If this casualty does not receive some ventilatory assistance within minutes to an hour, he will not survive. In a forward echelon, this care is not possible, nor is it possible to transport the casualty to a hospital within the critical period. A casualty with mild or moderate respiratory distress and physical findings of pulmonary edema must also be evacuated immediately (even though not triaged in the immediate treatment category because immediate *therapy* will not be provided). Capability for the immediate care that this moderately distressed individual needs is probably unavailable at the first echelons; if evacuation to a full-care MTF is not forthcoming in a reasonably short period, the prognosis becomes grim. Thus, with phosgene casualties, availability of both evacuation and further medical care is important in the triage decision.

Phosgene-induced pulmonary edema varies in severity; a casualty might recover with the limited care given at the unit-level MTF. The real dilemma for the triage officer is a casualty who complains of dyspnea but has no physical signs. One should keep in mind that malingering and “gas hysteria” were common in World War I. To evacuate this casualty might encourage others to come to the MTF with the same complaint, anticipating evacuation from the battle area; not to evacuate might preclude timely care and potentially cause an unnecessary fatality. To observe the individual until signs of illness appear might also postpone medical intervention until the damage is irreversible.

Knowledge about the following physical manifestations of phosgene intoxication[2] may be helpful to the triage officer if a reliable history of the time of exposure is available:

- The first physical signs of phosgene intoxication (crackles or rhonchi) occur at about half the time it takes for the injury to become fully evident. Thus if crackles (rales) are first heard 3 hours after exposure, the lesion will increase in severity for the next 3 hours.
- If there are no signs of intoxication within the first 4 hours, the chance for survival is good, although severe disease may ultimately develop. In contrast, if the first sign is within 4 hours of exposure,

the prognosis is not good, even with care in a medical center. The shorter the onset time, the more ominous the outlook.

Thus, if the triage officer sees a casualty with crackles or rhonchi 3 hours after exposure, the officer can assume that the casualty will be severely ill in 3 hours; within that time, the casualty must reach a medical facility where care can be provided. Even with optimal care, the chances of survival are not good. It should be emphasized that these guidelines apply only to objective signs, not the casualty's symptoms (such as dyspnea). In a contaminated area, it will not be easy and may not be possible to elicit these signs.

In a unit-level MTF, a phosgene casualty might be minimal or expectant, with a separate evacuation group for those who require immediate care if they can be evacuated in time to a facility that can provide it. In a large, higher-echelon MTF, phosgene casualties might be classified as minimal or immediate since there should be no delayed or expectant casualties at a facility in which full care can be provided.

Incapacitating Agents

Casualties showing the effects of exposure to an incapacitating agent may be confused, incoherent, disoriented, and disruptive. They cannot be held at the unit-level MTF but should not be evacuated ahead of those needing lifesaving care unless they are completely unmanageable and are threatening harm to themselves or others. A casualty may be only mildly confused from exposure to a small amount of such an agent, or his history may indicate that he is improving or near recovery. In such instances, the casualty may be held and reevaluated in 24 hours.

In a unit-level MTF, a casualty from exposure to an incapacitating agent might be minimal or delayed, with little need for high priority in evacuation. In a higher-echelon MTF, these casualties would be cared for on a nonurgent basis.

CATEGORIES FOR TRIAGE OF CHEMICAL CASUALTIES

The categories of triage for chemical casualties and the types of chemical casualties that might be placed in each group (Exhibit 14-2) follow.

Immediate

Nerve Agents

A casualty of nerve agents who is in severe distress would be classified as immediate. He may or may not be conscious. He may be in severe respiratory distress, or may have become apneic minutes before reaching the facility. He may not have convulsed, or he may be convulsing or immediately postictal. Often the contents of three MARK I kits (or more) plus diazepam and, possibly, short-term ventilatory assistance will be all that is required to prevent further deterioration and to save a life. In addition, a casualty with signs in two or more systems (eg, neuromuscular, gastrointestinal, respiratory—but excluding eyes and nose) should be classified as immediate and given the contents of three MARK I kits and diazepam.

Cyanide

A casualty of cyanide who is convulsing or became apneic minutes before reaching the medical station and has adequate circulation would be in the immediate group. If the circulation is still adequate, the administration of antidote may be all that is required for complete recovery. Since death may occur within 4 to 5 minutes of exposure to a lethal amount of cyanide unless treatment is immediate, this type of casualty is unlikely to be seen in the MTF.

Phosgene and Vesicants

Casualties of phosgene or vesicant agents who have moderate or severe respiratory distress should be placed in the immediate group when intense ventilatory and other support are immediately available. In a BAS or other unit-level MTF, these support systems will not be available immediately and probably will not be available during the hours required to transport this casualty to a large medical facility. In general, limited assets would best be used for other casualties more likely to benefit from them.

Delayed

Nerve Agents

Casualties who require hospitalization but have no immediate threat to life should be placed in the delayed group. This is generally limited to a casualty who has survived a severe nerve agent exposure, is regaining consciousness, and has resumed spontaneous respiration. This casualty will require further medical care but cannot be held in the unit-level MTF for the time necessary for recovery.

EXHIBIT 14-2
CHARACTERISTICS OF CHEMICAL CASUALTIES BY TRIAGE GROUP

Immediate

- *Nerve Agent*
  - Talking, not walking (severe distress with dyspnea, twitching, and/or nausea and vomiting); moderate-to-severe effects in two or more systems (*eg*, respiratory, gastrointestinal, muscular); circulation intact
  - Not talking (unconscious), not walking; circulation intact
  - Not talking, not walking; circulation not intact (if treatment facilities are available; if not, classify as *expectant*)
- *Cyanide*

  Severe distress (unconscious, convulsing or postictal, with or without apnea) with circulation intact
- *Vesicant*

  Airway injury; classify as immediate if help can be obtained (rare)
- *Phosgene*

  Classify as immediate if help can be obtained

Delayed

- *Nerve Agent*

  Recovering from severe exposure, antidotes, or both

- *Vesicant*

  Skin injury >5% but <50% (liquid exposure) of body surface area; any body surface area burn (vapor exposure); most eye injuries; airway problems starting >6 hours after exposure

- *Cyanide*

  Recovering; has survived more than 15 minutes after vapor exposure

Minimal

- *Nerve Agent*

  Casualty walking and talking; capable of self-aid; return to duty imminent

- *Vesicant*

  Skin injury <5% of body surface area in noncritical areas; minor eye injuries; minor upper-airway injury

Expectant

- *Nerve Agent*

  Not talking; circulation failed (with adequate treatment resources, should classify as *immediate*)

- *Vesicant*

  Over 50% body surface area skin injury from liquid; moderate-to-severe airway injury, particularly with early onset (<6 h after exposure)

- *Cyanide*

  Circulation failed

- *Phosgene*

  Moderate-to-severe injury with early onset

Vesicants

A casualty with a vesicant burn exceeding about 5% and less than 50% of body surface area (if by liquid) or with eye involvement will require hospitalization, but needs no immediate, lifesaving care. He must be observed for respiratory and hemopoietic complications, although, in general, respiratory complications occur at about the time the dermal injury becomes apparent.

Cyanide

After cyanide injury, a casualty will recover completely within the period that he can be held at the unit-level MTF (72 h) and need not be evacuated.

Phosgene

For casualties with significant phosgene injury, evacuation should not be delayed. Pulmonary edema can become life-threatening shortly after onset. If the casualty is to be saved, medical intervention must occur as

quickly as possible. As noted above, however, this may not be possible.

Incapacitating Agents

A casualty showing signs of exposure to an incapacitating agent (such as BZ; see Chapter 11, Incapacitating Agents) usually does not have a life-threatening injury, but will not recover within days and must be evacuated. A casualty who has had a very large exposure, however, and is convulsing or has cardiac arrhythmias might be an exception. He requires immediate attention if it can be made available.

Minimal

Nerve Agents

A nerve agent casualty who has only mild effects from the agent vapor (such as miosis, rhinorrhea, or mild-to-moderate respiratory distress) should be categorized as minimal. He can be treated satisfactorily with the contents of one or more MARK I kits if any treatment is indicated. A casualty who has administered self-aid for these effects may need no further therapy and can often be returned to duty in 24 hours or sooner.

Vesicants

A vesicant casualty with a small area of burn—generally less than 5% of body surface area in a noncritical site, but the area size depends on the site (see Chapter 7, Vesicants)—can possibly be cared for and returned to duty. Lesions covering larger areas or evidence suggesting more than minimal pulmonary involvement would place this casualty in another triage group.

Cyanide

A casualty who has been exposed to cyanide and has not required therapy will recover quickly.

Phosgene

A casualty exposed to phosgene rarely belongs in the minimal group. If development of pulmonary edema is suspected, the casualty is placed in a different triage group. On the other hand, if a casualty gives a reliable history of exposure several days before, reports mild dyspnea in the intervening time, and is now improving, the triage officer should consider holding the casualty for 24 hours for reevaluation, with the intent of

returning him to duty.

Incapacitating Agents

The evaluation of a casualty exposed to an incapacitating agent should be similar to that of a phosgene casualty. If the casualty's condition is worsening, evacuation is necessary. On the other hand, if there is a reliable history of exposure with an intervening period of mild effects and evidence of recovery, he could be observed for 24 hours on-site with the intent of returning him to duty.

Expectant

Nerve Agents

Any nerve agent casualty who does not have a palpable pulse or is apneic with the onset time of apnea unknown should be categorized as expectant. (However, as noted above, some of these casualties may survive if prolonged, aggressive care is possible.)

Cyanide

A cyanide casualty who does not have a palpable pulse belongs in the expectant group.

Vesicants

A vesicant casualty who has burns covering more than about 50% of body surface area from liquid exposure, or who has definite signs of more than minimal pulmonary involvement, will survive only with extensive medical care, which will not be forthcoming in forward echelons. This care might be available at rear echelons, but care there should be reserved primarily for those with greater chances of survival at a lower expenditure of medical assets.

Phosgene

A casualty with moderate or severe dyspnea and signs of advanced pulmonary edema from phosgene exposure requires a major expenditure of rear-area medical assets if evacuation could be accomplished quickly enough.

CASUALTIES WITH COMBINED INJURIES

Casualties with combined injuries not only have wounds that were

caused by conventional weapons but also have been exposed to a chemical agent. The conventional wounds may or may not be contaminated with chemical agent. Few experimental data on this topic exist, and little has been written specifically about these casualties from experiences in World War I or the Iran–Iraq War.

Some factors that might influence triage decisions at a unit-level MTF are discussed below. As noted above, most of these factors would not apply or would be ignored at a higher-level MTF that is relatively fully staffed and equipped, where the capability for medical care is not at a premium.

Nerve Agents

In a casualty with mild-to-moderate intoxication from exposure to nerve agent vapor, administering the contents of MARK I kits can rapidly and completely reverse the nerve agent effects. Further triage decisions and medical care should focus on the conventional wound.

In a casualty with severe systemic effects from agent exposure, the effects should be treated before all but the most emergent wound care is given. Of course, airway support (including removal of obstruction) must be given and bleeding controlled if the casualty is to be saved. Which is done first—airway management, bleeding control, or antidote administration—will depend on which problem, in the judgment of the emergency care provider, is the most immediate threat to life. (Immediate spot-decontamination or thorough flushing of the wound and surrounding skin, if these are possibly sites of exposure, must be done at once.) If the casualty is convulsing, bleeding might be difficult to control; on the other hand, his airway is probably at least minimally intact.

In general, if a casualty is walking and talking, the agent injury should not influence judgments about treatment of conventional injuries. If the casualty is talking but not walking because of the agent injury, the casualty is immediate because of the agent injury. He should be given the contents of three MARK I kits and diazepam immediately. His response will determine his further triage. Muscular paralysis or weakness, however, and its cause, inhibition of cholinesterase (pyridostigmine, the nerve agent pretreatment drug, also inhibits cholinesterase), might influence later decisions about anesthesia.[3]

If the casualty is not breathing because of nerve agent effects, attempting to provide ventilatory assistance might preclude the immediate care of a severe wound or other assistance in the contaminated area. If ventilation is marginal and the wound alone would classify the casualty as immediate, the time and effort required to stabilize ventilation might preclude timely wound care. The dual requirements might require more

care providers than are available.

A casualty who has a wound that needs immediate care, but who is unconscious and has impaired ventilation resulting from nerve agent intoxication might initially be considered expectant, particularly if other, more salvageable casualties exist. One should administer the contents of three MARK I kits and diazepam and reevaluate this casualty when time becomes available. A major medical facility would have the personnel to devote to simultaneous care of ventilation and the wound.

Mustard

In the front echelon, devoting a large effort to wound care in a patient whose long-term prognosis is poor from the effects of chemical agent exposure alone may not be warranted. A patient with a wound that would warrant a classification of immediate might become expectant with the addition of a significant skin lesion or more than minimal pulmonary effects from mustard exposure. Similarly, classifying a casualty as delayed on the basis of a wound may not be appropriate if liquid mustard burns are spread over more than 50% of his body surface or if the casualty has more than minimal pulmonary effects. Hemopoietic suppression may influence wound healing and will certainly decrease resistance to infection and the ability to recover from it. The long-term care of a major wound, whether initially classified as immediate or delayed, will often be unsuccessful in a patient with moderate or severe pulmonary signs and symptoms or with dermal involvement of more than 50% of body surface (from liquid) when first seen.

Phosgene

Several factors in the pathophysiology and natural course of phosgene intoxication suggest that a casualty with moderate-to-severe effects from phosgene exposure is not a good candidate to survive major wounding. The pulmonary edema causes hypoxia, which must be corrected before surgery. In addition, the fluid causing pulmonary edema is fluid lost from the circulation, which results in significant volume depletion, hypotension, and a large degree of hemoconcentration (*eg*, a hematocrit of 0.65–0.70). A wound with more than minimal blood loss further impairs the circulating volume, and the hypotension would be more difficult than usual to correct. The administration of fluid to correct the hypovolemia and hypotension potentially causes more fluid to leak through the alveolarcapillary membranes into the alveoli, which increases the pulmonary lesion and further reduces the capacity for oxygen and carbon dioxide exchange. Fluids must be given, however, to prevent failure of other organs. Even if it

were possible to repair the traumatic wound, several days later the lung would inevitably become the focus for bacterial colonization. The ensuing pneumonitis or pneumonia often becomes a nidus for sepsis, which might impair healing.

In the forward echelons, these problems cannot be corrected; the triage officer must judge whether the casualty can be evacuated to a higher-level MTF where they can be addressed, and whether evacuation can be carried out before the damage becomes irreversible. The treatment of phosgene injury alone requires a significant expenditure of assets. When that injury is complicated by factors that tend to worsen the pathophysiology, treatment becomes a major problem that might be insurmountable in all but the most fully staffed and equipped medical centers.

Cyanide

A casualty from exposure to a lethal amount of cyanide will die within a few minutes if he receives no therapy. If antidotes are given in time, he will recover with no serious adverse effects or sequelae to interfere with wound care. One of the antidotes, sodium nitrite, causes vasodilation and orthostatic hypotension, but these effects are short and should not be factors in overall patient care. If a casualty with a conventional wound and severe effects from cyanide poisoning presented at the unit-level MTF (or even at a major hospital), the procedure would be to give the antidote immediately. If the effects of cyanide are reversed, he should receive further care.

Incapacitating Agents

A serious problem in treating a casualty presenting with a major wound and intoxication by an incapacitating compound is that he might be delirious and unmanageable. If the compound is a cholinergic blocking agent, such as BZ (3-quinuclidinyl benzilate), the administration of the antidote physostigmine will calm him temporarily (the effects dissipate in 45–60 min) so that care can be given. At some stage of their effects, these incapacitating compounds cause tachycardia, suggesting that heart rate may not be a reliable indication of cardiovascular status. Otherwise, nothing known about these compounds suggests that they will interfere with wound healing or further care.

SUMMARY

Triage of casualties of chemical agents is based on the same principles as the triage of conventional casualties. The triage officer tries to provide

immediate care to those who need it to survive; he sets aside temporarily or delays treatment of those who have minor injuries or do not need immediate medical intervention; and he does not use limited medical assets on the hopelessly injured. At the first echelon of medical care on a battlefield, medical capabilities are very limited. When chemical agents are present or suspected, medical capabilities are further diminished because early care must be given while the medical care provider and casualty are in protective clothing. Decontamination, a time-consuming process, must be carried out before the casualty receives more definitive care, even at this level. At the rear echelons of care—or at a hospital in peacetime—medical capabilities are much greater and decontamination has already been accomplished before the casualty enters for treatment.

Triage is a matter of judgment by the triage officer. This judgment should be based on knowledge of medical assets, the casualty load, and, at least at unit-level MTFs, the evacuation process. Most importantly, the triage officer must have full knowledge of the natural course of an injury and its potential complications.

REFERENCES

1. Bowen TE, Bellamy RF, eds. *Emergency War Surgery NATO Handbook*. 2nd rev US ed. Washington, DC: Department of Defense, Government Printing Office; 1988.

2. Urbanetti JS. Clinical Assistant Professor of Medicine, Yale University School of Medicine, New Haven, Conn. Personal communication, 1989.

3. Keeler JR. Interactions between nerve agent pretreatment and drugs commonly used in combat anesthesia. *Milit Med*. 1990;155:527–533.

Chapter 15

DECONTAMINATION

CHARLES G. HURST, M.D.*

INTRODUCTION

METHODS OF DECONTAMINATION
- Physical Removal
- Chemical Methods
- Certification of Decontaminationn

WOUND DECONTAMINATION
- Initial Decontamination
- General Considerations
- Thickened Agents
- Off-Gassing
- Foreign Material
- Wound Contamination Assessment
- Dilute Hypochlorite Solution
- Wound Exploration/Debridement

BIOLOGICAL AGENT DECONTAMINATION
- Chemical Method
- Physical Method

SUMMARY

Colonel, Medical Corps, U.S. Army; currently, Special Assistant for Medical Programs, Office of the Deputy Assistant Secretary of Defense, Counterproliferation and Chemical/Biological Matters, Room 3E808, 3050 Defense Pentagon, Washington, D.C. 20301-3050; formerly, Commander, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425

INTRODUCTION

Decontamination is defined as the reduction or removal of chemical (or biological) agents so they are no longer hazards. Agents may be removed by physical means or be neutralized chemically (detoxification). Decontamination of skin is the primary concern, but decontamination of eyes and wounds must also be done when necessary. Decontamination can be further defined:

- *personal* decontamination refers to decontamination of oneself,
- *casualty* decontamination refers to the decontamination of casualties, and
- *personnel* decontamination usually refers to decontamination of noncasualties.

The most important and most effective decontamination after any chemical or biological exposure is that decontamination done within the first minute or two after exposure. This is personal decontamination. Early action by the soldier to decontaminate himself will make the difference between survival (or minimal injury) and death (or severe injury). Good training can save lives.

Decontamination of chemical casualties is an enormous task. The process requires dedication of both large numbers of personnel and large amounts of time. Even with appropriate planning and training, decontamination of casualties demands a significant contribution of resources. Liquids and solids are the only substances that can be effectively removed from the skin. It is generally not possible or necessary to decontaminate skin following vapor exposure. Removal from the atmosphere containing the vapor is all that is required.

Many substances have been evaluated for their usefulness in skin decontamination. The most common problems with potential decontaminants are irritation of the skin, toxicity, ineffectiveness, or high cost. An ideal decontaminant will rapidly and completely remove or detoxify all known chemical and biological warfare agents. Furthermore, a suitable skin decontaminant must have certain properties that are not requirements for decontaminants for equipment.[1,2] Recognized desirable traits of a skin decontaminant are shown in Exhibit 15-1. Decontamination issues have been explored since the beginning of modern chemical warfare. After years of research worldwide, simple principles that consistently produce good results are still recommended.

The first, which is without equal, is timely physical removal of the chemical agent. To remove the substance by the best means available is the primary objective. Chemical destruction (detoxification) of the offending agent is a desirable secondary objective. Physical removal is imperative because none of the chemical means of destroying these agents destroy them instantaneously. While decontamination preparations such as fresh hypochlorite (either sodium or calcium hypochlorite) react rapidly with some chemical agents (eg, the half-time for destruction of the nerve agent VX by hypochlorite at pH 10 is 1.5 min), the half-times of destruction of other agents, such as mustard, are much longer.[3] If a large amount of agent is present initially, a longer time is needed to completely neutralize the agent to a harmless substance.

EXHIBIT 15-1
DESIRABLE TRAITS OF A SKIN DECONTAMINANT

- Neutralizes all chemical and biological agents
- Is safe (nontoxic and noncorrosive)
- Is applied easily by hand
- Is readily available
- Acts rapidly
- Produces no toxic end products
- Is stable in long-term storage
- Is stable in the short term (after issue to unit individual)
- Is affordable
- Does not enhance percutaneous agent absorption
- Is nonirritating
- Is hypoallergenic
- Is easily disposed of

Sources: (1) Chang M. A Survey and Evaluation of Chemical Warfare Agent Contaminants and Decontamination. Dugway Proving Ground, Utah: Defense Technical Information Center; 1984. AD-202525. (2) Baker JA. COR Decontamination/Contamination Control Master Plan Users' Meeting. 11–13 September 1985. Unpublished.

Decontamination studies have been conducted using common household products. The goal of these studies was identification of decontaminants for civilians as well as field expedients for the soldier. Timely use of water, soap and water, or flour followed by wet tissue wipes produced results equal, nearly equal, or in some instances better than those produced by the use of fuller's earth, Dutch Powder, and other compounds.[4] (Fuller's earth [diatomaceous earth] and Dutch Powder [Dutch variation of fuller's earth] are decontamination agents currently fielded by some European countries.) Because no topical decontaminant has ever shown efficacy with chemical agent that has penetrated into the skin, and because chemical agents may begin penetrating the skin before

complete reactive decontamination (detoxification) takes place, early physical removal is most important.

Military personnel may be questioned for guidance by local civilian authorities or may deal with supply shortages in the field. Knowledge of the U.S. doctrinal decontaminating solutions may not suffice in these situations, and awareness of alternative methods of decontamination will prove very beneficial. What decontamination method is used is not as important as how and when it is used. Chemical agents should be removed as quickly and completely as possible by the best means available.

The M291 resin kit and 0.5% hypochlorite for chemical casualty decontamination are currently fielded by the U.S. military. The M291 kit is new, whereas hypochlorite solution has been around since World War I. The M291 kit is our best universal dry decontaminant for skin. Fresh 0.5% hypochlorite solution with an alkaline pH is our universal liquid decontaminating agent and is recommended for all biological agents.

Fig. 15-1. The six individual decontamination pads of the M291 kit are impregnated with the decontamination compound Ambergard XE-555 Resin, which is the black, free-flowing, resin-based powder. Each pad has a loop that fits over the hand. As the soldier holds the pad in one hand, he scrubs the pad over his contaminated skin. The chemicals are rapidly transferred into and trapped in the interior of the resin particles. The presence of acidic and basic groups in the resin promotes the destruction of trapped chemical agents by acid and base hydrolysis. Because the resin is black, the area that has been decontaminated is easy to see. Photograph: Courtesy of Michael R. O'Hern, Sergeant First Class, US Army (Ret) and Larry L. Harris, Sergeant First Class, US Army (Ret).

The M291 resin kit is best for spot-decontamination of skin (Figure 15-1). The dry, black resin rapidly adsorbs the chemical agent, with carbonaceous material physically removing the agent from skin contact. Later, an ion-exchange resin neutralizes the offending agent by chemical detoxification. Since the M291 kit is small and dry and easily carried by the soldier, it is well suited for field use.[5,6] Early intervention with the use of this kit will reduce chemical injury and save life in most cases.

Decontamination of the casualty using an M291 kit does not obviate the need for decontamination at a field medical treatment facility (MTF).

Chemical agent transfer is a potential problem that can be resolved by a second, deliberate decontamination. This thorough decontamination at the MTF prevents spread of the agent to areas of the body previously uncontaminated, contamination of personnel assisting the patient, and contamination of the MTF itself.

Liquids are best for decontaminating large or irregular surface areas. Hypochlorite solutions are well suited for MTFs with adequate water supplies. For hypochlorite to be most effective, it has to be relatively fresh (made daily or more frequently, particularly in a warm environment where evaporation will occur) and have a concentration of 0.5% at an alkaline pH (pH 10–11). Hypochlorite solutions are for use on skin and soft-tissue wounds only. Hypochlorite should *not* be used in abdominal wounds, in open chest wounds, on nervous tissue, or in the eye. Surgical irrigation solutions should be used in liberal amounts in the abdomen and chest. All such solutions should be removed by suction instead of sponging and wiping. Only copious amounts of water, normal saline, or eye solutions are recommended for the eye. Contaminated wounds are discussed later in this chapter.

METHODS OF DECONTAMINATION

Three basic methods of decontamination are physical removal, chemical deactivation, and biological deactivation of the agent. Biological deactivation has not been developed to the point of being practical.

Physical Removal

Several types of physical and chemical methods are at least potentially suitable for decontaminating equipment and material. Flushing or flooding contaminated skin or material with water or aqueous solutions can remove or dilute significant amounts of chemical agent. Scraping with a wooden stick (ie, a tongue depressor or Popsicle stick) can remove bulk agent by physical means. A significant advantage of most physical methods is their nonspecificity. Since they work nearly equally well on chemical agents regardless of chemical structure, knowledge of the specific contaminating agent or agents is not required.

Flushing With Water or Aqueous Solutions

When animal skin contaminated with the nerve agent GB was flushed with water at 2 minutes (a method in which physical removal predominates over hydrolysis of the agent), 10.6 times more GB was required to produce the same mortality rate as when no decontamination occurred.[7] In another

study, the use of water alone produced better results than high concentrations of hypochlorite (ie, 5% or greater, which is not recommended for skin). [8] Timely copious flushing with water physically removes the chemical agent and will produce good results.

Adsorbent Materials

Adsorption refers to the formation and maintenance of a condensed layer of a substance, such as a chemical agent, on the surface of a decontaminant, as illustrated by the adsorption of gases by charcoal particles and by the decontaminants described in this section. Some North Atlantic Treaty Organization (NATO) nations use adsorbent decontaminants in an attempt to reduce the quantity of chemical agent available for uptake through the skin. In emergency situations, dry powders such as soap or detergents, earth, and flour may be useful. Flour followed by wiping with wet tissue paper is reported [4] to be effective against the nerve agents soman (GD) and VX and against mustard.

M291 Resin

The current method of battlefield decontamination by the individual soldier involves the use of a carbonaceous adsorbent, a polystyrene polymeric, and ion-exchange resins (the M291 kit; see Figure 15-1). The resultant black powder is both reactive and adsorbent. The M291 kit has been extensively tested and has proven highly effective for skin decontamination.[5,6] It consists of a walletlike carrying pouch containing six individual decontamination packets. Each packet contains a nonwoven, fiberfill, laminated pad impregnated with the decontamination compounds. Each pad provides the individual with a single-step, nontoxic, nonirritating decontamination application, which can be used on the skin, including the face and around wounds. Instructions for use are marked on the case and packets.

Chemical Methods

Three types of chemical mechanisms have been used for decontamination: water/soap wash; oxidation; and acid/base hydrolysis.[9] Mustard (HD) and the persistent nerve agent VX contain sulfur molecules that are readily subject to oxidation reactions. VX and the other nerve agents (tabun [GA], sarin [GB], soman [GD], and GF) contain phosphorus groups that can be hydrolyzed. Therefore, most chemical decontaminants are designed to oxidize mustard and VX and to hydrolyze nerve agents (VX and the G series).[1]

Water and Water/Soap Wash

Both fresh water and sea water have the capacity to remove chemical agents not only through mechanical force but also via slow hydrolysis; however, the generally low solubility and slow rate of diffusion of chemical warfare agents in water significantly limit the agent hydrolysis rate.[10]

The predominant effect of water and water/soap solutions is the physical removal or dilution of agents; however, slow hydrolysis does occur, particularly with alkaline soaps. In the absence of hypochlorite solutions or other appropriate means of removing chemical agents, these methods are considered reasonable options.[4]

Oxidation

The most important category of chemical decontamination reactions is oxidative chlorination. This term covers the “active chlorine” chemicals like hypochlorite. The pH of a solution is important in determining the amount of active chlorine concentration. An alkaline solution is advantageous. Hypochlorite solutions act universally against the organophosphorus and mustard agents.[11]

Both VX and HD contain sulfur atoms that are readily subject to oxidation. Current U.S. doctrine specifies the use of a 0.5% sodium or calcium hypochlorite solution for decontamination of skin and a 5% solution for equipment.

Hydrolysis

Chemical hydrolysis reactions are of two types: acid and alkaline. Acid hydrolysis is of negligible importance for agent decontamination because the hydrolysis rate of most chemical agents is slow, and adequate acid catalysis is rarely observed.[11] Alkaline hydrolysis is initiated by the nucleophilic attack of the hydroxide ion on the phosphorus atoms found in VX and the G agents. The hydrolysis rate is dependent on the chemical structure and reaction conditions such as pH, temperature, the kind of solvent used, and the presence of catalytic reagents. The rate increases sharply at pH values higher than 8 and increases by a factor of four for every 10°C rise in temperature. Several of the hydrolytic chemicals are effective in detoxifying chemical warfare agents; unfortunately, many of these (eg, sodium hydroxide) are unacceptably damaging to the skin. Alkaline pH hypochlorite hydrolyses VX and the G agents quite well.[3,12]

Certification of Decontamination

Regardless of the method used to decontaminate, certification of chemical decontamination is accomplished by any of the following: processing through the decontamination facility; M8 paper; M9 tape; M256A1 ticket; or by the CAM (chemical agent monitor). (See Chapter 16, Chemical Defense Equipment, for a discussion of this detection equipment.) If proper procedure is followed, the possibility of admitting a chemically contaminated casualty to a field MTF is extremely small. The probability of admitting a dangerously contaminated casualty is minuscule to nonexistent. Fear is the worst enemy, not the contaminated soldier.

WOUND DECONTAMINATION

All casualties entering a medical unit after experiencing a chemical attack are to be considered contaminated unless there is certification of noncontamination. The initial management of a casualty contaminated by chemical agents will require removal of mission-oriented protective posture (MOPP) gear and decontamination with 0.5% hypochlorite before treatment within the field MTF.

Initial Decontamination

During initial decontamination in the decontamination areas, bandages are removed and the wounds are flushed; the bandages are replaced only if bleeding recurs. Tourniquets are replaced with clean tourniquets and the sites of the original tourniquets decontaminated. Splints are thoroughly decontaminated, but removed only by a physician. The new dressings are removed in the operating room and submerged in 5% hypochlorite or placed in a plastic bag and sealed.

General Considerations

Of the chemical agents discussed, only two types, the vesicants and the nerve agents, might present a hazard from wound contamination. Cyanide is quite volatile, so it is extremely unlikely that liquid cyanide will remain in a wound. A very large amount of liquid cyanide is required to produce vapor sufficient to cause effect.

Mustard converts to a cyclic compound within minutes of absorption into a biological milieu, and the cyclic compound reacts rapidly (ie, within minutes) with blood and tissue components.[13] These reactions will take place with the components of the wound—the blood, the necrotic tissue, and the remaining viable tissue. If the amount of bleeding and tissue damage is small, mustard will rapidly enter the surrounding viable tissue, where it will quickly biotransform and attach to tissue components (and its

biological behavior will be much like an intra-muscular absorption of the agent).

Although nerve agents cause their toxic effects by their very rapid attachment to the enzyme acetylcholinesterase, they also quickly react with other enzymes and tissue components. As they do with mustard, the blood and necrotic tissue of the wound will “buffer” the nerve agents. Nerve agent that reaches viable tissue will be rapidly absorbed, and since the toxicity of the nerve agents is quite high (a lethal amount is a small fraction of a drop), it is unlikely that casualties whose wounds are contaminated with much liquid nerve agent will survive to reach medical care.[14]

Potential risk to the surgeon from contaminated wounds arises from chemical agent on foreign bodies in the wound and from thickened agents.[15] Medical personnel treating biological casualties have only a minimal risk from secondary aerosolization of biological agents.

Thickened Agents

Thickened agents are chemical agents that have been mixed with another substance (commonly an acrylate) to increase their persistency. They are not dissolved as quickly in biological fluids nor are they absorbed by tissue as rapidly as other agents. VX, although not a thickened agent, is absorbed less quickly than other nerve agents and may persist in a wound longer than other nerve agents.

Thickened agents in wounds require more precautions. Casualties with thickened nerve agents in wounds (eg, from pieces of a contaminated battle-dress uniform or protective garment being carried into the wound tract) are unlikely to survive to reach surgery. Thickened mustard has delayed systemic toxicity and can persist in wounds even when large fragments of cloth have been removed. Although the vapor hazard to surgical personnel is extremely low, contact hazard from thickened agents does remain and should always be assumed.[14]

No country is currently known to stockpile thickened agents. In a chemical attack, the intelligence and chemical staffs should be able to identify thickened agents and to alert the medical personnel of their use.

Off-Gassing

The risk from vapor off-gassing from chemically contaminated fragments and cloth in wounds is very low and not significant. Further, there is no vapor release from contaminated wounds without foreign bodies. Off-gassing from a wound during surgical exploration will be negligible or zero. No eye injury will result from off-gassing from any of the

chemical agents. A chemical-protective mask is not required for surgical personnel.[14]

Biological agents can only be transmitted to medical personnel from secondary aerosolization from dry agents. Decontamination with 0.5% hypochlorite solution or flooding with water or saline will make this risk negligible. No protective equipment is necessary for surgical personnel other than standard barrier protections, unless the patient is infected with the plague bacillus, smallpox, or a hemorrhagic fever virus, or if procedures likely to generate bloody aerosols are employed. In such cases, wearing of a filtered respirator is recommended.

Foreign Material

The contamination of wounds with mustard or nerve agents is basically confined to the pieces of contaminated fabric in the wound tract. The removal of this cloth from the wound effectively eliminates the hazard. There is little chemical risk associated with individual fibers left in the wound. No further decontamination of the wound for unthickened chemical agent is necessary.[14]

Wound Contamination Assessment

The CAM can be used to assist in locating contaminated objects within a wound; however, 30 seconds are required to achieve a bar reading. The CAM detects vapor but may not detect liquid (a thickened agent or liquid on a foreign body) deep within a wound. A single-bar reading on CAM with the inlet held a few millimeters from the wound surface indicates that a vapor hazard does not exist.[14]

Dilute Hypochlorite Solution

Dilute hypochlorite (0.5%) is an effective skin decontaminant for patient use. The solution should be made fresh daily with a pH in the alkaline range (pH 10–11). Plastic bottles containing 6 ounces of calcium hypochlorite crystals are currently fielded for this purpose.

Dilute hypochlorite solution is contraindicated for the eye; it may cause corneal injuries. This substance is also not recommended for brain and spinal cord injuries. Irrigation of the abdomen with hypochlorite solution may lead to adhesions and is therefore also contraindicated. The use of hypochlorite in the thoracic cavity may be less of a problem, but the hazard is still unknown. Wound Exploration and Debridement Surgeons and assistants are advised to wear a pair of well-fitting (thin) butyl rubber gloves or double latex surgical gloves and to change them often until they are certain there are no foreign bodies or thickened agents in the wound.

Wound Exploration and Debridement

Surgeons and assistants are advised to wear a pair of well-fitting (thin) butyl rubber gloves or double latex surgical gloves and to change them often until they are certain there are no foreign bodies or thickened agents in the wound. Thin butyl rubber gloves will have no breakthrough for 60 or more minutes in an aqueous base. Double latex surgical gloves will have no breakthrough for 29 minutes in an aqueous medium; they should be changed every 20 minutes.[16] This is especially important where puncture is likely because of the presence of bone spicules or metal fragments.[14]

The wound should be explored with surgical instruments rather than with the fingers. Pieces of cloth and associated debris must not be examined closely but quickly disposed of in a container of 5% hypochlorite. The wound can then be checked with the CAM, which may direct the surgeon to further retained material. It takes about 30 seconds to get a stable reading from the CAM. A rapid pass over the wound will not detect remaining contamination.

The wound should be debrided and excised as usual, maintaining a no-touch technique. Removed fragments of tissue should be dropped into a container of 5% to 10% hypochlorite. Bulky tissue such as an amputated limb should be placed in a plastic or rubber bag (chemical proof), which is then sealed.[14]

Dilute hypochlorite solution (0.5%) may be instilled into deep, noncavity wounds following the removal of contaminated cloth. This solution should be removed by suction to a disposal container. Within a short time (ie, 5 min), this contaminated solution will be neutralized and rendered nonhazardous. Subsequent irrigation with saline or other surgical solutions should be performed.

Penetrating abdominal wounds caused by large fragments or containing large pieces of chemically contaminated cloth will be uncommon. Surgical practices should be effective in the majority of wounds for identifying and removing the focus of remaining agent within the peritoneum. When possible, the CAM may be used to assist.

Saline, hydrogen peroxide, or other irrigating solutions do not necessarily decontaminate agents but may dislodge material for recovery by aspiration with a large-bore suction tip. The irrigation solution should *not* be swabbed out manually with surgical sponges. Although the risk to patients and medical attendants is minuscule, safe practice suggests that any irrigation solution should be considered potentially contaminated. Following aspiration by suction, the suction apparatus and the solution should be decontaminated in a solution of 5% hypochlorite. Superficial

wounds should be subjected to thorough wiping with 0.5% hypochlorite and subsequent irrigation with normal saline or sterile water.

Surgical and other instruments that have come into contact with possible contamination should be placed in 5% hypochlorite for 10 minutes prior to normal cleansing and sterilization. Reusable linen should be checked with the CAM, M8 paper, or M9 tape for contamination. If found to be contaminated, the linen should be soaked in a 5% to 10% hypochlorite solution.

BIOLOGICAL AGENT DECONTAMINATION

Decontamination of personnel and equipment after a biological warfare attack is a lesser concern than after a chemical warfare attack because most biological warfare agents are not dermally active (the trichothecene mycotoxins are an exception). Still, decontamination remains an effective way to decrease the spread of infection from potential secondary aerosolization.

For biological agents, *contamination* is defined as the introduction of microorganisms into tissues or sterile materials, whereas *decontamination* is defined as disinfection or sterilization of infected articles to make them suitable for use (the reduction of microorganisms to an acceptable level). *Disinfection* is defined as the selective elimination of certain undesirable microorganisms to prevent their transmission (the reduction of the number of infectious organisms below the level necessary to cause infection), and *sterilization* is defined as the complete killing of all organisms. Biological warfare agents can be decontaminated by chemical and physical methods.

Chemical Method

Chemical decontamination renders biological warfare agents harmless by the use of disinfectants. Dermal exposure to a suspected biological warfare agent should be immediately treated by soap and water decontamination. Careful washing with soap and water removes a very large amount of the agent population from the surface. It is important to use a brush to ensure mechanical loosening from the skin surface structures, and then to rinse with copious amounts of water. This method is often sufficient to avert contact infection. The contaminated areas should then be washed with a 0.5% hypochlorite solution, if available, with a contact time of 10 to 15 minutes. The solution should be applied with a cloth or swab or can be sprayed on. As with hypochlorite in chemical decontamination, this solution should not be used in the eyes, abdominal cavity, or on nerve tissue. It will neutralize and render nonhazardous any biological agent within approximately 5 minutes.

For decontamination of fabric clothing or equipment, a 5% hypochlorite solution should be used. For decontamination of equipment, a contact time of 30 minutes prior to normal cleaning is required. Use of hypochlorite solution in this way is corrosive to most metals and injurious to most fabrics, so they should be rinsed thoroughly and metal surfaces should be oiled after completion.

An important point to remember is that soap and water washing followed by hypochlorite washing to decontaminate for biological agents should be prompt but should follow any needed use of decontaminants for chemical agents. Ampules of calcium hypochlorite granules are currently fielded in the chemical agent decontamination kit for mixing hypochlorite solutions. The 0.5% solution can be made adding one 6-ounce container of calcium hypochlorite granules to 5 gallons of water. The 5% solution can be made by adding eight 6-ounce containers of calcium hypochlorite granules to 5 gallons of water. These solutions evaporate quickly at high temperatures, so if they are made in advance, they should be stored in closed containers. The hypochlorite solutions should be placed in distinctly marked containers because it is very difficult to distinguish visually a 0.5% solution from a 5% solution.

Physical Method

Physical methods are concerned with rendering biological warfare agents harmless through such physical means as heat and radiation. To render agents completely harmless, dry heat requires 2 hours of treatment at 160°C. If steam is used at 121°C and 1 atm of overpressure (15 psi), the time may be reduced to 20 minutes, depending on volume. This last method is also known as autoclaving. The part of solar ultraviolet radiation that reaches the Earth’s surface has a certain disinfectant effect, often in combination with drying. Ultraviolet radiation is effective but hard to standardize into practical usage for disinfection or decontamination purposes.

SUMMARY

Decontamination at the MTF is directed toward (1) eliminating any chemical agent transferred to the patient during removal of protective clothing; (2) decontaminating or containing of contaminated clothing and personal equipment; and (3) maintaining an uncontaminated MTF.

Current doctrine specifies the use of 0.5% hypochlorite solution for chemical or biological skin contamination or the M291 kit for chemically contaminated skin. Fabric and other foreign bodies that have been introduced into a wound can sequester and slowly release chemical agent,

presenting a liquid hazard to both the patient and medical personnel. Dry biological agent could be a hazard through secondary aerosolization. Adequate liquid decontamination will mitigate this hazard. There is no vapor hazard, and protective masks are not necessary for surgical personnel.

REFERENCES

1. Chang M. *A Survey and Evaluation of Chemical Warfare Agent Contaminants and Decontamination.* Dugway Proving Ground, Utah: Defense Technical Information Center; 1984. AD-202525.

2. Baker JA. COR Decontamination/Contamination Control Master Plan Users' Meeting; 11–13 September 1985. Unpublished.

3. Yurow HW. Decontamination methods for HD, GB and VX: A literature survey. Aberdeen Proving Ground, Md: Army Armament Research and Development Command; Chemical Systems Laboratory; 1981. AD-B057349L.

4. van Hooidonk C. CW agents and the skin: Penetration and decontamination. In: Proceedings of the International Symposium on Protection Against Chemical Warfare Agents; June 6–9, 1983; Stockholm, Sweden. National Defense Research Institute.

5. Hobson D, Blank J, Menton R. *Comparison of Effectiveness of 30 Experimental Decontamination Systems and Evaluation of the Effect of Three Pretreatment Materials Against Percutaneous Application of Soman, Thickened Soman, VX, and Sulfur Mustard to the Rabbit.* Edgewood Area, Aberdeen Proving Ground, Md; 1985. MREF Task 85-12: Final Report.

6. Hobson D, Blank J, Menton R. *Testing of Candidate CSM Decontamination Systems.* Edgewood Area, Aberdeen Proving Ground, Md; 1986. MREF Task 86-25: Final Report.

7. Zvirblis P, Kondritzer AA. Protective ointment M5, water and bleaches as skin decontaminants for GB, I. In: *Studies on Skin Decontamination.* Army Chemical Center, Md: Medical Laboratories; 1953. Report 193.

8. Zvirblis P. Bleaching powder and related items as decontaminants for liquid GB, II. In: *Studies on Skin Decontamination*. Army Chemical Center, Md: Medical Laboratories; 1954. Report 307.

9. Gum R, Madsen J, Keeler J, Hurst C. Decontamination White Paper. Aberdeen Proving Ground, Md: US Army Medical Research Institute of Chemical Defense; 1991. Unpublished.

10. Chang AMH, Ciegler A. Chemical warfare, I. *Chemical Decontamination, NBC Defense and Technical International.* 1986;1(40):60.

11. Trapp R. *The Detoxification and Natural Degradation of Chemical Warfare Agents.* Stockholm, Sweden: Stockholm International Peace Research Institute

(SIPRI); 1985: 44–75.

12. Block F, Davis GT. *Survey of Decontamination Methods Related to Field Decontamination of Vehicles and Materiel.* Dugway Proving Ground, Utah: Defense Technical Information Center; 1978: 59–60. AD-B031659.

13. Papirmeister B, Feister A, Robinson S, Ford R. *Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological Implications.* Boca Raton, Fla: CRC Press; 1991: 92.

14. Cooper GJ, Ryan JM, Galbraith KA. The surgical management in war of penetrating wounds contaminated with chemical warfare agents. *J R Army Med Corps*. 1994;140:113–118.

15. Hobson D, Snider T. *Evaluation of the Effects of Hypochlorite Solutions in the Decontamination of Wounds Exposed to Either the Organophosphanate Chemical Surety Materiel VX or the Vesicant Chemical Surety Materiel HD.* Columbus, Ohio: Battelle Memorial Institute; 1992. Task 89-04.

16. Smith W, PhD. US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Md. Personal communication, 1996.

Chapter 16

CHEMICAL DEFENSE EQUIPMENT

MICHAEL R. O'HERN * ; THOMAS R. DASHIELL † ; AND MARY FRANCES TRACY ‡

INTRODUCTION

INDIVIDUAL PROTECTION EQUIPMENT
- Respiratory Protection
- Protective Clothing

DETECTION AND WARNING
- Chemical Detection and Warning
- Integrated Mobile Systems

COLLECTIVE PROTECTION

DECONTAMINATION EQUIPMENT

ADDITIONAL PATIENT PROTECTION AND TRANSPORT EQUIPMENT

SUMMARY

PSYCHOLOGICAL PROBLEMS ASSOCIATED WITH WEARING MISSION-ORIENTED PROTECTIVE POSTURE GEAR

- HISTORY
- THE CURRENT SITUATION
  - Modern Case Studies
  - Treatment
- SUMMARY

*Sergeant First Class, U.S. Army (Ret); PO Box 46, Aberdeen, Maryland 21010; formerly, Noncommissioned Officer in Charge, Chemical Casualty Care Office, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425

† Consultant, Life Sciences and NBC Defense, 504 Thomas Avenue, Frederick, Maryland 21701-6251; formerly, Director, Environmental and Life Sciences, Office of the Director of Defense Research, Office of the Secretary of Defense, Department of Defense, Washington, D. C.

‡ Research Scientist, Chemical Warfare/Chemical and Biological Defense Information Center (CBIAC): a Department of Defense Information Analysis Center operated by Battelle Memorial Institute and sponsored by Defense Technical Information Center, Aberdeen Proving Ground, Maryland 21010-5425

INTRODUCTION

A number of countries around the world have the capability to use chemical weapons. In fact, within the past decade, several events have been well documented where chemical weapons were used in armed conflict, most notably during the Iran–Iraq War. The most recent threat of such use was during the Persian Gulf War, where U.S. forces might have been exposed to the effects of both chemical and biological agents. An essential part of preparedness to continue operations in a chemical environment is adequate equipment.[1,2] Such equipment must encompass all areas of concern: detection and warning, personal protective equipment, decontamination and medical prophylaxis, and treatment. Only an integrated approach to the problem of protection can allow individuals to provide an effective response in a chemical warfare environment with a minimum degradation in human performance.

The primary item of protection is the personal respirator, designed to protect individuals against volatile agents and aerosols. The respirator must be carefully fitted on the face to ensure minimal leakage, and individuals must be well trained in the donning of masks (a maximum of ≤ 9 sec being desirable). In addition to the respiratory hazard, many chemical agents are dermally active. This requires that a proper overgarment, usually containing an activated charcoal layer to adsorb chemical agent, be donned, along with protective gloves and boots. The complete ensemble can seriously degrade individual performance; 50% reduction of mission-related task performance has routinely been measured in tests. (The physiological effects of wearing chemical and biological protective gear are discussed in detail in *Environmental Hazards of the Battlefield*, a forthcoming volume in the *Textbook of Military Medicine* series.) In addition to physical performance degradation, there are reports of psychological problems with some individuals while wearing the complete ensemble, owing to the claustrophobic effects.[3] This subject is discussed separately at the end of this chapter, in a section titled *Psychological Problems Associated With Wearing Mission-Oriented Protective Posture Gear*.

The rapid detection and warning of an opponent's use of chemical agents is critical to the protection of forces.[4,5] Usually, the chemical agent will be delivered via an aerial or missile attack, or an upwind release where the cloud of agent passes over a troop concentration. Timely detection is required to permit all potentially exposed forces to adopt an adequate posture, since the effects of agents can sometimes occur in less than a minute. Vesicant agents and some nerve agents (eg, VX and some of the G series), which can remain active for long periods of time, can affect individuals via the dermal route, thus requiring that a proper overgarment be part of the protective ensemble. Likewise, detection equipment is also used to confirm agent hazard reduction and facilitates reducing the mission-oriented protective posture (MOPP) level and the removal of protection equipment: the "all clear" signal.

Decontamination of equipment, facilities, and personnel is also required after an attack if effective military operations are to be maintained. Some of this decontamination burden can be mitigated by the use of effective collective protection equipment, which can allow continuing operations such as communications and medical care within protected facilities.

One criterion for the selection and use of protective equipment items is the need for joint service use, although there are some differences between the missions of air and ground crews that must be accommodated. This chapter is not intended to be all-encompassing in chemical defense equipment; rather, it is intended to describe the items and operations that are of greatest interest to the medical community.

The following sections address each of the protection areas described above in detail, with the current equipment items featured and items in development that are designed to overcome the deficiencies of present equipment briefly described. Sufficient technical data are included to allow the healthcare professional to become familiar with the operation, components, and the limitations of the present chemical defense equipment. Should the interested reader desire

more detail on chemical defense equipment, several sources are available. First, the written references and expert consultants to this chapter are sources of vast amounts of information. Possibly of more value to the healthcare professional is the nuclear, biological, and chemical (NBC) officer who is an integral part of each combat element and who is available to provide detailed advice as well as hands-on assistance.

Several tangential issues must be noted that impact on the area of chemical defense equipment, especially in the future. First, a continuing intelligence need exists to identify new agents that may be used against combat forces and ensure that the defense equipment meets the new threats. Second, it cannot be overemphasized that a viable, active, training program be maintained. And third, medical input into operations while participants are wearing protective equipment is vital to maintenance of a combat operation. Rest periods consonant with work loads and MOPP gear will allow continuing operations even in a contaminated environment. The development program will provide continuing improvements in the chemical defense equipment available to the forces, and updates will be required as new and better equipment comes on line.

INDIVIDUAL PROTECTION EQUIPMENT

The chemical–biological warfare threat can come in three possible physical forms: gas, liquid, and aerosol (ie, a suspension in air of liquid or solid particles). Protection against chemical agents disseminated as aerosols is especially difficult because the individual particles deliver a large amount of agent at a tiny site, thereby overwhelming the local capacity of the adsorbent.

Chemical agents can gain entry into the body through two broad anatomical routes: (1) the mucosa of the oral and respiratory tracts and (2) the skin. The icon of chemical warfare—the gas mask—protects the oral and nasal passages (as well as the eyes), while the skin is protected by the overgarment.

As noted earlier, total individual protection requires an integrated approach with the primary mechanism being respiratory protection which, when combined with an overgarment, gloves, and boots all properly fitted and used correctly, can provide excellent protection against chemical agents of all known types.

Respiratory Protection

Much of the basis of our understanding of the general principles of respiratory protection is contained in four source documents:

- *Chemical Warfare Respiratory Protection: Where We Were and Where We Are Going*, an unpublished report prepared for the U.S. Army Chemical Research, Development, and Engineering Center [6];
- *Jane's NBC Protection Equipment* (the most recent edition available), particularly the chapter titled "Choice of Materials for Use With NBC Protection Equipment" [7];
- *Basic Personal Equipment*, volume 5 of NATO's *NAIG Prefeasibility Study on a Soldier Modernisation Program*, published in 1994[8]; and
- *Worldwide NBC Mask Handbook*, published in 1992.[9]

Readers interested in greater detail can consult these sources and the authors of this chapter.

The fundamental question of protective mask design was first addressed in World War I: should the mask completely isolate the soldier from the poisonous environment or should the mask simply remove the specific threat substance from the ambient air before it can reach the respiratory mucosa? The first approach requires that a self-contained oxygen supply be provided. Because of a multitude of practical logistical constraints (eg, weight, size, expense), this approach is not used except for specialty applications in which the entire body must be

enclosed.

The more common practice has been to follow the second approach: to prevent the agent from reaching the respiratory mucosa by chemically destroying it, removing it in a nonspecific manner by physically adsorbing it, or both. Destruction by chemical reaction was adopted in some of the earliest protective equipment such as the “hypo helmet” of 1915 (chlorine was removed by reaction with sodium thiosulfate) and in the British and German masks of 1916 (phosgene was removed by reaction with hexamethyltetramine).[6] More commonly, the removal of the agent was brought about by its physical adsorption onto activated charcoal. (Due to its mode of formation, this substance has an extraordinarily large surface area, some 300–2,000 m^2 / g,[10] with a corresponding plethora of binding sites.) It was soon recognized that impregnation of the charcoal with substances such as copper oxide, which reacted chemically with certain threat agents, further increased protection.[6]

The effectiveness of modern masks depends on both physical adsorption and chemical inactivation of the threat agent. For example, in the M17 protective mask the adsorbent, known as ASC Whetlerite charcoal, is charcoal impregnated with copper oxide and salts of silver and chromium.[6] The M40 protective mask uses an ASZ impregnated charcoal, which substitutes zinc for the hexavalent chromium (CrVI). The Centers for Disease Control and Prevention and the National Institute for Occupation Safety and Health have identified CrVI as a potential human carcinogen.[11] A filter layer to remove particles and aerosols greater than 3 µm in diameter is also a component of all protective masks.

The location of the filters and adsorbent vis-à-vis the respiratory tract was also one of the questions that mask designers addressed in World War I. In the standard British mask (the small box respirator of 1916), the filter and the adsorbent were contained in a separate container worn around the soldier’s trunk and connected to the mask by a hose. By way of contrast, in the standard German mask introduced in late 1915, the filter and adsorbent, contained in a small can (canister), were attached directly to the mask. The advantages of the canister arrangement were lighter weight and reduced work of breathing. But these advantages were gained at the expense of a smaller protective capacity and a degree of clumsiness associated with motion of the head. The canister is attached directly to the mask in the majority of modern protective masks. The contents of a modern canister are shown in Figure 16-1.

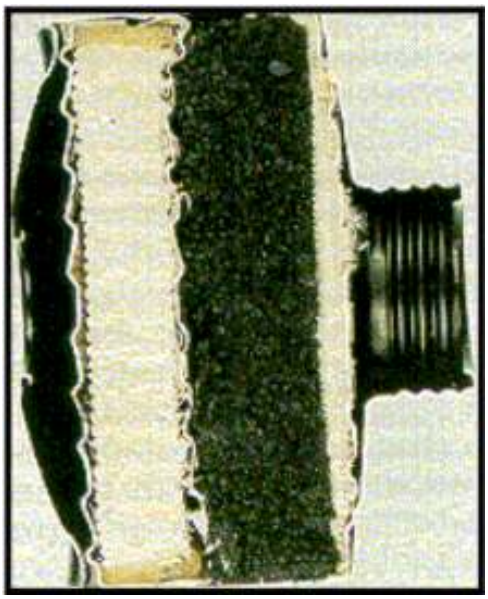


Fig. 16-1. The C2A1 canister is used with the M40 protective mask. After ambient air enters through the orifice on the left side, it passes first through the pleated white filter (where aerosols are removed), then through the layer of ASZ charcoal, then through a second filter (to remove charcoal dust), and finally exits the canister through the orifice on the right side. Photograph: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

Several of the essential features of modern protective mask design—features that might be thought to be more recent—also originated during World War I. For example, designing the inside of the mask so that inhaled air is first deflected over the lenses (which prevents exhaled air, saturated with water vapor, from fogging the lenses) and the use of separate one-way inlet and outlet valves (to minimize the work of breathing) were World War I–era inventions. The need of masked soldiers to be able to talk to one another was also recognized then. Interestingly, in the period after World War I, the U.S. Navy introduced the first useful solution to this problem: a moveable diaphragm held in place by perforated metal plates in the front of the mask. This device ultimately became the voicemitter found in today’s protective masks.[6]

An important question of mask design is the composition of the elastic material used to cover the face: the faceblank. The first masks introduced in World War I were made of rubberized cloth or leather. Subsequent masks used natural rubber, but recently, sophisticated synthetic polymers using silicone, butyl, and perfluorocarbon rubbers have been used.[6] Silicone rubber has the advantage of making possible a tight fit or seal between the mask and skin, with a correspondingly decreased potential for leaking (a factor said to be responsible for about 5% of mask failures).[12]

Unfortunately, silicone rubber offers rather low resistance to the penetration of common chemical agents. Perfluorocarbon rubber is very impermeable but is expensive and tears easily. Butyl rubber offers both good protection and seal and has therefore become the material of choice.[7] Even this description of materials used to construct the faceblank underestimates the complexity of actual mask design. In today's standard U.S. military masks, the faceblank consists of two separate layers: an inner layer made of silicone rubber (for maximum seal) and an outer layer made of butyl rubber for maximum protection (Figure 16-2).

Fig. 16-2. The M45 protective mask facepiece has two skins. The inner skin is composed of silicone rubber, and the outer skin is composed of butyl rubber. This arrangement maximizes both mask-to-skin seal and chemical agent impermeability. A similar design is used in the M40 protective mask. Photograph: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

Fig. 16-3. The nosecup of the M45 protective mask has a single, large hole in the center through which exhaled air is expelled on its way to the exit valve in the main mask. Inhaled air, which has passed through the canister, passes up and around the side of the nosecup, preventing fogging of the mask's lenses, after which it passes through the valve (seen on the reader's left) on its way to the soldier's respiratory tract. Photograph: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

The design of the modern protective mask is a sophisticated process. This is nowhere more apparent than in the designers' recognition of the dictates of respiratory physiology: specifically, the importance of dead space. The greater the space between the back of the mask and the face of the wearer in relation to the tidal volume, the smaller the proportion of inhaled air that will reach the alveoli. To minimize dead-space ventilation, modern protective masks have what is equivalent to a second mask—the nosecup—which is fitted separately from the mask proper and inserted between the main mask and the wearer's midface (Figure 16-3). The smaller volume encompassed by the nosecup, rather than the total volume enclosed by the entire mask, is responsible for most of the dead space added by the mask. Furthermore, the nosecup provides an extra seal against entry of threat agents.[6]

The work of breathing added by the mask is an important factor; it determines not only soldiers' acceptance of a given mask, but more importantly, the degree that a soldier's exercise tolerance is degraded. Since the pressure gradient that is required to move a given mass of air is flow-rate dependent, to make a quantitative comparison between the work of respiration of different masks, it is necessary to specify a specific flow rate. For example, at a flow rate of 85 L/min, a pressure gradient of about 8 cm H_2O is observed in World War II–vintage masks. At the same flow rate, the gradient for the M17 is 4.5 cm H_2O, and for the M40, 5 cm H_2O.[6] By way of contrast, breathing at a rate of 85 L/min without a mask requires a pressure gradient of 1.5 cm H_2O. [13] Some mask wearers perceive the 3-fold increase in the work of breathing as "shortness of breath."

Developmental objectives in personal respiratory protection equipment generally encompass factors such as personal comfort, breathing resistance, mask weight, and the ability to provide protection from new agents. Present equipment has met a number of these objectives but much remains to be done, especially in the area of new and improved chemical-resistant materials, manufacturing methods, and scratch-resistant lenses. All of these items must be integrated into a new, reliable, less cumbersome, and less degrading system.

Ground Crew Personal Protective Equipment

The equipment described below is generally suitable for use by all services, although oceanic environments may require that other construction materials be developed for the navy and marine corps. The masks protect against all known chemical and biological agents, whether in droplet, aerosol, or vapor form. However, a protective mask is only as good as its fit. In the past, the degree of fit was assessed by field-expedient qualitative indices (eg, the degree to which the mask collapsed with its inlet valve obstructed). The modern technology incorporated into the M41 Protection Assessment Test System allows the degree of fit to be quantitated.

M41 Protection Assessment Test System

The protective masks issued to members of the U.S. armed forces protect the individual's face, eyes, and respiratory tract from field concentrations of chemical–biological agents, toxins, and radioactive fallout particles. Several critical steps must be taken to ensure that an assigned mask will function properly in a toxic chemical environment:

- select the correct mask size,
- properly fit the selected mask,
- validate the mask protection,
- train the user in the proper wear and use of the mask, and
- perform preventive maintenance checks on the mask as required.

The M41 Protection Assessment Test System (PATS) was fielded to validate the protection afforded by the M40, M42, and M17 series masks (Figure 16-4). The PATS is a miniature, continuous flow, condensation nuclei counter. It samples particles from ambient air and compares them with particles in the air contained inside the wearer's mask. The resulting numerical values are then used to determine the protection factor (PF) of the mask. The result of the pass/fail test is determined by the mask's ability to provide a PF of 1,667 or greater, which is the minimum army requirement. The PATS ensures that the mask is the proper size for the individual wearer,

and that there are no critical leaks in the mask system due to missing or defective parts or improper maintenance.

Two PATS are fielded for each battalion-sized unit, and are located at the headquarters company. One PATS is fielded for each separate company-sized unit. To date, the PATS is used by the army and has been ordered for the marines and air force.

Fig. 16-4. The M41 Protection Assessment Test System (PATS). Ambient air is assessed through the green hose. Air inside the mask is assessed through the colorless hose, which couples with the protective mask by means of the drinking tube extension. For further information, see Department of the Army. *Protection Assessment Test System (PATS).* Washington, DC: DA; 14 January 1995. Training Circular 3-41.

Mask, Chemical–Biological: Field, M17A2

The blended natural rubber faceblank of the M17A2 Chemical–Biological Field Mask protects the wearer's face, eyes, and respiratory tract, while the attached M6A2 hood protects the exposed portions of the head and neck (Figure 16-5). The M17A2 protects the face, eyes, and respiratory tract from field concentrations of chemical and biological agents. When used together, the natural rubber facepiece and the M6A2 hood resist liquid chemical and biological agents. In fact, the hood was designed to completely cover not only the rubber components of the mask but also the head and neck so as to augment protection against liquid agents.

The M17A2 protective mask provides respiratory protection through the use of two M13A2 filter elements. Each filter element is "pork chop" shaped and is internally mounted within the cheek pouches of the mask. Each also consists of an activated charcoal gas filter paper and a particulate filter laminated together.

This mask can be used in any climatic condition, but the M4 winterization kit must be installed when used in temperatures of –20°F or below. A voicemitter outlet valve, provided on the front of the facepiece, transmits the user's voice outside the mask. A drinking tube assembly is attached just below the voicemitter and allows the user to drink while wearing the protective mask. The drink system couples with the M1 canteen cap (Figure 16-6).

The forehead straps, temple straps, and cheek straps come together at a head pad for ease of fitting. The M17A2 mask is manufactured in four sizes to accommodate all personnel: extra small, small, medium, and large. For personnel requiring vision correction, optical inserts are provided. The optical inserts are both prong-type and wire frame–types; the wire frame–type is easier to mount inside the mask.

The mask is compatible with shoulder-fired weapons, night-vision devices, and sighting devices. A variety of accessory items is available, including the M1 waterproof bag, the M4 winterization kit, the M6A2 hood, the M15A1 carrier, and optical inserts and outserts. This series of mask is currently being replaced by the M40 protective mask.[14,15]

a b c

Fig. 16-5. (a) The M17A2 Chemical–Biological Field Mask. **(b)** The M17A2 protective mask with hood. **(c)** This transparent version of the M17A2 protective mask was prepared in the hope that

wearers could recognize each other. Now this unique design, which was never fielded, serves only to show where the two pork chop–shaped M13A2 filter elements are located. Photographs (a) and (b): Reprinted from Brletich NR, Tracy MF, Dashiell TR. *Worldwide NBC Mask Handbook*. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Center; September 1992: 371. Photograph (c): Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

Mask, Chemical–Biological: Field, M40

The M40 Chemical–Biological Field Mask series represents the latest generation of protective mask to be issued to the U.S. military. The inner layer of the facepiece is composed of molded silicone rubber that fits tightly against the face, and has an inturned peripheral seal, which increases comfort and fit. The mask's two ridged eyelenses are approximately 35% larger than the type used in the M17A2, thus providing a better field of view (Figure 16-7). Filtration is provided in the M40 mask by one C2A1 filter canister, which, at the user's convenience, can be mounted on either cheek. Two canisters may be mounted on both cheeks for special-purpose activities such as explosive ordnance disposal or technical escort. The standard C2A1 canister will protect against 16 attacks of nerve and vesicant agents. For each exposure, the C*t* can be as great as 20,000 C*t* mg•min/m^3 (C*t* represents the product of concentration [in milligrams per cubic meter of air] and time [in minutes] of exposure to a gas or aerosol, and is discussed in other chapters in this textbook, in particular Chapter 5, Nerve Agents). Any other standard-thread canister issued by North Atlantic Treaty Organization (NATO) countries will fit the M40 mask.

Fig. 16-6. The M17A2 Chemical–Biological Field Mask with drinking tube assembly allows the soldier to drink without unmasking. Soldiers wearing mission-oriented protective posture (MOPP) gear must drink water to prevent heat stress. The drinking tube, essentially a flexible straw, couples with the canteen cap. The soldier holds the canteen upright and inverted, then sips water through the tube. After a few sips, the soldier needs to puff his own exhaled air back into the canteen to equalize the atmospheric pressure without introducing contaminated air. Then he can take a few more sips of water before he needs to equalize the pressure again. Photograph: Courtesy of Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md .

Fig. 16-7. The M40 Chemical–Biological Field Mask. Reprinted from Brletich NR, Tracy MF, Dashiell TR. Worldwide NBC Mask Handbook. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Center; September 1992: 385.

Fig. 16-8. The M42 Chemical–Biological Field Mask. Reprinted from Brletich NR, Tracy MF, Dashiell TR. Worldwide NBC Mask Handbook. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Center; September 1992: 383.

Communication is provided by two voicemitters. One is mounted in the front to allow face-to-face communication; the second is located in the cheek to permit the use of a radio telephone handset. A drinking system consists of internal and external drink tubes; the external tube has a quick-disconnect coupling that connects with the M1 canteen cap. A six-point, adjustable harness with elastic straps located at the forehead, temples, and cheeks comes together at a rectangular head pad.

The M40 mask comes in three sizes: small, medium, and large. Optical inserts are provided for vision correction and outserts are available to reduce fogging and sun glare and to protect against scratching. A check valve on the nosecup prevents exhaled air from fogging the lenses inside, and an air deflector directs inhaled air over the lenses, which also helps prevent fogging. Accessory items available include a carrier, a hood to protect the neck areas, and a waterproof bag.[16–18]

Mask, Chemical–Biological: Field, M42

The M42 Chemical–Biological Field Mask is the same series as the M40. The materials of construction and the basic features are identical, but the M42 protective mask is used by combat vehicle crews (Figure 16-8).

Filtration is provided by a C2A1 canister attached to the mask by a corrugated hose; the canister is housed in a specially designed canister carrier. The M42 integrates with the combat vehicle filtration protection system. The M42 also has a dynamic microphone that integrates with the combat vehicle via a microphone cable.[19,20]

Mask, Chemical–Biological: MCU-2/P

The MCU-2/P Chemical–Biological Mask is used by U.S. Air Force ground crews and aircrews when not in flight. This protective mask is constructed of molded silicone rubber facepiece material, and an integral, molded, polyurethane, one-piece panoramic lens is bonded to it (Figure 16-9).

Filtration is provided by one C2A1 canister mounted on either side of the facepiece. The primary voicemitter is located over the mouth area with a secondary voicemitter in the cheek area to utilize telephone handsets. The mask incorporates a drinking tube, which connects to the M1 canteen cap. The mask has a six-point, adjustable head harness suspension made of elastic, which comes together in the center head back into a rectangular patch of woven material. The mask comes in three sizes: small, medium, and large. Accessories include a carrier bag, a butyl-coated nylon cloth hood, outserts to protect the lens in storage, and a waterproof bag.

Aircrew Personal Protective Equipment

Each protective mask in current use is described in detail. There are some differences between the masks designed for helicopter use and high-performance aircraft, owing notably to the operational envelope. All masks protect against all known chemical and biological agents whether in droplet, aerosol, or vapor form.

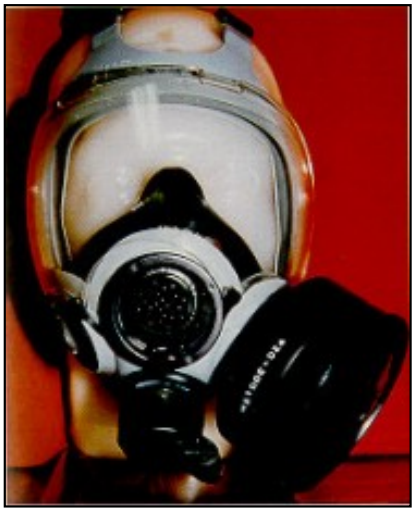


Fig. 16-9. The MCU-2/P Chemical–Biological Mask. Reprinted from Brletich NR, Tracy MF, Dashiell TR. *Worldwide NBC Mask Handbook.* Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Center; September 1992: 401.

Mask, Chemical–Biological: Aircraft, M43

The facepiece of the M43 Chemical–Biological Aircraft Mask is fabricated of bromo butyl and natural rubber with an integral butyl hood and skull-type suspension system (Figure 16-10). The M43 has two models, designated Type I and Type II. The two models are identical with the following exceptions: Type I has a notch in the right eyepiece that accommodates a special sighting device used by Apache helicopter pilots, and uses a different microphone for communication; Type II has two spherical lenses and uses a dynamic microphone. Both microphones interface with the helicopter communications systems.

Fig. 16-10. The M43 Chemical–Biological Aircraft Mask. Photograph: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

The mask is connected to two C2A1 canisters, which lower breathing resistance. A hose assembly that attaches to the two C2A1 canisters is located on the left cheek. The canisters are attached to a motor blower unit (capacity: 4 cu ft/min), which is powered either by aircraft electrical power or a battery. A constant overpressure is maintained within the mask by the motor blower unit.

The mask has an inhalation air-distribution assembly for regulating the flow of air to the mouth and nose, eyelenses, and hood assembly. The M43 mask has a drink capability which couples with the canteen cap. The mask is produced in four sizes from small to extra-large. Accessories include a mask carrier, vision correction outserts, winterization kit, nuclear hood, facepiece carrier, eyelens cushions, and a blower and harness assembly.

This new design effort was based on the need for little-to-no visual impairment. The requirement was met by placing the protective mask's eyelens 14 mm from the eye, which kept the spherical curvature equidistant from the corneal surface to eliminate

parallax. This lens configuration increased visual capability to within 4% of nonmasked vision in the same individual. Each mask is fitted to an individual crewman and remains with that crewman while he remains on flight status.[21,22]

Mask, Chemical–Biological: Aircrew MBU-I9/P

The MBU-19/P Chemical–Biological Aircrew Mask is the newest generation to be fielded by the U.S. Air Force exclusively for aircrews. This mask, dubbed the Aircrew Eye/Respiratory Protection (AERP) system, is issued in a helmeted version for fighter pilots and in a nonhelmeted version for aircrew and pilots of other types of aircraft (Figure 16-11).

Fig. 16-11. The MBU-19/P Chemical–Biological Aircrew Mask. Reprinted from Brletich NR, Tracy MF, Dashiell TR. *Worldwide NBC Mask Handbook.* Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Center; September 1992: 395.

The AERP mask-hood subsystem has a hood composed of bromo butyl coated fabric that incorporates standard MBU-19/P oxygen mask, clear plastic lens, neckdam, drinking facility, and communications systems. The MBU-19/P breathing subsystem consists of a chemical-resistant delivery hose, a chemical–biological canister, inline filter, and manifold assembly including an emergency oxygen filter. The breathing system will operate whether or not supplemental oxygen is present.

The blower system incorporates a variable-speed motor, battery, external power-supply cable, housing assembly, control switch, chemical–biological canister, and a means of securing the blower while the crew member is mobile. The mask receives filtered air from the blower unit, which also allows overpressure within the hood which defogs the lens and is vented through an exhaust valve. The communication system consists of the intercommunication unit, battery, electrical branch assembly, microphone, and bracket.

Developmental Respiratory Protection Equipment

The objective of development systems is to provide the next generation of respiratory protection equipment that will minimize mission degradation and assure compatibility with future weapons systems and equipment while maintaining protection levels. RESPO 21 is the latest generation wherein new materials and manufacturing technology are being investigated and evaluated. [23] New and improved filtration systems designed to remove or degrade new classes of agents are under evaluation. Systems designed to meet all service needs in one equipment item are in the design phases. It is hoped that these systems will overcome the deficiencies found in current equipment (eg, excessive weight and performance degradation).

Protective Clothing

An overgarment can be made to protect skin from chemical agents by either physical or chemical means:

1. The overgarment can be made of fabric that is impermeable to most molecules, even to air and water vapor.
2. The overgarment can be made of fabric that is permeable to most molecules, but that also chemically alters or physically removes chemical agents before they reach the skin.

In the first method, the chemical agent is totally excluded because the agent is physically

prevented from penetrating the substance of the overgarment. In the second method, the agent enters into the fabric of the overgarment but is absorbed before it can reach the skin. An overgarment made of an impermeable material such as Saran wrap or butyl rubber can offer complete protection against threat agents but at the unacceptable cost of causing heat injury. Cooling by sweating is not possible if water vapor cannot pass through to the ambient environment. Most fielded overgarments, therefore, depend on the fabric's ability to adsorb the threat agent. Activated charcoal is used for this purpose in U.S. military designs.

Placing a soldier into full chemical protective equipment—mask, overgarment, gloves, and boots—is a decision that appropriately considers not only the protection aspect but also the added heat stress and potential for dehydration. The heat stress problem must be recognized from the start.

Personnel must begin a drinking regimen prior to encapsulation to ensure that they do not become dehydrated quickly. The physical burden of a full ensemble can add 9 to 14 lb to a normal load; this added weight combined with heat stress, dehydration, and physical exertion can cause significant impairment to any mission.

Because of these factors, the completeness of protection is stratified by the anticipated magnitude of the threat from chemical–biological agents: that is, *the mission-oriented protective posture* (Figure 16-12). Five MOPP levels have been recognized previously, but with Change 2 to Field Manual 3-4, NBC Protection, the number was updated to seven in 1996 (Exhibit 16-1).[5] The two new MOPP levels are MOPP Ready and Mask-Only Command, but readers should be aware that MOPP levels are revised frequently to meet newly defined needs.

The MOPP level must be coordinated with the work load if troops are to remain effective. The over-garments in present use must be redesigned to reduce heat stress, reduce weight and bulk, and provide increased comfort as well as reduce the logistical burden. The present clothing will be described in detail except for the special-purpose equipment used by demilitarization personnel or special-purpose forces.

The sources for the following discussion are *Items of Combat Clothing and Equipment*, [24] and experts at the U.S. Army Natick Research, Development, and Engineering Center, Natick, Massachusetts, [25] whom interested readers can consult for greater detail.

Fig. 16-12. From left to right, the soldiers' gear is for mission-oriented protective posture (MOPP) levels 2, 3, and 4. Photograph: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

Protective Ensembles

Like various other armies of the world, the U.S. Army has chemical protective clothing available for individual protection. Several types are available, depending on the protection required to perform a specific mission and whether the protective clothing needs to be permeable or impermeable. Most troops use permeable protective clothing, which allows for air and moisture to pass through the fabric without hindering the chemical protection capabilities of the clothing. This type of permeable protective clothing is described in the following section.

Battledress Overgarment

The current standard A protective overgarment is the battledress overgarment (BDO). The BDO protects the wearer from all chemical agent vapors, liquid droplets, biological agents, toxins, and radioactive alpha and beta particles; however, the BDO does not stop either X or gamma radiation. For weartime and protective capabilities of the

BDO following removal from the protective bag, refer to Field Manual 3-4/Fleet Marine Force Manual 11-9, *NBC Protection*.[5] The BDO protects the wearer for 24 hours after contamination from chemical agent vapors, liquids, and droplets; and biological agents and toxins.

The effectiveness of the BDO is in its serviceability. Weartime of the BDO begins when it is removed from the sealed vapor-barrier bag and stops when it is returned to the vapor-barrier bag. *Wearing the BDO for any part of a day constitutes a day's wear*. The BDO becomes unserviceable if it is torn, ripped, a fastener is missing or broken, or petroleum, oils, or lubricants are splashed or spilled on the overgarment. This unserviceableness necessitates replacement.

The BDO is manufactured in two layers: a tightly woven outer layer and a charcoal-impregnated inner layer to adsorb agent liquid or vapor (Figure 16-13). The garment consists of a hip-length coat and trousers with appropriate fasteners and multiple pockets. It is manufactured in eight sizes ranging from XXX Small through XX Large. The BDO is not designed to be decontaminated or reimpregnated for reuse.

EXHIBIT 16-1
LEVELS OF MISSION-ORIENTED PROTECTIVE POSTURE (MOPP)

MOPP Ready	Soldiers carry their protective masks with their load-carrying equipment. The soldier's MOPP gear is labeled and stored no further back than the battalion support area and is ready to be brought forward to the soldier when needed. The time necessary to bring the MOPP gear forward should not exceed 2 hours. A second set of MOPP gear is available within 6 hours. Units at MOPP Ready are highly vulnerable to attacks with persistent agents and will automatically upgrade to MOPP Zero when they determine, or are notified, that chemical weapons have been used or that the threat for use of chemical weapons has risen. When a unit is at MOPP Ready, soldiers will have field-expedient items identified for use.
MOPP Zero	Soldiers carry their protective masks with their load-carrying equipment. The standard battledress overgarment and other individual protective equipment that make up the soldier's MOPP gear are readily available. "Readily available" means that equipment must either be carried by each soldier or be stored within the soldier's arms' reach (eg, within the work area, vehicle, or fighting position). Units at MOPP Zero are highly vulnerable to attacks with persistent agents and will automatically upgrade to MOPP 1 when they determine, or are notified, that persistent chemical weapons have been used or that the threat for use of chemical weapons has risen.
MOPP 1	When directed to MOPP 1, soldiers immediately don the battledress overgarment. In hot weather, the overgarment jacket may be unbuttoned and the battledress overgarment may be worn directly over the underwear. M9 or M8 chemical detection paper is attached to the overgarment. MOPP 1 provides a great deal of protection against persistent agents. The level is automatically assumed when chemical weapons have been employed in an area of operations or when directed by higher commands.
MOPP 2	Soldiers put on their chemical protective footwear covers, green vinyl overboots, or a field-expedient item (eg, vapor-barrier boots), and the protective helmet cover is worn. As with MOPP 1, the overgarment jacket may be left unbuttoned but the trousers remain closed.

MOPP 3	Soldiers wear the protective mask and hood. Again, flexibility is built into the system to allow the soldier relief at MOPP 3. Particularly in hot weather, soldiers may open the overgarment jacket and roll the protective mask hood for ventilation but he trousers remain closed.
MOPP 4	Soldiers will completely encapsulate themselves by closing their overgarments, rolling down and adjusting the mask hood, and putting on the NBC rubber gloves with cotton liners. MOPP 4 provides the highest degree of chemical protection, but it also has the most negative impact on an individual's performance.
Mask-Only Command	Only the protective mask is worn. The mask-only command is given in these situations: 1. When riot control agents are being employed and no chemical or biological threat exists. 2. In a downwind vapor hazard of a nonpersistent chemical agent. The mask-only command is not appropriate when blister agents or persistent nerve agents are present.

Adapted from Avery M. Major, Chemical Corps, US Army; US Army Chemical School, Doctrine Development Division, Fort McClellan, Ala. *New MOPP Levels and Peacetime Filter Changeout Criteria.* (Summary of Change 2 to Field Manual 3-4, 21 Feb 1996.) Internet Chemical-Doctrine discussion site, 24 Jul 1996.

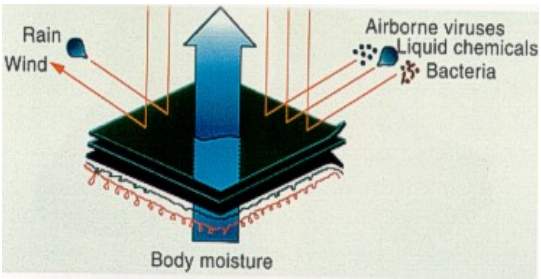


Fig. 16-13. A soldier wearing the battledress overgarment (BDO) and battledress uniform (BDU) is afforded five layers of protection. Although allowing body moisture to evaporate, the garments repel rain, wind, airborne viruses, liquid chemicals, and bacteria. (1) The BDO consists of two layers. The outer layer is a 50% nylon–50% cotton, twill weave, Quarpel-treated fabric in woodland camouflage pattern. (2) The inner layer of the BDO is made of 90-mil polyurethane foam impregnated with activated carbon and laminated on the inner side with nylon-tricotton knit. (3) The BDU for temperate-zone wear is made of 50% cotton–49% nylon–1% static dissipative fiber, twill weave fabric. (4) The drawers and undershirt are made of 100% cotton. (5) Human skin surface. Drawing: Courtesy of US Army Research Institute of Environmental Medicine, Biophysics and Biomedical Modeling Division, Natick, Mass.

Aircrew Uniform, Integrated Battlefield

The aircrew uniform, integrated battlefield (AUIB) is designed to replace the BDO, the chemical protective overgarment (CPOG), and the Nomex (a synthetic aramid polymer, manufactured by Du Pont Advanced Fiber Systems, Wilmington, Del.) flight suit for aircrews operating in a contaminated environment (Figure 16-14). It is also designed to protect against petroleum and oils. It provides flame resistance as well as NBC protection. The outer shell is a laminate of 95% Nomex/5% Kevlar (polypara-phenyleneterephthalamide, manufactured by Du Pont Advanced Fiber Systems, Wilmington, Del.), while the inner layer is a 90-mil, carbon-impregnated, flame-resistant foam/nylon laminate. The AUIB is designed as a two-piece garment with a coat and trousers with appropriate fasteners and is available in

woodland or desert camouflage. The heat stress burden of the AUIB is similar to that of the BDO. [5]

Fig. 16-14. The aircrew uniform, integrated battlefield (AUIB).

Chemical Defense Aircrew Ensemble

The chemical defense aircrew ensemble (CDAE) is the newest generation of aircrew protective clothing to be fielded by the U.S. Air Force. It is a one-piece garment consisting of the Nomex flight suit, a charcoal undergarment, and long cotton underwear. The CDAE incorporates carbon-sphere technology to adsorb chemical agent. It is basically two suits differing in color: the CWU-66/P is green and the CWU-77/P is brown. It may be laundered as many as 10 times prior to chemical agent exposure without destroying the protective capabilities of the coverall. [26]

Protective Boots and Gloves

A soldier wearing the chemical protective boots and gloves discussed here will soon realize that mobility is compromised by the boots and that tactile ability is degraded by the gloves. The present boots provide good protection against chemical warfare agents but are only an interim solution to the need for combined chemical protection, ease of decontamination, and safety. Wearers are at serious risk of falls due to the lack of adequate traction, and the weight of the boot contributes to the increased fatigue from complete protection ensemble wear.

The boots do not protect the wearer from heat or cold and in some cases may contribute to medical problems such as trench foot, frost bite, or other cold weather injuries. The protective gloves degrade tactility and again will not protect against heat or cold and may increase the chance of cold weather injuries if the work glove is not worn over the protective glove. The following descriptions of protective boots and gloves are based on information from *NBC Protection*. [5]

Green and Black Vinyl Overboots

The green vinyl overboot (GVO) and the black vinyl overboot (BVO) (Figure 16-15) are used to protect the individual's combat boots against all known chemical and biological agents, vectors, and radioactive (alpha and beta) particles. The overboots also provide protection from the environmental effects of snow, rain, and mud. (However, GVOs and BVOs issued and worn for environmental protection should not be used for NBC protection. A new pair should be issued with NBC protective gear.)[27] Following contamination by liquid agent, the boots will provide protection for a limited time. Following exposure to liquid agents, the boots should be decontaminated with a 5% household bleach–and-water or a 5% high-test hypochlorite (HTH)–and-water solution. This allows the overboots to be worn additional days before replacement. Should DS2 (Decontaminating Solution 2; diethylenetriamine, ethylene glycol monomethyl ether, sodium hydroxide; manufactured by Dalden Corp., Anaheim, Calif.) come into contact with the overboots during decontamination operations, they must be washed as soon as possible since it will soften and discolor the overboots. For additional information about weartimes and protective capabilities, refer to *NBC Protection*.[5]

Fig. 16-15. The black vinyl overboot (BVO).

Chemical Protective Footwear Cover

The chemical protective footwear cover (CPFC) is an impermeable black butyl rubber footwear cover that protects the combat boot from all agents (Figure 16-16). The CPFC has an unsupported butyl rubber sole and butyl rubber uppers with long laces, which fasten a front eyelet with side and rear eyelets. The CPFC can be decontaminated with 5% chlorine solution, then inspected and reused. If exposed to DS2, the CPFC should be washed since DS2 causes the rubber to deteriorate. The CPFC offers poor traction and the laces can cause a tripping hazard when the wearer is moving. Again, the CPFC offers no protection against cold; therefore, suitable precautions must be taken. Refer to *NBC Protection* [5] for protective capabilities.

Fig. 16-16. The chemical protective footwear cover (CPFC).

Chemical Protective Glove Set

The chemical protective glove set consists of an outer glove for chemical protection and an inner glove for perspiration absorption. The outer glove is made of impermeable butyl rubber and the inner glove is made of white cotton. The gloves come in three thicknesses: 7, 14, and 25 mil. Soldiers such as medical, teletypist, and electronic repair personnel, whose tasks require extreme tactility and sensitivity, and who will not expose the gloves to harsh treatment, will use the 7-mil glove set. Aviators, vehicle mechanics, weapons crews, and other soldiers whose tasks require tactility and sensitivity will use the 14-mil glove set (Figure 16-17). Soldiers who perform close combat tasks and other heavy labor tasks will use the 25-mil glove set.

All of the glove sets protect against liquid chemical agents and vapor hazards. However, if the 7- mil glove set is contaminated, it must be replaced or decontaminated within 6 hours after exposure. The 14-mil and 25-mil glove sets will provide protection following contamination for 24 hours. All three glove sets can be decontaminated with a 5% bleach-and-water solution or a 5% HTH-and-water solution, then inspected, and reused. All gloves will become sticky and soft if exposed to DS2 or petroleum-based fluids, and must be replaced. Replacement must occur following damage or degradation or both. Refer to *NBC Protection* [5] for protective capabilities.

Fig. 16-17. The 14-mil chemical protective glove set.

Developmental Whole-Body-Protection Equipment Items

The Joint Service Lightweight Integrated Suit Technology (JSLIST) program is developing the next generation of overgarment, which will be fielded in Fiscal Year 1997. The JSLIST program provides the future whole-body chemical–biological protective equipment for the joint services (U.S. Army, Navy, Air Force, and Marine Corps). The JSLIST program encompasses a lightweight garment (undergarment, overgarment, duty uniform) and improved chemical protective handwear and chemical protective overboot. It will provide less bulk and heat stress by being constructed of state-of-the-art materials (the exact materials are not yet known, however) and will be more durable and launderable than current

designs. The items in the JSLIST series are joint-service standardized items and are planned to be used by all services.[28,29]

In addition to the JSLIST, new agent-impermeable materials are being evaluated in conjunction with advanced fabrics to replace the carbon-impregnated fabrics, which have limited lifetimes. These new materials will be lighter, allow permeation of moisture while retaining protection, and cause less heat stress.

JSLIST Overgarment

The JSLIST Overgarment (OG) is a universal, lightweight, two-piece, front-opening garment that can be worn as an overgarment or as a primary uniform over personal underwear (Figure 16-18). It has an integral hood, bellows-type pockets, high-waist trousers, adjustable suspenders, adjustable waistband, and waist-length jacket. This design improves system compatibility, user comfort, and system acceptance, and maximizes individual equipment compatibility. The JSLIST OG provides optimum liquid, vapor, and aerosol protection and also flame protection.

JSLIST Aviation Overgarment

The JSLIST Aviation Overgarment (AVOG) is the aviator's version of the JSLIST OG and Duty Uniform (DU) configurations. It is a two-piece, front-opening, flame-resistant garment designed as a chemical protective overgarment or uniform. For cockpit compatibility, the integral hood and bellows-type pockets of the OG and the DU have been replaced with a crew-type collar and sewn-down pockets (Figure 16-19).

JSLIST Duty Uniform

The JSLIST Duty Uniform (DU) is a universal, lightweight, two-piece, front-opening garment that is worn as a primary uniform over personal underwear. It has an integral hood, bellows-type pockets, high-waist trousers, adjustable suspenders, adjustable waistband, and waist-length jacket (Figure 16-20). This improves system compatibility, user comfort, system acceptance, and ensures maximum individual equipment compatibility. The DU provides optimum liquid, vapor, and aerosol protection as well as flame protection.

JSLIST Vapor Protective Flame-Resistant Undergarment

The JSLIST Vapor-Protective, Flame-Resistant Undergarment (VPFRU) is a two-piece (jacket and drawers), front-opening, vapor-protective garment (Figure 16-21). It is configured with an integral form-fitting hood and detached vapor-protective, fire-resistant socks. Worn under standard duty uniforms, including the combat vehicle crewman coveralls and battle-dress uniform, the VPFRU is designed to provide the chemical vapor and biological agent protective layer. For Special Operations Forces and armor crews, the VPFRU is intended to provide maximum vapor and aerosol protection and MOPP flexibility.

Fig. 16-18. The Joint Service Lightweight Integrated Suit Technology Overgarment (JSLIST OG). Reprinted from US Marine Corps, Army, Navy, and Air Force. Joint Service Lightweight Integrated Suit Technology *Joint Service Lightweight Integrated Suit Technology (JSLIST) Program.* Columbus, Ohio: Battelle Memorial Institute; May 1996: unpaginated brochure.

Fig. 16-19. The Joint Service Lightweight Integrated Suit Technology Aviation Overgarment (JSLIST AVOG). Reprinted from US Marine Corps, Army, Navy, and Air Force. Joint Service Lightweight Integrated Suit Technology Program. *Joint Service Lightweight Integrated Suit Technology (JSLIST) Program.* Columbus, Ohio: Battelle Memorial Institute; May 1996: unpaginated brochure.

Fig. 16-20. The Joint Service Lightweight Integrated Suit Technology Duty Uniform (JSLIST DU). Reprinted from US Marine Corps, Army, Navy, and Air Force. Joint Service Lightweight Integrated Suit Technology Program. *Joint Service Lightweight Integrated Suit Technology (JSLIST) Program.* Columbus, Ohio: Battelle Memorial Institute; May 1996: unpaginated brochure.

Fig. 16-21. The Joint Service Lightweight Integrated Suit Technology Vapor-Protective, Flame-Resistant Undergarment (JSLIST VPFRU). Reprinted from US Marine Corps, Army, Navy, and Air Force. Joint Service Lightweight Integrated Suit Technology Program. *Joint Service Lightweight Integrated Suit Technology (JSLIST) Program.* Columbus, Ohio: Battelle Memorial Institute; May1996: unpaginated brochure.

Fig. 16-22. The Joint Service Lightweight Integrated Suit Technology Improved Chemical and Biological Protective Glove (JSLIST ICBPG). Reprinted from US Marine Corps, Army, Navy, and Air Force. Joint Service Lightweight Integrated Suit Technology Program. *Joint Service Lightweight Integrated Suit Technology (JSLIST) Program.* Columbus, Ohio: Battelle Memorial Institute; May 1996: unpaginated brochure.

JSLIST Improved Chemical and Biological Protective Glove

The JSLIST Improved Chemical and Biological Protective Glove (ICBPG) is designed to provide protection against chemical and biological agents in liquid, vapor, and aerosol form (Figure 16-22). Its protection performance is not degraded by exposure to petroleum, oil, and lubricants and to field decontaminants. To prevent excessive moisture buildup and improve user comfort, the ICBPG is semipermeable. The glove can be worn for up to 30 days without performance degradation and is flame resistant.

JSLIST Multipurpose Overboot

The JSLIST Multipurpose Overboot (MULO) is designed to be used for daily wear as required by the weather and is flame resistant.
It is a single-piece design with webbed straps, side-to-back chemical-resistant plastic buckle closures, and improved tread design (Figure 16-23). Protection is provided for environmental hazards as well as chemical and biological agents. Additionally, the resistance to agents is not degraded by exposure to petroleum, oil, and lubricants, and decontaminants.

Fig. 16-23. The Joint Service Lightweight Integrated Suit Technology Multipurpose Overboot (JSLIST MULO). Reprinted from US Marine Corps, Army, Navy, and Air Force. Joint Service Lightweight Integrated Suit Technology Program. *Joint Service Lightweight Integrated Suit Technology (JSLIST) Program.* Columbus, Ohio: Battelle Memorial Institute; May 1996: unpaginated brochure.

DETECTION AND WARNING

As noted in the introduction, timely detection and warning are critical to the protection of forces—especially since chemical agents act very quickly. Detection of an attack, with subsequent warning of affected forces downwind, can allow adoption of an effective protective posture and continuation of military operations with minimal degradation of operations. We discuss here those instruments most widely fielded; some special-purpose items are not discussed.

The army has recently fielded two new systems, the FOX and the Biological Integrated Detection System (BIDS), which are discussed below. Each of these new systems integrates a variety of detectors into a mobile, crew-served system; the composite detectors are vastly superior to any individual detector previously available.

Sources for this discussion are the *Worldwide Chemical Detection Equipment Handbook* [30] and experts at Aberdeen Proving Ground, whom readers who are interested in greater detail can consult.

Chemical Detection and Warning

This section briefly describes some of the fielded chemical detectors that may be of most use within the medical community. These detectors are divided into two groups: point detectors and standoff detectors.

Point Detectors

Point detectors sample the immediate area to determine the presence of chemical agents. The sample is most often taken from the atmosphere; however, specialized detection kits can be used to sample the soil or water. In addition to monitoring the atmosphere, the point detectors provide monitoring after an attack, identify the contaminated area, monitor collective protection areas, monitor effectiveness of decontamination, and identify chemical contamination during reconnaissance efforts.

M8 Chemical Agent Detection Paper

M8 Chemical Agent Detection Paper detects and identifies liquid chemical agents. It is tan in color and comes in a booklet containing 25 perforated sheets (2 in. x 3 in.), which are heat sealed in a polyethylene envelope. There are three sensitive indicator dyes suspended in the paper matrix. The paper is blotted on a suspected liquid agent and observed for a color change, which will occur within 30 seconds: VX turns the paper dark green, the G series of agents turn the paper yellow (see Chapter 5, Nerve Agents), and blister agent turns it red (Figure 16-24). M8 paper will change color with many interferents such as sodium hydroxide and petroleum products; thus, it is unreliable to use to check for completeness of personnel decontamination and should always be verified with another means of identification.

Fig. 16-24. M8 Chemical Agent Detection Paper. A drop of mustard (H) simulant from the vial has turned the paper red. Reprinted from Brletich NR, Waters MJ, Bowen GW, Tracy MF. *Worldwide Chemical Detection Equipment Handbook*. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Analysis Center; October 1995: 407. Photograph: Courtesy of US Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Md.

M9 Chemical Agent Detection Paper

M9 Chemical Agent Detection Paper is a portable, single roll of paper that comes with a Mylar adhesive- backed and-coated tape. It contains a suspension of an agent-sensitive dye in a green-colored paper matrix. The agent-sensitive dye will turn pink, red, reddish brown, or red-purple when exposed to agent but does not identify the specific agent. M9 paper is more sensitive to nerve and blister agents and reacts more rapidly than M8 paper, although it also reacts to a wide range of interferents such as petroleum products, brake fluid, aircraft cleaning compounds, DS2, insect repellent, defoliant, and antifreeze.

M9 paper, which is similar to masking tape, is used by attaching strips to the individual overgarment and to equipment such as vehicle controls. The strips are then inspected routinely for color change (Figure 16-25). The paper should not be attached to hot surfaces, as this will discolor the tape and lead to a false positive reaction.

Chemical Agent Monitor and Improved Chemical Agent Monitor

The chemical agent monitor (CAM) and improved chemical agent monitor (ICAM) are hand-held, soldier-operated devices designed for monitoring chemical agent contamination on personnel, equipment, and surfaces. They use ion mobility spectrometry technology to detect and discriminate between mustard and nerve agent vapor. The concentrations of agents detected by the CAM and ICAM areas are as follows: for sarin (GB), 0.03 mg/m^3; for VX, 0.1 mg/m^3; and for mustard (HD), 0.1 mg/m^3.

The units are simple to operate, can be held in either hand while the user is wearing chemical protective equipment, and operate day or night (Figure 16-26).

Relative vapor hazard and malfunction information is displayed by bars on a liquid crystal display. As an example, the bar readings for concentrations of the nerve

Fig. 16-25. Field use of M9 paper. Reprinted from Brletich NR, Waters MJ, Bowen GW, Tracy MF. *Worldwide Chemical Detection Equipment Handbook.* Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Analysis Center; October 1995: 417. Photograph: Courtesy of US Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Md.

agent sarin are shown in Table 16-1. The CAM and ICAM are point monitors only and cannot give an assessment of an area vapor hazard. The two may give false readings when used in enclosed spaces or when sampling near strong vapor sources such a dense smoke, aromatic vapors, cleaning compounds, exhausts from some rocket motors, and fumes from some munitions. Because of the technology employed, the CAM and ICAM are subject to saturation; they must be cleared to function properly.

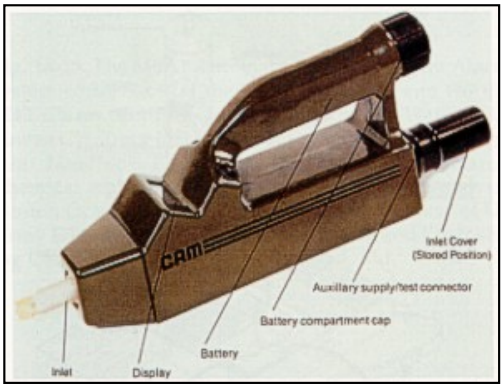


Fig. 16-26. The chemical agent monitor (CAM). Photograph: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

Specific information in this discussion of the CAM and ICAM is drawn from *Chemical Agent Monitor Employment*[31] and *Operator's and Organizational Maintenance Manual for the Chemical Agent Monitor (CAM)*,[32] which interested readers may wish to consult.

Chemical Agent Detector Kit

The M256A1 Chemical Agent Detector Kit is a portable, expendable item capable of detecting and identifying hazardous concentrations of nerve and blister agents and cyanide (Figure 16-27). The kit is used after a chemical attack to determine if it is safe for personnel to unmask. Each kit consists of 12 disposable plastic sampler-detectors (ticket or card), one booklet of M8 paper, and a set of instruction cards. Each ticket (card) contains laboratory filter paper test spots for the various agents. The technology used is wet chemistry, enzymatic substrate-based reactions, where the presence of agents is indicated by a specific color change. Response time is about 15 minutes. Some smokes, DS2, petroleum products, and high temperatures may produce false readings.

TABLE 16-1
BAR READINGS FOR CONCENTRATIONS OF SARIN (GB)

Bar Reading	Concentration (mg/m^3)
1	0.03
2	0.05
3	0.08
4	0.14
5	0.30
6	1.0
7	10.0
8	30.1

The detection limits for the M256A1 are as follows: for the G series of nerve agents, 0.005 mg/m^3; for VX, 0.02mg/m^3; for the vesicants mustard (HD) and Lewisite, above threshold concentrations of 3.0 mg/m^3 and 14 mg/m^3, respectively; and for hydrogen cyanide (AC), 11 mg/m^3, and cyanogen chloride (CK), 10 mg/m^3.

The M256A1 kit cannot be used to detect agent in water. It can, however, be used to check an area before a military unit moves in or to define clean areas or routes. Some chemical ingredients in the kit are considered possible carcinogens and should be handled as such. The emissions produced by this kit are also toxic; a mask and gloves must be worn while the kit is being used.

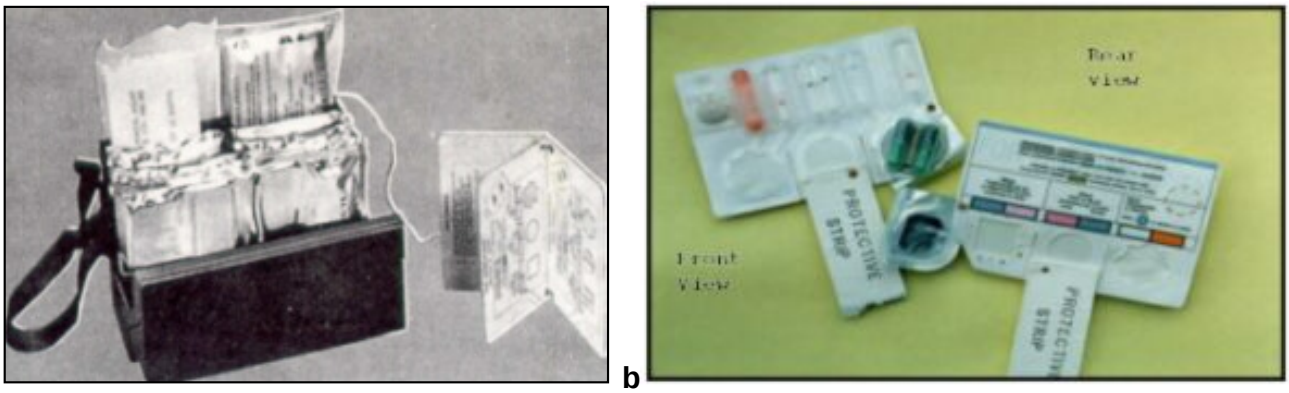


Fig. 16-27. (a) The M256A1 Chemical Agent Detector Kit and **(b)** the sampler/detector found inside the carrying case. Reprinted from Brletich NR, Waters MJ, Bowen GW, Tracy MF. *Worldwide Chemical Detection Equipment Handbook*. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Analysis Center; October 1995: 429. Photograph (a): Courtesy of Environmental Technologies Group, Inc, Baltimore, Md.

Chemical Agent Water Testing Kit

The M272 Chemical Agent Water Testing Kit is designed to detect and identify, via colorimetric reactions, hazardous levels of nerve agents, mustard, Lewisite, and cyanide in treated or untreated water (Figure 16-28). A full kit contains enough supplies to perform 25 tests for each agent, and simulants are included for training use. About 20 minutes is required to perform all four tests. All bodily contact should be avoided with the kit chemicals, as some can be very harmful and should only be handled while wearing protective gloves and equipment.

Detection limits are as follows: for the G-series nerve agents and VX: 0.02 mg/L; for the vesicants Lewisite (L) and mustard (H and HD): 2.0 mg/L; and for the cyanides (AC and CK), 20 mg/L.

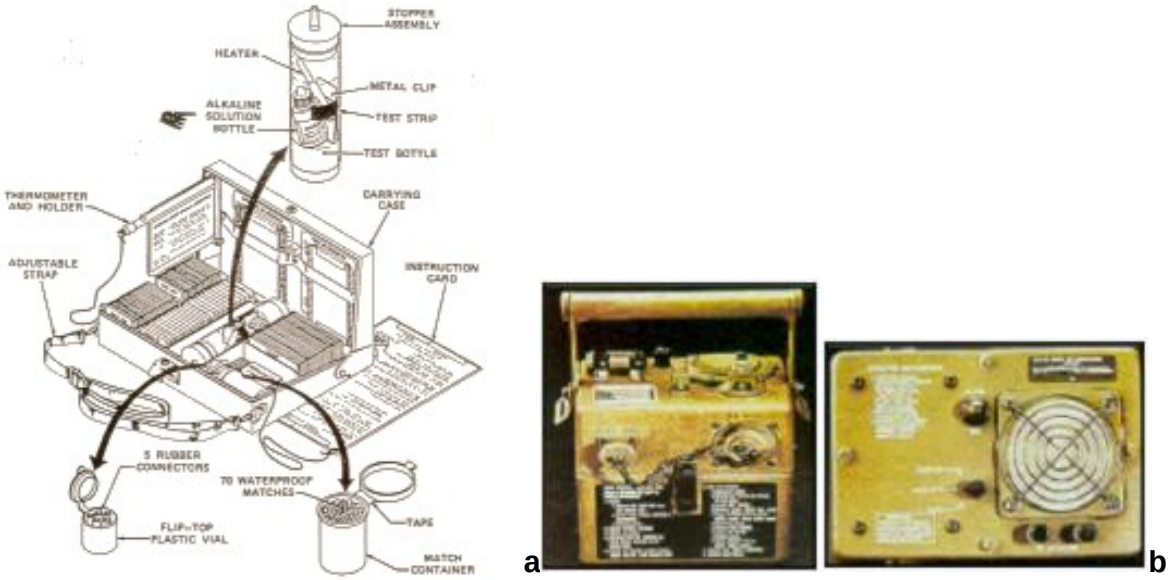


Fig. 16-28. The M272 Chemical Agent Water Testing Kit and its components. New kits have a test strip instead of a thermometer; this illustration shows both. Reprinted from Brletich NR, Waters MJ, Bowen GW, Tracy MF. *Worldwide Chemical Detection Equipment Handbook*. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Analysis Center; October 1995: 433. Drawing: Courtesy of US Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Md.

Fig. 16-29. The M8A1 Automatic Chemical Agent Alarm system consists of **(a)** the M43A1 Detector and **(b)** the M42 Alarm. Reprinted from Brletich NR, Waters MJ, Bowen GW, Tracy MF. *Worldwide Chemical Detection Equipment Handbook*. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Analysis Center; October 1995: 411. Photographs: Courtesy of US Army Edgewood Research, Development, and Engineering Center, Aberdeen Proving Ground, Md.

Automatic Chemical Agent Alarm

The M8A1 Automatic Chemical Agent Alarm is an automatic chemical agent detection and warning system designed to provide real-time detection of the presence of nerve agent vapors or inhalable aerosols. The M8A1 consists of the M43A1 detector and up to five M42 alarms, which will provide both an audible and a visible warning (Figure 16-29). The M43A1 is an ionization product diffusion/ion mobility type detector; it will sound a false alarm in the presence of heavy concentrations of rocket propellant smoke, screening smoke, signaling smoke, engine exhausts, and whenever a nuclear blast occurs.

The M8A1 can be located within a hospital complex, with alarm units placed to cover all critical care, treatment, and support areas. The M43A1 detects nerve agent vapors at concentrations of 0.2 mg/m^3 for sarin (GB) and 0.4 mg/m^3 for VX.

Standoff Detection

Early warning of chemical agents provides troops the necessary time to increase protective posture and to avoid contaminated areas. Standoff detectors provide this early warning at a distance of 1 to 5 km.

Optical remote sensing (ORS) technologies, employing infrared spectral analysis techniques, have been utilized in the development of chemical agent standoff detection technologies. Within the ORS technologies, there are two types of remote sensing systems: passive and active (laser). The section below only looks at the passive system, which employs a Fourier Transform Infrared (FTIR) spectrometer.

Alarm: Remote Sensing Chemical Agent Alarm M21

The M21 Remote Sensing Chemical Agent Alarm (RSCAAL) is an automatic scanning, passive, infrared sensor. The M21 detects nerve and blister agent clouds based on changes in the background infrared spectra caused by the presence of the agent vapor. It scans a horizontal 60° arc and can recognize agent clouds at line-of-sight ranges up to 5 km (Figure 16-30).

Usually, the M21 is placed facing into the wind. It measures and stores a background spectrum that is then compared by an onboard microcomputer, which makes agent/no agent decisions based on ambient radiance levels. Response time is 1 minute or less. The system is fielded to NBC reconnaissance units.

The sensitivity of the M21 for detecting nerve agents

(GA, GB, and GD) is 90 mg/m^3; and for vesicants is 500 mg/m^3 for Lewisite and 2,300 mg/m^3 for HD mustard.

Developmental Detection and Warning Items

In the area of chemical detection, the next developments are

1. standoff detection systems that use laser systems and can provide advance warning from 30 to 50 km distant, and
2. point detectors that will be placed on attended air vehicles, with warning sent back by radio or forward-emplaced point detectors with radio links to a headquarters or a central warning network.

Combined nuclear, chemical, and biological detectors, which could serve as joint detection and warning devices, are also being developed and fielded.

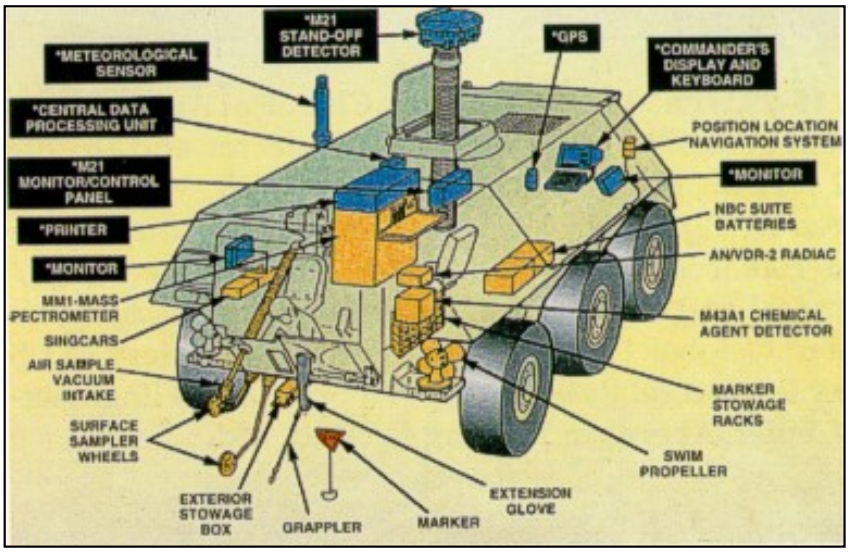
Fig. 16-30. Field use of the M21 Remote Sensing Chemical Agent Alarm (RSCAAL). Reprinted from Brletich NR, Waters MJ, Bowen GW, Tracy MF. *Worldwide Chemical Detection Equipment Handbook.* Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Analysis Center; October 1995: 425. Photograph: Courtesy of Brunswick Corporation (now Intellitec), DeLand, Fla.

Integrated Mobile Systems

M93A1 FOX Nuclear, Biological, Chemical Reconnaissance System

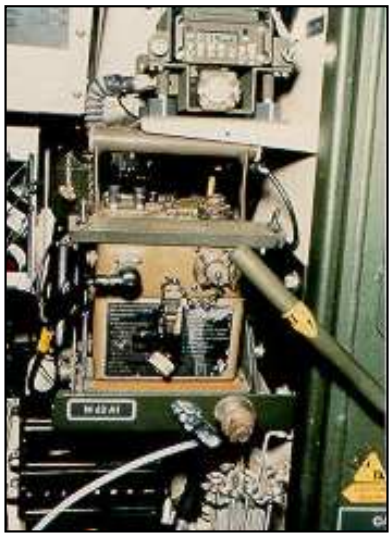
The M93A1 FOX Nuclear, Biological, Chemical Reconnaissance System (NBCRS) is a recently deployed, comprehensive solution to the problem of early recognition of NBC threats on the modern battlefield. Numerous sophisticated instruments have been mounted in a fast, mobile, 6x6 armored vehicle that weighs about 19 tons and is manned by a crew of three (Figure 16-31). The vehicle is of German origin, and the name FOX is a translation of Fuchs, for whom the design was named.

(a) The M93A1 FOX Nuclear Biological Chemical (NBC) Reconnaissance System. The M21 remote sensing chemical agent stand-off detector is not shown in its deployed configuration.



(b) The schematic shows important components of the FOX. Entries in black boxes are components that have been added to the original version. GPS indicates the location of the global positioning system instrument.

(c) A FOX NBC suite operator is seen controlling the M21 detector.



(d) The instrument at the top is the ANVDR2 Radiation Detector. Below it is the M43A1 Chemical Vapor Detector.

(e) Chemical agents present on the ground adhere to the silicone-tired sampling wheels. After sampling, the wheels are elevated into proximity of the vacuum port of the chemical sampling probe (black tubular object), which is connected to the MM1 mass spectrometer. Chemically contaminated terrain is marked by flags ejected from the FOX.

(f) Objects that are possibly contaminated are retrieved by a pair of tongs manually operated from inside the FOX. The sample is placed in the small box-like receptacle for latter analysis.

The FOX is instrumented to detect chemical contamination in its immediate vicinity with a variety of probes, and at a distance via a standoff detector (M21). Meteorological data are also sensed. Data are analyzed, synthesized, and transmitted to higher-echelon units by a secure, jam-resistant communication system. The local area is marked by warning markers ejected through a hatch in the rear of the vehicle. A global positioning system makes possible accurate marking of the contaminated locale. The interior of the vehicle is pressurized and offers collective protection against threat agents.

XM31 Biological Integrated Detection System

The XM31 Biological Integrated Detection System (BIDS) consists of a lightweight, multipurpose, collective protection shelter mounted on a heavy high-mobility, multipurpose, wheeled vehicle (HMMWV) and equipped with a biological detection suite (Figure 16-32). In its present configuration, the BIDS can

detect the bacteria *Bacillus anthracis* and *Yersinia pestis*, and the toxins botulinum toxin A and staphylococcal enterotoxin B.

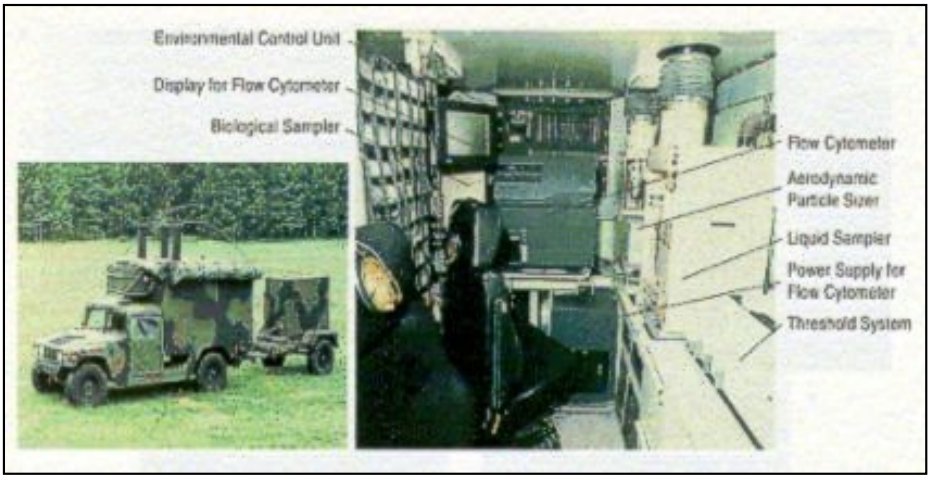
Several technological approaches are used sequentially to detect and confirm the presence of specific biological threat agents. Since biological threat agents are likely to be dispersed as aerosols, ambient air is continuously sampled and the background distribution of aerosol particles determined. Aerosol particles in the 2- to 10-µm diameter range are concentrated and then subjected to analysis for

1. adenosine 5'-triphosphate (ATP) by bioluminescence,
2. bacterial cells by flow cytometry, and
3. specific antigens by two different antigen-antibody reactions.

Individual BIDS are combined together into a corps-wide network by a secure communication system. An improved BIDS is being developed with the capability to detect two species of *Brucella, Francisella tularensis*, and ricin toxin. Detection capabilities for additional agents will no doubt be added to future models. [33]

(g) The gloved arm of a crew member is shown manipulating a thermal probe used to measure ground temperature. This information is valuable in estimating the vapor hazard from liquid agent. Photographs: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

a



b

Fig. 16-32. (a) The XM31 Biological Integrated Detection System (BIDS) in its present configuration. Electric power is provided by a towed 15-kW tactical quiet generator. An additional high-mobility, multipurpose, wheeled vehicle (HMMWV) is used as a support vehicle for the crew of four, two of whom are required to operate the equipment that comprises the biological detection suite. The stovepipe-like structures perform the aerosol-sampling function. **(b)** Interior view of the biological detection suite. Photographs: Courtesy of Visual Information Division, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md.

COLLECTIVE PROTECTION

Collective protection serves a vital role in the medical area since treatment of casualties must continue even in a contaminated environment, thus collective protection is required to allow this critical function to continue. In addition, it allows individuals to rest and eat, and provides temporary relief from the individual protection equipment thus allowing continuing military operations in the contaminated environment. Collective protection systems have been designed to be used in either a medical or a nonmedical application.

The sources for this discussion are experts at the U.S. Army Chemical and Biological

Defense Command, [34] and *Collective Protective Equipment*, U.S. Army Training Manual 34240-338-10,[35] which interested readers can consult for greater detail.

Medical Collective Protection Systems

The Medical Collective Protection Systems provide ample floor space and are accessible for litter patients through airlocks. Additionally, some of the systems provide airlocks through which ambulatory patients can pass. These options aid the medical community in its tasks when dealing with casualties in a chemically contaminated environment.

Chemically Protected Deployable Medical System

The chemically protected deployable medical system (CP DEPMEDS) consists of the M28 collective protection equipment (CPE), which is designed to protect critical areas within the hospital complex from chemical–biological contamination (Figure 16-33). The M28 CPE can only be used with the TEMPER (*t*ent, *e*xtendable, *m*odular, *per*)sonnel system. The entire composite hospital ensemble consists of expandable tentage, passageways, environmental control units, and ISO (International Organization for Standardization, from the French) shelters.

Fig. 16-33. The chemically protected deployable medical system (CP DEPMEDS).

The M28 CPE consists of the following items: end sections, center sections, and vestibule liner fabricated from a plastic film that is resistant to liquid and vapor agents; a protective entrance airlock for ambulatory personnel that is made from a butyl-coated material and hung in a collapsible aluminum frame, creating a triangular shape; a tunnel airlock for litter-borne patients, consisting of a collapsible frame with entry and exit doors at opposite ends fabricated from an NBC protective cover; the hermetically sealed filter canister and the accessory package, which support the purge requirement during collective protective entry; and the recirculation filter, which is a portable self-contained unit designed to filter any chemical agent vapors brought in through the entry or exit.

Chemically Hardened Air Transportable Hospital

The U.S. Air Force utilizes the Chemically Hardened Air Transportable Hospital (CHATH) in its operations. The CHATH is basically the same as the CP DEPMEDS. The air force is developing a chemically hardened air-management plant (CHAMP), which will provide 800 cu ft/min of filtered air, environmental control, and power generation integrated into a single (albeit very large) package. The CHAMP is intended to replace all M20/M28 filter blower sets. Although the CHAMP is intended to be used with the CHATH, it is competing with the air force's field deployable environmental control unit (FDECU) as the system to actually be applied to the CHATH.

Chemical and Biological Protected Shelter

The Chemical and Biological Protected Shelter (CBPS) is a direct replacement for the M51 C/B shelter, which eliminates the excessive erection/striking time, the insufficient floor space, lack of natural ventilation, and the unavailability of prime movers, which were the problems with the M51.

The CBPS can be set up or struck three times daily when operating as a Battalion Aid Station.

Fig. 16-34. The litter-patient airlock of the Chemical and Biological Protected Shelter. Treating casualties on a chemical-biological warfare battleground requires complicated procedures, even to get the casualty into a protected environment for examination. Special air locks for casualties and new procedures had to be developed. Photograph: Courtesy of Chemical and Biological Defense Command Historical Research and Response Team, Aberdeen Proving Ground, Md.

Setup times of the inflatable rib tent have been established at 15 to 20 minutes, and tear-down times at approximately 30 minutes. The CBPS consists of a power/support system and inflatable tent. The primary power source is the engine of a HMMWV variant and a backup generator mounted in a high-mobility multipurpose trailer. This system provides air conditioning or heating and electricity for lighting, equipment, and filter air.

The CBPS is staffed by a crew of four, who are carried in the HMMWV. The inflatable rib tent provides 300 sq ft of usable floor space, with a litter-patient airlock (Figure 16-34) and optional ambulatory-patient airlock. The CBPS has removable side entrances to allow side-to-side setup of additional CBPS.

Nonmedical Collective Protection

The nonmedical collective protection systems provide protection for two or more individuals from the effects of chemical agents present in the environment. These systems provide an area for individuals to perform their functions without experiencing deleterious effects.

M20 Simplified Collective Protection Equipment

The M20 Simplified Collective Protective Equipment (SCPE) is designed to provide a clean-air shelter for use in a contaminated environment, especially for command, control, and communication. The SCPE has a collapsible protective entrance, a hermetically sealed filter canister, a blower unit, and an accessory pack (Figure 16-35). It is designed to be employed inside an existing room of 200 sq ft of usable space, with or without a collapsible liner. The SCPE can be used without the liner only in rooms that are tightly sealed. [36]

Fig. 16-35. The M20 Simplified Collective Protective Equipment (SCPE).

Developmental Collective Protection Items

New developments in collective protection will center on

- improved adsorbents and impregnants as replacements for activated charcoal,
- methods to better determine filter lifetime, and
- new systems, such as pressure- and temperature-swing adsorption, which may provide significant improvements for collective protection applications in ships, aircraft, and armored vehicles.

Possible applications to military uses will be made of a number of civilian developments in environmental pollution abatement.

For these systems to provide environmentally controlled atmospheres, environmental control units are being developed to be compatible with collective protection systems. At the present, however, there are neither heaters nor air conditioners that can be used with collective protection equipment. The air force's FDECU, currently in late-stage development, is the primary candidate and is able to heat and cool.

DECONTAMINATION EQUIPMENT

The physical properties of chemical agents are highly variable. They range from nerve agent vapor, which usually dissipates in a few minutes to a few hours, to vesicants such as mustard, which can remain active for weeks (or in some cases, years: buried and recovered World War I mustard projectiles are often still quite toxic). It is imperative, then, that timely decontamination of the skin and personal equipment that has been exposed to agent, especially liquid agent, be completed. Skin decontamination should take place within 2 minutes if possible, and equipment decontamination should be completed within 1 hour.

To effectively perform complete personnel and equipment decontamination operations, decontamination units use truck-mounted tanks, pumps, and water heater units; and trailer-mounted pumps and water heater units. In these processes, reducing the exposure time of the individual or piece of equipment to the chemical contaminant is of the highest priority.

The sources for this discussion are *Decontamination of Chemical Warfare Agents*[37] and *NBC Decontamination*,[38] and experts at the U.S. Army Chemical and Biological Defense Command.[39] Readers interested in greater detail can consult these sources and the authors of this chapter.

Personnel Decontamination Items

Personnel decontamination is performed to reduce the level of contamination so it is no longer a hazard to the individual. Personnel decontamination consists of removal of clothing and decontamination of the skin. To expedite this procedure, personnel decontamination kits are used to remove the gross contamination. Complete decontamination, which is conducted by specialized decontamination units, is provided to troops to reduce the requirement for wearing complete NBC protective equipment. Additionally, when both crews and equipment are contaminated, combined complete personnel and equipment decontamination operations are scheduled as the situation and mission permit, bearing in mind the lengthy time required for such an operation. It is during this complete decontamination that commanders can give their soldiers rest and a change of personal protection equipment. [40]

The personnel decontamination items described below would be used to quickly decontaminate the skin of an exposed individual. Open wounds, however, should be decontaminated with water, saline, or dilute hypochlorite solutions.

M258A1 Skin Decontamination Kit

Fig. 16-36. The M258A1 Skin Decontamination Kit.

The M258A1 Skin Decontamination Kit is designed to remove and neutralize liquid chemical agents on the skin. The kit consists of three number 1 liquid packets and three number 2 liquid packets (Figure 16-36). The number 1 packet will neutralize G-series nerve agents by hydrolysis, and the number 2 packet will neutralize VX and mustard agents by oxidation. [41]

The contents of the kit are highly caustic and should not be used near the eyes or mouth or to decontaminate wounds. A training kit that contains only an alcohol and water solution, the M58A1, has been developed to be used in lieu of the M258A1.

M291 Skin Decontamination Kit

The M291 Skin Decontamination Kit is a soft package consisting of two flexible pockets, each of which contains three decontamination packets (Figure 16-37). Each of the packets contains a black resin (a mixture of a carbonaceous adsorbent, a polystyrene polymeric compound, and an ion-exchange resin) that is both reactive and adsorbent. The decontamination pad is made from a nonwoven fiberfill that is impregnated with the dry resin mixture. [42]

Fig. 16-37. The M291 Skin Decontamination Kit.

The decontamination is accomplished by merely opening the packet and scrubbing the skin surface with the applicator pad until an even coating of the resin is achieved. Use normal precautions to keep the powder from wounds, the eyes, and the mouth. The M291 kit is also used for training.

Equipment Decontamination Items

Equipment decontamination items are used to destroy, remove, or neutralize most of the NBC hazards from personal gear or unit equipment. Although all of the items that could be used for equipment decontamination are not listed below, those most useful to the medical community are described.

M295 Decontamination Kit, Individual Equipment

The M295 Decontamination Kit, Individual Equipment (DKIE) consists of a pouch containing four wipedown mitts, each enclosed in a soft protective packet (Figure 16-38). Each wipedown mitt is made of a dry, adsorbent, black resin (the same as in the M291 kit) contained within a nonwoven polyester material and a polyethylene film backing. This kit allows decontamination of exposed areas of the protective mask and hood, personal equipment, and protective boots. Decontamination is accomplished by adsorption of the liquid agent by the resin and the pad.

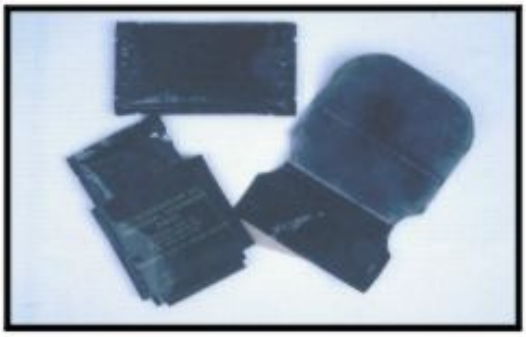


Fig. 16-38. The M295 Decontamination Kit, Individual Equipment (DKIE).

M11 Portable Decontamination Apparatus

The M11 Portable Decontamination Apparatus (PDA) is a hand-held, pressure-spray apparatus used to coat a surface with DS2 by equipment operators (Figure 16-39). The M11 contains 1 1/3 quarts of DS2 and will neutralize all agents within 30 minutes. It is pressurized using a nitrogen cylinder with a life of only 30 seconds. Any use of DS2 should be made with a protective mask and gloves, since it is highly irritating to the skin and can cause blindness. In addition, DS2 is highly flammable.

Fig. 16-39. The M11 Portable Decontamination Apparatus (PDA).

M17 Lightweight Decontamination System

The M17 Lightweight Decontamination System (LDS) is designed to draw water from any source and deliver it to the two installed spray wands at pressures up to 100 psi and at temperatures up to 120°C (Figure 16-40). The M17 LDS can be used to provide pressurized hot water before or after application of decontaminant at regulated pressures and temperatures. It has a liquid soap siphon hose attachment for use with mud, dirt, or grease removal (these may have absorbed chemical agent). The M17 LDS has a 3,000-gal collapsible water tank that can be prepositioned and filled for hot water showers or hospital use.

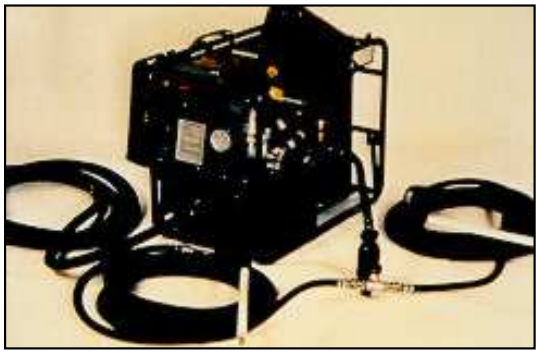


Fig. 16-40. The M17 Lightweight Decontamination System (LDS).

M12A1 Power-Driven Decontamination Apparatus

The M12A1 Power-Driven Decontamination Apparatus (PDDA) is used to apply decontamination solutions or hot soapy water and rinses during field decontamination operations. The M12A1 PDDA consists of a pump unit, 500-gal tank, personnel shower assembly, and M2 water heater, all of which is mounted on a 5- or 10-ton truck with drop sides (Figure 16-41). The pump assembly can deliver 50 gal of water or super tropical bleach (STB) decontaminating agent per minute at a pressure of 105 psi to the two spray wands.

Developmental Decontamination Items

There is a need for an effective and environmentally safe reactive decontaminant that does not harm equipment and personnel. Bacterial enzymes, catalytic-type compounds, and other stable decontaminants (eg, quaternary ammonium complexes) are under consideration. Sorbent compounds and nonaqueous decontaminants are also being investigated for use on electronic components and other sensitive equipment.

Fig. 16-41. The M12A1 Power-Driven Decontamination Apparatus (PDDA).

ADDITIONAL PATIENT PROTECTION AND TRANSPORT EQUIPMENT

Patient Protective Wrap

The patient protective wrap (PPW) is designed to protect a patient during evacuation after the BDO has been removed and the patient has received medical treatment (Figure 16-42). A patient can remain in the PPW for 6 hours. The protective mask is not needed inside the PPW, but it should be evacuated with the patient.

The PPW is for one patient only and weighs approximately 5.5 lb. The top of the PPW is made of a material similar to that used in the BDO, with a charcoal lining and in a camouflage pattern. The bottom is made of impermeable rubber. The PPW has a continuous zipper along the outer edge for ease of patient insertion; a large, transparent window in the top to view the patient (or for him to see out); and a pocket for medical records. The patient's breathing air comes through the permeable top of the PPW.

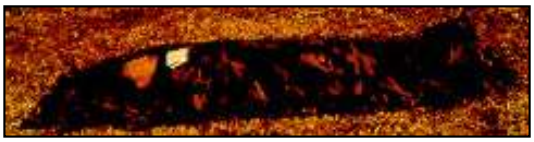


Fig. 16-42. A volunteer demonstrates the patient protective wrap (PPW).

Patient Transport: Decontaminable Litter

The decontaminable litter has been developed to meet the need for a litter that can withstand decontamination (Figure 16-43). The cover fabric is a honeycomb weave of monofilament polypropylene, which will not absorb agent and is not degraded by decontamination fluids. The cover fabric is flame retardant and rip resistant, and is treated to withstand weather and sunlight. It has aluminum poles, painted with chemical agent resistant coating, with round, grip-molded, retractable, black nylon handles. It conforms to all NATO standards and weighs about 15 pounds.

Fig. 16-43. The decontaminable litter.

SUMMARY

An integrated system of chemical defense equipment is required if we are to be successful in providing an adequate protective posture for all forces. The principal elements of that system include the following:

- Real-time detection and warning, preferably from remote sensors. This will provide more time, first, to assume a protective posture, and second, to identify the chemical agent.
- Personal protective equipment, consisting of a properly fitted mask and overgarment with gloves and boots as required. This equipment is the most critical component of chemical defense equipment, the first line of defense.
- Collective protection, which is necessary for optimal combat casualty care in a contaminated environment, whether the casualty's injuries are from exposure to chemical–biological weapons alone, or are combined with injuries from conventional weapons.
- Decontamination, which is required for personnel and equipment to maintain combat operations in a contaminated environment.

Medical treatment for chemical casualties has not been covered here since it is covered in other chapters, but it is essential that protective equipment be available to allow the proper treatment to be administered.

ACKNOWLEDGMENT

The authors thank the following experts for their technical assistance and generous help in illustrating and preparing this chapter: Major M. L. Malatesta, Chemical Corps, US Army, Deputy Program Director, Office of the Program Director for Biological Defense Systems; Bryan J. Keirn, Engineering Technician for the Program Manager, Nuclear, Biological, Chemical Defense Systems; John A. Scavnicky, Project Manager for Nuclear, Biological, Chemical Defense Systems, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Maryland; and Sergeant First Class Larry L. Harris, US Army (Ret).

REFERENCES

1. US Army Armament Munitions and Chemical Command. *Chemical Defensive Equipment (General Information Booklet)*. Rock Island, Ill: Materiel Management Directorate, HQ, US Army Armament Munitions and Chemical Command; October 1984.
2. Department of the Army. *NBC Handbook*. Washington, DC, DA; September 1990. Field Manual 3-7.
3. Ramirez TL, Rayle ME, Crowley PA, Derringer, CV. *The Thermal Effects of the Chemical Defense Ensemble on Human Performance.* US Air Force, Human Systems Division, Brooks Air Force Base, San Antonio, Tex; April 1988. CB-010358. ADB131 710.
4. Department of the Army. *NBC Contamination Avoidance.* Washington DC: DA; November 1992. Field Manual 3-3.
5. Department of the Army. *NBC Protection*. Washington, DC: DA; February 1996: Chap 2. Field Manual 3-4 With Change 2. Fleet Marine Force Manual 11-9.
6. Weiss RA, Weiss MP, Church JK Jr, Strawbridge JB, Decker RW II, Martin FA. *Chemical Warfare Respiratory Protection: Where We Were and Where We Are Going*. Aberdeen Proving Ground, Md: US Army Chemical Research, Development, and Engineering Center; n.d. Unpublished report.
7. Gander TJ, ed. *Jane's NBC Protection Equipment*, 1992–93. Alexandria, Va: Jane's Information Group Inc; 1992: 21–25.
8. North Atlantic Council, NATO Industrial Advisory Group, Sub-Group 48. *Basic Personal Equipment*. Vol 5. In: *NIAG Prefeasibility Study on a Soldier Modernisation Program*. Brussels, Belgium: NIAG; September 1994. NATO unclassified document NAIG-D(94)3.

9. Brletich NR, Tracy MF, Dashiell TR. *Worldwide NBC Mask Handbook*. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Center; September 1992.

10. McHenry R, ed. *The New Encyclopædia Britannica*. Vol 3, Micropædia. 15th ed. Chicago, Ill: Enclycopædia Britannica, Inc; 1993: 98.

11. Bender TR, MD, MPH. Director, Division of Safety Research, Centers for Disease Control, National Institute for Occupation Safety and Health, Morgantown, WVa. Letter to all Respirator Manufacturers; June 15, 1990.

12. Klenke WJ. Lieutenant Colonel, Medical Service, US Army. Medical NBC Defense Staff Office, Office of The Surgeon General. Personal communication, June 1996.

13. Phillips YY. Colonel, Medical Corps, US Army; Chief, Department of Medicine, Walter Reed Army Medical Center, Washington, DC. Personal communication, July 1996.

14. Masks, Chemical-Biological, Field, M17A1. Military Specification MIL-M-51282D(EA). November 1986.

15. Church JK. Chemical Engineer, US Army Chemical and Biological Defense Command, Edgewood Area, Aberdeen Proving Ground, Md. Letter to MF Tracy, May 1996.

16. Mask, Chemical–Biological, Protective Field, M40. US Army Chemical Research, Development and Engineering Center Purchase Description EA-M-1377A. Edgewood Area, Aberdeen Proving Ground, Md; January 1990.

17. Mask, Chemical–Biological, Protective Field, M40. US Army Chemical Research, Development and Engineering Center Purchase Description EA-M-1377A, Amendment 1. Edgewood Area, Aberdeen Proving Ground, Md; September 1990.

18. Department of the Army. *Operator's Manual Chemical–Biological Mask: Field*, M40. Washington, DC: DA; June 1988. Training Manual 3-4240-300-l0-l.

19. Mask, Chemical–Biological Protective, Combat Vehicle, M42. US Army Chemical Research, Development and Engineering Center Purchase Description EA-M-1389A. Edgewood Area, Aberdeen Proving Ground, Md; December 1990.

20. Department of the Army. *Operator's Manual Chemical–Biological Mask: Combat Vehicle, M4*2. Washington, DC: HQ, DA; August 1988. Training Manual 34240-300-10-2.

21. Mask, Chemical–Biological Protective Aircraft, M43. US Army Chemical Research, Development and Engineering Center Purchase Description EA-M-1352A. Edgewood Area, Aberdeen Proving Ground, Md; January 1988.

22. Department of the Army. *Operator's and Organizational Manual: Mask, Chemical–Biological: Aircraft M43*. Washington, DC: HQ, DA; June 1988. Training Manual 34240 312-12&P.

23. Grove CM, Scavnicky JA. *Concept Development Studies for Respiratory Protection System 21*. Edgewood Area, Aberdeen Proving Ground, Md: US Army Edgewood Research Development and Engineering Center; September 1995.

24. US Army Natick Research, Development, and Engineering Center. *Items of Combat Clothing and Equipment*. Natick, Mass: US Army Natick Research, Development, and Engineering Center; September 1991. Natick P-32-1.

25. Wajda DJ. US Army Natick Research, Development and Engineering Center, Natick, Mass. Letter to MF Tracy, May 1996.

26. Winfield Manufacturing Co. *Chemical Protective PBI Saratoga CWU/66P Air Force Flight Coverall*. New York, NY: Winfield Manufacturing Co; June 1989. Technical Bulletin SAr3.

27. US Department of the Army and US Marine Corps. *Operator's Manual for Individual Chemical Protective Clothing*. Washington, DC: HQ, DA, USMC; March 1993. Training Manual 10-8415-209-10. Fleet Marine Force Manual 8415 10/2.

28. US Marine Corps. *Joint Operational Requirement Document for a Lightweight Integrated Nuclear, Biological, and Chemical (NBC) Protective Garment.* Washington, DC: USMC; June 1995. NBC 215.2.1.

29. US Marine Corps, Army, Navy, and Air Force. Joint Service Lightweight Integrated Suit Technology Program. *Joint Service Lightweight Integrated Suit Technology (JSLIST) Program.* Columbus, Ohio: Battelle Memorial Institute; May 1996.

30. Brletich NR, Waters MJ, Bowen GW, Tracy MF. *Worldwide Chemical Detection Equipment Handbook*. Edgewood, Md: Chemical Warfare/Chemical and Biological Defense Information Analysis Center; October 1995.

31. Department of the Army. *Chemical Agent Monitor Employment*. Washington, DC: DA; July 1991. TC 34-1.

32. Department of the Army. *Operator's and Organizational Maintenance Manual for the Chemical Agent Monitor (CAM)*. Washington, DC: DA; June 1992. Training Manual 3-6665-331-10.

33. Malatesta ML. Major, Chemical Corps, US Army. Deputy Program Director, Office of the Program Director for Biological Defense Systems, US Army Chemical and Biological Defense Command, Aberdeen Proving Ground, Md. Personal communication, June 1996.

34. Blewett WK. Chemical Engineer, Team Leader for Ventilation Kinetics, US Army Chemical and Biological Defense Command Edgewood Area, Aberdeen Proving Ground, Md. Letter to MF Tracy, May 1996.

35. Department of the Army. *Collective Protection Equipment*. Washington, DC: DA; July 1991. Training Manual 34240-338-10.

36. Department of the Army. *Operator's and Organizational Manual for the Simplified Collective Protective Equipment, M20*. Washington, DC: DA. August 1987. Training Manual 4240-288-12&P.

37. Edgewood Research Development and Engineering Center. *Decontamination of Chemical Warfare Agents*. Aberdeen Proving Ground, Md: Edgewood Research Development and Engineering Center; December 1992. ERDEC-TR-004.

38. Department of the Army. *NBC Decontamination*. Washington, DC: DA; June 1985. Field Manual 3-5.

39. Christian IL. Quality Assurance Engineer, US Army Chemical and Biological Defense Command, Edgewood Area, Aberdeen Proving Ground, Md. Letter to MF Tracy, May 1996.

40. Department of the Army. *Nuclear, Biological, and Chemical (NBC) Reconnaissance and Decontamination Operations*. Washington, DC: DA; February 1980. Field Manual 3-87.

41. Department of the Army. *Operator's Manual for Decontaminating Kit, Skin: M258A1 and Training Aid, Skin Decontaminating: M58A1.* Washington, DC: DA; October 1989. Training Manual 34230-216-10.

42. Department of the Army. *Operator's Manual for Skin Decontamination Kit*, *M291.* Washington, DC: DA; October 1989. Training Manual 34230-229-10

PSYCHOLOGICAL PROBLEMS ASSOCIATED WITH WEARING MISSION-ORIENTED PROTECTIVE POSTURE GEAR

Prepared for this textbook by Elspeth Cameron Ritchie, M.D., Major, Medical Corps, U.S. Army, Department of Psychiatry, Walter Reed Army Medical Center, Washington D. C. 20307-5001

Personal respirators or protective masks, incorrectly but commonly called gas masks, have been worn since the Germans used gas in World War I. Now they are a routine part of a soldier's field gear, and in planning for combat, the military relies on the putative saving graces of protective masks. However, a few soldiers are unable to tolerate wearing a gas mask for a few minutes even in a peaceful garrison setting. Most soldiers have decrements in cognitive and physical functioning when the mission-oriented protective posture (MOPP) gear is worn for a few hours. How well would these soldiers tolerate wearing MOPP 4 gear for hours or days in a chemical environment?

There are three principal psychological reasons to avoid wearing gas masks: (1) "gas mask phobia," a form of claustrophobia; (2) malingering; and (3) feeling embarrassed at being thought a coward. "Gas hysteria," the panicked feeling that one has been gassed, can also occur.

Gas mask phobia may be categorized as a form of claustrophobia occasioned by the wearing of a protective mask. In psychiatric terms, it could be defined as a simple phobia.[1] Symptoms include hyperventilation, sweating, and panic. These usually cause the eyepieces to fog up, leading to an inability to see, move, shoot, and communicate. Many soldiers either break the seal and lift the mask off the face, or remove the mask completely. Prevention and initial treatment of gas mask phobia and malingering should be command issues, handled though the NBC (nuclear, biological, and chemical) officers; recalcitrant cases may be referred to the mental health service for further evaluation and desensitization techniques.

Prevention of fear of the mask is an important training issue. Consideration should be given to having soldiers in basic training wear the mask in a relaxed setting the first few times. Soldiers then need to train in MOPP gear to learn *(a)* how to handle communication obstacles, and *(b)* what they should do if they or their buddy develop difficulties with the equipment. Often proper fit is not given adequate attention when the mask is issued. If women wear their hair pinned up or if men have facial hair, for example, a tight fit is very difficult to obtain.

An ethical dilemma is raised with having soldiers in a potentially toxic environment who cannot wear the mask. Should these soldiers be removed from the combat environment, thereby risking an epidemic of soldiers claiming that they cannot wear the mask? Or should the soldiers be kept in that environment, risking their injury or death if gas is used?

Not discussed here but a factor to be considered in any potential deployment of soldiers in a biological or chemical environment is the intense fear of that form of warfare. The feelings of helplessness in the face of a ubiquitous and unseen killer can be overwhelming.

HISTORY

Gas was first used extensively in early 1915 by the Germans against both the French and the British. In the accounts of that war, over and over poor discipline is recorded as a significant source of gas casualties.[2] The American Expeditionary Forces used primarily the British small-box respirator, with the French M2 mask as a reserve. Both masks were very uncomfortable, and soldiers took them off at the first opportunity, often while gas still lingered in the area. Many casualties were sustained.[3] "In the recent gas attack practically all casualties had been caused by ignorance of the officers concerning the persistency of mustard, premature removal of masks, and failure to evacuate the camp promptly."[4(p11)]

Shame was another source of casualties:

> It is almost unbelievable nowadays that at one time one of the chief sources of

> these constantly occurring casualties was shamefacedness at being seen in a mask. Men would not protect themselves until absolutely forced to do so, for fear others would regard them as being too easily frightened....They (the soldiers) were met with jeers from some of the supporting troops who shouted “Hello, got the wind up?” and in this way induced the corporal, really against his better judgement, to order masks off. Not more than twenty or thirty yards further along the party ran into a particularly bad pocket of Green Cross and the corporal and several of his men were so badly gassed that they had to be sent to the rear. [5(pp166–167)]

Malingering and gas hysteria were two other sources of casualties. In World War I, many soldiers were evacuated to the aid stations who probably were suffering from gas hysteria. A Division Medical Gas Officer

> capped this account with his report that from 1–13 November a total of 763 men came in to aid stations and field hospitals as gassed. Of these, 339 were not considered gassed and were returned to their units via the casual camp set up in the rear. [6(p73)]

How many of these were judged malingerers is not recorded.

In World War II, gas was not a significant threat. The first volume of *Neuropsychiatry in World War II*, part of the official history of World War II issued by the Office of The Surgeon General, does not discuss difficulties with the protective mask as a problem leading to psychiatric disability.[7] Similarly in the Korean and Vietnam wars, gas posed little danger. Until 1990 and the outbreak of the Persian Gulf War, if a soldier in a routine job could not wear the protective mask, that fact was often ignored by the chain of command.

THE CURRENT SITUATION

American soldiers typically first learn to put on a mask during basic training, in a tear gas–filled tent. The environment is tense, and soldiers are burdened with hot, unfamiliar gear. They may be told to remove their masks long enough to get a whiff of tear gas, causing watery eyes and coughing. When soldiers put their masks back on, they are then sweating and teary, adding to their psychological and physical distress. In many cases, the mask is then worn in strenuous, hot conditions, such as on a road march or a field exercise. In practice, the mask interferes with verbal and visual communication, adding to a sense of isolation. Real casualties from heat stroke or injury are often suffered.[8–10]

Respirators are used in many occupations in the civilian world, including firefighting, and there are similar problems associated with industrial use. However, the self-selection process may eliminate those who cannot tolerate a mask.[11]

In the military, donning the rest of the MOPP gear causes additional physiological and psychological difficulties. The wearing of the suit increases body temperature, which may be compounded by doing tasks in the hot sun. Numerous studies [12–18] have documented the decrement in function caused by wearing MOPP 4 for extended periods. Studies have also tested the effects on soldier performance of administering the nerve agent–antidotes atropine sulfate and pralidoxime chloride (2-PAM Cl)[19] and pyridostigmine bromide[20,21]; the combination of atropine and 2-PAM Cl significantly shortened endurance time for heat sessions for soldiers wearing MOPP 4 gear.[19]

Modern Case Studies

The following three case studies are of soldiers who could not tolerate wearing their protective masks during the Persian Gulf War. Little on the phenomenon had been published in the

medical or military literature by the early 1990s; it is probable that many more cases were undiagnosed. These three previously published[22,23] cases highlight two issues: the question of trying to get out of combat by not being able to wear the mask, and the reluctance of motivated or senior soldiers to admit that they have had a problem with the MOPP gear.

Case 1. A 19-year-old single white male, stationed at the Demilitarized Zone in Korea, was unable to finish a 2-mile road march while wearing his protective mask. His chief complaint was “It’s embarrassing to be unable to finish the march.” This soldier had difficulty wearing his mask for more than a few minutes. After the first mile of a road march, he experienced confusion, shortness of breath, blurry vision, fainting sensations, and intense thirst. His background, past psychiatric history, and medical history were unremarkable. He did remember an incident when he was 6 years of age, when he and his friends were playing “mummy.” He felt hot and agitated and scared; later his grandmother told him that he had been hyperventilating. A diagnosis of simple phobia was made. Treatment principles outlined below were followed. Initially he was told to wear his mask while listening to music. He did, but still felt “confined.” He would try to adjust his mask constantly by moving it around, and getting cool air underneath. Gradually he felt more confident while wearing the mask and could increase the time he wore it. He was able to wear his entire MOPP gear during the annual “Team Spirit” exercise.[22(p105)]

Case 2. An officer with 20 years of military service was referred with depressive symptoms. These followed in the wake of a chronic gas mask phobia that had gone untreated for years because the officer had been too ashamed to admit it to anyone. He had remained on the front lines, enduring several gas mask alerts, and was identified when he finally sought assistance. He was eventually evacuated because his depression and phobia could not safely be treated in the desert environment.[23(ppA10–A11)]

Case 3. A specialist was brought to the hospital for treatment of gas mask phobia, which had been refractory to systemic desensitization by a unit medic. The treatment had been notable for the patient’s overall compliance but insufficient “effort.” She would remove the mask frequently and inexplicably during low subjective anxiety, or with sudden incongruous elevation in anxiety unaccompanied by any objective signs. It was decided to send the patient to the division rear to provide some improved safety, given that malingering could not be judged as certain.[23(pA-11)]

Treatment

The basic techniques of treating gas mask phobia are similar to those of treating other phobias, modified for the military culture and environment. They are desensitization, relaxation, and flooding. To avoid malingering, any secondary gain (ie, other benefits accrued by not being able to wear the mask, such as evacuation from the field environment) should be avoided.

In desensitization, the soldier needs to wear the mask enough that it becomes routine. Secondly, he needs to relax while wearing it. Thus, soldiers should begin by wearing the mask while watching television, ironing, or doing other household or barrack tasks. They should start wearing it for short periods (5 min), then lengthen the time (30 min).

Flooding, the next step, refers to the technique of having a patient imagine wearing a mask in tense situations. Gradually, the soldier actually wears the mask in more-tense and -fatiguing situations. To avoid secondary gain, benefits of not being able to wear a mask should be minimized. Therefore, soldiers should not be excused from road marches, field exercises, or other duties because they are unable to wear MOPP gear.[24]

Other primary strategies include *(a)* simulation training; *(b)* modeling training (ie, observation of live peer models); *(c)* self-management training (ie, training in self-control techniques); and *(d)* inoculation training, both as a soldier and as a casualty. Secondary prevention strategies include: *(a)* self-care; *(b)* buddy care; *(c)* leader management, to minimize the risk of symptom spread; and *(d)* medic care.[18]

SUMMARY

As threats of chemical and biological warfare become more routine, it is imperative that soldiers can wear their protective gear. Frequent practice should increase comfort and decrease problems with claustrophobia and embarrassment. In those who cannot initially tolerate the gas masks, treatment should be initiated as close to the front lines as possible, to minimize casualties in training and in combat.

REFERENCES

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed. Washington DC: APA Press, 1994.
2. Spencer EW. *The History of Gas Attacks Upon the American Expeditionary Force During the World War*. Book 1, Part I. Carlisle Barracks, Pa: Military Historical Institute; 1928: 146–150. Bound typescript.
3. Spencer EW. *The History of Gas Attacks Upon the American Expeditionary Force During the World War*. Book 3. Carlisle Barracks, Pa: Military Historical Institute; 1928: 409–415. Bound typescript.
4. Cochrane RC. *Gas Warfare at Chateau Thierry*. Carlisle Barracks, Pa: Military Historical Institute; July 1956. Bound typescript.
5. Auld SJM. *Gas and Flame in Modern Warfare*. New York, NY: George H Doran Co; 1918.
6. Office of the Chief Chemical Officer. *The 92nd Division in the Marbache Sector*. Washington, DC: US Army Chemical Corps Historical Office; 1918.
7. Carter J, Cammermeyer M. Biopsychological responses of medical unit personnel wearing chemical defense ensemble in a simulated chemical warfare environment. *Milit Med.* 1985;150(5):234–248.
8. Glass AJ, Bernucci RJ, eds. *Zone of Interior*. Vol 1. In: Mullins WS, Glass AJ, eds. *Neuropsychiatry in World War II*. Washington, DC: US Department of the Army, Office of The Surgeon General; 1966.
9. Carter BJ, Cammermeyer M. Emergence of real casualties during simulated chemical warfare training under high heat conditions. *Milit Med*. 1985;150(12):657–663.
10. Cole RD. Heat stroke during training with nuclear, biological and chemical protective clothing: Case report. *Milit Med*. 1983;148:624–625.
11. Morgan WP. Psychological problems associated with the wearing of industrial respirators: A review. *Am Ind Hyg Assoc J*. 1983;44(9):671–676.
12. Boer LC, vd Linde LC. *Psychological Fitness With NBC Clothing*. Soesterberg, The Netherlands: Netherlands Organization for Applied Scientific Research; 1990.
13. Rauch TM, Banderet LE, Tharion WJ, Munro I, Lussier AR, Shukitt B. *Factors Influencing the Sustained Performance Capabilities of 155mm Howitzer Sections in Simulated Conventional and Chemical Warfare Environments*. Natick, Mass: US Army Research Institute of Environmental

Medicine; 1986. Technical Report TII/86.

14. Munro I, Rauch TM, Tharion WJ, Banderet LE, Lussier AR, Shukitt B. *Factors Limiting Endurance of Armor, Artillery and Infantry Units Under Simulated NBC Conditions.* Natick, Mass: US Army Research Institute of Environmental Medicine; 1986. Presentation.

15. Kelley JL. *The Effects of Chemical Protective Gear, Heat and Cold on Cognitive Performance*. Natick, Mass: Army Research Laboratory; July 1994. ARL-TN-36.

16. Kelly TL. *Annotated Bibliography for Gas Mask and Chemical Defense Gear Related Papers*. San Diego, Calif: Naval Health Research Center; 1988. Technical Report 88-7.

17. Ryman DH, Kelly TL, Englund CE, Naitoh P, Sinclair M. *Psychological and Physiological Effects of Wearing a Gas Mask or Protective Suit Under Non Exercising Conditions*. San Diego, Calif: Naval Health Research Center; 1998.

18. Brooks FR, Ebner DG, Xenakis SN, Balson PM. Psychological reactions during chemical warfare training. *Milit Med*. 1983;148:232–235.

19. Kobrick JL, Johnson RF, McMenemy DJ. Effects of nerve agent antidote and heat exposure on soldier performance in the BDU and MOPP-IV ensembles. *Milit Med*. 1990;155:250–254.

20. Cook JE, Kolka MA, Wenger CB. Chronic pyridostigmine bromide administration: Side effects among soldiers working in a desert environment. *Milit Med*. 1992;157:159–162.

21. Arad M, Varssano D, Moran D, Arnon R, Vazina A, Epstein Y. Effects of heat-exercise stress, NBC clothing, and pyridostigmine treatment on psychomotor and subjective measures of performance. *Milit Med*. 1992;157:210–214.

22. Ritchie EC. Treatment of gas mask phobia. *Milit Med*. 1992;157(2):104–106.

23. Sandman L. Gas mask phobia. *Milit Med*. 1992;157:A10–A11. Letter.

24. Deakins DE. Further cases of gas mask phobia. *Milit Med*. 1993;158:A-4. Letter.

Chapter 17

HEALTHCARE AND THE CHEMICAL SURETY MISSION

ROBERT GUM, D.O., M.P.H.*

INTRODUCTION

CHEMICAL PERSONNEL RELIABILITY PROGRAM

HEALTH SURVEILLANCE FOR CHEMICAL WORKERS
- Preplacement Examination
- Baseline Data for Future Exposures
- Periodic Medical Examinations
- Termination Examination

HEAT STRESS

HEALTH EDUCATION FOR CHEMICAL WORKERS

MANAGEMENT OF THE CONTAMINATED PATIENT

CHEMICAL ACCIDENT OR INCIDENT RESPONSE AND ASSISTANCE

DEMILITARIZATION OF CHEMICAL WARFARE AGENTS

SUMMARY

* *Lieutenant Colonel, Medical Corps, U.S. Army; Combat Casualty Care Office, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010-5425*

INTRODUCTION

Medical officers with assignments to U.S. Army depots or other installations storing chemical warfare agents face a number of unique challenges. Not only will newly assigned general medical officers provide patient care to both military and civilian workers, they will also have a myriad of additional duties unique to chemical weapons storage sites.

The depot may be physically isolated and a considerable distance away from the Medical Center (MEDCEN) or Medical Department Activity (MEDDAC) responsible for providing medical support and consultation. The preventive/occupational medicine physicians are usually responsible for providing this support and are a source of information and guidance. Other governmental agencies have also been identified to assist medical personnel in acquiring solutions to unfamiliar medical problems related to chemical exposure (Exhibit 17-1).

EXHIBIT 17-1
ADVISING AGENCIES FOR TREATMENT OF CHEMICAL AGENT INJURY

The Preventive or Occupational Medicine department of the supporting Medical Department Activity or Medical Center	U.S. Army Medical Research Institute of Chemical Defense ATTN: SGRD-UV-ZM Aberdeen Proving Ground, Maryland 21010-5425
U.S. Army Center for Health Promotion and Preventive Medicine ATTN: HSHB-MO Aberdeen Proving Ground, Maryland 21010-5422	U.S. Army Materiel Command ATTN: AMCSG 5001 Eisenhower Avenue Alexandria, Virginia 22333-0001
Office of The Surgeon General Chemical Surety Consultant ATTN: SFIL-CMS Aberdeen Proving Ground, Maryland 21010-5401	U.S. Army Depot System Command ATTN: AMSDS-SU Chambersburg, Pennsylvania 17201-4170

Physicians assigned to installations with a chemical *surety* mission (the term encompasses safety, security, and reliability) must be able to recognize and treat a wide variety of chemically related diseases and injuries. Time or assets are seldom available, however, to train a general medical officer in the unique occupational setting of depot operations. At the present time, newly assigned general medical officers are required to complete the Medical Management of Chemical Casualties Course given at Aberdeen Proving Ground, Maryland. This course provides the basic concepts needed to recognize the clinical signs and symptoms of a chemical agent exposure and the appropriate therapeutic interventions used in treating and managing chemical agent casualties. In addition, the Office of The Surgeon General sponsors the Toxic Chemical Training for Medical Support Personnel Course, which is conducted at the Chemical Demilitarization Training Facility at the Edgewood Area of Aberdeen Proving Ground. This training course has incorporated presentations on medical diagnosis and treatment that are essential to managing the health-related concerns of the chemical surety mission. These orientation courses provide essential information to the medical officer beginning his atypical assignment.

As used in this chapter, a *chemical agent* is defined as a chemical substance intended for use in military operations to kill, seriously injure, or incapacitate a person through its physiological effects. Riot control agents, chemical herbicides, smoke, and flame are not officially defined as chemical agents.

Although the chemical agents discussed are unique to the military, the hazards to the workers are common to many industries. Examples include pesticide workers who are exposed to acetylcholinesterase inhibitors (the operative mechanism of nerve agents) and carbonyl chloride (phosgene), which is used in the production of foams and plastics. Both are transported daily on the nation’s highways. In addition to these chemical threats, many physical hazards found in the chemical storage depot are shared by other types of operations. The operation of forklifts, the presence of excessive noise, heat stress, lifting, and other chemical exposures (in addition to chemical warfare agents) are only a few of the more common hazards.

The intended use, packaging, and storage of chemical munitions, however, present different hazards and therefore require different controls. The system of controls, procedures, and actions that contribute to the safety, security, and reliability of chemical agents and their associated weapon systems throughout their life cycle without degrading operational performance is known as chemical surety.

An integral part of a physician’s practice is addressing the occupational

healthcare needs of the patients. This responsibility includes identification of occupational and environmental health risks, treatment of disease and injury, and patient counseling concerning preventive behavior. This task by itself is time-consuming and presents demands that, in part, can be performed by the occupational health nurse, the industrial hygienist, and other clinic staff members.

Although industrial hygienists are often not assigned to the health clinic, they are an integral part of the healthcare team. The industrial hygienist maintains a hazard inventory that contains conventional hazards as well as a list of chemical agents located at the installation. He routinely designs primary prevention strategies and frequently oversees hearing conservation, respiratory protection, and occupational vision programs. The information he provides is necessary to evaluate the work environment and to determine the appropriate frequency of periodic medical examinations. Close and frequent coordination with this individual is imperative for developing a knowledge of the worksite and the subsequent development of a medical surveillance program.

Just as it is imperative to work closely with industrial hygiene and safety personnel, medical personnel must also work in accord with the command, supervisors, personnel officers, and the workers. Maintaining these relationships is frequently difficult, but by identifying and addressing concerns of both the management and the individual workers, medical personnel can establish a basis for formulating appropriate preventive medical measures.

CHEMICAL PERSONNEL RELIABILITY PROGRAM

The Chemical Personnel Reliability Program (CPRP) is a management tool used within the army to identify chemical surety duty positions and to manage the persons assigned to these positions. It also provides a way to assess the reliability and acceptability of personnel being considered for and assigned to chemical duty positions. Chemical surety material is defined in Army Regulation 50-6, *Chemical Surety*, as “chemical agents and their associated weapon system, or storage and shipping containers, that are either adopted or being considered for military use.”[1(p43)]

The program was established to ensure that the personnel assigned to positions involving access to or responsibility for the security of chemical surety material are emotionally stable, loyal to the United States, trustworthy, and physically fit to perform assigned duties. The certifying official is the commander’s representative for the CPRP and ultimately responsible for its administration. The decision to qualify or disqualify personnel for CPRP duties is made by the certifying official, with input from the personnel officer and medical personnel. The certifying official

must also determine the appropriate medical surveillance category for each worker (see below for a discussion of the four categories) based on the worker's potential for exposure.

The CPRP requires both preassignment screening and continuing evaluation. This screening and evaluation is performed when an individual is assigned initial CPRP duties, when a new assignment is being considered by the certifying official, and once every 5 years thereafter. The CPRP screening/evaluation consists of an initial interview with the certifying official, personnel records screen, medical evaluation, and a final evaluation and briefing by the certifying official.

During each portion of the screening process, evaluators look for any evidence of potentially disqualifying factors that may affect personnel reliability or suitability for CPRP duties. The potential disqualifying factors of medical relevance include: alcohol abuse, drug abuse, inability to wear protective clothing and equipment required by the assigned position, or any significant physical or mental condition that in the judgment of the certifying official may be prejudicial to the reliable performance of CPRP duties. Factors that restrict the wearing of protective clothing include: *(a)* the inability to obtain a seal with the protective mask, *(b)* an allergy to protective clothing and equipment, *(c)* any medical condition that precludes correct wear and use of protective clothing, and *(d)* poor visual acuity that requires the use of glasses unless mask optical inserts are used. Contact lenses are not permitted to be worn with the protective mask because they can concentrate agent beneath the lens, or, more commonly, a foreign body will become lodged beneath a contact lens, necessitating immediate removal. This cannot be done in a chemical environment!

Any medical conditions, including the use of any prescribed medications, that may detract from an individual's ability to perform assigned chemical surety duties must be communicated to the certifying official by oral notification and confirmed *in writing.* In addition, the physician must provide a recommendation as to the suitability of the worker to continue CPRP duties. Documentation of these communications should be included on the Standard Form 600. As in all healthcare, documentation is extremely important and, in this case, subject to examination during a Chemical Surety Inspection (CSI). Exhibit 17-2 lists the administrative documentation necessary to support a CSI.

EXHIBIT 17-2
ADMINISTRATIVE DOCUMENTATION TO SUPPORT A CHEMICAL SURETY INSPECTION

Army Regulations

AR 11-34, 15 Feb 90	*The Army Respiratory Protection Program*
AR 40-2, 15 Mar 83	*Army Medical Treatment Facilities*
AR 40-3, 15 Feb 85	*Medical, Dental and Veterinary Care*
AR 40-5, 15 Oct 90	*Preventive Medicine*
AR 40-13, 1 Feb 85	*Medical Support: Nuclear/Chemical Accidents and Incidents*
AR 40-63, 1 Jan 86	*Ophthalmic Services*
AR 40-66, 20 Jul 92	*Medical Record Administration*
AR 40-68, 20 Dec 89	*Quality Assurance Administration*
AR 40-400, 1 Oct 83	*Patient Administration*
AR 50-6, 12 Nov 86	*Chemical Surety*
AR 385-10, 23 May 88	*Army Safety Program*
AR 385-32, 1 May 84	*Protective Clothing and Equipment*
AR 385-40, 1 Apr 87	*Accident Reporting and Records*
AR 385-64, 22 May 87	*Ammunition and Explosives Safety Standards*
AR 600-85, 21 Oct 88	*Alcohol and Drug Abuse Prevention and Control Program*
HSC-R 10-1, 25 Sep 91	*Organization and Functions Policy*
HSC-R 40-5, 1 Sep 87	*Ambulatory Primary Care*
HSC Supplement 1 to AR 40-2, 3 Jun 91	*Army Medical Treatment Facilities*
HSC Supplement 1 to AR 40-3, 1 May 92	*Medical, Dental and Veterinary Care*
HSC Supplement 1 to AR 50-6, 10 Feb 88	*Chemical Surety*
AMC-R 385-131, 9 Oct 87	*Safety Regulation for Chemical Agents H, HD, HT, GB, and VX*
7th MEDCOM-R 40-8, 24 Apr 87	*Medical and Dental Management of the Personnel Reliability Program*

Department of the Army Pamphlets and Technical Bulletins Medical

DA PAM 40-8, 4 Dec 90	*Occupational Health Guidelines for the Evaluation and Control of Occupational Exposures to Nerve Agents GA, GB, GD, and VX*
DA PAM 40-173, 30 Aug 91	*Occupational Health Guidelines for the Evaluation and Control of Occupational Exposures to Mustard Agents H, HD, and HT*
DA PAM 40-501, 27 Aug 91	*Hearing Conservation*
DA PAM 50-6, 17 May 9	*Chemical Accident or Incident Response and Assistance (CAIRA) Operations*
HSC PAM 40-2, June 83	*Occupational Health Program*
TB MED 502, 15 Mar 82	*Respiratory Protection Program*
TB MED 503, 1 Feb 85	*Industrial Hygiene Program*
TB MED 506, 15 Dec 81	*Occupational Vision*
TB MED 507, 25 Jul 80	*Prevention, Treatment, and Control of Heat Injury*
TB MED 509, 24 Dec 86	*Spirometry in Occupational Health Surveillance*

Field Manuals

	NBC Decontamination
FM 3-5, 24 Jun 85	*Treatment of Chemical Agent Casualties*
FM 8-285, 28 Feb 90	*and Conventional Military Chemical Injuries*

Personnel Documents

- Table of Distribution and Allowances with mission statement for medical treatment facility or activity
- Intraservice Support Agreement between tenant health clinic and the host installation
- Job descriptions with performance standards (or support forms for active duty)
- Scopes of practices
- Individual or categorical credentials for health care practitioners
- Current certificates of licensure for physicians and nurses
- Advanced Trauma Life Support/Advanced Cardiac Life Support certification for physicians (nurses optional)
- Basic Life Support certification for all personnel with patient care responsibilities
- Certificate of completion of Medical Management of Chemical and Biological Casualties Course for physicians

Memorandums of Understanding and Mutual Aid Agreements

- With local civilian hospitals or ambulance services
- With the supporting medical center or medical department activity
- Between Health Services Command and Army Materiel Command (or other major army commands, if appropriate)

Standing Operating Procedures

- Spirometry
- Audiometry
- Vision screening
- Optical insert program for protective masks
- Medical surveillance examination (agent-specific)
- Pregnancy surveillance/reproductive hazards
- Medical screening of Personnel Reliability Program records
- Illness absence monitoring vis-à-vis Personnel Reliability Program records
- Incorporation of air monitoring results into the medical record
- Interface with Alcohol and Drug Abuse Prevention and Control officer
- Ambulance operation and stockage
- Preparation and review of first aid briefings
- Chemical accident and incident response
- Handling of contaminated casualties at the clinic

Medical Directives

- Administration of nerve agent antidotes in the clinic
- Administration of intravenous solutions
- First aid for minor illnesses or injuries

Other

- *Medical Management of Chemical Casualties Handbook*, September 1994

(Available from Chemical Casualty Care Office, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010)

AMC: Army Materiel Command; HSC: Health Services Command; MEDCOM: Medical Command; NBC: nuclear, biological, and chemical

While the medical officer does not decide the suitability of a candidate for CPRP duties, the certifying official makes a decision based on the medical information and recommendations he provides. The recommendation should state: *(a)* no restriction, *(b)* restrictions or limitations on duties, *(c)* temporary disqualification, or *(d)* permanent disqualification. Potentially disqualifying information is provided in a sealed envelope marked “EXCLUSIVE FOR” the certifying official. Temporarily disqualified personnel remain in the CPRP; therefore, their medical records must be treated in the same manner as the medical records of other personnel in the program.

A chemical-duty position roster lists all individuals assigned to chemical-duty positions in the CPRP by name, social security number, and job title. This roster also contains the name of the certifying official, the organization, and the medical surveillance exposure category of each worker. The roster must be periodically reviewed to verify that a change in duty position that requires a change in category is incorporated into the medical record, and that periodic surveillance is changed to match. Medical records for personnel in the CPRP are required to be identified in accordance with Army Regulation 40-66, *Medical Record Administration.*[2] These medical records are required to be segregated from records of personnel not in the CPRP.

HEALTH SURVEILLANCE FOR CHEMICAL WORKERS

Medical surveillance is the systematic collection, analysis, and dissemination of disease data on groups of workers. It is designed to detect early signs of work-related illness.[3] A chemical work site medical program should provide the following surveillance: preplacement screening, periodic medical examinations (with follow-up examinations, when appropriate), and termination examinations. Additional follow-up examinations are required if an individual has been exposed or if a potential exposure has occurred. An efficient medical surveillance program will assist in detecting a relationship between exposure to a hazard and a disease. In addition, the medical surveillance system will assist in identifying an occupational disease at an early stage, when medical intervention can be beneficial.

Since the purpose of medical surveillance is to identify work-related disease at an early stage, it may be considered a type of screening. *Screening* is the search for a previously unrecognized disease or pathophysiological condition at a stage when intervention can slow, halt, or reverse the progression of the disorder.[3] Screening for the CPRP must be performed by a physician or other qualified medical staff member (physician’s assistant, dentist, or dental assistant) who has been officially

designated to perform this function.

Additional examinations that are independent of medical surveillance will be required. These include fitness evaluations for personal protective equipment and evaluation of a potential worker's ability to meet the functional requirements of the job.

Engineering and individual protective measures are the primary disease prevention methods; medical screening is a tertiary measure. The importance of engineering and individual protective measures must continually be stressed. An individual that shows signs or complains of symptoms of occupationally related illness should be identified as a possible sentinel case. Not only must the individual be treated, but the cause must also be investigated thoroughly by the Installation Medical Authority (IMA), the industrial hygienist, and the safety personnel. The cause may be related to improper work practices of the affected individual or it may be related to a failure of engineering devices or personal protective measures. In the latter case, further morbidity can be avoided if identification of the problem is prompt.

The IMA, or contract physician, is responsible for establishing and supervising the medical surveillance system. Not all individuals working at the installation, or even in a particular work area, need to be on the same surveillance program. The type of work, work area, and required personal protective equipment are factors in determining the type and frequency of surveillance.

For additional information and direction concerning the development of an occupational medicine program, the installation medical officer is encouraged to seek advice from the regional MEDCEN or MEDDAC. In addition, the Occupational and Environmental Medicine Division of the U. S. Army Center for Health Promotion and Preventive Medicine may be of assistance.

Preplacement Examination

Prior to evaluating a patient history and completing a physical examination, the physician should acquire an accurate and *current* job description listing the specific tasks the worker will be required to accomplish. The type of respiratory protection and protective clothing required must also be ascertained, because these will affect an individual's ability to perform his job.

Not all individuals are required to wear protective clothing all the time; the frequency of use, the exertion level associated with the personal protective clothing, and the environmental conditions in which they are worn will have a dramatic influence on how well an individual will perform. Changing environmental conditions must be considered; a worker at

Tooele, Utah, may be very comfortable in the winter in protective clothing and unable to tolerate the same level of protection in the heat of the summer. Work–rest cycles become very important.

Preplacement examination has two major functions: (1) determination of an individual's fitness for duty, including the ability to work while wearing protective equipment, and (2) provision of baseline medical surveillance for comparison with future medical data.[4] The chemical agent worker must be evaluated to ensure that he is not predisposed to physical, mental, or emotional impairment, which may result in an increased vulnerability to chemical warfare agent exposure. This examination is performed at no cost to the applicant. Abnormalities identified during the course of the preplacement examination, however, need to be followed up by the applicant, at his expense, with a private physician.

An occupational and medical history questionnaire is the first step in acquiring necessary information from the prospective worker. A thorough review, by the medical officer, is required to identify past illnesses and diseases that may prevent the individual from satisfactory performance of job requirements. It is particularly important to inquire about atopic dermatitis, pulmonary disease, and cardiovascular disease.

A review of symptoms will enable the medical officer to evaluate the ability of an individual to work in protective ensemble. Questions concerning shortness of breath or labored breathing on exertion, asthma, other respiratory symptoms, chest pain, high blood pressure, and heat intolerance will provide helpful information. Questions about allergic reactions to rubber products and cold-induced bronchospasm should be asked and a brief psychiatric history directed toward the individual's ability to be encapsulated in personal protective equipment should be taken. Questions about panic attacks, syncopal episodes, or hyperventilation will also offer valuable information.

For those who are not clearly disqualified by their medical history and physical examination, it is necessary for the medical officer to determine their ability to function while wearing respiratory protective equipment. This can be done by either pulmonary function testing or a "use" test. The former is effective, although costly; the latter provides necessary information and can be performed safely by the majority of applicants. Caution must be exercised, however, in requiring an individual to perform a use test. A worker with a questionable history (eg, angina or previous myocardial infarction) should not be required to complete a use test prior to pulmonary function testing. Input from the industrial hygienists concerning the required tasks will produce more useful results than a generic use test. The outcome of either test must be documented in the individual's medical record.

Contact lenses must be replaced by optical inserts whenever a full-face

respirator is worn. Personnel who require glasses must also have optical inserts. Permitting a worker to begin work in a chemical environment without appropriate optical inserts, or while wearing contact lenses, places both the worker and the coworkers at an unacceptable risk for accidents.

The physical examination should be comprehensive and focus on the skin, cardiovascular, pulmonary, and musculoskeletal systems. Obesity, lack of physical strength, and poor muscle tone are indicators of increased susceptibility to heat injury, a condition which will be amplified by working in chemical protective clothing. Factors such as facial hair, scarring, dentures, and arthritic hands or fingers can affect a worker's ability to wear or don a respirator and protective clothing.

Baseline Data for Future Exposures

Baseline data acquired during the preplacement screening can be used following a subsequent exposure event to determine the extent of the exposure. It can also be used to verify the engineering controls in effect. Additionally, baseline data may be used to determine if the worker has been adversely affected by the exposures. Red blood cell cholinesterase (RBC-ChE) baseline levels are essential for workers assigned in areas in which nerve agent munitions are stored. Workers are categorized by the area they are assigned to and the frequency with which they are in a chemical environment. The frequency of follow-up examinations are determined by the category in which prospective workers are placed. These categories are discussed in the following section.

Periodic Medical Examinations

Periodic medical examinations should be developed and used in conjunction with preplacement screening examinations.[4] Comparing the data obtained through periodic monitoring with the preplacement baseline data is essential for identifying early signs of occupationally induced diseases. The primary purpose of the periodic medical examination is to identify conditions for which early interventions can be initiated, so that progression of the adverse effects can be curtailed prior to significant injury or disease.

The interval medical history and physical should focus on changes in health status, illness, and possible work-related signs and symptoms. The examining physician must be aware of the work environment and potentially hazardous exposures in order to identify work-related conditions or disease. Unlike patients seen in a private office, chemical surety workers who show a change in health status in the periodic evaluation make an evaluation of the work site necessary. Additional workers may require examination on the basis of conditions identified. At a

minimum, coordination must be made with industrial hygiene personnel to determine if there has been a change in the work environment that could be causally related.

The frequency and extent of the periodic medical examination will be determined by the toxicity of the potential or actual exposures, frequency and duration of contact, and the information obtained in the preplacement history and physical examination. The data obtained from these periodic examinations can serve as a guide to the future frequency of physical examinations or tests. Data consistently within acceptable limits for several months may indicate that the frequency can be safely decreased, provided that the work situation remains constant.

Biological monitoring for nerve agent exposure consists of RBC-ChE measurement. Determining who will be monitored, and the frequency, is the responsibility of the IMA. The certifying official is responsible for supplying information concerning the duties the worker performs; an accurate job description is essential. The surety officer and the safety officer may provide advisory input to the monitoring strategy for nerve agent exposures.

In accordance with Department of the Army Pamphlet 40-8, *Occupational Health Guidelines for the Evaluation and Control of Occupational Exposure to Nerve Agents GA, GB, GD, and VX,* the following four categories of personnel are required to have their RBC-ChE measured[5]:

1. Category A: personnel with a high risk of potential exposure due to the nature of the agent operations being conducted. Examples of such operations might include (but are not limited to) storage monitoring inspections of M55 rockets, periodic inspections, toxic chemical munitions maintenance operations that involve movement of munitions from storage locations, work in known contaminated environments, and first-entry monitoring. Category A personnel may be routinely required to work for prolonged periods in areas with high levels of nerve agents where the use of either of the following are required:

    - toxicological agent protective (TAP) ensembles, or
    - protective ensembles with a self-contained or supplied-air breathing apparatus.

2. Category B: personnel with both

- a low risk or infrequent potential exposure to nerve agents in routine industrial, laboratory, or security operations (examples of such operations might include but are not limited to daily site security checks and accident/incident response by initial response force members), and
- job requirements involving the prolonged wearing of protective ensembles during training and emergency responses.

3. Category C: personnel with minimal probability of exposure to nerve agents, even under accident conditions, but whose activities may place them in close proximity to agent areas.

4. Category D: transient visitors to agent areas where a potential for exposure exists and who are not included in the medical surveillance program for nerve agents at the visited installation.

An individual in category A must have a monthly determination of the RBC-ChE level; an individual in category B will have an annual RBC-ChE determination. Inaccurate categorization of workers will either fail to provide adequate surveillance or cause exorbitant cost and effort without benefits.

Termination Examination

At the termination of employment or at the termination of duty in a chemical surety position, a worker should have a medical examination. Unless otherwise specified by a local regulation, this examination may be done within 30 days before or after termination of employment. In the event the worker is exposed after his termination examination, it will be necessary to evaluate for and thoroughly document that specific exposure. In most cases, exposure is not expected to occur, and completing the termination examination within the 30 days before the worker departs is advisable. Medical personnel must be aware that although it is in the worker’s best interest to have a termination examination, it can be difficult for him return to his former place of employment to complete a medical examination once employment is terminated.

HEAT STRESS

Heat stress is a constant and potentially severe health threat to the worker in toxicological protective clothing. The combination of exposure to solar radiant energy or enclosed areas with high temperatures, metabolic

heat production, and the use of impermeable clothing (which prevents evaporative cooling) place the chemical worker at high risk for heat injury.

Encapsulating uniforms increase the heat strain associated with most environments and work rates by creating a microenvironment of small volume around the worker. The impermeability to vapor of the suit (which is, after all, the characteristic that makes it protective) creates high local humidity, restricting evaporative cooling and conductive/convection cooling. In effect, the suit creates an environment at the body surface hotter and wetter under almost any circumstances than the environment outside the suit. Moderating the heat strain associated with an encapsulating ensemble is accomplished in the following ways:

- microclimate cooling: direct removal of heat, water vapor, or both from the worker's microenvironment;
- heat sinks in the suit: ice vests;
- increasing the temperature gradient across the suit: shielding workers from radiant heat sources, cooling the work space or, in dry environments, wetting the surface of the suit; and
- work–rest cycles to permit cooling and rehydration.

Heat-induced occupational injury or illness occurs when the total heat load from the environment and metabolism exceeds the cooling ability of the body. The resultant inability to maintain normal body temperature results in heat strain (the body's responses to total heat stress).[6]

The reduction of adverse health effects can be accomplished by the proper application of engineering and work-practice controls, worker training and acclimatization, measurements and assessment of heat stress, medical supervision, and proper use of heat-protective clothing and equipment.[6] Worker training and adequate supervision are basic requirements that need constant reinforcement. The occurrence of heat-induced illness or injury is an indication that *(a)* the worker has engaged in a careless act that should have been avoided and detected by adequate training and supervision, *(b)* the individual's medical status has changed and requires further or more frequent evaluation, or *(c)* supervisory enforcement of work–rest cycles or of adequate rehydration is lacking. In all cases, the healthcare provider must investigate the cause. If the individual's health status has changed, further medical evaluation is indicated. The worker may require temporary duties commensurate with his present health status or a permanent change of duties if his medical condition warrants. Should the injury appear to be a result of carelessness or lack of attention to changing environmental conditions, further training is indicated. Eliciting the worker's support may be necessary to acquire the

appropriate support of intermediate supervisors.

Numerous textbooks and other sources discuss thermoregulation and physiological responses to heat; healthcare providers may benefit from a review of these subjects. This chapter will address the evaluation of heat stress and preventive measures.

The preplacement physical examination is designed for workers who have not been employed in areas exposed to heat extremes. It should be assumed that such individuals are not acclimatized to work in hot climates. The physician should obtain the following information[6]:

- A medical history that addresses the cardiovascular, respiratory, neurological, renal, hematological, gastrointestinal, and reproductive systems and includes information on specific dermatological, endocrine, connective tissue, and metabolic conditions that might affect heat acclimatization or the ability to eliminate heat.
- A complete occupational history, including years of work in each job, the physical and chemical hazards encountered, the physical demands of these jobs, intensity and duration of heat exposure, and nonoccupational exposures to heat and strenuous activities. The history should identify episodes of heat-related disorders and evidence of successful adaptation to work in heat environments as part of previous jobs or in nonoccupational activities.
- A list of all prescribed and over-the-counter medications used by the worker. In particular, the physician should consider the possible impact of medications that potentially can affect cardiac output, electrolyte balance, renal function, sweating capacity, or autonomic nervous system function. Examples of such medications include diuretics, antihypertensive drugs, sedatives, antispasmodics, anticoagulants, psychotropic medications, anticholinergics, and drugs that alter the thirst (haloperidol) or sweating mechanism (phenothiazines, antihistamines, and anticholinergics).
- Information about personal habits, including the use of alcohol and other social drugs.
- Data on height, weight, gender, and age.

The direct evaluation of the worker should include the following[6]:

- Physical examination, with special attention to the skin and cardiovascular, respiratory, musculoskeletal, and nervous systems.
- Clinical chemistry values needed for clinical assessment, such as fasting blood glucose, blood urea nitrogen, serum creatinine, serum

electrolytes (sodium, potassium, chloride, bicarbonate), hemoglobin, and urinary sugar and protein.
- Blood pressure evaluation.
- Assessment of the ability of the worker to understand the health and safety hazards of the job, understand the required preventive measures, communicate with fellow workers, and have mobility and orientation capacities to respond properly to emergency situations.

A more detailed medical evaluation may be required. Communication between the physician performing the preplacement evaluation and the worker's private physician may be appropriate and is encouraged.

Follow-up evaluations may be warranted during the acclimatization period for selected workers. The phenomenon of heat acclimatization is well established, but for an individual worker, it can be documented only by demonstrating that after completion of an acclimatization regimen, the worker can work without excessive physiological heat strain in an environment that an unacclimatized worker could not withstand. The IMA needs to be intimately involved in developing the acclimatization program for the installation.

Annual or periodic examinations should monitor individuals for changes in health that might affect heat tolerance and for evidence suggesting failure to maintain a safe working environment. Education of the workers and supervisors, however, is the single most important preventive measure in avoiding heat casualties.

Personnel required to wear toxic-agent protective clothing are also at high risk for dehydration, which is a contributing factor for developing heat injury. The thirst mechanism is not adequate to stimulate a worker to consume as much as a liter of water per hour that may be lost in sweat. If weight loss exceeds 1.5% to 2.0% of body weight, heart rate and body temperature increase, and work capacity (physical and psychological) decreases.[7] Workers should be required to consume at least 8 oz of cool water at each break period; for moderate work in greater than 80°F wet bulb globe temperature (WBGT), the average male should plan on 1 qt of fluid per hour; more water may be required depending on the ambient temperature and humidity.

The average diet in the United States provides adequate salt intake for the acclimatized worker. The unacclimatized worker may excrete large amounts of salt: another reason that he will need close monitoring while adjusting to the evaluated temperatures and decreased evaporative cooling. Individuals on medications that further deplete sodium (ie, diuretics) will need even closer monitoring and medical follow-up. The judicious use of sodium replacement may be required during the acclimatization period.

HEALTH EDUCATION FOR CHEMICAL WORKERS

All personnel entering an area where chemical munitions are stored must recognize and understand the potential hazards to their health and safety associated with chemical agents. Workers must be required to recognize signs and symptoms of exposure to these agents. They must be totally familiar with the procedures to assist a coworker and to summon assistance in the event of an accident. Visitors must be briefed on basic procedures that will permit them to complete their visit safely. Visitors must also be evaluated to ensure they can wear a mask appropriately should escape become necessary.

The objectives of training programs for chemical workers are to provide awareness of the potential hazards they may encounter and to provide the knowledge and skills necessary to perform the work with minimal risk. Additional requirements are to make workers aware of the purpose and limitations of safety equipment and to ensure that they can safely avoid or escape during an emergency situation.

Although the IMA may be requested to present a discussion of medical topics, he is responsible for reviewing the training program's lesson plans and the SOPs to ensure the correctness and comprehensiveness of the medical aspects. The level of training should be commensurate with the workers' job function and responsibilities, which will necessitate a modification of training material and techniques to accommodate the audience. The training programs should consist of both classroom didactic instruction and hands-on practice, when feasible.

Although this chapter primarily addresses the principles of occupational medicine as they apply to working in a chemical environment, it should be recognized that other workplace hazards exist. Training programs may focus on chemical warfare agents, but they should also address any additional physical and chemical hazards. A number of these hazards may be obvious and directly related to the primary mission; for example, the heat stress associated with wearing chemical protective clothing. Additionally, certain occupational medical hazards are common to all industrial operations (eg, low-back strain), which may produce excessive absenteeism and disability. By working closely with management, medical officers can minimize the impact of these additional safety and industrial hazards.

Special consideration should be given to training workers in the recognition of signs of exposure in a coworker wearing chemical agent protective clothing. Describing fasciculations and localized diaphoresis will be of limited value because the coworker will be wearing full protective clothing. Alerting the workers to watch for lack of coordination,

inappropriate activity, and pinpoint pupils would be of far greater value. Moreover, discussions of the early symptomatology will give the workers the capability of recognizing chemical agent exposure early enough to permit evaluation prior to the onset of serious injury. These signs and symptoms are discussed at length in other chapters of this text.

Each employee should be thoroughly familiar with the requirements for providing effective self-aid and buddy-aid. The first rule of protection—to protect oneself from injury—must be emphasized. There are numerous case reports of individuals or groups attempting to assist someone exposed to toxic compounds only themselves to become casualties. Workers will require training in proper lifts and carries, both with and without a litter.

All workers should know the procedure for requesting medical assistance. Many installations have one “hotline” for medical, technical escort unit, and security support. Workers should be aware of any set format for reporting emergencies that will expedite the report and response time. Once assistance has arrived, the support personnel should be given accurate and complete information about the accident or incident. Teaching the worker a logical format in which to present this information is extremely helpful. Their reports should include the nature of the accident or incident (ie, the agent involved and number of casualties), what has been done for the victims to that point (eg, the number of MARK I injectors administered), and whether personnel are missing. Support personnel can ask for additional information as the situation progresses.

Decontamination procedures must be well known to all chemical workers. The training class should present the M258 and M291 kits and their contents and make clear the use of household bleach in the decontamination process. Current doctrine specifies that in a tactical environment 0.5% bleach be used for skin decontamination. In depot operations, however, 5% bleach is used. This stronger concentration may be used because workers exposed at the depot will be decontaminated and then thoroughly rinsed in a fixed facility in a relatively short time. Soldiers in the field, however, may be decontaminated several times and not be rinsed thoroughly for several hours. Repeated applications of 5% bleach without a complete and thorough rinse will cause skin injury.

The bleach used for decontamination should be stored in airtight containers and dated. Bleach deteriorates and may not be as effective after several months.

Decontamination includes removal of contaminated clothing and the decontamination of skin using the bleach solution. Care must be used to avoid putting bleach in open wounds and the eyes. These areas must be rinsed with copious quantities of water. The bleach requires a contact time of approximately 15 minutes to be fully effective. Small areas can be

decontaminated by removing the contaminated section of clothing and following the directions on the M258 or M291 kits. Medical evaluation, treatment, or both is always required.

Several additional decontaminants are used at the depot. They are generally very caustic and are not to be used on the skin. They include super tropical bleach (STB), high-test hypochlorite (HTH), 10% sodium carbonate, and 10% sodium hypochlorite. Healthcare personnel must be aware what decontaminants are stocked and what they are used for in case they are used inappropriately and a worker develops a medical problem. The industrial hygienist should be able to furnish this information.

Outergarments should never leave the installation, even for laundering. If the clothing is contaminated, it will pose a chemical agent exposure hazard to the launderer. The use of disposable outer garments or decontamination prior to washing will generally solve this problem; however, a change in contractor or new personnel involved in the transportation or laundering process must be addressed.

MANAGEMENT OF THE CONTAMINATED PATIENT

Clinics located at depots with a chemical surety mission should have an area designated for the decontamination of exposed patients. Generally the treatment area for these patients is separate from the normal patient treatment areas. These facilities are rarely used for an actual chemically contaminated patient, however. A conscious effort must be made to keep these rooms at 100% operational capability. To maintain this capability, the medical staff must develop standing operating procedures (SOPs) that are comprehensive and detailed.

The planning phase is essential to a successful operation, but the plan is useless if the personnel involved are not totally familiar with their responsibilities. Planning is an ongoing process that must be kept current in an ever-changing world. If the planning and updating process stops, the resulting document loses its usefulness. Unfortunately, many SOPs are written, only to be placed in a file for months without being reviewed by assigned personnel, only a few of whom may have been involved in initially producing the document. A routinely scheduled review and update of the clinic’s SOPs not only keeps the document current but, more importantly, requires that the healthcare personnel think about the plan and refamiliarize themselves with the operating procedures.

In addition to producing viable internal SOPs, external coordination dictates Memorandums of Agreement (MOAs) with local agencies. The nature of the chemical agents being stored or demilitarized requires that preparations be made for receiving and treating casualties beyond the capability of the installation clinic. While stabilization may be done at the

clinic, hospitalization will require outside facilities. The specter of chemical casualties may make local hospitals needlessly reluctant to accept chemical casualties even after decontamination. Existing MOAs will make the transfer much smoother and will stimulate the local hospital to do preaccident planning and training themselves.

Much of the coordination required for outside agreements will be handled through command channels. The medical officer and medical administrator can accomplish much, however, by interpersonal contact with the medical facilities and the emergency medical personnel who will respond to an installation emergency. Coordination and interaction between civilian and military medical resources should be a continuous process. The IMA must take the lead to ensure the limited post resources are adequately augmented by off-post medical facilities.

Staffing and treatment capabilities of off-site emergency medical facilities should be verified to ensure appropriate resources are available. Training of civilian resources is coordinated through the Chemical Stockpile Emergency Preparedness Program (CSEPP); the Program Director for CSEPP is located at the Edgewood Area of Aberdeen Proving Ground, Maryland. Unfortunately, the many demands placed on the IMA limits the amount of time he can devote to coordinating with local healthcare providers and administrators. Communicating with local supporting agencies, however, will be extremely valuable should an incident occur.

The physician assigned as the IMA should have attended the Toxic Agent Training Course and the Medical Management of Chemical Casualties Course prior to reporting for duty. Enlisted personnel and civilian healthcare providers will require training by the medical officer. Evacuation plans, coordination with off-post civilian medical facilities, MOAs, and periodic inventories (with restocking of supplies and equipment) are the responsibility of the IMA. As individual training continues, collective training in the form of drills should become a routine part of the clinic schedule. Only the successful completion of all of the above will ensure readiness for proper management of a chemically contaminated patient.

CHEMICAL ACCIDENT OR INCIDENT RESPONSE AND ASSISTANCE

Each installation with a chemical surety mission is required to develop detailed plans and procedures to be implemented by the emergency actions community in response to a Chemical (Surety Material) Accident or Incident (CAI). Health services support during Chemical Accident or Incident Response and Assistance (CAIRA) operations involves personnel with a wide range of medical expertise who will be involved in providing

emergency care.

A decontamination area must be a part of the early medical care to limit the degree of exposure to the casualty. Emergency medical care will, initially, be provided by nonmedical workers who are responsible for removing the casualties from the site of injury through a personnel decontamination station and to the waiting medical team. Further evacuation may be required for one or more victims, either to the Installation Medical Facility (IMF) or to an off-post medical treatment facility (MTF). Civilian medical facilities may be required to receive the injured personnel, and they also will need their own supplies, equipment, and training appropriate for treating these casualties.

The fundamental pathophysiological threats to life (namely, airway compromise, breathing difficulties, and circulatory derangement [the ABCs]) are the same for chemical casualties as they are for casualties of any other type. Because these are chemical agent casualties, all personnel involved must be provided additional training. The IMA, whether military or civilian, must be very proactive in developing medical teams, medical training programs, and strong community relations.

A list of chemical agents, the number of personnel involved, the location of the work area, a summary of work procedures, and the duration of the operation is necessary to develop appropriate emergency medical plans. This information is available through the installation commander or the certifying official. In addition, the most probable event (MPE) and maximum credible event (MCE) must be defined to determine the anticipated casualty loads in either situation. An MPE is the worst potential event likely to occur during routine handling, storage, maintenance, or demilitarization operations that results in the release of agent and exposure of personnel. An MCE is the worst single event that could reasonably occur at any time, with maximal release of agent from munitions, bulk container, or work process as a result of an accidental occurrence. The Office of The Surgeon General will develop guidance for use by installations in estimating the chemical agent casualties expected from an MPE or an MCE.

For planning purposes, medical staffing requirements are based on the MPE for the installation. Because an MCE is expected to exceed the capabilities of the Installation Medical Facility, medical contingency plans and coordination with local, state, and federal emergency medical authorities is essential. The IMA is responsible for developing and periodically updating MOAs with local civilian hospitals and supporting military MTFs to augment the installation medical treatment capabilities.

The IMA must actively participate in training both medical and nonmedical personnel. Nonmedical workers require training in self-aid and buddy-aid as a minimum. The Installation Response Force (IRF) is

responsible for providing the immediate safety, security, rescue, and control at the chemical accident or incident site to save lives and reduce exposure to hazards. The IMA must approve the training program for both workers and the IRF and must review their lesson plans for accuracy and completeness. The essentials of this training include recognizing signs and symptoms of agent exposure, first aid, self-aid, buddy-aid, individual protection, personnel decontamination (including decontamination of a litter patient), and evacuation of casualties. Active participation in the training by the IMA will ensure that the personnel understand their role, and that the medical care given by people who are not healthcare professionals meets acceptable standards.

Healthcare providers, as well as local officials, are concerned about the spread of contamination. The procedure for decontamination of litter patients can be found in Appendix E of U.S. Army Field Manual 8-10-4, *Medical Platoon Leaders' Handbook: Tactics, Techniques and Procedures.*[8] The IRF will decontaminate patients and pass them across a hotline to the Medical Response Team (MRT). At that point the casualty should be completely clean. Civilian officials may require a casualty "certified clean" before moving the patient off the military installation. This requirement may be avoided through adequate coordination and training prior to an exercise or an actual chemical accident or incident. Building confidence in the civilian sector through education and communication is essential in providing a rapid and adequate medical response.

Chemical Accident or Incident Response and Assistance encompasses actions taken to save life and preserve health and safety. This support involves a continuum of medical care, ranging from self-aid/buddy-aid in the field to treatment at a tertiary care facility. Due to the nature of some chemical warfare agents, proper care and adequate decontamination must be provided early in the care to avoid serious injury or death. The levels of medical care include the following:

- Level I: composed of IRF nonmedical installation personnel. The local commander appoints the IRF members and ensures they are provided initial and ongoing training as described in Department of the Army Pamphlet 50-6, *Chemical Accident or Incident Response and Assistance (CAIRA) Operations.*[9] The Office of The Surgeon General and the U.S. Army Medical Department Center and School are developing a list of essential medical tasks for this group. Additional tasks may be added at the discretion of the IMA or the local commander.
- Level II: the MRT, composed of on-post medical personnel. The leader of the MRT is a physician and is responsible for training the

team in triage, treatment, stabilization, and evacuation of casualties from the accident site to the appropriate MTF. The MRT must have adequate personnel, supplies, and equipment to provide healthcare to casualties generated by a MPE. The specific tasks for the MRT leader and members are specified in DA PAM 50-6, Tables 6-3 and 6-4.[9] One member of the MRT should be issued toxicological agent protective gear so he may cross the hotline and provide emergency medical care to casualties as required. The remaining members of the MRT should be available on the clean side of the hotline to perform triage and provide immediate care.

- Level III: the Medical Augmentation Team (MAT), provided by the MEDDAC or MEDCEN to an installation having chemical surety missions. This team must have the capability to augment the MRT in the event of an MCE. The MAT leader's responsibilities are also delineated in DA PAM 50-6, Table 6-5.[9]
- Level IV: The Chemical Casualty Site Team (CCST) is provided by the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) located at Aberdeen Proving Ground, Maryland. This team provides clinical consultation and subject-matter experts in chemical casualty care. In addition, a veterinarian may be a designated member of this team. During the initial phases of an exercise, concern is primarily for casualties. In previous service response force exercises, however, many questions have also been asked about the safety of live-stock, pets, and wildlife. The veterinarian has proven to be an extremely valuable source of information and an asset to this team.

The installation commander looks initially to the IMA for medical support and advice. If the chemical accident or incident exceeds the capability of the installation, a Service Response Force (SRF) is provided to assume control of the situation. The SRF surgeon assumes operational control of the MRT, the MAT, and the Medical Chemical Advisory Team (MCAT) at the accident site.

MOAs are required with local MTFs, local emergency medical services, ambulance services, and regional or state emergency medical services officials. The MOAs and frequent coordination with these agencies are necessary to ensure that appropriate off-post resources will be available for support during a chemical accident or incident.

Because of the unique nature of chemical agents, training, as defined in an MOA, must be provided to the supporting civilian agencies. A proactive stance in giving and sustaining education will enhance the relationship with the civilian community. Many civilian medical personnel and officials are very supportive and willing to play an active role in

Chemical Accident or Incident Response and Assistance exercises. Assisting them in training and providing them with appropriate supplies and equipment will go far in enlisting their future support and allaying some of their fears of the unknown.

DEMILITARIZATION OF CHEMICAL WARFARE AGENTS

The U.S has produced and stored a stockpile of chemical warfare agents since World War I. These projectiles, rockets, mines, and ton containers have been maintained at eight depots in eight states: Aberdeen Proving Ground, Maryland; Anniston Army Depot, Alabama; Blue Grass Army Depot, Kentucky; Newport Army Ammunition Plant, Indiana; Pine Bluff Arsenal, Arkansas; Pueblo Army Depot Activity, Colorado; Tooele Army Depot, Utah; and Umatilla Army Depot, Oregon. In addition, two additional states could possibly be affected should there be a large release of agents: Washington and Illinois. The majority of chemical agents are stored in bulk containers that do not have explosive components.

Leaking chemical agents have not presented a health threat to areas surrounding these depots. However, continuing to store the aging munitions may present a risk of chemical agent exposure. The M55 rocket is the most hazardous of the chemical munitions. The rocket contains propellant and a stabilizer that could degrade and form reaction products that might cause ignition.

In 1985, the U.S. Congress initiated a program to dispose of our entire stockpile of lethal chemical agents. There are multiple reasons for destroying these chemical warfare agents:

- Congress has required that the U.S. Army destroy the chemical stockpile by the year 2004,
- ratification of a multilateral chemical arms control treaty requires the destruction of the weapons,
- the need for the stockpile no longer exists, and
- the stockpile is slowly deteriorating with age; although the risk of continued storage is small, it will increase with time.

The prototype destruction plant for lethal agents was erected on Johnston Island: the Johnston Atoll Chemical Agent Destruction System (JACADS). A second destruction facility was built at Tooele Army Depot, Tooele, Utah, as a pilot plant for other facilities to be located at the remaining depots.

Incineration has been determined to be the process that will safely treat all components of the weapons. The destruction facilities were built with

back-up systems to prevent environmental release of agent. The U.S. Public Health Service reviews plans and monitors operations of these chemical destruction plants. The appropriate state environmental authorities must issue permits prior to beginning the incineration process.

Despite the extensive precautions in building the destruction plants, the U.S. Army and the Federal Emergency Management Agency (FEMA) are working with local emergency responders to enhance their capabilities. Training in the medical management of chemical agent casualties specific to the installation is provided frequently to first responders and emergency management officials through CSEPP.

Critics of the army's high-temperature incineration on Johnston Island have found the method to be very controversial and undesirable. The disagreement among scientific experts concerning the incineration process and the emotional concerns of populations surrounding the eight U.S. depots have created numerous debates over the chemical agent destruction program. This controversy has presented the army with numerous challenges in risk communication and preparation to complete the destruction mission.

Extensive security and safety measures have been adopted to ensure that an accident or incident involving the chemical warfare agents and chemical surety material is avoided. The containers are typically stored in an igloo (ie, a storage building topped with 3–4 ft of earth and concrete) and transported in large overpack containers (ie, a container within a heavy container) designed to withstand an explosion.

The agent is destroyed at 2,700°F. Metal parts are also incinerated. Exhaust gases are passed through extensive pollution-control systems. Munitions are destroyed in small quantities in thick-walled rooms that are designed to withstand detonation. The likelihood of an accident that results in exposure of surrounding off-post areas is extremely remote in day-to-day operations.

The solid residue remaining from ash, fiberglass, and wooden dunnage are evaluated for contamination and are transported to approved landfills. Brine (a by-product waste) is packaged and also sent to approved landfills. There is no water discharge resulting from the incineration process.

Stack effluent must meet all requirements of the Clean Air Act,[10] especially the amendments that were passed in 1970,[11] 1977,[12] and 1990[13]; (these last three versions were codified in the United States Code in 1990[14]). In addition to carbon dioxide and oxygen, small quantities of sulfur dioxide, oxides of nitrogen, carbon monoxide, and particulate are discharged. Special precautions have been taken to reduce and eliminate the formation of furans and dioxans from the incineration process. Discharges from the stack are continuously monitored to ensure that the requirements of the Clean Air Act are met. Even though the possibility of

an event leading to the contamination of an area surrounding a community is remote, extensive planning and preparation have been accomplished. The U.S. Army and FEMA have jointly enhanced the emergency preparedness of these communities.

SUMMARY

The unique challenges of chemical warfare agents, aging munitions, and protecting worker health in a chemical environment can prove a rewarding experience for healthcare providers. The personnel reliability program places numerous safety and administrative demands that require that the physician acquire knowledge in occupational medicine that many physicians never experience. Unlike many clinicians, the IMA is thrust into an environment that requires interaction with multiple professional groups. Coordination with industrial hygienists and safety officers will result in an awareness of the workplace and the work conditions that is seldom appreciated by other physicians.

Designing a medical surveillance program to prevent illness and injury is seldom attempted by most physicians in clinical practice. This secondary preventive measure will augment and reinforce the primary preventive efforts of safety and industrial hygiene measures. Appropriate surveillance requires a thorough knowledge of the chemical agents. Requisite information is available through mandatory courses and on-the-job training.

The chemical demilitarization process places additional demands on U.S. Army Medical Department personnel. In addition to the many responsibilities inherent to the chemical surety mission, the IMA may be challenged with risk communication. Many of the civilians living near depot storage facilities do not approve of the plan to incinerate the 30,000 tons of agents stored at these sites. Healthcare providers can play an important role in providing information and building confidence in the U.S. Army's ability to safely destroy these agents through incineration.

REFERENCES

1. US Department of the Army. *Chemical Surety.* Washington, DC: DA; 1986. Army Regulation 50-6.

2. US Department of the Army. *Medical Record Administration.* Washington DC: DA; 1992. Army Regulation 40-66.

3. McCunney RJ. *Handbook of Occupational Medicine.* Boston, Mass: Little Brown; 1988.

4. National Institute of Occupational Safety and Health. *Occupational Safety and Health Guidance Manual for Hazardous Waste Site Activities.* Cincinnati, Ohio: US Department of Health and Human Services, Public Health Service; 1985.

5. US Department of the Army. *Occupational Health Guidelines for the Evaluation and Control of Occupational Exposure to Nerve Agents GA, GB, GD, and VX.* Washington, DC: HQ, DA; 1990. DA Pamphlet 40-8.

6. National Institute of Occupational Safety and Health. *Occupational Exposure to Hot Environments. Revised Criteria.* Cincinnati, Ohio: US Department of Health and Human Services; 1986.

7. Greenleaf JE, Harrison MH. Water and electrolytes. In: Layman DK, ed. *Exercise, Nutrition and Health.* Washington, DC: American Chemical Society; 1986: 107–123.

8. US Department of the Army. *Medical Platoon Leaders' Handbook: Tactics, Techniques and Procedures.* Washington, DC: November 1990. Field Manual 8-10-4.

9. US Department of the Army. *Chemical Accident or Incident Response and Assistance (CAIRA) Operations.* Washington, DC: HQ, DA; May 1991. DA Pamphlet 50-6.

10. Clean Air Act of 1963. Pub L No. 88-206.

11. Clean Air Act of 1970. Pub L No. 91-604.

12. Clean Air Act of 1977. Pub L No. 95-95.

13. Clean Air Act of 1990. Pub L No. 101-549.

14. Clean Air Act. 42 USC § 7401–7671 (1990).

Chapter 18

HISTORICAL OVERVIEW OF BIOLOGICAL WARFARE

EDWARD M. EITZEN, JR., M.D., M.P.H., FACEP, FAAP*; AND ERNEST T. TAKAFUJI, M.D., M.P.H.†

INTRODUCTION

EARLY USE

DURING AND AFTER WORLD WAR II

THE 1972 BIOLOGICAL WEAPONS CONVENTION

RECENT EVENTS: 1972 TO 1994

CONCLUSIONS

* *Colonel, Medical Corps, U.S. Army; Chief, Operational Medicine Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*
† *Colonel, Medical Corps, U.S. Army; Commander, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. 20307-5100*

INTRODUCTION

The possibility that biological weapons will be used against us is no longer unthinkable. Until recently, medical officers and other healthcare practitioners may have considered this topic more suitable for academic than practical pursuit. The fact is, however, that biological agents have been used as weapons since antiquity, and the threat that modern weapons will be used is real. In fact, Saddam Hussein's aggression in the Persian Gulf War may have provided our nation with a wake-up call. The importance of education regarding this unpalatable subject cannot be overestimated. Before our soldiers deploy again against an aggressor likely to use biological weapons, our military healthcare providers need to be confident that they understand both the threat and the medical countermeasures to the threat. This chapter and the ones that follow will help meet that need.

EARLY USE

Warfare with biological weapons has been attempted many times dating back to antiquity. It is appropriate that a discussion of early use of biological agents begin with the accomplishment of Hannibal, the great Carthaginian leader, who, in preparation for a naval battle against King Eumenes of Pergamum in 184 BC, ordered that earthen pots be filled with "serpents of every kind."[1(p12)] During the heat of battle, Hannibal hurled the earthen pots onto the decks of the puzzled Pergamene warriors, who remained amused only until they saw their ships crawling with serpents. The battle was won by Hannibal's forces, as the Pergamene soldiers battled two enemies.[1]

Recognition of the devastating impact that infectious diseases could have on an army resulted in the often crude but ingenious use of disease organisms and poor sanitation to weaken the enemy. The use of corpses of men and animals to pollute wells and other sources of water of the opposing forces was a common strategy. The fouling of water supplies continued to be used through the many European wars, the American Civil War, and into the 20th century.[1,2] In his *Memoirs,* General W. T. Sherman expressed discontent with Confederate troops, who were deliberately shooting farm animals in ponds so that their "stinking carcases"[1(p12)] would compromise the water supplies of the Union forces. Not only did such actions have a demoralizing impact on the enemy, but the consumption of contaminated water probably also accounted for many undocumented epidemics of gastrointestinal disease.

Military leaders during the Middle Ages recognized that victims of infections could become weapons in themselves. Gabriel de Mussis, a notary, saw the Tatar attack on Caffa, a well-fortified, Genoese-controlled seaport (modern Feodosiya, Ukraine), in 1346. De Mussis described how the plague-weakened aggressors catapulted victims of plague into the town:

> [The Tatars], fatigued by such a plague and pestiferous disease, stupefied and amazed, observing themselves dying without hope

> of health ordered cadavers placed on their hurling machines and thrown into the city of Caffa, so that by means of these intolerable passengers the defenders died widely.[3(p180)]

An epidemic of plague followed, forcing a retreat of the Genoese forces. The exported disease continued to spread in Europe.[4,5]

During the Black Plague, which killed 25 million Europeans in the 14th and 15th centuries, bodies of dead soldiers and “2,000 cartloads of excrement”[6(p59)] were hurled into the ranks of the enemy at Carolstein in 1422. A similar strategy was used in 1710, when Russian troops battling Swedish forces in Reval resorted to throwing plague victims over the city walls.[2]

On several occasions, smallpox has been used as a biological weapon in the New World. Pizarro is said to have presented indigenous peoples of South American with variola-contaminated clothing in the 15th century, and the English did the same when Sir Jeffery Amherst provided Indians loyal to the French with smallpox-laden blankets during the French and Indian War (1754–1767). Native Americans defending Fort Carillon sustained epidemic casualties that directly contributed to the loss of the fort to the English.[7] In 1763, Captain Ecuyer of the Royal Americans, out of concern that an Indian attack was possible in the near future and under the pretense of friendship, deliberately distributed two variola virus–contaminated blankets and a handkerchief from a smallpox hospital to enemy Indian forces.[5,8,9] This was followed several months later by large outbreaks of smallpox among various Indian tribes in the Ohio region. A similar strategy (deliberately infecting adversaries with variola virus) was used during the Revolutionary War by smallpox-immune colonists, whose vaccinations against smallpox had been made mandatory by General George Washington.[2,10]

Biological warfare became more sophisticated during the 1900s—against both humans and animals. During World War I, reports circulated of attempts by the Germans to ship horses and cattle inoculated with disease-producing bacteria, such as *Bacillus anthracis* (the bacterium that causes anthrax) and *Pseudomonas pseudomallei* (the bacterium that causes glanders in livestock), to the United States and elsewhere.[2] This accusation was difficult to substantiate, since glanders was widespread in Europe at the time. However, a German saboteur, who supposedly infected 4,500 mules with glanders, was arrested in 1917 in Mesopotamia.[2,5] Other allegations of attempts by Germany to spread cholera in Italy and plague in St. Petersburg, Russia, in 1915 followed; the dropping of contaminated fruit, chocolate, and children’s toys into Romanian cities such as Bucharest by German planes was also alleged.[2]

Germany denied all allegations, including the accusation that biological bombs were being dropped over British positions. In 1924, a subcommittee of the Temporary Mixed Commission of the League of Nations, in support of Germany, stated that, in contradistinction to the chemical arm, there was no hard evidence that the bacteriological arm had been employed in war.[2]

On 17 June 1925, the Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or Other Gases and of Bacteriological Methods of Warfare, commonly called the Geneva Protocol of 1925, was signed. This was the first multilateral agreement that extended prohibition of chemical agents to biological agents.[2,5] Since viruses were not differentiated from bacteria at the time, they were not specifically mentioned in the protocol. However, subsequent interpretations of the agreement considered the term “bacteriological” to include viruses, rickettsiae, and fungi, and to be synonymous with the term “biological.” A total of 108 nations, eventually including the five permanent members of the United Nations Security Council, signed the agreement, which became known as the Geneva Protocol.

Nations currently implicated with chemical and biological weapons (ie, Iraq and Libya) also signed the protocol, raising questions about the agreement’s true effectiveness. Verification of compliance was not addressed.

DURING AND AFTER WORLD WAR II

Events during and after World War II were clouded by charges and countercharges of experimentation with biological warfare agents. The Japanese were accused of using biological agents against the Soviet Union and Mongolia in 1939, against Chinese civilians from 1940 to 1944, and against Chinese troops in 1942.[2,5] In October 1940, a Japanese plane supposedly scattered contaminated rice and fleas over the city of Chuhsien in Chekiang province. This event was soon followed by an outbreak of bubonic plague, a disease never recorded previously in Chuhsien. Several other mysterious flights of Japanese aircraft over at least 11 Chinese cities—with the dropping of grain (wheat, rice, sorghum, or corn), strange granules containing Gram-negative bacilli, and other materials suspected of being contaminated with the plague organism—took place through August 1942. Thousands are estimated to have been hospitalized and 700 became victims of artificially spread plague bacilli.[5] However, despite compelling evidence, testimony, and documents, failure to associate directly the isolation of plague bacilli in the laboratory with actual materials dropped by the planes made prosecution difficult. It is worth noting that a Japanese document dated 1 October 1941, *“Defense and Security Intelligence Report No. 8: Chinese Employment of Chemical and Bacteriological Warfare Against the Japanese,”* revealed a paranoia about secret Chinese initiatives:

> There is evidence that during the China Incident the enemy has skillfully and secretly carried out chemical and bacteriological warfare activities against personnel, animals, natural resources, water and food supplies. It may be presumed that the enemy will become increasingly active in such methods. Therefore, security and defense measures must be thorough during advances and halts.[2(p221)]

At least 3,000 prisoners of war (including Chinese, Koreans, Mongolians, Soviets, Americans, British, and Australians) are alleged to have been used as guinea pigs by Japan's Imperial Unit 731.[2,11] Conservatively, more than 1,000 of these prisoners are estimated to have died in experiments with agents causing anthrax, botulism, brucellosis, cholera, dysentery, gas gangrene, meningococcal infection, and plague[2]. Experiments with tetrodotoxin (highly poisonous fugu toxin) were also conducted. These experiments were later considered to be "most regrettable from the view point of humanity"[11(p11)] by the Japanese government.

In fact, Japan had been conducting experiments on biological and chemical warfare in occupied Manchuria from approximately 1932 until the end of World War II. There were actually several special Japanese experimental units in Manchuria, including the more infamous Units 731 and 100.[12] Unit 731 was formed to prepare for biological warfare, and Unit 100's purpose was to ready bacteriological weapons for use.[13] Experimental work on human prisoners was carried out in Ping Fan outside Harbin (Unit 731), Changchun, Nanking, and other sites. The number of people exterminated at Ping Fan alone is estimated to have been more than 3,000. Subjects either died in the experiments or were "sacrificed" when they were no longer useful to the Japanese.[12] The Japanese death factories may also have led to the epidemics of plague that occurred in the Harbin area after World War II, possibly due to the release of thousands of infected animals during the Japanese evacuation in 1945.[12] No prisoner left Unit 731 alive.[13]

In December 1949, 12 Japanese prisoners of war, including the Commander in Chief of the Japanese Kwantung Army, were tried by a Soviet military tribunal in Khabarovsk, USSR, for preparing and using biological weapons, including agents causing plague, typhoid, paratyphoid, and typhus.[11] Major General Kawashima, former head of Unit 731's First, Third, and Fourth Sections, testified that no fewer than 600 prisoners were killed *yearly* at Unit 731.[12] The Japanese, in turn, accused the Soviets of experimentation with biological warfare agents, citing, as an example, glass bottles and ampules containing *Shigella* (bacillary dysentery), *Bacillus anthracis* (anthrax), and *Vibrio cholerae* (cholera) organisms recovered from Russian spies.[2]

Although German medical researchers during World War II experimentally infected prisoners with disease-producing organisms such as *Rickettsia prowazeki, R mooseri,* hepatitis A virus, and malaria, no charges were pressed at the conclusion of the war. In December 1941, the British reported finding Colorado beetles in areas of the United Kingdom in which they were not normally found, and suggested that they might have been released by the Germans.[14] In May 1945, apparent intentional fecal pollution of a large reservoir in northwestern Bohemia caused an outbreak of dysentery.[2] However, an offensive biological warfare program by Nazi Germany could never be documented, although some in the Third Reich were interested in developing an adequate defense against biological agents.[2]

On the other hand, the Germans also accused the Allies of using biological weapons, causing a widespread plague of Colorado beetles on their potato crops.[14] Dr. Joseph Goebbels, German Minister of Propaganda, also accused the British of attempting to introduce yellow fever into India by transporting infected mosquitoes from West Africa.[7] This was believable to many, for the British were indeed experimenting with at least one biological agent during 1941 and 1942. British trials with *Bacillus anthracis* were held on Gruinard Island off the coast of Scotland. The small bomb experiments resulted in heavy contamination: persistent anthrax spores contaminated parts of the island for many years.[8,15] Winston Churchill is said to have seriously considered using anthrax if Nazi Germany used biological agents against Britain.[5]

During the years immediately following World War II, newspapers were filled with articles of disease outbreaks supposedly caused by foreign agents armed with biological weapons.[2] Outbreaks of cholera in Egypt in 1947 were reportedly caused by Zionist infiltrators. In 1951, a Soviet navy newspaper reported that the United States had tested biological weapons against Eskimos in Canada, leading to an epidemic of plague in 1949. In 1950, East Germany accused the United States of spreading Colorado beetles over parts of Germany.

During the Korean War, the Soviet Union, China, and North Korea accused the United States of using biological warfare against North Korea and China.[2,16] In 1952, an international group of scientists—formed as a result of North Korean complaints—concluded that tests of bacteriological weapons were being conducted against North Korea and China. These experiments supposedly included mosquitoes carrying yellow fever virus and other means of disseminating infectious agents. The United States admitted that it had the capability to produce biological agents but denied conducting germ warfare. The International Red Cross suggested that a special commission be created to investigate the charges, but the request was refused by the Chinese and the Koreans.[8,17]

There is a total lack of scientific basis for these allegations, and at least one American military historian[18] believes that the charges were blatant propaganda. Epidemics occurred in North Korea and China not because biological warfare attacks were perpetrated by the United States but because the devastation of "war caused a collapse of the meager health system. The [biological warfare] propaganda was an attempt to conceal the inability to control epidemics."[18(p97)]

Moreover, recent access to material from the Korean War era, contained in the Archives of the Polish Academy of Science, sheds additional new light on allegations that biological warfare attacks were launched against North Korea and China. Among the official documents now available to western investigators is a letter, written when the allegations of biological warfare were at their height, containing a request from the Korean government that Polish funds be redirected. Amazingly, the Korean request was that Polish funds be used to pay for clothing instead of the sera and vaccines that the Koreans had been receiving. “This letter, dated August 1952, at the height of the [biological warfare] propaganda, casts doubt on the widely advertised existence of biological warfare at that time.”[17(p98)]

Other events and allegations included the following[2] :

- accusations by the Eastern European press that Britain used biological agents in Oman in 1957;
- Brazilian landowners’ deliberate infection of Indian tribes in 1970, to remove them from parts of the Amazon;
- Chinese accusations that the United States started a cholera epidemic in Hong Kong in 1961;
- accusations in July 1964 by the Soviet newspaper *Pravda* that Colombian troops and the U.S. Military Commission in Colombia had used biological agents against the peasants of Colombia and Bolivia; and
- accusations in 1969 by Egyptians that the “imperialist aggressors” had used biological warfare agents in the Middle East, specifically cholera in Iraq in 1966.

In 1970, South Korea maintained that North Korea was planning to launch a biological warfare attack, based on a North Korean facility’s placing a large order from a Japanese trading firm for anthrax, cholera, and plague bacteria. Although the situation was peacefully resolved, biological warfare merely provided one more issue on which North and South Korea could disagree and distrust each other.

During the Vietnam War in the 1960s, many considered the use of fecally contaminated spear traps (“pungi sticks”) to be the Vietcong’s adaptation of biological warfare. Emphasis in that conflict was largely on conventional warfare and special operations in the jungles of Southeast Asia, although concern continued over endemic and artificially introduced infectious agents. “Yellow rain” (which is discussed in Chapter 34, Trichothecene Mycotoxins) became an issue later, along with controversies surrounding use of the chemical herbicide Agent Orange (an approximately 50%–50% mixture of dichlorophenol [2,4-D] and trichlorophenol 2,4,5-T, with trace amounts of the contaminant 2,3,7,8 tetrachlorodibenzo-*p*-dioxin [TCDD]).

In November 1969, the World Health Organization of the United Nations issued a report on chemical and biological weapons. This report (and an earlier

report by the 18-Nation Committee on Disarmament) described the unpredictability of biological warfare weapons and the attendant risks and lack of control when such weapons are used. The effectiveness of biological weapons was not questioned; estimated casualty figures were staggering.[19] In July 1969, Great Britain submitted a recommended statement to the Conference of the Committee on Disarmament prohibiting the "development, production, and stockpiling of bacteriological (biologic) and toxin weapons."[20(p116)] Then in September 1969, the Soviet Union unexpectedly recommended a disarmament convention to the United Nations General Assembly.

THE 1972 BIOLOGICAL WEAPONS CONVENTION

As a followon to the 1925 Geneva Protocol, the 1972 Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction, commonly known as the Biological Weapons Convention, was convened.[2,5] Agreement was eventually reached among the 103 co-signing nations

> never to develop, produce, stockpile, or otherwise acquire or retain microbial or other biological agents or toxins, whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes; and weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict.[5(p135)]

The agreement went into effect in March 1975 and reduced the concerns that some nations had over the development and use of biological agents. However, problems with verification and the interpretation of "defensive" research continued.

Every year, signatories to the agreement are required to submit to the United Nations information on

- facilities where biological defense research is being conducted;
- scientific conferences that are held at specified facilities;
- exchanges of scientists or information; and
- disease outbreaks.

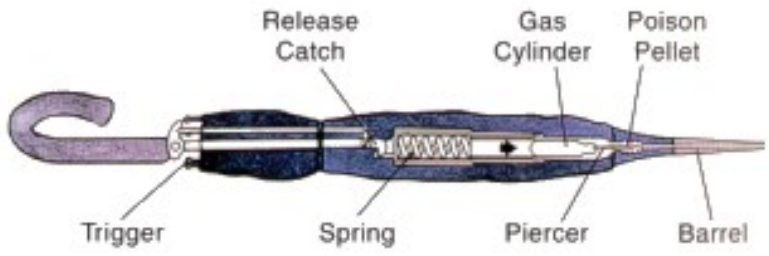
The Security Council, of which the United States and Russia are members, reserves the right to veto any request for an investigation, should any allegations of infractions be lodged with the United Nations. In 1986 and again in September 1991, review conferences were held to resolve continuing problems.

RECENT EVENTS: 1972 TO 1994

Since the signing of the Biological Weapons Convention in 1972, the U.S. intelligence community has identified many significant events and emerging threats in the area of offensive biological warfare. The number and identity of countries engaged in offensive biological warfare work is classified; however, we can accurately state that the number of state-sponsored programs of this type has increased significantly. Also, several terrorist or assassination attacks have been documented.

On April 3, 1979, a mysterious explosion at the Soviet Institute of Microbiology and Virology in Sverdlovsk raised questions about the effectiveness of any weapons-control agreements.[5,21,22] At least 66 persons, most of them civilians (the area affected was downwind of the military compound), are believed to have been killed (most with inhalation anthrax), and many more to have been infected with *Bacillus anthracis*. For years, the Soviets maintained that this incident had not been due to an accidental release of anthrax from the military research facility, but instead was due to ingestion by the local residents of contaminated animal products. Controversy raged back and forth in the lay press over the incident. Finally, in 1992, the President of Russia, Boris Yeltsin, admitted that there had, in fact, been an accidental airborne release of anthrax spores from the research facility in question, confirming the long-held belief of many in the United States.[23] (This incident is discussed more fully in Chapter 22, Anthrax.)

In 1978, before the Sverdlovsk incident, a Bulgarian exile named Georgi Markov was attacked in London, England, with a device disguised as an umbrella (Figure 18-1). This weapon discharged a tiny pellet (Figure 18-2) into the subcutaneous tissue of his leg while he was waiting for a bus. He died several days later. On autopsy, the pellet, cross-drilled as if designed to be filled with another material, was found. This assassination, it was later revealed, was carried out by the communist Bulgarian government, and the technology to commit the crime was supplied to the Bulgarians by the Soviet Union[7]. (This incident is discussed more fully in Chapter 32, Ricin Toxin.)



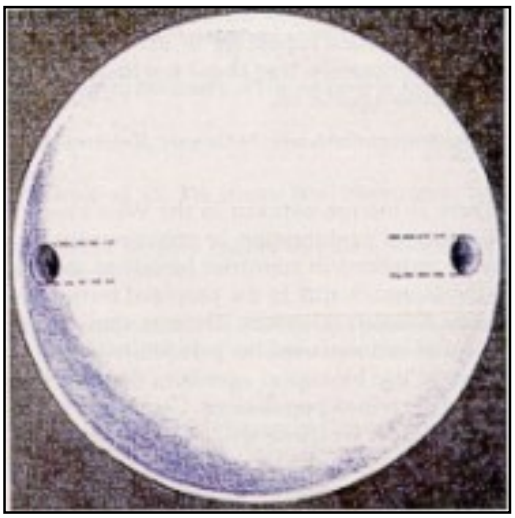


Fig. 18-1. An umbrella gun of this type was the clandestine weapon used to assassinate Bulgarian exile Georgi Markov in London in 1978. The weapon consisted of a spring-loaded piston, which would drive a carbon dioxide cartridge forward into a firing pin. The gas would then propel a poison projectile out of the hollow tip of the umbrella gun, through the clothing, and into the flesh of the intended victim. Reprinted from van Keuren RT. *Chemical and Biological Warfare, An Investigative Guide.* Washington, DC: Office of Enforcement, Strategic Investigations Division, US Customs Service; October 1990: 89.

Fig. 18-2. A pellet of this type, designed to contain ricin toxin, was used to assassinate Georgi Markov in London and in the attempt on the life of Vladimir Kostov in Paris. The tiny, platinum–iridium pellet—the size of the head of a pin (0.068 in. diameter)—was cross-drilled with 0.016-in. holes in which ricin (or another toxin) could be placed. Reprinted from van Keuren RT. *Chemical and Biological Warfare, An Investigative Guide.* Washington, DC: Office of Enforcement, Strategic Investigations Division, US Customs Service; October 1990: 90.

Another attempted killing of another Bulgarian exile, Vladimir Kostov, had occurred in Paris, France, 10 days before the Markov assassination. Kostov was a defector who worked for Radio Free Europe, broadcasting opposition to the Bulgarian government. As he was leaving a metro stop in Paris, Kostov felt a sharp pain in his back. Turning quickly, he observed a man with an umbrella running away.[24] Only the heavy clothing worn by Kostov prevented the pellet shot at him from penetrating any deeper than the subcutaneous tissue of his back.[25]

The pellet remained in Kostov's back until after he learned of Markov's death, about 2 weeks later, whereupon French doctors examined his back. They removed a similar pellet, which was made from an exotic alloy of iridium and platinum and contained the toxin ricin, in time to save Kostov's life. The ricin was kept in the pellet by a wax plug designed to melt at body temperature. Fortunately for Kostov, the wax did not melt.

Similar pellet-firing weapons may have been responsible for at least six assassinations in recent years[26]. In addition, an attempted assassination with ricin may have occurred in the United States—in a shopping mall parking lot in Tyson's Corner, Virginia[27] —although this incident is less well documented than the two previous ones.

In the late 1970s, there were indications that inhabitants of Laos and Kampuchea may have been attacked by planes and helicopters delivering aerosols of several colors. After being exposed, people and animals allegedly became disoriented and ill, and a small percentage of those stricken died. Some of these clouds were thought to be comprised of trichothecene toxins (in particular, T-2 mycotoxin). These attacks are lumped under the label "yellow rain." A great deal of controversy has raged over whether these clouds were in fact biological warfare agents. Some[28] in the scientific community have summarized the available evidence and concluded that the yellow rain was most likely the fecal matter of wild honeybees dropped during their "cleansing flights." Others believe that "there is enough evidence to make agent use in these reported attacks highly probable."[7(p100)] The controversy remains

unresolved. For a more complete discussion, see Chapter 34, Trichothecene Mycotoxins.

During Operation Desert Shield, the buildup phase of the Persian Gulf War (fall and winter, 1990) after Iraq invaded and occupied Kuwait, the United States and the coalition of allies faced the threat of biological and chemical warfare. Fortunately, Saddam Hussein did not use unconventional weapons, but the allies believe that he retained this capability after his defeat.

In August 1991, the first United Nations inspection of Iraq's biological warfare capabilities was carried out in the aftermath of the Persian Gulf War. On 2 August 1991, representatives of the Iraqi government announced to leaders of United Nations Special Commission Team 7 (of which one of the authors, E.M.E., was a member) that Iraq had conducted research into the offensive use of *Bacillus anthracis,* botulinum toxins, and *Clostridium perfringens* (presumably one of its toxins). This was the first open admission in recent memory of biological weapons research by any country, and it publicly verified many of the concerns of the U.S. intelligence community. Iraq had extensive and redundant research facilities at Salman Pak, Al Hakam, and other sites, only some of which were destroyed during the Persian Gulf War.

Subsequent United Nations inspections have further elucidated Iraqi biological warfare intentions, identified some of the Iraqi capabilities, and, we hope, decreased the likelihood that Iraq will use biological warfare in future conflicts. However, since the defection of Iraqi General Hussein Kamal Hassan on 7 August 1995, it has become clear that the Iraqi biological warfare program was even further advanced than United Nations inspectors had suspected. Iraq has disclosed in the wake of Hussein's defection that it had actually filled biological warfare agents into weapons immediately before the outbreak of the Persian Gulf War. These weapons included the following[29]:

- 166 bombs (100 botulinum toxin, 50 anthrax, 16 aflatoxin);
- 25 Scud/A1 Hussein missile warheads (13 botulinum toxin, 10 anthrax, 2 aflatoxin);
- 122-mm rockets filled with anthrax, botulinum toxin, and aflatoxin;
- spray tanks capable of being fitted to a fighter aircraft or remotely piloted aircraft, and spraying 2,000 L over a target; and
- artillery shells.

It is now clear that U.S. forces faced a significant biological warfare capability in the desert in this decade. Fortunately, Iraq chose not to use biological weapons against us.

CONCLUSIONS

The threat of biological warfare has increased over the past 2 decades, with a number of countries working on offensive use of these agents. The extensive

program of the former Soviet Union is now controlled largely by Russia. Admitting that a biological warfare program existed until early 1992—nearly 20 years after the USSR signed the Biological Weapons Convention in 1972—Russian president Boris Yeltsin has stated[23] that he will put an end to further offensive biological research. However, the degree to which the program has been scaled back is not known.

There is intense concern in the West about the possibility of proliferation or enhancement of offensive programs in countries hostile to the western democracies, due to the potential hiring of expatriate Russian scientists. There is also a certain amount of concern over the possibility that terrorists might use biological agents to threaten either military or civilian populations. Certainly the threat that biological weapons will be used against U.S. military forces is broader and more likely in various geographic scenarios now than it has been at any point in our history.

REFERENCES

1. Rothschild JH. *Tomorrow's Weapons.* New York, NY: McGraw-Hill; 1964.
2. Stockholm International Peace Research Institute (SIPRI). *The Rise of CB Weapons*. Vol 1. In: *The Problem of Chemical and Biological Warfare.* New York, NY: Humanities Press; 1971.
3. De Mussis G. *Historica de Morbo s. Mortalitate quae fuit Anno Dni MCCCXLVIII.* Cited in: Derbes VJ. De Mussis and the great plague of 1348: A forgotten episode of bacteriological warfare. *JAMA*. 1966;196(1):179-182.
4. Derbes VJ. De Mussis and the great plague of 1348: A forgotten episode of bacteriological warfare. *JAMA.* 1966;196(1):59–62.
5. Geissler E, ed. *Biological and Toxin Weapons Today*. Oxford, England: Oxford University Press, Stockholm International Peace Research Institute; 1986.
6. Varillas. *Historie de l'Hérésie de Viclef, Jean Hus, et de Jerome de Prague*. Vol 2. Lyon, France: Chez Iean Certe. 1682: 117. Quoted in: Derbes VJ. De Mussis and the great plague of 1348: A forgotten episode of bacteriological warfare. *JAMA.* 1966;196(1):59–62.
7. US Army Medical Research Institute of Infectious Diseases. *Medical Management of Biological Casualties Handbook*. 2nd ed. Fort Detrick, Frederick, Md: USAMRIID; 1996.
8. Cole LA. *Clouds of Secrecy*: *The Army's Germ Warfare Tests Over Populated Areas.* Totowa, NJ: Rowman and Littlefield; 1988.
9. Cartwright FF. *Disease and History*. New York, NY: New American Library; 1974.
10. Bayne-Jones S. *The Evolution of Preventive Medicine in the United States Army, 1607–1939.* Washington, DC: Department of the Army, Office of The Surgeon General; 1968.

11. Hersh SM. *Chemical and Biological Warfare: America's Hidden Arsenal*. Indianapolis, Ind: Bobbs-Merrill; 1968.

12. Harris SH. *Factories of Death*. New York, NY: Routledge; 1994.

13. Tomilin VV, Berezhnai RV. Exposure of criminal activity of the Japanese military authorities regarding preparation for bacteriological warfare. *Voen Med Zh.* 1985;8:26–29.

14. Harris R, Paxman J. *A Higher Form of Killing.* New York, NY: Hill and Wang; 1982: 87, 99.

15. Manchee R, Stewart W. The decontamination of Gruinard Island. *Chem Br.* 1988;July:690–691.

16. Cowdrey AE. *The Medics' War.* Washington, DC: Center of Military History, US Army; 1987.

17. Rolicka M. New studies disputing allegations of bacteriological warfare during the Korean War. *Milit Med*. 1995:160:97–100.

18. Cowdrey AE. Germ warfare and public health in the Korean conflict. *J Hist Med All Sci.* 1984;39:179–268. Cited in: Rolicka M. New studies disputing allegations of bacteriological warfare during the Korean War. *Milit Med.* 1995:160:97–100.

19. Report of a WHO Group of Consultants. *Health Aspects of Chemical and Biological Weapons.* Geneva, Switzerland: World Health Organization; 1970.

20. Bernstein BJ. The birth of the US biological-warfare program. *Sci Am*. 1987;256:116–121.

21. Defense Intelligence Agency. *Soviet Biological Warfare Threat.* Washington, DC: Department of Defense, DIA; 1986. DST-1610F-057-86.

22. Meselson M, Guillemin J, Hugh-Jones M, et al. The Sverdlovsk anthrax outbreak of 1979. *Science*. 1994; 266(5188):1202–1208.

23. Smith JR. Yeltsin blames '79 anthrax on germ warfare efforts. *Washington Post.* 1992;June 16:A-1.

24. van Keuren RT. *Chemical and Biological Warfare, An Investigative Guide.* Washington, DC: Office of Enforcement, Strategic Investigations Division, US Customs Service; 1990.

25. Murphy S, Hay A, Rose S. *No Fire No Thunder: The Threat of Chemical and Biological Weapons.* New York, NY: Monthly Review Press; 1984: 145.

26. Livingstone NC, Douglass JDJ. CBW: *The Poor Man's Atomic Bomb*. Cambridge, Mass: Institute for Foreign Policy Analysis, Inc.; 1984.

27. Douglas JDJ, Livingstone NC. *America the Vulnerable.* Lexington, Mass: Lexington Books; 1987.

28. Robinson J, Guillemin J, Meselson M. Yellow rain: The story collapses. *Foreign Policy*. 1987;68:101–117.

29. Secretary-General of the United Nations. Note by the Secretary-General. New York: NY: United Nations Security Council; 11 October 1995. S/1995/864. Original in English.

Chapter 19

THE U.S. BIOLOGICAL WARFARE AND BIOLOGICAL DEFENSE PROGRAMS

DAVID R. FRANZ, D.V.M., PH.D.*; CHERYL D. PARROTT†; AND ERNEST T. TAKAFUJI, M.D., M.P.H.‡

INTRODUCTION

A SECRET BIOLOGICAL WARFARE PROGRAM
- The Secret Program Is Acknowledged
- Field Testing in the United States

AN EXPANDED DEFENSE PROGRAM

A COMPREHENSIVE MEDICAL BIOLOGICAL DEFENSE PROGRAM
- Safety in Research and Patient Care
- A National Resource

SUMMARY

* *Colonel, Veterinary Corps, U.S. Army; Commander, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*
† *Formerly, Technical Writer, Public Affairs Office, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702; currently, Biomedical Writer, Office of Communications, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892*
‡ *Colonel, Medical Corps, U.S. Army; Commander, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. 20307-5100*

INTRODUCTION

Biological agents have been used in war for centuries. After World War I, Major Leon Fox, Medical Corps, U.S. Army, prepared a lengthy report[1] that concluded that biological warfare was no longer a concern because of the development of modern sanitary procedures. However, as he wrote, the Japanese were already developing an offensive biological warfare program involving an extensive list of biological agents, capable of causing diseases such as anthrax, tularemia, plague, botulinum, smallpox, glanders, and typhoid.

The United States conducted a second review of the potential of biological warfare during 1941 and 1942 and implemented its program to develop biological weapons in 1943. The biological warfare program of the United States was conducted under military auspices and was characterized during its early years by a high degree of secrecy and controversial testing programs. By the 1960s, U.S. scientists had clearly established that the development of biological weapons was feasible and that their use on the battlefield could be effective.

The purpose of the U.S. program in the early years was to deter the use of biological agents against the United States and its military forces, and to retaliate only if deterrence was unsuccessful. The program was characterized by an aggressive offensive and defensive research and development effort that would be modified to one based on maintaining a strong defense against biological agents.

When the biological warfare program was established, the United States was fighting World War II on two fronts. After the war ended, the Cold War developed and our security was still threatened. The United States maintained an active offensive biological warfare program until it unilaterally renounced the use of biological weapons in two National Security Memoranda in 1969 and 1970. The United States ratified the Biological Weapons Convention in 1975. Although capabilities of the world's military forces have changed significantly in the years following the disestablishment of the U.S. biological warfare program—and despite the Biological Weapons Convention—a biological warfare threat still exists; therefore, the United States maintains a program for medical defense against biological warfare agents.

A SECRET BIOLOGICAL WARFARE PROGRAM

In 1941, Secretary of War Henry L. Stimson asked the National Academy of Sciences to evaluate the feasibility of biological warfare. The academy concluded that biological warfare was feasible and recommended that steps be taken to reduce U.S. vulnerability and also to

conduct research to explore the offensive potential of bacteriological weapons. In April 1942, Stimson recommended to President Franklin D. Roosevelt the creation of a civilian advisory group that would coordinate governmental and privately owned institutions in a biological warfare effort.[2,3] (What he did not tell Roosevelt was that the Army Chemical Warfare Service had begun its own biological warfare research in 1941.)

The idea of biological weaponry was controversial, since little was known about the predictability or effectiveness of biological weapons in wartime. President Roosevelt approved the plan in 1942, and the War Reserve Service, headed by George W. Merck, was established and attached to the Federal Security Agency, a New Deal agency of the Department of Agriculture. The War Reserve Service started out in mid 1942 with a budget of $200,000. Secret work began under Merck's direction at 28 American universities, including Harvard, Stanford, and other top schools. This agency received consultative advice from national scientific committees and organizations, including the National Academy of Sciences and the National Research Council.

The War Reserve Service also empowered the U.S. Army's Chemical Warfare Service to greatly expand its efforts in regard to biological weapons. The army's efforts were better funded than those of the War Reserve Service: in 1942 and 1943, the Chemical Warfare Service received millions of dollars to build research facilities. Several locations were selected for the army's biological research, with the main headquarters at Camp Detrick, Frederick, Maryland, a small National Guard airfield (designated Fort Detrick in 1956). The army also made plans to build a manufacturing plant near Terre Haute, Indiana, and built a 2,000-acre field test site on Horn Island in Pascagoula, Mississippi. It is ironic that much of the United States's biological warfare effort during World War II was in response to a perceived threat from Germany, when in fact the Japanese were much more actively building their biological warfare capability.[2]

In the spring of 1942, President Roosevelt and British Prime Minister Winston Churchill announced policies limiting the use of biological weapons to retaliation only, closely paralleling previous decisions, such as the Geneva Protocol of 1925, on the limited use of chemical weapons. But these new policies did not prevent the United States and Great Britain from beginning to amass arsenals of biological weapons.[4] By 1943, the research center and pilot plant at Camp Detrick employed approximately 3,800 military and 100 civilian personnel. In 1944, Dugway Proving Ground, Utah, was established to replace the Mississippi site, and the production plant was constructed near Terre Haute, Indiana.[2]

The United States exchanged information with Great Britain and Canada, two other nations concerned about the biological warfare threat,

but the general public was unaware of a biological warfare program in the United States until 4 months after the war was over. During World War II, the United States worked primarily on anthrax and botulism; however, brucellosis, psittacosis, tularemia, and glanders were also studied. There was also considerable work on agents for use against plants, and records show that there were plans drawn up to decimate Japan's rice crops.[2]

At the end of World War II, construction and testing slowed to a stop, and the effort on biological warfare development was largely limited to research. The production plant in Indiana was sold to the Charles A. Pfizer Company for commercial use. Although the highly classified program was initially defensive, and closely tied with the chemical weapons program, research continued on developing an independent retaliatory capability using various disease agents.

The Secret Program Is Acknowledged

Since 1937, Japan had conducted a large biological warfare program, including human testing, at its Unit 731 in Manchuria.[5] After the war, the United States granted amnesty to Japanese scientists who had participated in the research; however, a condition of the amnesty was full disclosure of research information. Two scientists from Camp Detrick, Dr. Edwin Hill and Dr. Joseph Victor, went to Japan in 1945 and interviewed 22 scientists. They learned that many of the classical biological warfare agents had been studied, and that approximately 1,000 autopsies had been performed in Unit 731, most of these on humans who had been exposed to anthrax. They also learned that the Japanese had stockpiled 400 kg of anthrax spores, which were to be used in a specially designed fragmentation bomb.

In January 1946, the War Department made public for the first time the fact that the United States had been conducting biological warfare research and testing. The press release emphasized the high priority placed on safety:

> In all work on biological warfare carried on in the United States, extreme care was taken to protect the participating personnel from infection. Many new techniques were devised to prevent infection and proved highly successful. Hospitals and dispensaries were maintained at all installations, staffed with both Army and Navy personnel and were equipped to treat accidental infections. As the result of the extraordinary precautions taken, there occurred only sixty cases of proven infection caused by accidental exposure to virulent biological warfare agents which required treatment. Fifty-two of these

> recovered completely; of the eight cases remaining, all were recovering satisfactorily. There were, in addition to the sixty proven cases, 159 accidental exposures to agents of unknown concentrations. All but one of these received prompt treatment and did not develop any infection. In one instance, the individual did not report exposure, developed the disease, but recovered after treatment.[3(vol 1, p1-4)]

Mr. Merck, the head of the War Reserve Service, in his final report[6] to the secretary of war noted that although remarkable achievements had been made, the potential of biological warfare had by no means been completely measured. He recommended that the program be continued on a sufficient scale to provide an adequate defense.

In 1948, the Research and Development Board (then under the secretary of defense), which had been given the responsibility to supervise the governmental research program, requested an evaluation of biological agents as weapons of sabotage. The Committee on Biological Warfare was formed, and the Baldwin Report[7] prepared by the committee stated that the United States was particularly vulnerable to covert attack with biological agents. It also stated that the current research and development program was “not now authorized to meet the requirements necessary to prepare the defensive measures against special [biological warfare] operations.”[7(p1)]

The Baldwin Report recommended[7] that

- means be developed to detect and identify biological warfare agents;
- methods be developed for decontamination, protection, prophylaxis, and treatment; and
- methods be assessed for dissemination of biological agents, with emphasis on application to special operations.

Specifically recommended were research programs, such as the testing of “innocuous organisms”[7(p7)] in ventilation systems, subway systems, and public water supplies. This guidance influenced several subsequent administrations over the next 20 years, and the United States conducted a sequence of highly classified scientific tests on unknowing populations throughout the country, with agents and materials believed to be nonpathogenic. In fact, not until early 1977 was the extent of the military biological weapons testing program publicly disclosed before Congress.[3,4]

The biological warfare research program in the early 1940s and 1950s involved antipersonnel, anticrop, and, for a brief period, antianimal

studies.[8] Field trials included open-air vulnerability testing, and contamination of public water systems with live organisms such as *Serratia marcescens*. Covert programs were conducted by the Central Intelligence Agency. Pathogenic organisms were also tested in Florida and the Bahamas in the 1940s. Chemical anticrop studies evaluated defoliation and crop destruction. Explosive munitions tests with pathogens were begun in 1949.

In 1950, the first open-air tests with biological simulants were conducted in various locales, one of which was off the coast of Norfolk, Virginia. This was followed by limited zinc cadmium sulfide dispersal tests in Minneapolis, Minnesota, and St. Louis, Missouri, in 1953; and *Bacillus subtilis* var *niger* dispersal in the New York City subway system in 1966.[3,4] The Special Operations Division at Camp Detrick conducted much of the research on possible methods of covert attack and sabotage, and many environmental studies—often without informing local or state governmental agencies or the general population.

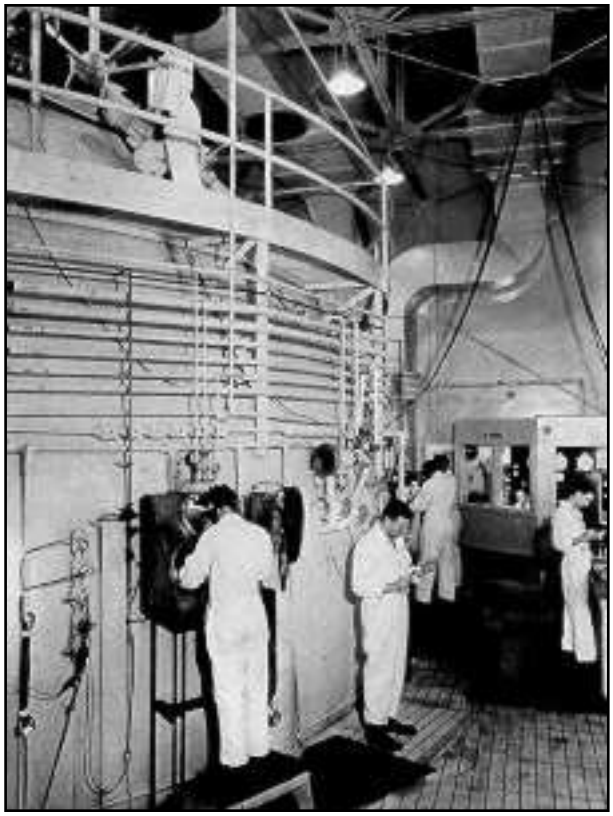


Fig. 19-1. These workers are standing outside the “8-Ball,” a 1-million-liter sphere used for testing static aerosols of biological agent preparations during the United States’s offensive biological warfare program. The building enclosing the 8-Ball and its supporting infrastructure were destroyed by fire in 1974. The sphere remains today as a historical monument at Fort Detrick, Frederick, Maryland. Photograph: Public Affairs Office, Fort Detrick, Frederick, Md. circa 1968.

Between 1948 and 1950, several reviews were conducted by the Research Review Board of the biological, chemical, and radiological warfare programs. Recommendations included the creation of a specific biological warfare production facility, continued field tests with biological warfare agents and munitions, and expansion of the overall program. In 1949, an enclosed, 1-million-liter steel test sphere was built at Camp Detrick, and biological warfare explosive munitions tests with agents were begun (Figure 19-1).

During the early 1950s, Major General George E. Armstrong, The U.S.

Army Surgeon General (1951–1955) became concerned about medical defense issues. Lieutenant Colonel Abram S. Benenson, a medical officer from the Walter Reed Army Institute of Research, was appointed medical liaison with the biological warfare laboratories at Fort Detrick. A joint agreement was signed, and beginning in 1953, studies on medical defense against biological weapons were conducted cooperatively by the Chemical Corps and the U.S. Army Medical Department. In 1954, a congressionally approved medical volunteer program, designated “Project Whitecoat,” was established after a series of meetings with representatives of the General Conference of the Seventh-Day Adventist Church and The Surgeon General, U.S. Army.

Field Testing in the United States

The Korean War, which began in June 1950, added justification for continuing the biological warfare program, when the possible entry of the Soviet Union into the war was feared. Concerns over the Soviet Union were justified, for the Soviet Union would pronounce in 1956 that chemical and biological weapons would, indeed, be used for mass destruction in future wars.[9] In October 1950, the secretary of defense approved continuation of the program, based largely on the Soviet threat and a belief that the North Korean and Chinese communists would use biological weapons.[10]

The first large-scale aerosol vulnerability test was conducted in the San Francisco Bay area in September 1950, using two species of bacteria (*Bacillus globigii* and *Serratia marcescens*) and fluorescent particles. Various *Bacillus* species were used in many experiments because of their spore-forming capabilities and their similarities to *Bacillus anthracis*. *S marcescens* was used because its red pigment made it readily identifiable. What was unexpected was the increased number of cases of *Serratia* infections over the next few years in communities that had been sprayed earlier with the organisms.[4] The military considered the situations coincidental, but many civilian physicians believed them to be directly related. Other limited-scale field tests with pathogenic organisms were conducted at Dugway Proving Ground, Utah. Antianimal studies were conducted at Eglin Air Force Base, Florida.

The biological warfare research facilities at Camp Detrick were expanded, and a biological warfare production facility was created at Pine Bluff Arsenal, Arkansas, in 1951. The first limited, biological warfare retaliatory capability was achieved when an anticrop bomb was developed, tested, and placed in production for the U.S. Air Force. Anticrop–agent production sites were carefully selected for safety with the coordination and approval of the U.S. Department of Agriculture. This

marked the first peacetime biological weapons production by the United States.[11]

By 1954, the Pine Bluff laboratory produced *Brucella suis* (the causative agent of brucellosis, also called undulant fever) and *Francisella tularensis* (tularemia, or rabbit fever). Hardware for antipersonnel biological cluster bombs was delivered to Pine Bluff for filling with *Brucella suis* to support air force requirements. By 1955, the accelerated program was producing stocks of *B suis* and *F tularensis* as biological warfare agents. While many of the efforts involved military researchers, others from the Public Health Service, other Federal departmental agencies, and civilian scientific institutions were also involved in the research.

The general public was uninformed of these on-going studies, especially the environmental and open-air experiments that were being conducted. A controversial environmental test occurred in 1951, when army researchers deliberately exposed a disproportionate number of black citizens to the fungus *Aspergillus fumigatus*, to see if African Americans were more susceptible to such infection, like they were already known to be to coccidioidomycosis (*Coccidioides immitis*). Some in the scientific community believed that such knowledge would assist in preparing defenses against a more virulent form of this fungus. Similarly, in 1951, unsuspecting workers at the Norfolk Supply Center, Norfolk, Virginia, were exposed to crates contaminated with *A fumigatus* spores.

Needless to say, there was a public outcry several years later when much of this information was released, and the biological warfare research program would be forever tainted as operating within “clouds of secrecy.”[4] The first lawsuit against the U.S. government was filed by family members of an individual who had died, allegedly as a result of the San Francisco experiments in 1950. The court decided that the U.S. government could not be sued (under the Federal Tort Claims Act), since the decision to spray *S marcescens* was a part of national defense planning. Several of the organisms (such as *S marcescens* and *A fumigatus*), which were considered at one time to be innocuous, are now recognized to cause infections in humans, on occasion. Immunocompromised or debilitated persons appear to be at greatest risk. Early experiments conducted with such organisms involving subjects or populations who were unaware of the ongoing experiments may have posed a health risk to highly susceptible persons.

During the two decades following the second World War, laboratories for biological and chemical warfare research continued to increase in size, and programs were expanded with a multimillion dollar budget. The Fort Detrick research program was complemented by contractual civilian institutions; for example, Ohio State University was tasked with making vaccines. Human volunteers were used in many of the studies. Vaccines

against diseases, such as Q fever and tularemia, were developed.

AN EXPANDED DEFENSE PROGRAM

With expansion of the biological warfare retaliatory program, the scope of the defensive program was nearly doubled. Data were obtained on personnel protection, decontamination, and immunization. Early detection research produced prototype alarms for use on the battlefield, but progress was slow, apparently limited by technology.

The U.S. Army Medical Unit, under the direction of The U.S. Army Surgeon General, began formal operations in 1956. One of the Unit's first missions was to manage all aspects of Project CD-22, the exposure of volunteers to aerosols containing a pathogenic strain of *Coxiella burnetii*, the etiologic agent of Q fever. The volunteers were closely monitored and antibiotic therapy was administered when appropriate. All volunteers recovered from Q fever with no adverse aftereffects. One year later, the Unit submitted to the U.S. Food and Drug Administration an Investigational New Drug application for a Q fever vaccine.

The United States was now accumulating invaluable data on personnel protection, decontamination, and immunization; and, in the offensive program, on the potential for mosquitoes to be used as biological vectors. A new Department of Defense Biological and Chemical Defense Planning Board was created in 1960 to establish program priorities and objectives. Preventive approaches toward infections of all kinds were funded under the auspices of biological warfare. As concern increased over the biological warfare threat during the Cold War, so did the budget for the program: to $38 million by fiscal year 1966.

The U.S. Army Chemical Corps was given the responsibility to conduct biological warfare research for all of the services.[3] In 1962, the responsibility for the testing of promising biological warfare agents was given to a separate Testing and Evaluation Command. Depending on the particular program, different test centers were used, such as the Deseret Test Center at Fort Douglas, Utah, the headquarters for the new biological and chemical warfare testing organization. In response to increasing concerns over public safety and the environment, the Testing and Development Command implemented a complex system of approval of its research programs that included the U.S. Army Chief of Staff, the Joint Chiefs of Staff, the Secretary of Defense, and the President of the United States.

During the last 10 years of the offensive research and development program, many scientific advances were made that proved that biological warfare was clearly feasible, although dependent on careful planning, especially with regard to meteorological conditions. Large-scale

fermentation, purification, concentration, stabilization, drying, and weaponization of pathogenic microorganisms could be done safely. Furthermore, modern principles of biosafety and containment were established at Fort Detrick that have greatly facilitated biomedical research; still today, these are copied throughout the world. Arnold G. Wedum, M.D., Ph.D., a civilian scientist who was Director of Industrial Health and Safety at Fort Detrick, was the leader in the development of containment facilities (Figure 19-2).

Fig. 19-2. Technicians in Class III hood lines are seen working with preparations of biological agents at Fort Detrick, Frederick, Maryland, circa 1968, when the United States had an offensive biological warfare program. The completely enclosed hood lines provide total containment of hazardous organisms. Hoods were maintained at a pressure negative to ambient by constantly drawing air out through high-efficiency

particulate air (HEPA) filters. Workers wore heavy rubber gloves to access material inside the hoods. Photograph: Public Affairs Office, Fort Detrick, Frederick, Md.

During the 1960s, the country experienced a philosophical change, and attention was now directed toward biological agents that could incapacitate but not kill. In 1964, research programs involved staphylococcal enterotoxins capable of causing food poisoning. Research initiatives also included new therapy and prophylaxis. Pathogens studied included the agents causing anthrax, glanders, brucellosis, melioidosis, plague, psittacosis, Venezuelan equine encephalitis, Q fever, coccidioidomycosis, and a variety of plant and animal pathogens.[10,12]

Particular attention was directed at chemical and biological detectors during the 1960s. The first devices were primitive field alarms to detect chemicals. Although the development of sensitive biological warfare agent detectors was at a standstill, two systems were, nonetheless, investigated. The first was a monitor that detected increases in the number of particles sized 1 to 5 µm in diameter, based on the assumption that a biological agent attack would include airborne particles of this size. The second system involved the selective staining of particles collected from the air. Both systems lacked enough specificity and sensitivity to be of any practical use.[8]

But in 1966, a research effort directed at detecting the presence of adenosine triphosphate (a chemical found only in living organisms) was begun. By using a fluorescent material found in fireflies, preliminary studies indicated that it was possible to detect the presence of a biological agent in the atmosphere. The important effort to find a satisfactory detection system continues today, for timely detection of a biological attack would allow the attacked force to use its protective masks effectively, and identification of the agent would allow any pretreatment regimens to be instituted.

The army also experimented with and developed highly effective barrier protective measures against both chemical and biological agents. Special impervious tents and personal protective equipment were developed, including individual gas masks even for military dogs.

During the late 1960s, funding for the biological warfare program decreased temporarily, to make up for the accelerating costs of the Vietnam War. The budget for fiscal year 1969 was $31 million, decreasing to $11.8 million by fiscal year 1973. Although the offensive program had been stopped in 1969, both offensive and defensive programs continued to be defended. John S. Foster, Director of Defense Research and Engineering, responded to a query by Congressman Richard D. McCarthy:

> It is the policy of the U.S. to develop and maintain a defensive chemical-biological (CB) capability so that our military forces could operate for some period of time in a toxic environment, if necessary; to develop and maintain a limited offensive capability in order to deter all use of CB weapons by the threat of retaliation in kind; and to continue a program of research and development in this area to minimize the possibility of technological surprise.[13(pp153–154)]

On 25 November 1969, President Nixon visited Fort Detrick to announce a new policy on biological warfare. In two National Security Memoranda,[14,15] the U.S. government renounced all development, production, and stockpiling of biological weapons and declared its intent to maintain only small research quantities of biological agents, such as are necessary for the development of vaccines, drugs, and diagnostics.

Ground was broken in 1967 for construction of a new, modern laboratory building at Fort Detrick. The building would open in phases during 1971 and 1972. With the disestablishment of the biological warfare laboratories, the name of the U.S. Army Medical Unit, which was to have been housed in the new laboratories, was formally changed to U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in 1969. The institute's new mission was stated in General Order 137, 10 November 1971 (since superseded):

> Conducts studies related to medical defensive aspects of biological agents of military importance and develops appropriate biological protective measures, diagnostic procedures and therapeutic methods.[16]

The emphasis shifted away from offensive weapons to development of vaccines, diagnostic systems, personal protection, chemoprophylaxis, and rapid detection systems.

A COMPREHENSIVE MEDICAL BIOLOGICAL DEFENSE PROGRAM

In response to President Nixon's decision in 1969, all antipersonnel biological warfare stocks were destroyed between 10 May 1971 and 1 May 1972. The laboratory at Pine Bluff Arsenal, Arkansas, was converted to a toxicological research laboratory, and was no longer under the direction or control of the Department of Defense. Biological anticrop agents were destroyed by February 1973. Biological warfare demilitarization continued through the 1970s, with input provided by the U.S. Department of Health, Education and Welfare; Department of the Interior; Department of Agriculture; and the Environmental Protection Agency. Fort Detrick and other installations involved in the biological warfare program took on new identities, and their missions were changed to biological defense and the development of medical countermeasures. The necessary containment capability, Biosafety Levels 3 and 4 (BL-3 and BL-4, which are discussed below) continued to be maintained at USAMRIID.

In 1984, the Department of Defense requested funds for the construction of another biological aerosol test facility in Utah. The proposal submitted by the army called for BL-4 containment, although maintaining that the BL-4 inclusion was based on a possible need in the future and not on a current research effort. The proposal was not well received in Utah, where many citizens and government officials still recalled the secretive projects of the military: the areas on Dugway Proving Ground still contaminated with anthrax spores, and the well-publicized accidental chemical poisoning of a flock of sheep in Skull Valley, Utah, in March 1968.[10] Questions arose over the safety of the employees and the surrounding communities, and a suggestion was even made to shift all biological defense research to a civilian agency, such as the National Institutes of Health. The plan for a new facility was revised to utilize a Biosafety Level 3 (BL-3) facility, but not before congress had instituted more surveillance, reporting, and control measures on the army to ensure compliance with the Biological Weapons Convention of 1972.

Safety in Research and Patient Care

Currently, the medical biological defense research effort (part of the U.S. Army's Biological Defense Research Program [BDRP]) is concentrated at USAMRIID at Fort Detrick. The army maintains state-of-the-art containment laboratory facilities at USAMRIID, with more than

10,000 ft^2 of BL-4 and 50,000 ft^2 of BL-3 laboratory space. BL-4, the highest containment level, includes laboratory suites that are isolated by internal walls and protected by rigorous entry restrictions, air-locks, negative-pressure air-handling systems, and filtration of all out-flow air through high-efficiency particulate air (HEPA) filters. Workers in BL-4 laboratories also wear filtered positive-pressure total body suits, which isolate the worker from the internal air of the laboratory. BL-3 laboratories have a similar design, but do not require that personnel wear positive-pressure suits. Workers in BL-3 suites are protected immunologically by vaccines. U.S. governmental standards provide guidance as to which organisms may be handled under various containment levels in laboratories such as USAMRIID.[17]

The unique facilities available at USAMRIID also include a 16-bed clinical research ward capable of BL-3 containment, and a 2-bed patient care isolation suite where ICU-level care can be provided under BL-4 containment. Here, healthcare personnel wear the same positive-pressure suits as are worn in BL-4 research laboratories. The level of patient isolation required depends on the infecting organism and the risk to healthcare providers. Patient care can be provided at BL-4. There is no patient-care category analogous to BL-3; humans who are ill as a result of exposure to BL-3 agents are cared for in an ordinary hospital room with barrier nursing procedures.

USAMRIID guidelines have been prepared to determine which level of containment should be employed for individual patients who require BL-4 isolation or barrier nursing care (Exhibit 19-1). Staff augmentation for BL-4 critical care expertise comes from Walter Reed Army Medical Center, Washington, D.C., under an existing Memorandum of Agreement. Patients can be brought directly into the BL-4 suite from the outside through specialized ports with unique patient-isolation equipment.

EXHIBIT 19-1
ISOLATION PROCEDURES FOR PATIENT CARE AT USAMRIID, BY DISEASE AGENT OR TYPE OF EXPOSURE

Biosafety Level 4 (BL-4) isolation suite admission; care providers in positive-pressure protective suits

Ebola virus
Marburg virus
Crimean-Congo hemorrhagic fever virus
Variola (smallpox) and monkeypox viruses

The patient is presumed to be a victim of biological agent attack until definitive diagnosis is made

Biosafety Level 4 (BL-4) isolation suite admission; barrier nursing procedures*

Yersinia pestis (pneumonic form)†
Lassa fever virus
Argentine hemorrhagic fever (Junin) virus
Bolivian hemorrhagic fever (Machupo) virus
Venezuelan hemorrhagic fever (Guanarito) virus

Normal hospital room; barrier nursing procedures* or secretion precautions,‡ depending on the agent

Tick-borne encephalitis complex
Yellow fever virus§
Venezuelan equine encephalitis virus§
Rift Valley fever virus§
Chikungunya virus§
Dengue virus§
Brucella species
Vibrio cholerae
Bacillus anthracis (pulmonary or cutaneous forms)
Francisella tularensis (pulmonary form)
Yersinia pestis (bubonic or septicemic form)

Normal hospital room; no special precautions
Eastern equine encephalitis virus
Western equine encephalitis virus
Hemorrhagic fever with renal syndrome (Hantaan, Seoul, Puumala viruses)
Japanese encephalitis virus
Sandfly fever viruses
Coxiella burnetii (Q fever)
Chlamydia psittaci
Botulinum toxin
Staphylococcal enterotoxin B
Ricin toxin
Saxitoxin
Trichothecene mycotoxins

---

*Barrier nursing procedures: wearing gown, gloves, and surgical mask, but caring for patients in isolation suites.
†Pneumonic plague initially requires respiratory protection: full-face respirator or Racal hood (manufactured by Racal Health and Safety, Inc, Frederick, Md).
‡Secretion precautions: wearing gown and gloves; special handling of potentially infectious dressings, drainage, and/or excreta.
§The patient must be protected from potential arthropod vectors: windows should be screened and/or closed.
USAMRIID: U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md.

Finally, USAMRIID maintains a unique evacuation capability called the Aeromedical Isolation Team (AIT). Led by a physician and a registered nurse, each of the two teams consists of eight volunteers who train intensively to provide an evacuation capability for casualties suspected of being infected with highly transmissible, life-threatening BL-4 infectious diseases (eg, hemorrhagic fever viruses). The unit uses special adult-sized Vickers isolation units (Vickers Medical Containment Stretcher Transit Isolator, manufactured by Isolators Ltd., Shropshire, U.K.) (Figure 19-3). These units are aircraft transportable and isolate a patient placed inside from the external environment. The AIT can transport two patients simultaneously; obviously, it is not designed for a mass casualty situation. During the 1995 outbreak of Ebola fever in Zaire, the AIT remained on alert to evacuate any Americans who might have become ill while working to control the disease in that country.

Fig. 19-3. Members of the Aeromedical Isolation Team (AIT) prepare to transfer a patient from a stretcher isolator into the Biosafety Level 4 (BL-4) isolation suite at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland. The interior of the isolator is maintained at a pressure negative to the external environment by a high-efficiency particulate air (HEPA) filtered blower. The isolator can be attached directly to a transfer port, visible on the external wall of the building, to allow movement of the patient into the suite without exposing the environment to the patient. Team members are seen wearing protective suits and positive-pressure, HEPA-filtered Racal hoods (manufactured by Racal Health & Safety, Inc, Frederick, Md). Photograph: Public Affairs Office, Fort Detrick, Frederick, Md.

Some biological defense research also continues at the U.S. Army Medical Research Institute of Chemical Defense, Edgewood Arsenal,

Maryland, and the Walter Reed Army Institute of Research, Washington, D.C. USAMRIID and these laboratories conduct basic research in support of the medical component of the Biological Defense Research Program, which develops strategies, products, information, procedures, and training for medical defense against biological warfare agents. The products include diagnostic reagents and procedures, drugs, vaccines, toxoids, and antitoxins. Emphasis is placed on protecting personnel *before* any potential exposure to the biological agent occurs.[18]

A National Resource

Since biological warfare agents are often etiologic agents for naturally occurring diseases, the military research effort provides substantive benefits for civilian populations also. Products produced or being developed through military research include

- vaccines to prevent tularemia, Q fever, Rift Valley fever, Venezuelan equine encephalitis, Eastern and Western equine encephalitis, chikungunya fever, Argentine hemorrhagic fever, the botulinum toxicoses, and anthrax;[18,19]
- antitoxins for diseases such as botulism;
- human immune globulin preparations (passive antibody protection) against various bacteria and viruses; and
- antiviral drugs against multiple viral agents.

Some vaccines also have applicability for diseases of domestic animals (eg, Rift Valley fever and Venezuelan equine encephalitis). In addition, vaccines are provided to persons who may be occupationally exposed to such agents (eg, laboratory workers, entomologists, and veterinary personnel) throughout government, industry, and academe.

USAMRIID also provides diagnostic and epidemiological support to federal, state, and local agencies and foreign governments. Examples of assistance rendered to civilian health efforts by the former U.S. Army Medical Research and Development Command (renamed the U.S. Army Medical Research and Materiel Command in October 1994) include

- the massive immunization program instituted during the Venezuelan equine encephalitis outbreak in the Americas in 1971;
- the laboratory support provided to the U.S. Public Health Service during the outbreak of Legionnaire’s disease in Philadelphia, Pennsylvania, in 1976;

- the management of patients suspected of having African viral hemorrhagic fever in Sweden during the 1980s;
- international support during the outbreak of Rift Valley fever in Mauritania in 1989;
- assistance with the outbreak of Ebola infections among monkeys imported to Reston, Virginia, in 1990; and
- epidemiological and diagnostic support to the World Health Organization–Centers for Disease Control and Prevention field team that studied the Ebola outbreak in Zaire in 1995.

The current research effort combines new technological advances, such as genetic engineering and molecular modeling, applying them toward development of prevention and treatment of diseases of military significance. The program is conducted in full compliance with requirements set forth by the U.S. Food and Drug Administration, U.S. Public Health Service, Nuclear Regulatory Commission, U.S. Department of Agriculture, Occupational Safety and Health Administration, and Biological Weapons Convention.[18]

Even though the United States stopped all offensive biological warfare research in 1969, the Biological Defense Research Program must remain strong in view of

- evidence that some countries are not complying with the Biological Weapons Convention;
- the difficulty of verifying compliance with the Convention;
- the potential use of biological warfare by terrorists;
- the increased possibility of new threat agents based on advances in biotechnology; and
- the belief that a strong defense serves as a deterrent.

While some of the military's biological defense programs remain classified, based on worldwide threats and uncertainties, the medical Biological Defense Research Program is unclassified and continues to be an invaluable resource for the nation.

SUMMARY

Although biological agents have been used in warfare for centuries to produce death or disease in humans, animals, or plants, the United States did not begin a biological warfare offensive program until 1941. It was concern about the Japanese biological warfare threat that motivated the United States to begin to develop biological weapons. During the next 28

years, the United States initiative evolved into an effective, military-driven research and acquisition program, shrouded in controversy and secrecy. Most research and development was done at Fort Detrick, Maryland, while production and testing occurred at Pine Bluff, Arkansas, and Dugway Proving Ground, Utah. Field testing was done secretly and successfully with simulants and actual agents disseminated over wide areas. A small defensive effort paralleled the weapons development and production program.

With the presidential decision in 1969 to halt offensive biological weapons production, and the agreement in 1972 at the international Biological Weapons Convention never to develop, produce, stockpile, or retain biological agents or toxins, the program became entirely defensive, with medical and nonmedical components. The U.S. Biological Defense Research Program exists today, conducting research to develop physical and medical countermeasures to protect service members and civilians from the threat of modern biological warfare.

REFERENCES

1. Fox LA. Bacterial warfare: The use of biologic agents in warfare. *Milit Surg*. 1933;72(3):189–207.

2. Bernstein BJ. The birth of the US biological-warfare program. *Sci Am*. 1987;256:116–121.

3. Department of the Army. Special Report to Congress. *US Army Activity in the US Biological Warfare Programs, 1942–1977*. Vols 1 and 2. Washington, DC: DA. 24 Feb 1977. Unclassified.

4. Cole LA. *Clouds of Secrecy: The Army's Germ Warfare Tests Over Populated Areas*. Totowa, NJ: Rowman and Littlefield; 1988.

5. Williams P, Wallace D. *Unit 731: Japan's Secret Biological Warfare in World War II*. New York, NY: Free Press; 1989.

6. Report to the Secretary of War by Mr. George W. Merck, Special Consultant for Biological Warfare, 3 Jan 1946. Cited in: Department of the Army. Special Report to Congress. *US Army Activity in the US Biological Warfare Programs, 1942–1977*. Vol 2, annex 1. Washington, DC: DA. 24 Feb 1977. Unclassified.

7. Baldwin IL. *Special BW Operations*. Washington, DC: The National Military Establishment Research and Development Board; 5 Oct 1948. Memorandum for Executive Secretary, Research and Development Board. Unclassified.

8. Hersh SM. *Chemical and Biological Warfare: America's Hidden Arsenal*. Indianapolis, Ind: Bobbs-Merrill; 1968.

9. Geissler E, ed. *Biological and Toxin Weapons Today* (Stockholm International

Peace Research Institute). Oxford, England: Oxford University Press; 1986.

10. Harris R, Paxman J. *A Higher Form of Killing: The Secret of Chemical and Biological Warfare.* New York, NY: Hill and Wang; 1982.

11. Cowdrey AE. *The Medics' War*. Washington DC: Center of Military History, US Army; 1987.

12. Stockholm International Peace Research Institute (SIPRI). *The Rise of CB Weapons*. Vol 1. In: *The Problem of Chemical and Biological Warfare.* New York, NY: Humanities Press; 1971.

13. Foster JS, Director of Defense Research and Engineering, US Department of Defense. Letter dated 15 April 1965 to Honorable Richard D. McCarthy, US House of Representatives. Cited in: McCarthy RD. *The Ultimate Folly: War by Pestilence, Asphyxiation, and Defoliation*. New York, NY: Alfred A. Knopf; 1969: 153–154.

14. Kissinger HA. N.S. Decision Memorandum 35. 25 November 1969.

15. Kissinger HA. N.S. Decision Memorandum 44. 20 February 1970.

16. Department of the Army. General Order 137. Washington, DC: Headquarters, DA; 10 November 1971.

17. Centers for Disease Control and Prevention. *Biosafety in Microbiological and Biomedical Laboratories*. 3rd ed. Washington, DC: US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. May 1993. HHS Publication (CDC) 93-8395.

18. Huxsoll DL, Parrott CD, Patrick WC III. Medicine in defense against biological warfare. *JAMA*. 1989;265:677–679.

19. Takafuji ET, Russell PK. Military immunizations: Past, present and future prospects. *Infect Dis Clin North Am*. 1990;4:143–157.

Chapter 20

USE OF BIOLOGICAL WEAPONS

EDWARD M. EITZEN, JR., M.D., M.P.H., FACEP, FAAP*

INTRODUCTION

REQUIREMENTS FOR AN IDEAL BIOLOGICAL WARFARE AGENT
- Availability or Ease of Production
- Incapacitation and Lethality
- Appropriate Particle Size in Aerosol
- Ease of Dissemination
- Stability After Production
- Susceptibility and Nonsusceptibility

METHODS OF DELIVERY

ADVANTAGES AND DISADVANTAGES OF USING BIOLOGICAL WEAPONS
- Advantages
- Disadvantages
- Psychological Factors

STRATEGIC AND TACTICAL CONCERNS

UNCONVENTIONAL WARFARE AND TERRORISM

DETECTING BIOLOGICAL WARFARE AGENTS
- Environmental Detection and Monitoring
- Epidemiological Surveillance
- Medical Diagnostics

SUMMARY

* *Colonel, Medical Corps, U.S. Army; Chief, Operational Medicine Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

INTRODUCTION

During the Persian Gulf War (1990–1991), the United States and her coalition partners were faced with the credible and realistic threat that weapons of mass destruction, including biological agents, might be used by Iraq against allied forces. It is now known that Iraq had actually put offensive biological agents into several weapons systems for delivery (see Chapter 18, Historical Overview of Biological Warfare). These revelations, combined with the fact that terrorist groups such as the Aum Shinrikyo in Japan have sought to produce biological agents, have catapulted the United States into an era where the use of biological warfare agents against either our military forces or against civilian populations by terrorists is a possibility that we cannot ignore.

Defending against these agents requires us to understand how an adversary might use them. Biological agents must be considered in terms of an evolving world, where advances in modern technology and weapons delivery systems (eg, long-range cruise missiles with multiple warheads) have overcome some of the earlier physical limitations. A biological warfare agent need no longer be highly lethal to be effective, for incapacitation and confusion may be all the disruption necessary to cause the intended effects. Biological weapons may also be used in combination with other types of weapons, or to add to the disruption produced by conventional weaponry. Finally, it would appear from the way conflict is evolving that nonconventional or terrorist use of biological agents is becoming more rather than less likely. The method for delivery of biological warfare agents may be as simple and inconspicuous as attaching an off-the-shelf spray device to a car, truck, boat, or airplane which appears harmless and normal to all who might observe the delivery vehicle.

Since initial symptoms resulting from a biological warfare agent may often be indistinguishable from those produced by endemic infections, a biological weapon may be capable of overcoming a military force before the presence of the agent is even suspected. When one member of a unit falls victim, others may still be incubating the disease. Troops deployed to foreign lands are at greater risk for exotic endemic disease agents, since they may lack natural immunity. Therefore, a biological warfare attack may not even be suspected after the first casualties have presented to medical

personnel. Finally, the psychological and demoralizing impact of the sinister use of a lethal infection or toxin cannot be underestimated.

Many biological agents, including bacteria, viruses, and toxins, can be used as biological weapons. The respective chapters in this textbook contain detailed discussions of these pathogens and toxins as biological weapons.

REQUIREMENTS FOR AN IDEAL BIOLOGICAL WARFARE AGENT

Although many pathogens and toxins cause disease or intoxication of humans, relatively few would actually be effective if employed as biological weapons (Exhibit 20-1).

For a number of reasons, some that might be useful on a small scale, such as an assassination weapon or a terrorist weapon, would normally not be applicable on a large scale. The key factors that make a biological pathogen or toxin suitable for a large-scale biowarfare attack include: *(a)* availability or ease of production in sufficient quantity; *(b)* the ability to cause either lethal or incapacitating effects in humans at doses that are achievable and deliverable; *(c)* appropriate particle size in aerosol; *(d)* ease of dissemination; *(e)* stability (while maintaining virulence) after production in storage, weapons, and the environment; and (f) susceptibility of intended victims with nonsusceptibility of friendly forces.

We will examine each of these requirements for a suitable agent in greater detail below. However, healthcare providers and medical planners must always remember that biological agents could be used in a wide variety of scenarios. What may look like an ordinary outbreak of diarrheal disease early in a deployment could be, instead, a

EXHIBIT 20-1
POSSIBLE BIOLOGICAL WARFARE AGENTS

Bacteria

- *Bacillus anthracis* (anthrax)
- *Yersinia pestis*(plague)
- *Francisella tularensis*(tularemia)
- *Brucella* species (brucellosis)
- *Coxiella burnetii* (Q fever)

Viruses

- Variola virus (smallpox)
- Equine encephalitis viruses (viral equine encephalitides)
- Arenaviruses, bunyaviruses, filoviruses, flaviviruses (hemorrhagic fevers)

case of sabotage of food or water supplies with an infectious agent. This type of operation might even be carried out by enemy special forces against our soldiers in garrison at home or deep behind friendly lines.

Toxins

- Staphylococcal enterotoxin B
- Ricin
- Botulinum toxins
- Trichothecene mycotoxins
- Saxitoxin

Availability or Ease of Production

Many replicating agents (bacteria and viruses) can be produced in large quantities with modern fermentation and viral production technologies. Some toxins, like ricin, are widely available because their source in nature is ubiquitous and the process necessary to harvest the toxin is technically straightforward. On the other hand, some replicating agents are very difficult to grow in quantity or to a high-enough titer, and many toxins are produced in nature in such low quantities that harvesting them is impractical (shellfish toxins are a good example). Some of the rare toxins, like saxitoxin, could be used on a very limited scale to target one or a few individuals but currently could not be used as weapons of mass destruction. Some of these limitations could possibly be overcome by future adversaries, who might use recombinant techniques to amplify production of some of these compounds. To be adequate weapons for large-scale use, some toxins of low toxicity or high LD_{50} (ie, the dose that is lethal to 50% of the exposed population) would have to be used in ton quantities to be effective over a wide area—which limits their potential utility. This subject is discussed in greater detail in Chapter 30, Defense Against Toxin Weapons (see Figure 30-1).

Incapacitation and Lethality

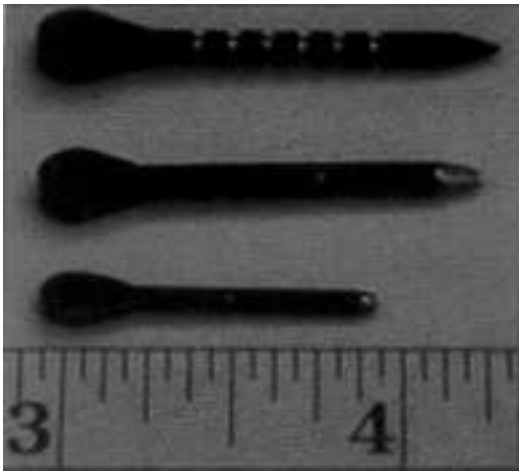
Biological warfare agents are likely to be selected for their ability to either incapacitate or kill the human targets of the attack. A biological warfare agent does not necessarily have to be lethal to be useful as a military weapon. An agent such as Venezuelan equine encephalitis (VEE) virus could render entire military units mission-ineffective by incapacitating large numbers of unit personnel. If one of an adversary's aims is to overload our field medical care systems, an incapacitating agent such as VEE virus might be chosen preferentially to a lethal biological agent. VEE casualties may require hospitalization, tying up beds and medical resources needed for the care of battle-injured soldiers.

If lethality is desired, agents such as *Bacillus anthracis* (anthrax); the viruses that cause Ebola hemorrhagic fever, Marburg disease, and

Crimean-Congo hemorrhagic fever; or the plague bacillus, *Yersinia pestis*, might be used against U.S. forces. Inhalational anthrax, pneumonic plague, and certain viral hemorrhagic fevers have high case-fatality rates once infection is established in nonimmune hosts. Viruses may be particularly attractive as biological warfare agents because specific treatments are not available for many of them. Agents that require low doses to produce their intended effects whether lethality, incapacitation, or intoxication would, for logistical and tactical reasons, have greater utility as biological weapons.

Although in general a shorter incubation time to onset of deleterious effects is desirable, this characteristic would vary according to the aims of the aggressor. Very short incubation periods might be more useful in a tactical setting, whereas longer incubation times would work to the advantage of the terrorist, assassin, or special forces unit, to enable them to escape prior to the onset of the effects of the attack.

Very few biological agents are immediately lethal. Some of the most toxic toxins, such as saxitoxin, are available only in small quantities but an adversary could use these as assassination or antipersonnel weapons. Certain penetrating devices designed to be used with such toxins, such as flechettes, have been developed in the past (Figure 20-1).



An ideal biological weapon would also cause disease or intoxication when inhaled, as the most likely and effective mode of dissemination of a biological warfare agent is by aerosolization of the toxins or bacterial or viral organisms. The characteristics of such aerosols are discussed further below.

Appropriate Particle Size in Aerosol

Biological agents may enter the human body via several routes:

- entering the lungs as an aerosol (the inhalational route),
- being ingested in food or water (the oral route),
- being injected through the skin into the body (the percutaneous route), and
- being absorbed through the skin

or placed on the skin to do damage to the integument (the dermal route).

The inhalational or aerosol route of entry into the body is by far the most important to consider when planning defenses against biological warfare attacks. An ideal biological warfare agent would be of a particle size that would allow it to be (1) carried for long distances by prevailing winds and (2) inhaled deeply into the lungs of the unsuspecting victims. The size range of particles that meets both of these conditions is 1 to 5 µm in diameter. Particles larger than this would either settle out onto the ground or be filtered out in the upper respiratory tract of those who inhale them. Particles in this size range are invisible to the human eye; thus, a cloud of such particles would not generally be detected by those attacked, even if such a cloud were to be carried through their position. An adversary designing a biological weapon would therefore seek to produce such a small particle size, and would require the equipment and technology to do so. From the standpoint of military medical personnel, the lack of a fielded standoff (ie, remote) detection capability for such a biological “cloud” makes clinical and laboratory diagnostics critical for early diagnosis of the initial casualties of a biological attack. The separate issues of detection and medical diagnosis will be addressed later in this chapter.

Fig. 20-1. Sharp flechettes designed for antipersonnel purposes can be used with either chemical or biological agents. The agent can be applied to the flechette’s exterior grooves, or the flechette can be drilled and filled with either a liquid or a dry agent. These types of weapons would not be permissible under the Biological Weapons Convention; however, an adversary of the United States might ignore such restrictions, as has occurred in the past. Reprinted from van Keuren RT. *Chemical and Biological Warfare, An Investigative Guide*. Washington, DC: Office of Enforcement, Strategic Investigations Division, US Customs Service; 1990: 87.

Ease of Dissemination

An ideal biological warfare agent would be easily disseminated in the open air by using off-the-shelf devices such as industrial sprayers or other types of aerosol-producing devices. These could be mounted on an airplane, boat, car, or other moving vehicle, or even placed in a stationary position. An alternative method would be to disseminate the agent in an enclosed space (eg, a building), where it could more efficiently infect or intoxicate humans living or working in the area. In addition to having the proper particle size, an ideal agent might also be dried (by freeze-drying or spray-drying), which would make it easier to disseminate widely and over longer distances. As noted above, some toxins, although inherently very

toxic, have low-enough lethality that massive (ton) amounts would be required to cover any significant area. Delivering such amounts to a battlefield might be logistically very difficult, if not impossible. Although logistical difficulties mitigate against using toxins of low lethality as open-air weapons, they could be used on a smaller scale (eg, in an enclosed space such as a building, or as an assassination or terrorist weapon). Such uses may become more likely considering the kinds of limited conflicts and terrorist scenarios that we face in the post–Cold War world. Medical personnel must be alert to the possibility of such “surprise” attacks, which could occur even within our own borders.

Stability After Production

Once a biological warfare agent is produced in quantity by an adversary, it must be fairly stable—either in bulk storage or once put into a weapon or a delivery system. It must, therefore, retain its viability and virulence or toxicity during production, storage, transportation, and delivery. Agents that have a very short shelf life or those that are subject to rapid degradation in the environment once released would, by definition, be poor biological weapons. On the other hand, those with long shelf lives, or those that are very slowly degraded by environmental influences such as temperature extremes, dryness, or ultraviolet radiation, would be more useful as weapons, assuming other requirements are met. Anthrax spores are one example of an ideal agent from this standpoint, as they are both very hardy in the environment and stable in storage. Studies[1] on Gruinard Island off the northwest coast of Scotland, where the British conducted field trials with anthrax in the early 1940s, showed that in the right conditions anthrax spores can remain viable for 50 years or more. However, even anthrax spores are susceptible to breakdown by high ultraviolet (bright sunlight) exposure.

Susceptibility and Nonsusceptibility

The ideal biological warfare agent would be one to which the target force is known to be susceptible (ie, not immunized against) but to which the aggressor force possesses high levels of immunity, usually via vaccination. When used as aerosols, biological warfare agents are only as predictable as the weather: a shift in wind direction could blow the agent cloud back over the aggressor’s position, which could be catastrophic if his forces were unprotected against the agent. It is assumed, therefore, that an aggressor will attempt to immunize his forces against agents that he is planning to use tactically, although this is not necessarily the case for all countries with offensive biowarfare programs. It is also desirable that an

infectious biological warfare agent not produce an uncontrollable epidemic that might ultimately infect friendly forces due to person-to-person spread of disease. With toxins, obviously, this is not a consideration.

METHODS OF DELIVERY

Biological agents may be delivered in either wet or dry form. Dry powders composed of very small particles tend to have better dissemination characteristics, and have advantages in storage. Dried agents require an increased level of technological sophistication to produce, although freeze drying or spray drying technology has been available in industry for a number of years.

Biowarfare agents might be released by an aggressor against our forces or against civilian populations by means of sprays, explosive devices, and contamination of food and water. Most commonly, delivery methods use aerosolized agent.

The agent can be dispersed by attaching a spray device to a moving conveyance. An industrial insecticide sprayer designed to be mounted on an aircraft is an example. A line of release would then occur while the sprayer is operating. This is known as a *line source*, and is sprayed perpendicular to the direction of the wind, upwind of the intended target area. Up to a certain range, anyone downwind of such a line source would theoretically be at risk. The range that the infectious or toxic agent would reach depends on a number of factors, including wind speed and direction, atmospheric stability, and the presence of inversion conditions; and on characteristics of the agent itself (eg, stability to desiccation or ultraviolet light). Studies[2] using computer models have shown that clouds of hardy organisms such as anthrax spores can be infectious more than 200 km from the source of the aerosol.

A second type of aerosol source is a *point source*, which is a stationary device for aerosolization of the agent, such as a stationary sprayer. A modified point source would be a group of spray devices, such as specially designed bomblets dispersed in a pattern on the ground by a missile or artillery shell designed to release such bomblets. Many such devices have been designed; an example is the Flettner rotor (Figure 20-2). Several types of spherical bomblets have also been widely tested, and most biological bomblets are spherical (Figure 20-3). Bomblets may be designed to disseminate on impact or at a predetermined altitude above the ground. They may be released from missiles or aircraft, and may have special designs to improve their aerodynamics or pattern in the target area.[3]

Other types of delivery systems for biological agents have been designed by various countries. These include bombs or bomblets that

release the agent by exploding (generally very inefficient delivery systems), land and sea mines, pipe bombs, and many other special devices.[3] *Clandestine* means of delivering biological warfare agents are potentially available to terrorists or special forces units; these include devices that penetrate and carry the agent into the body via the percutaneous route, such as pellets or flechettes, or means to contaminate food or water supplies so that the agent would be ingested.

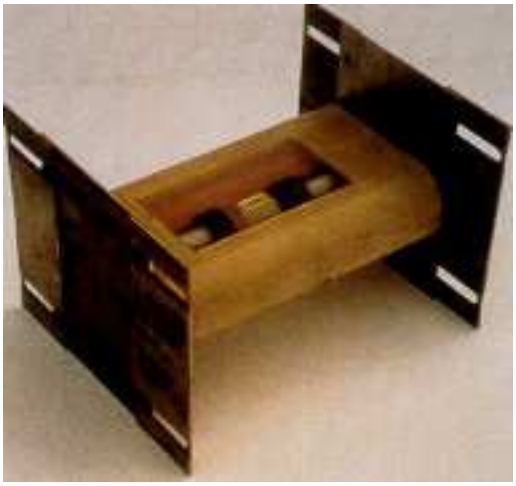
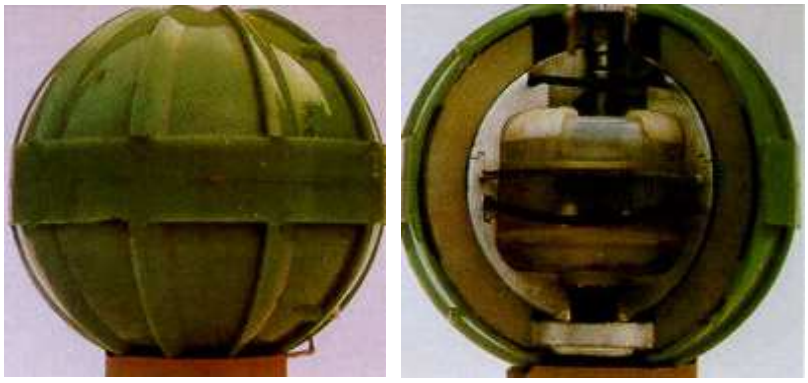


Fig. 20-2. The Flettner rotor, a 7-in.-long winged biological bomblet design, was a test design that was never standardized (ie, selected to be mass produced). It could carry either liquid or dry biological agents. This type of bomblet was designed to be released from cluster bombs, dispensers, or missile warheads, and spray out agent on impact. The fins were designed to extend by centrifugal force in flight to stabilize the bomblet's flight path to the target area. Reprinted from van Keuren RT. *Chemical and Biological Warfare, An Investigative Guide*. Washington, DC: Office of Enforcement, Strategic Investigations Division, US Customs Service; 1990: 85.



a **b**

Fig. 20-3. The E120 biological bomblet was one of a number of spherical biological bomblets that were developed before the United

States discontinued its offensive program in the 1970s. **(a)** The vaned outer shell of this spherical bomblet was designed to provide rotation during flight. On impact, the outer shell would shatter; the bomblet was asymmetrically weighted so that agent would then be sprayed from the top of the bomblet. **(b)** Cutaway view of the E120 biological bomblet. Reprinted from van Keuren RT. *Chemical and Biological Warfare, An Investigative Guide*. Washington, DC: Office of Enforcement, Strategic Investigations Division, US Customs Service; 1990: 79.

ADVANTAGES AND DISADVANTAGES OF USING BIOLOGICAL WEAPONS

Biological weapons offer many potential advantages to an aggressor who chooses to use them. However, some of the advantages may be counter-balanced by several key disadvantages, most of which also apply to the aggressor force (Exhibit 20-2). In addition, the threat of impending attack with biological warfare agents can create psychological effects out of proportion to the military threat.

Advantages

An attack using biological weapons may be more sinister than an attack using conventional, chemical, or nuclear weapons, where effects are more immediate and obvious. By the time the first casualty is recognized, the agent may have already been ingested, inhaled, or absorbed by many others and more casualties may be inevitable despite medical countermeasures. Minute particles can silently pass through the air supply systems of ships, vehicles, command headquarters, sleeping quarters, and even hospitals.

EXHIBIT 20-2
ADVANTAGES AND DISADVANTAGES OF BIOLOGICAL WEAPONS

Advantages

- The potential deadly or incapacitating effects on a susceptible population
- The self-replicating capacity of some biological agents to continue proliferating in the affected individual and, potentially, in the local population and surroundings
- The relatively low cost of producing many biological weapons
- The insidious symptoms that can mimic endemic diseases
- The difficulty of immediately detecting the use of a biological agent, owing to the current limitations in fielding a multiagent sensor system on the battlefield, as well as to the prolonged incubation period preceding onset of illness (or the slow onset of symptoms) with some biological agents
- The sparing of property and physical surroundings (compared with conventional or nuclear weapons)

Disadvantages

- The danger that biological agents can also affect the health of the aggressor forces
- The dependence on prevailing winds and other weather conditions on effective dispersion
- The effects of temperature, sunlight, and desiccation on the survivability of some infectious organisms
- The environmental persistence of some agents, such as spore-forming anthrax bacteria, which can make an area uninhabitable for long periods
- The possibility that secondary aerosols of the agent will be generated as the aggressor moves through an area already attacked
- The unpredictability of morbidity secondary to a biological attack, since casualties (including civilians) will be related to the quantity and the manner of exposure
- The relatively long incubation period for many agents, a factor that may limit their tactical usefulness
- The public’s aversion to the use of biological warfare agents

Biological weapons are also unique in their ability to inflict large numbers of casualties over a wide area—with minimal logistical requirements and by means that can be virtually untraceable.[4] In 1970, the World Health Organization estimated[5] that if 50 kg of anthrax spores were dispersed upwind of a population center of 500,000 people in optimal weather conditions, almost half of the population of that area would be either disabled or killed in such an attack (Table 20-1). This highlights the mass casualty–producing potential of these agents.

The ease and low cost of producing an agent, the difficulty in detecting its presence and protecting (and treating) its intended victims, and the potential to selectively target humans, animals, or plants make defense against this class of weapon particularly difficult.[4] An enemy may also be able to deny the use of such a weapon after casualties occur, claiming that a natural outbreak of an endemic disease with similar symptoms is to blame for the occurrence of disease in friendly forces.

Another advantage of biological warfare to an enemy is that defensive programs always tend to lag behind the current offensive threat. This is due to three major factors[6]:

1. Defensive efforts are not initiated until the threat is evident.

2. Defensive systems generally require longer development times in comparison to offensive systems.

3. Medical defenses may be even more problematic and take even longer to field due to important regulatory requirements that impose strict guidelines on the development of vaccines and prophylactic drugs for use in human beings.

Table 20-1 is not shown due to copyright restrictions, please refer to the textbook.

The United States remains vulnerable to the strategic, tactical, and terrorist use of biological weapons. There may a tendency on the parts of some potential adversaries of this country to overcome their conventional military disadvantages by choosing weapons of mass destruction that can be produced easily and cheaply, and with technology already available to them. The cost advantage of biological weapons was clearly illustrated by a 1969 United Nations report[4] that estimated the cost of operations against civilian populations at $1.00 US/km^2 for biological weapons, versus $600.00/km^2 for chemical, $800.00/km^2 for nuclear, and $2,000.00/km^2 for conventional armaments.

Thus, from several standpoints, including cost, availability, relative technological feasibility, ease of dissemination, difficulty of detection, deniability, and ability to cause mass casualties, biological weapons may be very attractive as weapons of mass destruction for adversaries of the United States or its allies.

Disadvantages

Many of the disadvantages to using biological warfare agents—both to the target population and to the aggressor forces—have been presented in testimony to the U.S. Congress and in public documents,[7–9] but it should be noted that biological warfare organisms or toxins have never been used against U.S. forces. Therefore, we have no practical experience with their use.

A major disadvantage to the use of biological weapons is that, since they are widely viewed as weapons of mass destruction and out of the ordinary, their use may bring about the escalation of a military conflict (ie, in the type of retaliatory attack). Since there are no biological weapons in the current arsenal of the United States, and since the effect of chemical weapons is so much more limited in scale and area covered, we can only speculate on the response of the United States if our military forces or civilian populations were attacked with biological weapons. Such concerns may be one reason why Iraq elected not to use chemical or biological weapons in the Persian Gulf War.

Certain types of biological weapons systems may be more disadvantageous than others to aggressor forces. For example, bomblets delivered on a target leave a definable biological warfare signature, and up to 5% of the agent may remain as residue after the contents have been expended. Therefore, the remaining agent could be identified and used to prove culpability. The residua could also be used to tailor medical treatment or postexposure prophylaxis, thus mitigating the intended results of the attack. [3]

Psychological Factors

By virtue of their invisibility and undetectability, biological agents may be more psychologically disruptive than conventional weapons, especially to an unprepared military unit. The prospect of dying from an incurable, potentially painful, and highly communicable disease can create a panic among unprotected soldiers trained to fight against conventional weapons only. Most military organizations have little experience in dealing with casualties of biological warfare, and facing an unknown threat can give rise to considerable anxiety and fear. Even the prospect of facing such a threat can create a great deal of concern. These fears were seen in numerous instances in Saudi Arabia during the Persian Gulf War.

Concerns over biocontamination may affect or even halt operations at key bases or facilities. Medical personnel may be even more susceptible to concerns over biowarfare, as they are more likely to understand the effects that would follow enemy use of such weapons. This was seen in the Gulf War, as individual personnel in medical units had a better appreciation for the potential vulnerability that a lack of adequate stocks of vaccine might create.

It is not only the threat of use of biological warfare agents that creates psychological stress, however. The tremendous difficulties involved with continuing unit operations in a biological or chemical environment may have a profound psychological impact. Medical care in such an environment may require the use of individual protection of varying degrees, group or collective protection, decontamination, transfer of contaminated patients or medical supplies through a particular corridor (eg, from an air force base to an army hospital), and contained operations for extended periods.[10] This increases the difficulty of what is already hard enough: providing modern medical care in an austere field environment. The stress of trying to do so may be psychologically overwhelming, particularly if medical units have not trained to operate in nuclear, biological, and chemical (NBC) environments. All military units, including medical units, must be prepared to perform their mission-essential tasks under NBC conditions.[11] The only way to assure that our medical units are ready to do so is to train under realistic conditions. Medical planners must ensure that such training occurs regularly and often.

STRATEGIC AND TACTICAL CONCERNS

Knowledge and understanding of enemy strategy, tactics, and doctrine are essential parts of waging a successful military campaign. This understanding includes an appreciation of whether the enemy possesses biological weapons and in what quantity, what might compel him to use

them, and in what manner he would employ them. Biological weapons have the potential of inflicting heavy casualties in selected areas, contaminating personnel and equipment, causing disruption of troop movement and maneuvers, impacting on resources in forward and rear areas, and restricting or denying the use of terrain by friendly and enemy forces.

Biological weapons, when compared with nuclear weapons, are less likely to cause wide-spread physical devastation. Likely scenarios of use include large-scale attacks against fixed rear areas and forces, such as supply points, ammunition dumps, airbases, command and control assets, and fixed medical facilities; or application on a more-limited scale to cause disruption rather than annihilation. As a force is demoralized and reduced by disease and strange illnesses, attrition may become a more significant factor. It is less likely that an enemy would attempt to use biological warfare weapons against tactical maneuver units, owing to those units' high mobility and the fact that effects on such units may not occur quickly enough to be decisive in the enemy's favor.

A proper defense against biological weapons thus requires *(a)* an understanding of the enemy and his likely objectives for a biological attack, and *(b)* the adoption of effective personal protective measures to minimize their impact. Biological defenses and future detection efforts should be emphasized in areas of the battlefield where an enemy attack is most likely. However, since an adversary may attempt to use biological weapons when and where such an attack is least expected, all efforts should be made to prepare our forces in depth for the possibility of a biological attack. This preparation should include the continued development of better vaccines and prophylactic drugs to protect U.S. military forces deployed to areas where intelligence indicates that an attack with biological agents is likely.

Biological warfare agents, by themselves, are not ideal tactical weapons, owing to their unpredictability and delayed effects (long incubation times). They are also viewed as inhumane by many, and their "first use" would generate significant world criticism. Their tactical importance may increase, however, as more is learned about the predictability of damage from specific biological agents. But the U.S. military must be prepared to defend against biological attack at all levels of conflict. Biological warfare agents in combination with other weapons systems must also be anticipated. With the development of new missile delivery systems, even intercontinental delivery of biological agents is possible, and the use of low-flying, long-range cruise missiles or remotely piloted drones may be the best way to generate a dense cloud of biological warfare agents close to the ground. It has been estimated that under suitable conditions, a cruise missile could deliver anthrax spores

over an area of the same magnitude as the lethal fallout from a ground-burst nuclear warhead.[3] However, much more subtle delivery vehicles (such as an agricultural sprayer mounted on a truck, boat, or other, more conventional platform) could be used to deliver biological agents anywhere in the world.

Simply to maintain a defensive posture against attack is not adequate, however. The U.S. military must be able to sustain an offensive campaign in a biologically contaminated environment. To do otherwise is to invite use of such weapons by the enemy. The impact of infectious diseases on military units has been well documented in past wars, but the potential fielding of highly lethal agents by adversaries for use as biological warfare weapons makes personal protective measures and command-driven discipline even more important for today's army.

While the more accurate conventional weapons systems that are currently fielded by some military forces produce less collateral damage, an aggressor using biological or chemical weapons may use multiple weapons or dissemination devices to cover a large area. Biological weapons could be effective if the enemy's goal was to preserve logistical materiel; this presupposes the enemy use of captured friendly weapons and infrastructure, as opposed to mass physical destruction, thereby making biological weapons more attractive to an enemy than nuclear weapons to accomplish this purpose.[12,13]

UNCONVENTIONAL WARFARE AND TERRORISM

The U.S. Department of State estimates that more than 6,000 terrorist incidents have occurred in the world since 1980, with more than 4,000 people killed and 11,000 wounded.[14] Many terrorist groups have been supported by countries with substantial research, development, and weapons capabilities. Countries like Iran and Iraq, which are known to have links to terrorist groups, have long been suspected of having offensive biological warfare programs. Assassinations with biological agents such as ricin have been carried out effectively (see Chapter 32, Ricin Toxin).

Biological weapons may, in fact, be much more effective if used against unsuspecting, unprotected, and nonimmune civilian populations than against a fast-moving military organization. This was certainly true when the Japanese attempted to use biological weapons against Chinese and Soviet troops in World War II. The objectives of a terrorist group may not be typical military objectives; therefore, biological weapons may be better suited to their purposes.[15]

Many factors may make biological agents particularly attractive to

terrorists. The availability of less-sophisticated biological weaponry has been mentioned earlier in this chapter. Biological weapons can be employed effectively as a means of terrorism or sabotage, especially in rear-echelon areas, port or staging sites, and industrial and storage areas. Biological weapons could also be used against civilian populations, creating panic.

The contamination of logistical supplies is a well-recognized possibility, and security in all areas of the battlefield is of great importance when dealing with an enemy with such capabilities. The stealth with which these weapons may be employed is a factor, especially if the terrorist wishes to escape detection before the results of the attack are obvious.

The use of biological warfare in rear areas close to local civilian populations also might create large numbers of civilian casualties. How would military medical personnel respond to civilians seeking care at medical treatment facilities? And how can civilian casualties be factored into medical planning? These are only two of many unanswered questions relating to development of realistic medical doctrine for management of biological casualties.

Terrorist or criminal use of biological agents may also not be limited to the battlefield or a theater of operations. As the World Trade Center bombing in New York illustrated in January 1993, terrorist attacks within the borders of the United States are certainly possible. Knowledge of microbiology and its potential applications is widespread, and development of a crude biological weapon does not require extensive expertise.

Several attempts at biological sabotage have occurred within the United States. Even an attack that turns out to be a hoax may have significant public impact: the 1992 case of a Tyson’s Corner, Virginia, man, who sprayed his neighbors with a fluid he claimed contained anthrax is illustrative. This incident caused local hazardous material teams to be deployed, the house involved to be quarantined, and numerous individuals to go to local hospitals for care.[16]

An incident on a larger scale could create a panic and cause hundreds or even thousands of people to seek medical care. The potential impact on health care facilities in the area of an attack or even a threatened attack is tremendous. Emergency departments may be “overrun” with patients, open hospital beds may become scarce, intensive care units may be filled, and antibiotic stocks may be depleted.

Possible scenarios for criminal or terrorist attacks with biological agents include four basic types[15]; the examples demonstrate that terrorist acts have already occurred using chemical agents:

1. product tampering (eg, the Tylenol tampering cases of the 1980s);
2. attacks on specific ethnic population groups within a country, who are perceived to be in opposition to terrorist goals;
3. sabotage of specific food groups or industries, such as contamination of an imported food product with a toxin, pathogenic bacteria, or poison (eg, the lacing with cyanide of Chilean grapes in March 1989); and
4. attacks directed at one of a country's institutions, agencies, or departments such as the military, a stock market, or a major communications center.

Because of the variety of possible scenarios and the likely similarity of the medical effects of such attacks to endemic disease outbreaks, medical personnel will have to be extremely alert to differentiate the initial cases resulting from biological warfare terrorism from naturally occurring disease.

Biological terrorism could range from the use of sophisticated biological warfare weapons such as dried anthrax spores or botulinum toxins to unsophisticated agents such as *Salmonella* or other common bacteria. The agent used and the mechanism of delivery may depend to a great extent on whether the terrorists are sponsored by a government. Although cutting-edge biotechnology research requires an infrastructure of sophisticated laboratories, and production of sophisticated biological weapons may require specialized equipment, some effective biological pathogens or toxins may be produced or harvested using relatively primitive techniques.[17] A state-sponsored terrorist group is, however, much more likely to have the wherewithal to produce casualties on a large scale; the unsupported single operative may still have a devastating impact, but on a much smaller scale.

Consider, for example, the difference between the hypothetical potential casualties produced by a state-sponsored terrorist spraying dried anthrax from a crop duster airplane northwest of Washington, D.C., versus an unsponsored, disgruntled employee sprinkling a species of *Salmonella* in broth culture on the salad bar in the company cafeteria. The difference in potential medical impact is obvious. Countries suspected to have biological programs that may support terrorist activities include Iran, Iraq, Libya, and North Korea.[17] The operative question may not be *whether* biological agents will be used as terrorist weapons against the United States, but rather *when*.

DETECTING BIOLOGICAL WARFARE AGENTS

A satisfactory battlefield biological defense requires a sensitive

environmental monitoring system to be employed, one that can detect the presence of toxic or infectious biological materials in the environment in a manner similar to chemical agent detectors.[12] A distinction must be made here between detection of biological agents in the environment (detection, monitoring, or standoff detection) and medical diagnostics (detection of biological agents, components of biological agents, or antibodies to biological agents in tissue samples such as blood or other body fluids). Environmental biological detectors must be reliable and sensitive to be useful, and also must be able to determine when a previously contaminated area is safe. Medical diagnostics depend on fieldable laboratory testing.

Environmental Detection and Monitoring

Once a biowarfare agent has been dispersed, detection of the aerosol prior to its arrival over the target, in time for personnel to don their protective equipment, is the best way to minimize or prevent casualties. When properly fitted and worn, currently issued chemical protective equipment is also effective in protecting personnel from biological agents (see Chapter 16, Chemical Defense Equipment). However, there are no currently fielded means of detecting biological agents in the environment in real time, and until reliable detectors are fielded, the first indication of a biological attack against unprotected soldiers will likely be one or more ill soldiers. The situation may be as simple as a few patients with respiratory illness, or as complex as a mass casualty situation.

Evolving detector systems are an area of intense interest with the highest priority within the research and development community. A Biological Integrated Detection System (BIDS) is planned for fielding within the next several years (see Figure 16-32 in Chapter 16, Chemical Defense Equipment). This system will be vehicle mounted and will test environmental air samples, by concentrating appropriate aerosol particle sizes in the air samples, then subjecting the sample to analytical- and antibody-based detection for selected agents. Also planned for future use is the Tactical Biological Standoff Detection System, which, when fielded, will provide for the first time a biological standoff detection capability to both provide early warning and activate the NBC Warning and Reporting System. The Tactical Biological Standoff Detection System will employ ultraviolet laser and laser-induced fluorescence to detect biological aerosol clouds at a standoff distance of up to 5 km. This system will be available for fixed-site applications or inserted into various transport platforms such as fixed-wing or rotary aircraft, tactical vehicles, or unmanned aerial vehicles. The other standoff detection system now in the research and

development phase is the Strategic Biological Stand-off Detection System, which will provide a long range/large area aerosol detection, tracking, and mapping capability. This system has the potential to detect aerosol clouds out to ranges of 100 km. The information will be used to provide early warning, enhance contamination avoidance efforts, and cue tactical-level (ie, unit-level) detection assets. The strategic standoff detection system is envisioned for theater use in existing fixed- and rotary-wing aircraft.[18]

The principal difficulty in detecting biological agent aerosols stems from differentiating the artificially generated biological warfare cloud from the background of organic matter normally present in the atmosphere. Therefore, medical protection (vaccines and other prophylactic measures), intelligence, medical surveillance and medical diagnostics, and physical protection during times of high threat must be relied on as primary defenses in the absence of currently fielded environmental biological detectors.[18]

Epidemiological Surveillance

An epidemiological surveillance system that closely monitors unusual illnesses or outbreaks of disease is also extremely important. It is difficult for command medical advisors to know if a disease outbreak is consistent with a biowarfare attack unless background rates of disease for an area of operations are known. A theaterwide epidemiological surveillance program is therefore a necessity for informed advice to the theater commander. Surveillance programs must be specifically tailored to both the mission and the geographical area where the mission is based, and they must be focused toward specific, diagnosable disease entities. Generic surveillance systems that lump disease and injury into broad categories such as “respiratory” or “dermatological” are nearly useless as epidemiological indicators of a biological warfare attack. Early and rapid analysis of specific epidemiological surveillance data may be the first clue, however, that such an attack has occurred.

In addition to specific clinical clues that suggest illness from biological threat agents (eg, a widened mediastinum in the terminal phases of inhalational anthrax), certain epidemiological indicators may suggest that a biological warfare attack has occurred (Exhibit 20-3).[19] All medical surveillance teams and healthcare providers need to keep these indications in mind; findings such as these should heighten suspicion that a biological attack has occurred and provoke specific and vigorous diagnostic steps aimed at isolating the cause of the outbreak.

Medical Diagnostics

Medical diagnostics are also extremely important. A field medical laboratory capable of identifying live agents or toxins quickly and accurately in medical samples is an absolute necessity if the use of biological weapons by the enemy is a possibility. Viral agents and toxins present the most difficult diagnostic problems for current diagnostic tests, which are based on polymerase chain reaction (PCR), radioimmunoassays, or enzyme-linked immunosorbent assays (ELISAs). Laboratory capabilities must be tailored carefully to intelligence information on enemy biological warfare capabilities and also to information on specific endemic diseases that are likely to occur in the theater of operations.[18]

EXHIBIT 20-3
INDICATIONS OF POSSIBLE BIOWARFARE ATTACK

- A disease entity that is unusual or that does not occur naturally in a given geographic area, or combinations of unusual disease entities in the same patient populations
- Multiple disease entities in the same patients, indicating that mixed agents have been used in the attack
- Large numbers of both military and civilian casualties when such populations inhabit the same area
- Data suggesting a massive point-source outbreak
- Apparent aerosol route of infection
- High morbidity and mortality relative to the number of personnel at risk
- Illness limited to fairly localized or circumscribed geographical areas
- Low attack rates in personnel who work in areas with filtered air supplies or closed ventilation systems
- Sentinel dead animals of multiple species
- Absence of a competent natural vector in the area of outbreak (for a biological agent that is vector-borne in nature)

Source: Wiener SL, Barrett J. Biological warfare defense. In: *Trauma Management for Civilian and Military Physicians*. Philadelphia, Pa: WB Saunders; 1986: 9.

Both ELISA-based testing and specific testing for minute amounts of specific genetic material of biological agents in medical samples using PCR techniques are feasible for deployment and use in a field setting.[20] Both epidemiological surveillance and field laboratory capabilities are receiving a great deal of attention currently as the U.S. Army Medical Department prepares for the next conflict in which both endemic disease and biological warfare are legitimate threats.

SUMMARY

Biological warfare could be used against the United States in a theater of operations or against our civilian populations in any number of realistic scenarios. The medical consequences of such use are potentially catastrophic unless measures are taken to minimize the potential impact of biological warfare agents on our people. Proper planning for terrorist as well as military scenarios, better and more realistic training under NBC conditions, adequate environmental detection and monitoring, improved vaccines and prophylactic drugs for our military, and sensitive and specific medical diagnostics are all pathways toward defending against the possible nightmare of biological attack.

REFERENCES

1. Manchee R, Stewart W. The decontamination of Gruinard Island. *Chem Br*. 1988;July:690–691.
2. McNally RE, Morrison MB, Stark M, et al. *Effectiveness of Medical Defense Interventions Against Predicted Battlefield Levels of* Bacillus anthracis. Joppa, Md: Science Applications International Corp; 1993.
3. Patrick WC. Overview of Biological Warfare. Frederick, Md; October 30, 1992. Unpublished manuscript.
4. North Atlantic Treaty Organization. *NATO Handbook on the Medical Aspects of NBC Defensive Operations*. A-Med-P6, part 2, Biological. June 1992. Final draft.
5. World Health Organization. *Health Aspects of Chemical and Biological Weapons*: *Report of a WHO Group of Consultants*. Geneva, Switzerland:

WHO; 1970.

6. Huxsoll D, Patrick WC, Parrott C. Veterinary services in biological disasters. *JAVMA*. 1987;190(6):714–722.

7. Department of the Army. Special Report to Congress. *US Army Activities in the US Biological Defense Programs*, *1942–1977*. Vols 1 and 2. Washington, DC: DA. 24 Feb 1977. Unclassified.

8. Special Subcommittee on the National Science Foundation of the Committee on Labor and Public Welfare, US Senate. *Chemical and Biological Weapons: Some Possible Approaches for Lessening the Threat and Danger*. Washington, DC: US Government Printing Office; May 1969.

9. Special Subcommittee on the National Science Foundation, Committee on Labor and Public Welfare. *Chemical and Biological Weapons*. Washington, DC: US Government Printing Office; May 1969.

10. Ursano R. Combat stress in the chemical and biological warfare environment. *Aviation Space & Environmental Medicine*. 1988;59(12):1123–1124.

11. Mojecki JA. Mission-essential training and weapons of mass destruction. *Military Review*. 1994;74(10):91–93.

12. Geissler E, ed. *Biological and Toxin Weapons Today*. Stockholm International Peace Research Institute. Oxford, England: Oxford University Press; 1986.

13. Douglas JD Jr, Livingstone NC. *America the Vulnerable: The Threat of Chemical and Biological Warfare*. Lexington, Mass: Lexington Books; 1987.

14. US Department of State. *International Terrorism*. Washington, DC: DOS; 1989. GIST.

15. Zilinskas RA. Terrorism and biological weapons: Inevitable alliance? *Perspect Biol Med*. 1990;34(1):44–72.

16. Bates S. Fairfax man accused of anthrax threat. *Washington Post*. 3 March 1992;C-3.

17. Kupperman RH, Smith, DM. Coping With Biological Terrorism. Washington, DC; 22 Aug 1991. White paper; unpublished.

18. US Army Medical Research Institute of Infectious Diseases. *Medical Management of Biological Casualties Handbook*. Fort Detrick, Frederick, Md: USAMRIID; August 1993.

19. Wiener SL, Barrett J. Biological warfare defense. In: *Trauma Management for Civilian and Military Physicians*. Philadelphia, Pa: WB Saunders; 1986: Chap 35: 507–528.

20. Caudle L. Major, Medical Corps, US Army. Officer in Charge, Problem Definition and Assessment Team, Operation Vigilant Warrior. Personal communication, 1994.

Chapter 21

THE BIOLOGICAL WARFARE THREAT

LESTER C. CAUDLE III, M.D., M.T.M. & H.*

INTRODUCTION

EVIDENCE OF A SOVIET BIOLOGICAL WARFARE PROGRAM
- Indirect Evidence
- Direct Evidence
- Current Evidence

PROLIFERATION OF BIOLOGICAL WEAPONS
- Military Incentives
- Technical Incentives
- Economic Incentives
- Political Incentives

NONHUMAN TARGETS OF ATTACK
- Animals
- Crops
- Material

THE CURRENT THREAT
- North Korea and China
- Iraq
- Foreign and Domestic Terrorism

SUMMARY

* *Lieutenant Colonel, Medical Corps, U.S. Army; formerly, Command Surgeon, On-Site Inspection Agency, P.O. Box 17498, 201 West Service Road, Dulles International Airport, Washington, D.C. 20041-0498; currently, Assistant Chief, Department of Retrovirology, U.S. Army Medical Component, Armed Forces Research Institute of*

Medical Sciences, APO Area Pacific 96546, 315/6 Rajvithi Road, Bangkok 10400, Thailand

INTRODUCTION

Biological warfare agents may be more potent than the most lethal chemical warfare agents and provide a broader area of coverage per pound of payload than any other weapons system. The proliferation of technology and the scientific progress in biochemistry and biotechnology have simplified production requirements and provided the opportunity for creation of exotic agents.[1]

Genetic engineering holds perhaps the most dangerous potential. Pathogenic microorganisms capable of creating a novel disease, perhaps on an epidemic scale, could be tailor-made. Suppose that an adversary inserted a gene lethal to humans into a virus or bacterium. This agent could then spread a disease that could overwhelm the diagnostic, therapeutic, and preventive capacity of a country's health service.[2] The threat posed by new biological and chemical weapons requires our urgent attention:

> Certainly it is of great importance that new and insidious biochemical weapons are being developed and deployed, in total disregard for existing treaties, while the perpetrators routinely deny all charges and, in turn, counter that their accusers are fabricating the allegations as a propaganda device. It seems clear that the highest priority should be afforded this issue for efforts toward its resolution.[3(p498)]

The use of biological agents in future wars and terrorist attacks is a realistic concern. The difficult issues of degraded troop performance and healthcare delivery are serious threats, and resources must be allocated to develop an effective response plan. Countering this threat will require that the highest levels of our government find ways to[1]

- strengthen and verify international arms control agreements,
- maintain the United States's ability to respond with a broad range of alternatives against any aggressor who attempts to proliferate biological weapons, and
- maintain a robust biological defense effort for our U.S. armed forces, one that would equally and effectively

> apply to our civilian population in case of use by terrorists.

Unfortunately, biological weapons are considered by some nations to be part of their military armamentarium, and there are military, technical, economic, and political incentives for nations to develop and maintain such a program. A goal of this chapter is to present the evidence in such a way that the reader can conclude that the threat is real and significant; it is neither in the realm of science fiction nor confined to our own nation. As a Russian writer stated in 1993:

> I have been gathering information on bacteriological weapons (BW) for several years. Out of all the means of mass destruction, this kind can be considered as the most mysterious.[4(p15)]

EVIDENCE OF A SOVIET BIOLOGICAL WARFARE PROGRAM

We have known for many years that the Soviet Union maintained an offensive biological warfare program in violation of the 1972 Biological Weapons Convention, which they initiated and signed without reservation. This knowledge has been reported to the U.S. Congress and the American public since 1984, and our government has repeatedly raised this subject, first with the Soviet leadership, and more recently with the current Russian leadership.[5]

Indirect Evidence

Beginning in the latter 1970s and continuing throughout the 1980s, U.S. intelligence agencies repeatedly alleged the existence of a Soviet biological weapons program. Then in April 1979, a major outbreak of anthrax in the city of Sverdlovsk (now Yekaterinburg) caused the death of a number of Soviet citizens from pulmonary anthrax.[1] (This event is also discussed in Chapter 22, Anthrax.) Details about this epidemic were not disseminated by the Soviets, and it was not until much later that the Western communications media became aware of it.

On February 13, 1980, the widely circulated German magazine *Bild Zeitung* carried a story describing an accident in a military settlement in Sverdlovsk in which an anthrax cloud resulted, which prevailing winds carried into the outskirts of the city. The magazine article went on to state that only a chance change in wind direction prevented the cloud from passing through the main section of the city. When this story was published, major Western newspapers finally began to take an interest in

the event.

Several weeks later, the U.S. government asked the Soviet government for an explanation of the epidemic—specifically, whether this was a biological warfare experiment gone awry. Moscow denounced the suggestion as slanderous propaganda but did acknowledge the outbreak of anthrax. They insisted that the outbreak was caused by poor food control, which had somehow allowed contaminated meat to be dispensed to the population of Sverdlovsk.[6]

However, a recently released report[7] on a series of 42 autopsies, done on what is thought to be the majority of the fatalities from this outbreak, consistently revealed pathological lesions diagnostic of inhalational anthrax: hemorrhagic necrosis of the thoracic lymph nodes involved in the lymphatic drainage of the lungs, and hemorrhagic mediastinitis. These are not findings seen in gastrointestinal anthrax associated with contaminated meat.

The U.S. government found Moscow's explanation disturbing for several reasons, including strong U.S. intelligence reporting that listed Sverdlovsk as a site of a biological warfare establishment; the fact that shortly after 3 April 1979, D.F. Ustinov, then the Soviet Minister of Defense, visited Sverdlovsk; and the difficulty in imagining an anthrax epidemic attributable to contaminated meat in a developed nation with a highly effective and functioning public health service.[6] Last and perhaps even more compelling, satellite photography had revealed some time earlier that the compound in which the accident took place (Military Compound 19) was heavily guarded, had special security precautions, and had ventilation and animal pens typical of a biological weapons facility.[8]

In February 1992, Russian President Boris Yeltsin admitted that the 1979 outbreak of anthrax in Sverdlovsk was indeed linked to an accident at a secret biological weapons facility. He went on to reveal that the former Soviet Union had conducted an illegal biological warfare program in violation of the 1972 Biological Weapons Convention. He further acknowledged that the Soviet Union had failed (1) to implement its commitment under the convention to destroy existing biological weapons and (2) to conduct research only for defensive purposes. He also said that "past military efforts had crossed the line set by international treaties."[1(p16)]

The Washington Post published an article in August 1992 that stated that shortly after Yeltsin's admission, a confidential report on the extent of the Russian biological weapons program had been prepared by Anatoly Kuntsevich, a retired Russian general and a former director of Soviet research on chemical arms. Kuntsevich stated in the report that the military had illicitly developed aerial bombs and rocket warheads. These

warheads were capable of carrying several biological warfare agents, including anthrax, tularemia, and Q fever. General Kuntsevich revealed that the biological weapons effort had been maintained through at least 1990 but had been scaled down during the 6 years of Mikhail Gorbachev's presidency.[9]

In April 1992, Yeltsin issued a decree that outlawed all activities that were prohibited by the 1972 Convention. General Kuntsevich stated, following Yeltsin's decree, that all offensive biological weapons programs in territorial Russia had been banned and placed under firm control of the government; that the large biological test area near the Aral Sea, Vozrozhdeniya Island, had been closed; and that Russia had no stockpiles of biological weapons.

Unfortunately, that same month, Russia failed to meet the deadline set forth for all Convention signatories to declare their total inventory, stockpiles, and production sites for offensive and defensive biological weapons programs. This requirement had been established in the set of confidence-building measures that had been agreed upon at the Third Review Conference on the Biological Weapons Convention in September 1991, at which Russia was a participant. The Russian government finally submitted their report in late July 1992, but, according to U.S. officials, the report lacked key details. This delay only strengthened our heightened concerns about the extent of the former or still-existent Soviet program.[9]

The Soviet offensive biological weapons program was monitored by the United States for decades. We know that scientific personnel at a number of microbiological research institutes in the former Soviet Union are capable of performing research and development with highly infectious disease agents and very potent plant, animal, and microbial toxins. Likewise, their considerable work in aerobiology, cloud physics, airborne infections, and disease-agent stabilization has direct application to biological warfare. Much of the knowledge and technical expertise at these institutes was in the recent past and possibly still is funded and utilized by the Ministry of Defense for offensive purposes as well as for defensive and protective aspects of biological warfare.[10]

Direct Evidence

Much of our current knowledge concerning the former Soviet Union's biological warfare program has been gleaned from Vladimir Pasechnik, a microbiologist who defected to the West in 1989 while attending a scientific conference in London, England. For years, Pasechnik had pled with Soviet officials to allow him to switch some of his research effort to civilian purposes, for which it had originally been established.

Pasechnik was born in 1939 in Stalingrad, a city that was largely

destroyed during World War II. He became one of the brightest young scientists in the Soviet Union, determined to use his talent working for peace and never allowing his research in biophysics to be used to further a means of destruction that had cost the lives of his family. In 1974, a general from the Soviet Ministry of Defense offered Pasechnik the opportunity to set up and run his own biotechnology laboratory. The offer included an unlimited budget to buy Western equipment and the ability to recruit the best scientists in the Soviet Union.

Pasechnik was not concerned that the offer had come from the Ministry of Defense, as the military was the driving force behind most research conducted in the Soviet Union, even projects that had strictly civilian application. He built up his laboratory over the better part of the next decade, hiring 400 well-credentialed scientists to staff the facility, acquiring a relatively unlimited budget that gave his staff access to the latest Western biotechnology. The laboratory began operating in 1981, and during the next 2 years Pasechnik realized the truth: far from running a state-of-the-art civilian research operation, he was part of a vast network of laboratories and factories working on deadly new weapons of war. A newspaper story quoted Pasechnik as stating:

> Officially we were involved in vaccine development and in producing preparations for protecting crops, but in fact, we were developing methods of production and equipment for a huge biological warfare program.[11(p1)]

He revealed a network of nominally civilian research institutes known as Biopreparat. This organization was created in 1973 by the Soviet Central Committee and the Council of Ministers as a cover for the existing military program. Biopreparat was a huge organization, employing more than 25,000 people at 18 or more research and development facilities, including six mothballed production plants and a major storage complex in Siberia.[12] Their budget in the early 1980s was 200 million rubles and tens of millions of U.S. dollars per year.

One of the goals was to genetically alter known pathogens in the hope of making the pathogens resistant to Western drugs. The institutes were also directed to produce new strains of diseases, more powerful than those previously known to science, and to produce them in aerosol form. In 1983, at a Biopreparat research institute in Obolensk, 60 miles south of Moscow, scientists developed their first superplague: a new strain of tularemia. Testing revealed such favorable results that Moscow gave permission for full-scale development and production. Then in 1985, scientists at the same institute were asked to develop a more deadly strain of pneumonic plague.

Pasechnik's role was to refine the production process to make the development more efficient. He claims that by 1987 the Soviet Union had sufficient industrial capability within Biopreparat to manufacture 200 kg of the superplagues every week, enough to kill up to 500,000 people. Owing to the fairly short half-life of the agent, these agents were never produced on this scale; in fact, only enough for testing was produced.

The fact remains, however, that the Biopreparat network stood ready to begin full production when told, at a time in the future designated as Day X. The Soviet Defense Ministry built these biological weapons, known as Weapons of Special Designation, into their military planning. They were to be used not only as a last resort but also as support weapons in a conventional (nonnuclear) conflict, to attack the enemy's troop reserves and hamper his operations at ports and rail centers.[11]

Pasechnik had first-hand knowledge of the program: he was a director of the Biopreparat organization known as the Institute of Especially Pure Biopreparations in Leningrad, and was also a member of the Scientific and Technical Committee, which was composed of institute heads. An article in the popular press[12] noted that, according to Pasechnik, Biopreparat officials had discussed putting weaponized forms of these agents into terrorists hands.

Current Evidence

Vladimir Pasechnik's story has been confirmed by another well-placed defector from the Biopreparat program whom the Central Intelligence Agency (CIA) brought out in late 1992. This second defector even stated that the research and development of new strains of genetically engineered superweapons were continuing up to the time of his defection, despite Yeltsin's decree that the program be dismantled. Again in the fall of 1993, a third defector from the Biopreparat group spoke to British intelligence and confirmed the stories of both previous defectors.[11]

Sound, compelling evidence from these recent defectors; confrontations among the leaders of the United States (President Bush), United Kingdom (Prime Minister Thatcher, Prime Minister Major), and the Soviet Union (President Gorbachev); and confrontations between U.S. President Clinton and Russian President Yeltsin have all failed to alleviate U.S. concerns about the current Russian biological warfare program. Invited by the Russians, a joint United States–United Kingdom delegation visited several Russian sites in 1990. The trip was intended to be reassuring but had the opposite effect. The team discovered a large aerosol test chamber that was designed to spray aerosols on animals tied down at various locations within the chamber. They also discovered a

chamber used to test delivery systems for biological weapons. At another site, they saw row after row of huge fermentation vessels. All this evidence corroborates what Vladimir Pasechnik told.[11]

In 1992, in an attempt to meet the concerns expressed by the United States and the United Kingdom, Russia emphasized its commitment to comply with the Biological Weapons Convention officially, in a three-way agreement with the United States and the United Kingdom. The agreement, officially known as the Trilateral Agreement, was struck after negotiations in Moscow between U.S. Undersecretary of State Frank Wisner and senior British and Russian officials. This meeting resulted from an official request by the U.S. State Department on 31 August 1992 to the Russians for reassurance that the germ weapons program of the former Soviet Union has been terminated.[13]

The Trilateral Agreement (1) is intended to build confidence that the Russians will dismantle the former Soviet Union's offensive biological weapons program; (2) commits Russia to *(a)* open suspect facilities to inspection, *(b)* convert biological warfare facilities to civilian production, and *(c)* end biological weapons projects except for defensive programs; and (3) provides for mutual inspection of biological sites in the United States, the United Kingdom, and Russia. U.S. and British inspectors may visit any suspect site and conduct inspections as intrusive as necessary to resolve concerns. Russian inspectors will be permitted to visit U.S. and British civilian biological research facilities once the initial round of inspections within Russia is completed.[1] Military facilities will be open to inspection after the suspect civilian facilities have been inspected.

In 1991, a United States–United Kingdom delegation visited the St. Petersburg Institute of Especially Pure Biopreparations, which Vladimir Pasechnik had said was the site of work on biological weapons. The first visit under the Trilateral Agreement occurred in the fall of 1993, after a year of talks concerning the protection of proprietary information during the visits to the three participating countries. The delegation to this visit toured two sites: an installation at Berdsk, a town near Novosibirsk, Siberia, and Pokrov, a site outside Moscow. In January 1994, the United States–United Kingdom delegation visited two more sites in the former Soviet Union; later that year, a Russian delegation visited one nonmilitary site in the United Kingdom and three in the United States. Anatoly Kuntsevich, director of Russia's Presidential Committee on Problems of Chemical and Biological Disarmament, stated that these visits should put the Western concerns to rest; however, American officials say that ambiguities remain and that a working group should be formed to discuss the two sides' past biological warfare programs.[14]

Not fully alleviating the United States's concerns about the continuation of the former Soviet program, but a positive step forward, was Yeltsin's

firing of Kuntsevich in April 1994. Kuntsevich had played a leading role in development of the former Soviet Union's chemical arsenal. He was regarded by some in Washington as an obstacle to progress on the germ weapon issue, and, in fact, was one of the main persons behind the attempt to hide the continuation of the Russian program from Yeltsin after Yeltsin had stated that it would be dismantled. President Yeltsin's office attributed Kuntsevich's dismissal to a "gross violation of his duties."[15(pA-28)] A news brief reported on 7 April 1994 by the Information-Telegraph Agency of Russia–Telegraph Agency of the Soviet Union (ITAR-TASS) stated that a Moscow daily newspaper, *Kommersant*, speculated that a connection existed between Western press accusations of continuing Russian work on biological warfare and the firing of Anatoly Kuntsevich.

Doubts linger in the West about Russia's claims that no biological weapons were ever produced and that all activities have been halted, never to be restarted. Moreover, the West worries that the political leadership in Moscow may be unable to ever get a full disclosure or a firm commitment to cease all biological warfare activities beyond those permitted by the Biological Weapons Convention of 1972.[16] In light of all these revelations, it is clear that the United States must maintain a strong Biological Defense Research Program.

PROLIFERATION OF BIOLOGICAL WEAPONS

International proliferation of biological weapons programs broadens the range of agents that U.S. forces may encounter. The modernization of many Third World nations with the subsequent development of industrial, medical, pharmaceutical, and agricultural facilities needed to support these advancing societies also provides the basis for development of a biological weapons program should that nation decide to pursue such an endeavor. A biological weapons program can be easily concealed within legitimate research and development and industrial programs within those countries that seek such capabilities.[1] In December 1991, Robert Gates, then Director of Central Intelligence, stated in testimony before the U.S. House of Representatives Armed Services Committee's Defense

EXHIBIT 21-1
INTERNATIONAL BIOLOGICAL WEAPONS PROGRAMS

Known
- Iraq
- Former Soviet Union

Policy Panel:

> The accelerating proliferation of nuclear, biological, and chemical weapons in other countries around the world is probably of gravest concern. The more countries that possess such weapons—even if acquired for deterrent purposes—the greater the likelihood that such weapons will be used.[1(p2)]

Probable

- China
- Iran
- North Korea
- Libya
- Syria
- Taiwan

Possible

- Cuba
- Egypt
- Israel

Source: Committee on Armed Services, House of Representatives. Special Inquiry Into the Chemical and Biological Threat. *Countering the Chemical and Biological Weapons Threat in the Post-Soviet World.* Washington, DC: US Government Printing Office; 23 Feb 1993. Report to the Congress.

A report issued by the Committee on Armed Services, U.S. House of Representatives, on its inquiry into the chemical and biological threat noted that 11 nations possess or could develop an offensive biological weapons capability (Exhibit 21-1). These nations are in addition to the 31 that already possess or could develop an offensive chemical weapons capability.[1] While many in government, intelligence, and diplomatic circles express grave concern about the proliferation of biological weapons, there has been relatively little carryover into the general public. In part, the relative lack of interest in the proliferation of biological weapons can be attributed to the view that the Biological Weapons Convention successfully deters nations from pursuing a biological warfare capability. But another explanation is that the facts are hidden from the public domain: much of the information on biological weapons and proliferation is classified, and only those with a “need to know” have access.

An analysis of the incentives associated with a biological weapons program may offer the best insight into the current proliferation problem. Such a program has military, technical, and economic, as well as political, incentives.[17] Unless positive international controls can be established, the proliferation of biological weapons will probably proceed unchecked.

Military Incentives

From a military viewpoint, the ability of biological warfare to produce large numbers of casualties makes these weapons highly attractive for long-range targeting of populations. Detailed in a report from the World Health Organization on the health aspects of the use of these weapons, the enormous impact these weapons would have on a population can readily be seen. According to this study, if a biological agent such as anthrax were used on an urban population of approximately 5 million people in an economically developed country such as the United States, an attack on a large city from a single plane disseminating 50 kg of the dried agent in a suitable aerosol form would affect an area far in excess of 20 km downwind, with approximately 100,000 deaths and 250,000 being incapacitated or dying. In the same scenario but using a different agent (such as Q fever), we would expect to find only several hundred deaths but the same number of people temporarily incapacitated.[18]

The U.S. military confirmed the magnitude of the effects on a population such as that described above when it looked at the combat effects of major biological agents in studies conducted at Dugway Proving Ground, Utah.[19] We cannot help but ask: Is our medical system (civilian or military) ready for such a casualty situation—from either the purely medical patient-management perspective or the psychological perspective that the mass casualty situation would incite?

For the sake of argument, we might agree at this point that if nuclear weapons are not attainable, then biological weapons could be a feasible and economical alternative, filling the strategic role of nuclear weapons. Biological weapons could very easily fill an intimidation role. Further, if the agent and means of delivery were both carefully selected, biological weapons could also play a tactical role against a wide array of battlefield targets, including communications and logistical centers as well as areas of high troop concentrations. Because some biological agents (eg, Q fever, staphylococcal enterotoxin) are likely to incapacitate more victims than they kill and do not cause substantial collateral destruction, they have some significant advantages over nuclear weapons.[17]

It seems logical to conclude that countries might want to add biological warfare weapons to their military armamentaria out of fear, believing that they may be at a military disadvantage if their enemies have these weapons. Robert Gates, former Director of Central Intelligence, stated, in a speech delivered in San Francisco, California, to the Comstock Club on 15 December 1992, that for some,

> these weapons represent symbols of technical sophistication

> and military prowess—and that acquiring powerful weapons has become the hallmark of acceptance as a world power.[1(p4)]

Technical Incentives

In analyzing the biological weapons proliferation problem by scrutinizing the technical incentives that might attract a country to pursue such a program, we quickly see the comparative ease with which production of many biological warfare agents can be accomplished. Virtually all the technology needed to support a biological weapons program is dual use, obtainable off the shelf for a variety of legitimate purposes, and widely available.[16] This technology is very different from nuclear warfare technology, which requires dedicated facilities, or chemical warfare technology, where the agents have little if any civilian application. In addition, both nuclear and chemical technologies require raw materials that make innocent and legitimate use difficult to explain to the international community.

On the other hand, the equipment and technology used for offensive biological weapons research is no different from that used in legitimate biomedical research anywhere in the world. Equipment for processing and production of such agents is found in such diverse industries as wine and beer manufacture, pharmaceutical research and development, and the food and agriculture industries.[17] Few items need to be purchased from the “outside,” and simple fermentation media are easy to make.[20]

Advanced capabilities have proliferated as Western universities have produced an abundance of graduate students in the biological sciences with training and expertise on the leading edge of biotechnology. Third-world facilities engaged in biological research are rapidly becoming more advanced as the dissemination of scientific information is at an all-time high. Dissemination equipment that could be purchased and used as off-the-shelf items, with few or minor modifications, may be found in the agriculture industry (sprayers) and hardware stores (paint sprayers), to name just a few.[17]

The potential agents may *themselves* be considered dual-use items. For example, botulinum toxin has been used investigationally for many years to treat ocular muscle disorders, and was approved in 1990 by the U.S. Food and Drug Administration (FDA) for intramuscular treatment of strabismus and blepharospasm.[21] As another example, saxitoxin has been widely used for decades to study the sodium channel in nerve tissue, and algal toxins are used in research as potential antineoplastic agents. Stating this concept another way, *all* of the infectious agents (bacteria,

> fungi, and viruses) as well as the toxins can be considered dual use in this sense: they can be used purposefully to inflict disease in humans, animals, and plants. Therefore, research needs to be done (and is, in fact, ongoing) on these agents so that better means of detection, prevention, and treatment of disease may be devised. Thus, we must acknowledge that potential biological warfare agents are currently found worldwide in laboratories and medical centers.[17]

Economic Incentives

In analyzing the biological weapons proliferation problem through economic incentives that might attract a country to pursue such a program, we realize that the start-up costs of biological weapons programs are not prohibitive. This becomes a major incentive, especially relative to the cost of embarking on a nuclear weapons program.[17] The cost of a biological program is much less than either a nuclear or chemical program: estimates vary from $2 billion to $10 billion for a nuclear program; to tens of millions for a chemical program; to less than $10 million for a biological program.[20] From a purely economic standpoint, we can understand why biological weapons, according to a famous saying, are a poor man's nuclear bomb and can be produced by Third World countries.[4]

Even the weapons used to deliver these agents are relatively cost-effective. A group of chemical and biological experts, appearing before a United Nations panel in 1969, estimated that

> for a large-scale operation against a civilian population, casualties might cost about $2,000 per square kilometer with conventional weapons, $800 with nuclear weapons, $600 with nerve-gas weapons, and $1 with biological weapons.[22(p16)]

Unfortunately, long-range delivery systems are also proliferating. Aging ballistic missiles that the superpowers have discarded as obsolete are being acquired by Third World nations. The lighter biological warhead seems to extend the range of these missiles, and inaccuracy is a lesser problem with biological weapons, as dissemination of the agent depends on environmental conditions.[23]

Political Incentives

Two distinct political incentives might persuade a country to pursue a biological weapons program: (1) domestic and international status and (2) a favorable risk–benefit ratio. First, a country's ability to threaten its enemies with a weapon capable of inflicting mass casualties offers some tangible advantages.[17] W. Seth Carus, Director for Defense Strategy on the Policy

Planning Staff in the Office of the Secretary of Defense, summarizes this political incentive in the following statement:

> The perceived need for deterrence or compellence [sic] capabilities, a desire to influence the political-military calculations of potential adversaries, the search for national status, and even bureaucratic and personal factors can play a role in the initiation of such programs.[16(p22)]

Second, detecting a clandestine biological warfare program is difficult. The risk is relatively low that biological weapons research and development will be uncovered and confirmed—unlike a nuclear or chemical weapons program. Because virtually all of the equipment associated with biological weapons can be used for legitimate purposes, there is no easily discriminated, unambiguous signature.[16]

A country can undertake many illicit biological warfare activities—save actual use—toward developing a sophisticated offensive biological warfare program without provoking inquiries from the international community.[17] Even signatories to the Biological Weapons Convention can investigate, for public health and commercial purposes, the properties of bacteria, viruses, and toxins that are considered to be biological warfare agents without ever violating the convention. Legitimate production facilities can produce such agents for offensive purposes on short notice, and with no more than a temporary slowdown on their routine production output. Because biological weapons can be configured out of preexisting conventional or chemical munitions that have already been declared to international groups, such weapons do not always require extensive testing. Thus, biological weapons can be a nation's "silent equalizer" but not cost a large percentage of the country's gross national product.

Weapons of mass destruction should be compared from such perspectives as technology, cost, signature, effectiveness on troops—protected and unprotected—and tactics as well as strategy (Table 21-1). We can see from looking at Table 21-1 that a nuclear weapons program requires a very high level of technology and a significant investment in a unique and distinctive infrastructure for research, development, production, and support of such weapons on the battlefield.[1] The outlay of facilities that are associated with a nuclear weapons program usually results in a distinctive and readily observable signature. Chemical and biological weapons programs, on the other hand, usually require lower levels of technology and monetary investment. The infrastructure for development and production of chemical and biological agents can be embedded in the industrial chemical and pharmaceutical infrastructure of any modern or developing state, thus resulting in a nondistinctive and readily observable signature.

TABLE 21-1
COMPARISON OF WEAPONS OF MASS DESTRUCTION

				Effectiveness			
				Protected Troops		Unprotected Troops	
Type	**Technology**	**Cost**	**Signature**	**Tactical**	**Strategic**	**Tactical**	**Strategic**
Nuclear	++	++	++	++	++	++	++
Chemical	+	+	+	-	-	++	+
Biological	+	-	-	-	-	+	++

++: very high; +: high; –: lower
Adapted from Committee on Armed Services, House of Representatives. Special Inquiry Into the Chemical and Biological Threat. *Countering the Chemical and Biological Weapons Threat in the Post-Soviet World.* Washington, DC: US Government Printing Office; 23 Feb 1993: 7. Report to the Congress.

In trying to ascertain these weapons' effectiveness, we must keep in mind that nuclear weapons have immediate and decisive effects, whether employed tactically on the battlefield or strategically against rear areas, and regardless of whether personnel in the area are protected or not. On the other hand, protective measures can provide a high level of protection against chemical and biological weapons if they are in use at the time these weapons are employed. This requires an effective and timely detection system, as these protective measures cannot continually be in place. Finally, the delayed effect of most biological weapons (as a result of the incubation period before becoming ill) causes us to question the tactical utility of biological weapons.[1]

NONHUMAN TARGETS OF ATTACK

We must remember that humans need not be the only target of biological weapons. These weapons might be meant to attack a country's animal herds, crops, or even a material supply that is vital for revenue. For example, if purposely introduced into a large pig population, swine fever virus, also known as wart hog disease, could destroy the herd. This attack could be carried out with relative ease and virtually no immediately visible signature. The usual method of controlling such widespread disease—mass slaughter of the infected animals—can be economically devastating to many countries.[22]

The main purpose of this use of biological agents is to affect humans indirectly by limiting their food supply.[24] Such use of biological agents could initiate a longer-term decline in food stores, which could then add the prospect of starvation of a population to the immediate devastating effects of conventional weapons. A population dependent on a staple crop such as rice could be rendered helpless by the introduction of a specific disease that devastated their unprotected rice crop.[25]

Likewise, a purposely introduced organism that degrades specific metals and renders them useless could also be devastating for a small country's economy. With increasing technology, specific antimaterial agents could be designed and could create enormous problems.

Animals

The purposeful spreading of infectious agents that attack cattle or other domestic animals can lead to serious consequences for a country's food supply or export of animal products (hides, wool, fats, and biological medicinal products such as adrenalin, insulin, pituitary extracts, cortisone, vaccines, and antisera).[26] The first 20th-century allegations of the use of biological warfare were made against the Germans, this being in the form of antianimal use. In 1915, the Germans were alleged to have inoculated horses and cattle with disease-producing bacteria before the animals were shipped from the United States to our allies in Europe. The following year, the Germans again allegedly inoculated horses with glanders and cattle with anthrax, this time in Bucharest, Romania.[27] Then in 1917, they supposedly employed similar tactics to infect 4,500 mules in Mesopotamia. As expected, Germany issued official denials of these allegations.[28]

An example of economic setback (albeit as a result of unfortunate natural circumstances) caused by disease among animals occurred when African swine fever was spread from Africa to the Iberian peninsula during the latter 1950s. The annual loss amounted to $9 million as a result of

reduced pig production.[29]

A much more recent occurrence was in January 1984, in the state of Queensland, Australia, when an extortionist threatened to infect the livestock with foot-and-mouth disease if certain prison reforms were not undertaken. Authorities could not afford to take this threat lightly: Queensland is home to 60% of the Australian beef industry. The Australian government sought urgent and highly secret consultations with the United States and some of its other allies. The threat was ultimately determined to be a hoax perpetrated by a local convict; however, the economic implications of such an attack are obvious, and even the Australian authorities concluded that it would have been almost impossible to prevent.[22]

It seems reasonable that aggressor forces will use this deliberate spreading of infectious agents—either in peacetime or immediately before an outbreak of war—with the intention of initiating economic breakdowns. From the very beginnings of our biological warfare program, our scientists felt confident of their abilities to cause catastrophic epidemics in domestic animal populations with such viral diseases as foot and mouth, rinderpest, wart hog disease, fowl pest, and hog cholera. Each disease had devastating mortality and could render a predictable epidemic-producing agent in the animal population, much more certain than the predictability of agents inducing epidemics in humans.[30]

Antianimal research began in 1942 in the United States and was initially concerned with developing methods for protecting our large livestock population against biological warfare attack. This research resulted in the development of vaccines to protect against rinderpest, a deadly cattle disease and Newcastle disease, a serious poultry affliction. Research was initially carried out at Camp Detrick (now Fort Detrick), Frederick, Maryland, but when research on a larger scale was needed, a facility was established at Camp Terry on Plum Island, New York. Two field tests of potential antianimal agents were conducted using hog cholera virus and Newcastle virus. The program at Camp Detrick was terminated in 1954. By agreement between the secretaries of defense and agriculture, the Department of Agriculture assumed responsibility for the defense of our livestock against biological warfare attack, and the Plum Island facilities were transferred to that agency.[31]

Crops

Plants, like man, have been plagued with numerous diseases, and history is dotted with recordings of human suffering caused by naturally occurring plant epidemics (epiphytotics). Biological antiplant agents (ie,

living organisms that cause disease or damage to plants; note that this definition includes insects and other crop-eating pests in addition to plant diseases), may be used intentionally by an enemy to attack food or economically valuable crops, thereby reducing a nation's ability to resist aggression.[26]

Attacks with biological weapons on a country's agriculture could have serious economic consequences and, if international sanctions or an embargo were in place, could prove devastating in the production of foodstuffs. Modern agricultural methods dictate that large areas be planted with genetically identical crops. This genetic homogeneity leaves entire regions susceptible to attack with an antiplant agent to which the crop is not resistant. Entire crops are thus susceptible to being wiped out during a single harvest season.[29] Resistant crops could eventually be planted, but enough seeds could not be produced in a short enough time to prevent dire economic consequences.

Speculations as to how to destroy a nation's agricultural base are not idle fantasy. Both the U.S.[32] and the British[33] governments sponsored extensive anticrop research programs during World War II. Not only were powerful herbicides developed but, in addition, fungi capable of eradicating specific crops were identified, and efforts were made to mass-produce them. In 1944 and 1945, serious consideration was given to destroying the Japanese rice crop using the fungus *Helminthosporium oryzae van Brede de Haan.* That this did not happen reflects not so much legal or moral factors but practical problems, notably the relative delay with which the desired effect would be obtained against the Japanese, compared to the effect of more conventional weapons such as firebombs.[33]

Research on biological warfare agents included strain selection, evaluation of nutritional requirements, development of optimal growth conditions and harvesting techniques, and preparation in a form suitable for dissemination.[31] Much of our knowledge in this area of anticrop biological warfare came from the British. In fact, the United States is said to have provided the money and resources, and the British provided the brains, as they had begun experimenting with this form of warfare much earlier than the United States.[33] Between 1951 and 1969, the U.S. Army carried out at least 31 anticrop tests, and rice and wheat blast fungi were stored at Fort Detrick and at Edgewood Arsenal, both in Maryland, and the Rocky Mountain Arsenal, Denver, Colorado. Experiments showed that 3 g of the rice blast fungi per hectare could infect between 50% and 90% of the crops exposed.[30]

The CIA was also involved in antiplant agents about the same time as the U.S. Army. In materials provided to the U.S. Senate Select Committee

on Intelligence Activities by the CIA during the mid 1970s, the agency acknowledged that it had developed “methods and systems for carrying out a covert attack against crops and causing severe crop loss.”[34(p16)] The CIA denied that it had ever employed such systems, and there is no evidence that it has ever used herbicides or biological agents against the crops of another nation.[34]

Some have wondered whether the United States may have been a target of bioterrorism as recently as the late 1980s. A very peculiar pattern had emerged in the spread of the Mediterranean fruit fly, a major threat to agriculture in California. In 1989, a panel of scientists led by Roy Cunningham, U.S. Department of Agriculture, met in Los Angeles, California, to discuss possible reasons for this unexpected ecological anomaly. Despite heroic attempts to eradicate this insect, new infestations repeatedly appeared in odd and unexpected places. Some members of the panel questioned whether a person or group was purposely breeding and releasing Mediterranean fruit flies. This scenario might have seemed totally farfetched had not the mayor of Los Angeles, Tom Bradley, received several letters during this time frame from a group calling itself The Breeders, which claimed to be spreading the insect to protest California’s agricultural practices.[35]

Material

Antimaterial biological warfare might be attempted by an adversary because biological agents are hard to detect and identify, are readily concealed, and can plausibly be denied. Antimaterial agents are organisms that degrade some item of material. Most of the material damage done by microorganisms is a result of natural contamination that grows only under special conditions of temperature and relative humidity. Fungi, for example, damage fabrics, rubber products, leather goods, and foodstuffs. Some bacteria produce highly acidic compounds that cause pitting in metals; these agents could create potential problems with stockpiled material.[24]

Other bacteria can use petroleum products as an energy source, producing organic residues that might clog fuel or oil lines. Imagine the disastrous implications for the military if such a residue-producing bacterial agent were purposely introduced into a jet fighter aircraft’s fuel system. If the residue were to clog the fuel line, the fuel pumps would be rendered inoperable.[24]

THE CURRENT THREAT

Despite the end of the Cold War, the United States still faces a range of

serious national security issues. One at the forefront is the issue of the proliferation of biological weapons, and the accelerated development of the capabilities to design and produce biological weapons on the part of many Third World nations.[17] The Committee on Armed Services of the U.S. House of Representatives, in their Special Inquiry Into the Chemical and Biological Threat, concluded that despite the demise of the Soviet Union, with its sizable chemical and biological arsenal, the threat has increased in terms of widespread proliferation, technological diversity, and the probability of use.[1]

Of the nations currently believed to have an offensive biological warfare program, only a few are candidates for a direct armed conflict with the United States. The most likely route for the United States or our allies to become involved in a biological conflict would be as third parties in regional conflicts, whether as members of a United Nations peacekeeping force or through an act of terrorism.[17] This is due to our increased participation in low-intensity operations, however, and does not diminish the threat that biological warfare poses to our forces fighting a conventional war.

North Korea and China

During the 1990s, hostilities have mounted in North Korea over allegations that evidence of nuclear weapons activities is being hidden from international inspectors. This is the kind of future conflict that could involve the United States. In 1993, the Russian Foreign Intelligence Service, successor to the Soviet Union's KGB, released a statement that said, in part:

> North Korea is performing applied military-biological research in a whole number of universities, medical institutes and specialized research institutes. Work is being performed in these research centers with inducers of malignant anthrax, cholera, bubonic plague and smallpox. Biological weapons are being tested on the island territories belonging to the DPRK (Democratic Peoples Republic of Korea).[36(pA-10)]

Mr. Gordon Oehler, director of the CIA's Non-Proliferation Center, confirmed this Russian report.[36] The use of smallpox in a weapon, whether the use be tactical or strategic, would have disastrous effects, as the military and civilians are not immunized against this disease nor have they been for many years.

In his last message to congressional leaders on 19 January 1993, outgoing President George Bush revealed intelligence reports (previously highly classified) that China still has an active biological warfare program—despite their having signed the international treaty banning such weapons.[37] Even as recently as May 1994, a reporter investigating China's military–industrial complex stated that arms sales, especially unreported sales and transfers of weapons of mass destruction to some of the most dangerous governments in the world, are part of the dark side of China's commercial activities. The newspaper report went on to state: "China has violated every non-proliferation pledge it has ever made, including its pledge not to engage in an offensive biological warfare program."[38(pC-3)] This is alarming, especially as we consider the close relationship between China and North Korea.

Iraq

As reported by the department of defense in 1992,[39] by the time Iraq invaded Kuwait, Iraq had developed the most extensive biological warfare program in the Arab world (despite having been a signatory to the 1972 Biological Weapons Convention outlawing such endeavors). After the Persian Gulf War (1990–1991), Iraqi authorities admitted the program included anthrax bacteria and botulinum toxin.[1] Then in August 1995, Iraq revealed to United Nations inspectors that it had had a far more extensive and aggressive biological warfare program prior to the Persian Gulf War than had previously been admitted. The Iraqi authorities claimed production of thousands of liters of botulinum toxin and of *Bacillus anthracis*. Additionally, they stated that unspecified amounts of both agents were loaded on Scud missile warheads and aerial bombs. In this same revelation to United Nations inspectors, the Iraqis claimed to have destroyed the agents during the January–February 1991 time frame, but they have yet to produce evidence to support their claim.[40]

Iraq's biological capabilities, described as "a sizable stockpile,"[41(pA-1)] by William Webster, former Director of Central Intelligence, caused the coalition forces to procure large quantities of special biological warfare vaccines (against anthrax and botulinum) for their military personnel. These vaccines were in extremely limited supplies, forcing commanders to allocate vaccine only to troops they believed were at greatest risk to the

use of these agents. Situations like this require commanders to make ethical decisions, as it was not known definitively who was at greatest risk.

The anthrax vaccine, which is licensed by the FDA, was given to approximately 150,000 military personnel (25%–30% of the deployed U.S. forces).[42] The botulinum toxoid vaccine was given to approximately 8,000 military personnel (1% of deployed U.S. forces).[42] The biological weapons threat posed by Iraq during this crisis has been characterized as the most serious such threat faced by U.S. forces since World War I.[1] During January 1991, the image of a scared, shaking television reporter wearing a gas mask and the trembling of his voice as he reported the detonation of an incoming Iraqi Scud missile near Tel Aviv, Israel, brought home to all a frightening view of the increasing sophistication of the chemical and biological threat and the apparent vulnerability of soldiers and civilians alike.

The reasons why Iraq did not use these weapons during the Persian Gulf War, the subject of much speculation, will probably never be known with certainty. However, this Iraqi threat highlighted the problems posed by the proliferation of weapons of mass destruction among Third World nations and the potential threat posed to U.S. forces as well as other nations. On 3 April 1991, the United Nations Security Council passed Resolution 687, which required that

- Iraq's chemical, biological, and nuclear weapons be registered;
- these weapons and all production and research facilities be destroyed, rendered unusable, or removed;
- Iraq disclose all its holdings and programs and allow unhindered on-site inspections, and destroy the weapons-making potential on its territory itself or permit those weapons to be destroyed.

In conjunction with a longer-term verification regime established by United Nations Resolution 715, Iraq was also required to renounce the possession of any weapons of mass destruction. On 6 April 1991, Iraq accepted the terms of Resolution 687. However, the Iraqi government has steadfastly refused to accept Resolution 715 and regards the resolution as an unwarranted infringement on its sovereignty.

The Security Council entrusted implementation of the resolution to a special commission known as UNSCOM (*U*nited *N*ations *S*pecial *Com*mission), which was set up by the United Nations Secretary General in May 1991. UNSCOM has carried out a series of inspections of Iraqi facilities, but relations with Iraq have been marked by frequent instances

of misinformation, concealment, lack of cooperation, and obstruction and harassment of the inspection teams. Before the 1995 admission by Iraq of having weaponized biological agents, inspections into two suspected key Iraqi biological warfare facilities—Salmon Pak and Al Hakam—found no conclusive evidence of an Iraqi offensive biological program. However, evidence (such as Iraq's inability to explain the use of tons of growth media suitable for biological agent production) strongly pointed to the existence of a program. Furthermore, the inspection teams had only obtained an admission by Iraqi officials that Iraq had carried out a program of biological research for military purposes.[1] The UNSCOM experience underscores the difficulty of monitoring compliance with the Biological Weapons Convention.

Foreign and Domestic Terrorism

Although biological warfare is most often discussed in terms of weapons of mass destruction, usually in the context of war, terrorist use of biological agents cannot be excluded. Biological warfare agents are, for the most part, inexpensive and readily obtainable, and "cookbook" approaches are readily available. One such book is *Silent Death*, by an author who calls himself Uncle Fester.[43] Other titles from the same publisher include *The Poisoner's Handbook* and *Crimes Involving Poisons.*

Unfortunately, recent examples of possible intent to use are not difficult to find. A laboratory in a safe house of the Red Army Faction in Paris, France, was found to have made quantities of botulinum toxin; it is believed that none was used.[16] More alarming were the actions of the Aum Shinrikyo cult in Japan in early 1995. In addition to releasing the nerve agent sarin in the Tokyo subway on 18 March 1995, cult members were preparing vast quantities of *Clostridium difficile* bacterial spores for terrorist use.[44] Although possibly not producing large numbers of casualties, the use of even small amounts of such an agent would almost assuredly create major panic.

In March 1989, the most intensive food safety investigation in FDA history took place when a terrorist threatened to poison this nation's fresh fruit supply, to focus attention on the living conditions of the lower classes in Chile. The terrorist made good on his phone call to the FDA, as two grapes were found laced with small amounts of cyanide. Fortunately no one was poisoned, but the incident cost millions of dollars to investigate in this country, and had a significant impact on Chile's national economy, where fruit and vegetable exports are second in importance only to copper. [45] A biological toxin could have been used just as easily as cyanide in this instance.

The amounts of toxin needed to obtain the desired effect are exceedingly small. “For example, about 30 grams of the toxin ricin, easily concealed in a pocket, would be sufficient to lethally poison one batch of 150 pounds of meat, enough to produce 1,500 hot dogs.”[46] The threat is real. And the knowledge required is not esoteric:

> To engage in bioterrorism requires only the type of knowledge that Kateuas found in his herbals—that is, a sophisticated understanding of the properties of various edible plants, medicinal herbs, toxins and venoms, and infectious and pharmaceutical agents.[35(p45)]

The use of biological agents in domestic terrorism has been attempted on several occasions in recent years, causing alarm and illustrating the ease of obtaining these agents. In May 1995, the American Type Culture Collection (ATCC), a nonprofit organization in Rockville, Maryland, that supplies biological specimens to scientists all over the world, shipped a package containing three vials of *Yersinia pestis* to the home of a white supremacist in Ohio.[47] (This organism, which causes plague, killed one quarter of the population when it struck Europe in the 14th century and wreaked havoc in India as recently as 1994.[48]) The American Type Culture Collection has tightened its controls on whom it will provide hazardous materials to, but even terrorists can produce legitimate request documents: the white supremacist is a qualified microbiologist. When his house was searched, police reported finding hand grenade triggers, homemade explosive devices, and detonating fuzes.[47] Fortunately, he was caught prior to any intentional use.

Two members of the Minnesota Patriots Council were convicted in 1995 for planning to use a lethal biological agent against U.S. marshals and Internal Revenue Service agents. According to trial testimony, they planned to poison U.S. agents by placing ricin toxin on doorknobs.[49]

Lastly, in March 1992, a Tyson’s Corner, Virginia, man was arrested and charged with malicious wounding in a hoax in which he sprayed his roommates with a fluid that he claimed contained anthrax bacteria. The house was placed under quarantine immediately after the incident, and while awaiting test results, 20 people—one a pregnant woman—were treated for possible exposure to anthrax.[50]

The threat that terrorists will use biological agents cannot be ignored and we have to remain strong, vigilant, and determined to defeat this form of terrorism as we do every other act of terrorism.

SUMMARY

International proliferation of biological warfare programs broadens the range of agents that members of the U.S. armed forces may encounter. Ironically, the modernization of many Third World nations—with the subsequent development of industrial, medical, pharmaceutical, and agricultural facilities needed to support these advancing societies provides the basis for development of a biological weapons program, should a nation decide to pursue such an endeavor. A biological weapons program can easily be concealed within legitimate research-and-development and industrial programs, even by countries that are signatories to the 1972 Biological Weapons Convention. Actions such as these are grave threats to our national security.

Biological warfare agents may be more potent than the most lethal chemical warfare agents, and provide a broader area coverage per pound of payload than any other weapons system. The proliferation of technology and of scientific progress in biochemistry and biotechnology has simplified production requirements and provided the opportunity for the creation of exotic agents. This could involve the tailoring of pathogenic microorganisms capable of creating a novel disease, perhaps on an epidemic scale. Humans need not be the only target of biological weapons. These weapons might be meant to attack a country's revenue or food crop, animal herds, or even a supply of a material that is vital for revenue or defense.

Despite the end of the Cold War, the United States still faces serious national security issues. One at the forefront is the issue of the proliferation of biological weapons, especially in Third World nations that have compelling military, technical, economic, and political incentives to pursue this capability. The use of biological agents in future wars and actions by terrorists—foreign and domesticis a legitimate issue of concern. Resolution of this problem should be given the highest priority.

REFERENCES

1. Committee on Armed Services, House of Representatives. Special Inquiry Into the Chemical and Biological Threat. *Countering the Chemical and Biological Weapons Threat in the Post-Soviet World.* Washington, DC: US Government Printing Office; 23 Feb 1993. Report to the Congress.

2. Chemical and bacteriological weapons in the 1980s. *Lancet*. 1984;Jul 21:141–143.

3. Harruff RC. Chemical-biological warfare in Asia. *JAMA.* 1983;250(4):497–498.

4. Leskov S; Kogan V, trans. Military bacteriological programs in Russia and USA are strictly secretive and represent a terrible threat to the world. *Izvestiya.* 26 Jun 1993;15.

5. Department of State. *Joint US/UK/Russian Statement on Biological Warfare.* Washington, DC: USSD, Office of the Assistant Secretary/Spokesmen, 14 Sep 1992. Statement for Immediate Release by Richard Boucher, Spokesman.

6. Zilinskas RA. Anthrax in Sverdlovsk? *Bull At Sci*. 1983;Jun/Jul:24–27.

7. Abramova FA, Grinberg LV, Yampolskaya OV, Walker DH. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979. *Proc Natl Acad Sci*. 1993;90(Mar):2291–2294.

8. Storella MC. *Poisoning Arms Control: The Soviet Union and Chemical/Biological Weapons.* Cambridge, Mass: Institute for Foreign Policy Analysis, Inc; 1984.

9. Smith RJ. Russia fails to detail germ arms. *Washington Post.* 3 Aug 1992;A-1.

10. Defense Intelligence Agency. *Soviet Biological Warfare Threat*. Washington, DC: Department of Defense, 1986. Report DST-1610F-057-86.

11. Adams J. The untold story of Russia's secret biological weapons. *Sunday Times (London).* 27 Mar 1994;4-1.

12. Barry J. Planning a plague? *Newsweek.* 1993;Feb 1:40–41.

13. Smith RJ. Russia agrees to inspection of biological research facilities. *Washington Post*. 15 Sep 1992;A-14.

14. Gordon MR. Moscow is making little progress in disposal of chemical weapons. *New York Times*. 1 Dec 1993;A-1.

15. Smith RJ. US wary of Russian germ arms. *Washington Post*. 8 Apr 1994;A-1.

16. Roberts B. *Biological Weapons: Weapons of the Future?* Washington, DC: The Center for Strategic and International Studies; 1993.

17. Spertzel RO, Wannemacher RW, Linden CD. *Biological Weapons Proliferation.* Fort Detrick, Frederick, Md: US Army Medical Research Institute of Infectious Diseases; 1993. Defense Nuclear Agency Report DNA-TR-92-116.

18. Report of a WHO Group of Consultants. *Health Aspects of Chemical and Biological Weapons.* Geneva, Switzerland: World Health Organization; 1970: 98–99.

19. Tomich N, ed. Medicine in the Gulf War: Policy and policymakers. *US Med.* 1991;27(15, 16):6–18.

20. Huxsoll DL. The nature and scope of the BW threat. *Director's Series on Proliferation, 4*. Lawrence Livermore National Laboratory, University of California; May 23, 1994. Report UCRL-LR-114070-4.

21. Abramowicz M, ed. Botulinum toxin for ocular muscle disorders. *Med Lett.* 1990:32(830):100–101.

22. Douglass JD Jr, Livingston NC. *America the Vulnerable: The Threat of Chemical/Biological Warfare.* Lexington, Mass: DC Heath; 1987.

23. Orient JM. Chemical and biological warfare: Should defenses be researched and deployed? *JAMA.* 1989;262(5):644–648.

24. Department of the Army. *Potential Military Chemical/Biological Agents and Compounds.* Washington, DC: DA; 1990. Field Manual 3-9.

25. Gander TJ, ed. *Jane's NBC Protection Equipment, 1992–93*. Alexandria, Va: Jane's Information Group Inc; 1992.

26. Department of the Army. *Technical Aspects of Biological Defense*. Washington, DC: DA; 1971. Technical Manual 3-216.

27. Somani SM. *Chemical Warfare Agents*. San Diego, Calif: Academic Press; 1992.

28. Poupard JA, Miller LA. History of biological warfare: Catapults to capsomeres. In: Zilinskas RA, ed. The microbiologist and biological defense research: Ethics, politics, and international security. *Ann N Y Acad Sci.* 1992;666:9–18.

29. Gripstad B, ed. *Biological Warfare Agents*. Stockholm, Sweden: Swedish National Defense Research Institute; 1986.

30. Beckett B. *Weapons of Tomorrow.* New York, NY: Plenum Press; 1983.

31. Department of the Army. *US Army Activity in the US Biological Warfare Programs 1942–1977*. Vol 2. Washington, DC: DA; 1977.

32. Bernstein BJ. The birth of the US biological-warfare program. *Sci Am.* 1987; 256:116–121.

33. Harris R, Paxman J. *A Higher Form of Killing: The Secret Story of Chemical and Biological Warfare*. New York, NY: Hill and Wang, The Noonday Press; 1982.

34. Livingstone NC, Douglass JD. CBW: The poor man's atomic bomb. *National Security Papers*. Vol 1. Cambridge, Mass: Institute for Foreign Policy Analysis, Inc; 1984.

35. Root RS. Infectious terrorism. *Atlantic Monthly*. 1991;May:44–50.

36. Fialka J. CIA says North Korea appears active in biological, nuclear arms. *Wall Street Journal*. 25 Feb 1993;A-10.

37. Fenyvesi C, ed. Washington whispers. *US News World Rep*. 22 Feb 1993:22.

38. Triplett WC. A look at Asia past and present: Inside China's scary new

military–industrial complex. *Washington Post.* 8 May 1994;C3.

39. US Department of Defense. *Conduct of the Persian Gulf War.* Washington, DC: DOD; Apr 1992.

40. Office of the Secretary of Defense. *Proliferation: Threat and Response.* Washington, DC: US Government Printing Office; 1996. Document ISBN 0-16-048591-6.

41. Moore M. Iraq said to have supply of biological weapons. *Washington Post*. 29 Sep 1990;A1,A24.

42. Persian Gulf Veterans Coordinating Board. *Summary of the Issues Impacting Upon the Health of Persian Gulf Veterans.* Washington, DC: PGVCB; 18 Apr 1994: Version 2.2.

43. Uncle Fester. *Silent Death*. Port Townsend, Wash: Loompanics Unlimited; 1989.

44. Garrett L. The return of infectious disease. *Foreign Affairs.* 1996;Jan/Feb:66–79.

45. Grigg B, Modeland V. The cyanide scare: A tale of two grapes. *FDA Consumer*. Jul–Aug 1989;7–11.

46. Zilinskas RA. Terrorism and biological weapons: Inevitable alliance? *Perspect Biol Med.* 1990;34(1):44–72.

47. Birch D. Bubonic plague sample delivered to white racist. *Baltimore Sun*. 18 May 1995;A-4.

48. Reuter. Plague reaches Calcutta's millions. *Toronto Star*. 29 Sep 1994;A-3.

49. Smith RM, Parker M, eds. Poison probe. *Newsweek*. 1995;May 29:4. Periscope.

50. Bates S. Fairfax man accused of anthrax threat. *Washington Post*. 3 Mar 1992;C-3.

Chapter 22

ANTHRAX

ARTHUR M. FRIEDLANDER, M.D.*

INTRODUCTION AND HISTORY

THE ORGANISM

EPIDEMIOLOGY

PATHOGENESIS

CLINICAL DISEASE
- Cutaneous Anthrax
- Inhalational Anthrax
- Oropharyngeal and Gastrointestinal Anthrax
- Meningitis

DIAGNOSIS

TREATMENT

PROPHYLAXIS
- Prophylactic Treatment After Exposure
- Active Immunization
- Side Effects

SUMMARY

* *Colonel, Medical Corps, U.S. Army; Chief, Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011; and Clinical Associate Professor of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799*

INTRODUCTION AND HISTORY

Anthrax, a zoonotic disease caused by *Bacillus anthracis*, occurs in domesticated and wild animals—primarily herbivores, including goats, sheep, cattle, horses, and swine. Humans usually become infected by contact with infected animals or contaminated animal products. Infection occurs most commonly via the cutaneous route and only very rarely via the respiratory or gastrointestinal routes.

Anthrax has a long association with human history. The fifth and sixth plagues described in Exodus may have been anthrax in domesticated animals followed by cutaneous anthrax in humans.

The disease that Virgil described in his *Georgics* is clearly anthrax in domestic and wild animals.[1] And during the 16th to the 18th centuries in Europe, anthrax was an economically important agricultural disease. Anthrax was intimately associated with the origins of microbiology and immunology, being the first disease for which a microbial origin was definitively established, in 1876, by Robert Koch.[2] It also was the first disease for which an effective live bacterial vaccine was developed, in 1881, by Louis Pasteur.[3] During the latter half of the 19th century, a previously unrecognized form of anthrax appeared for the first time, namely, inhalational anthrax.[4] This occurred among woolsorters in England, due to the generation of infectious aerosols of anthrax spores under industrial conditions, from the processing of contaminated goat hair and alpaca wool. It probably represents the first described occupational respiratory infectious disease.

Owing to the infectiousness of anthrax spores by the respiratory route and the high mortality of inhalational anthrax, the military's concern with anthrax is with its potential use as a biological weapon. This concern was heightened by the revelation that the largest epidemic of inhalational anthrax in this century, in Sverdlovsk, Russia, in 1979, occurred after anthrax spores were released from a military research facility located upwind from where the cases occurred. Cases were also reported in animals located more than 50 km from the site.[5,6]

THE ORGANISM

Bacillus anthracis is a large, Gram-positive, spore-forming, nonmotile bacillus (1–1.5 µm × 3–10 µm). The organism grows readily on sheep blood agar aerobically and is nonhemolytic under these conditions. The colonies are large, rough, and grayish-white, with irregular, curving outgrowths from the margin. Both in vitro in the presence of bicarbonate and carbon dioxide, and in tissue in vivo, the organism forms a prominent capsule. In tissue, the encapsulated bacteria occur singly or in chains of two or three bacilli (Figure 22-1). The organism does not form spores in living tissue; sporulation occurs only after the infected body has been opened and exposed to oxygen. The spores, which cause no swelling of the bacilli, are oval and occur centrally or paracentrally (Figure 22-2). They are very resistant and may survive in the environment for decades in certain soil conditions. Bacterial

identification is confirmed by demonstration of the protective antigen toxin component, lysis by a specific bacteriophage, detection of capsule by fluorescent antibody, and virulence for mice and guinea pigs. Additional confirmatory tests to identify toxin and capsule genes by the polymerase chain reaction have also been developed as research tools.

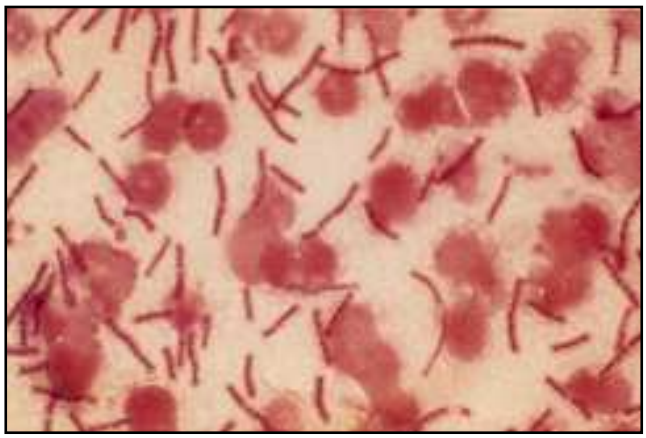


Fig. 22-1. Gram's stain of peripheral blood smear from a rhesus monkey that died of inhalational anthrax.

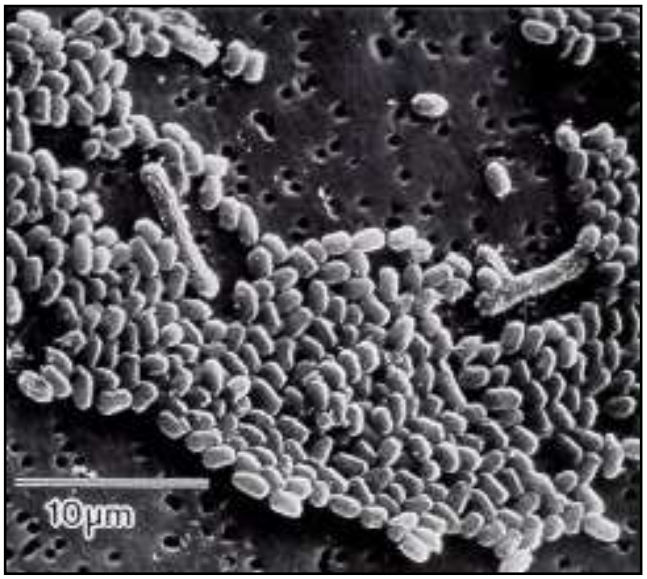


Fig. 22-2. Scanning electron micrograph of a preparation of *Bacillus anthracis* spores. Two elongated bacilli are also present among the oval-shaped spores. Original magnification **x** 2620. Photograph: Courtesy of John Ezzell, Ph.D., US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md.

EPIDEMIOLOGY

Anthrax occurs worldwide. The organism exists in the soil as a spore. The question remains unsettled as to whether its persistence in the soil is due to significant multiplication of the organism in the soil, or if it is due solely to cycles of bacterial amplification in infected animals whose carcasses then contaminate the soil.[7,8] The form of the organism in infected animals is the bacillus. Only when the organism in the carcass is exposed to air does sporulation occur.

Animals, domestic or wild, become infected when they ingest spores while grazing on contaminated land or eating contaminated feed. Environmental conditions such as drought, which may promote trauma in the oral cavity on grazing, are thought to increase the chances of acquiring anthrax, as Pasteur originally reported.[9] Spread from animal to animal by mechanical means—by the biting flies,[10] and from one environmental site to another by the nonbiting flies and by vultures[8]—has been suggested.

Anthrax in humans is associated with agricultural, horticultural, or industrial

exposure to infected animals or contaminated animal products. In the less-developed countries, primarily in Africa, Asia, and the Middle East, disease occurs from contact with infected domesticated animals or contaminated animal products. This includes handling contaminated carcasses, hides, wool, hair, and bones; and ingesting contaminated meat. Cases associated with industrial exposure, rarely seen today, occur in workers processing contaminated hair, wool, hides, and bones. Direct contact with contaminated material leads to cutaneous disease, while ingestion of infected meat gives rise to oropharyngeal or gastrointestinal forms of anthrax. Inhalation of a sufficient quantity of spores, usually seen only during generation of aerosols in an enclosed space associated with processing contaminated wool or hair, gives rise to inhalational anthrax.

Unreliable reporting makes it difficult to estimate with accuracy the true incidence of human anthrax. It was estimated in 1958 that worldwide between 20,000 and 100,000 cases occurred annually.[11] In more recent years, anthrax in animals has been reported from 82 countries, and human cases continue to be reported from Africa, Asia, Europe, and the Americas.[12] In the United States, the annual incidence of human anthrax has steadily declined—from about 127 cases in the early years of this century to about one per year for the past 10 years. The vast majority of cases have been cutaneous. Under natural conditions, inhalational anthrax is exceedingly rare, with only 18 cases having been reported in the United States in the 20th century.[13] In the early years of this century, cases of inhalational anthrax were reported in rural villagers in Russia who worked with contaminated sheep wool inside their homes.[14] Five cases of inhalational anthrax occurred in woolen mill workers in New Hampshire in the 1950s.[15] During times of economic hardship and disruption of veterinary and human public health practices, such as occurs during war, there have been large epidemics of anthrax. The largest reported epidemic of human anthrax occurred in Zimbabwe from 1978 through 1980, with an estimated 10,000 cases. Essentially all were cutaneous, with very rare cases of gastrointestinal disease and eight cases of inhalational anthrax, although no autopsy confirmation was reported.[16]

PATHOGENESIS

B anthracis possesses three known virulence factors: an antiphagocytic capsule and two protein exotoxins, called the *lethal* and the *edema* toxins. The role of the capsule in pathogenesis was demonstrated in the early 1900s, when anthrax strains lacking a capsule were shown to be avirulent.[17] In more recent years, the genes encoding synthesis of the capsule were found to be encoded on a 110-kilobase (kb) plasmid. Molecular analysis revealed that strains cured of this plasmid no longer produced the capsule and were attenuated,[18] thus confirming the critical role of the capsule in virulence. The capsule is composed of a polymer of poly-D-glutamic acid, which confers resistance to phagocytosis and may

contribute to the resistance of anthrax to lysis by serum cationic proteins.[19]

It was Koch, in his initial studies on anthrax, who first suggested the importance of toxins. In 1954, Smith and Keppie[20] demonstrated a toxic factor in the serum of infected animals that was lethal when injected into other animals. The role of toxins in virulence and immunity was firmly established by many workers in the ensuing years.[21–23] Advances in molecular biology in the last decade have produced a more complete understanding of the biochemical mechanisms of action of the toxins and have begun to provide a more definitive picture of their role in the pathogenesis of the disease.

The genes encoding the synthesis of the two protein exotoxins are located on a 60-kb plasmid, distinct from that encoding for the capsule. In an environment of increased bicarbonate and carbon dioxide and increased temperature, such as is found in the infected host, there is increased transcription of the genes for synthesis of the two toxins,[24–26] as well as for the capsule.[27]

The anthrax toxins, like many bacterial and plant toxins, possess two components: a cell-binding, or B, domain; and an active, or A, domain that has the toxic and, usually, the enzymatic activity (Figure 22- 3). The B and A anthrax toxin components are synthesized from different genes and are secreted as noncovalently linked proteins. The two toxins are unusual in that the B protein, called *protective antigen* (MW 83,000), is shared by both toxins. Thus the lethal toxin is composed of the protective antigen combined with a second protein, which is known as the lethal factor (MW 90,000). The lethal toxin is lethal for experimental animals[28,29] and the lethal factor has been shown to possess homology to metalloproteases, although no direct enzymatic activity has yet been discovered.[30]

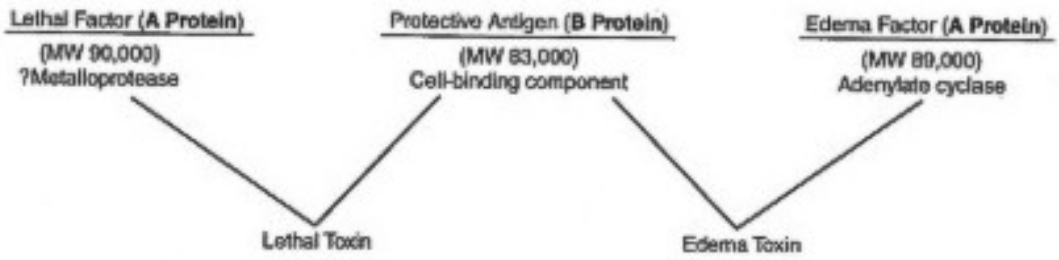


Fig. 22-3. Composition of anthrax lethal and edema protein toxins.

The edema toxin, consisting of the same protective antigen together with a third protein, edema factor (MW 89,000), causes edema when injected into the skin of experimental animals.[28,29] The edema factor is a calmodulin-dependent adenylate cyclase, which elevates intracellular cyclic adenosine monophosphate, and which is likely to be responsible for the marked edema often present at the site of bacterial replication.

Each of the three toxin proteins—the B protein and both A

proteins—individually is without biological activity. The critical role of the toxins in pathogenesis was established when it was shown that deletion of the toxin encoding plasmid[18,31] or the protective antigen gene alone[32] attenuates the organism. The lethal toxin also appears to be more important for virulence in a mouse model than the edema toxin.[33]

Recent studies in cell culture models have given a clearer understanding of the molecular interactions of the toxin proteins. Protective antigen first binds, most likely by a domain at its carboxy-terminus,[34,35] to a specific cell receptor.[36] Once bound, it is cleaved by a protease located on the cell surface,[37,38] resulting in retention on the cell surface of a 63-kilodalton (kd) fragment of protective antigen. This cleavage creates a binding site on the protective antigen to which either the lethal factor or the edema factor can bind with high affinity. The complex is then internalized and passes through an acidic vesicle and is translocated to the cell cytosol, where it expresses its toxic activity.

The situation in the infected animal may be somewhat different, since the toxin proteins may exist in the serum as a complex of protective antigen and lethal factor.[39] It is possible that the proteolytic activation of protective antigen necessary to form lethal or edema toxin may occur in interstitial fluid or serum rather than on the cell surface. The lethal or the edema toxin may then bind to target cells and be internalized.

Infection begins when the spores are inoculated through the skin or mucosa. It is thought that spores are ingested at the local site by macrophages, in which they germinate to the vegetative bacillus with production of capsule and toxins. At these sites, the bacteria proliferate and produce the edema and lethal toxins that impair host leukocyte function and lead to the distinctive pathological findings: edema, hemorrhage, tissue necrosis, and a relative lack of leukocytes. In inhalational anthrax, the spores are ingested by alveolar macrophages, which transport them to the regional tracheobronchial lymph nodes, where germination occurs.[40]

Once in the tracheobronchial lymph nodes, the local production of toxins by extracellular bacilli gives rise to the characteristic pathological picture: massive hemorrhagic, edematous, and necrotizing lymphadenitis; and mediastinitis (the latter is almost pathognomonic of this disease).[41] The bacilli can then spread to the blood, leading to septicemia with seeding of other organs and frequently causing hemorrhagic meningitis. Terminally, toxin is present in high concentrations in the blood,[21] but both the site of toxin action and the molecular mechanism of death remain unknown. Death is the result of respiratory failure associated with pulmonary edema, overwhelming bacteremia, and, often, meningitis.

Crude toxin preparations have been shown to impair neutrophil chemotaxis,[42] phagocytosis,[19] and killing.[43] More recent work has shown that purified edema toxin impairs phagocytosis[44] and priming for the

respiratory burst[45] in neutrophils, and also inhibits the production of interleukin-6 (IL-6) and tumor necrosis factor (TNF) by monocytes, which may further impair host resistance.[46] The lethal toxin is directly cytolytic for macrophages,[47] causing release of the potentially toxic cytokines IL-1 and TNF.[48] Experimentally, animals can be protected against death from lethal toxin by depleting them of macrophages or blocking the effect of IL-1,[48] but the role of these cytokines in death from infection remains to be established.

CLINICAL DISEASE

The military's interest in anthrax is with defense against its use as an inhalational biological weapon. However, other forms of the disease are far more likely to be seen by medical officers—particularly when deployed to Third World countries—and are therefore included for completeness.

Cutaneous Anthrax

More than 95% of cases of anthrax are cutaneous (Figure 22-4). After inoculation, the incubation period is 1 to 5 days. The disease first appears as a small papule that progresses over a day or two to a vesicle containing serosanguinous fluid with many organisms and a paucity of leukocytes. The vesicle, which may be 1 to 2 cm in diameter, ruptures, leaving a necrotic ulcer. Satellite vesicles may also be present. The lesion is usually painless, and varying degrees of edema may be present around it. The edema may occasionally be massive, encompassing the entire face or limb, and is described by the term "malignant edema." Patients usually have fever, malaise, and headache, which may be severe in those with extensive edema. There may also be local lymphadenitis. The ulcer base develops a characteristic black eschar and after a period of 2 to 3 weeks the eschar separates, often leaving a scar. Septicemia is very rare, and with treatment mortality should be less than 1%.

a **b**

Fig. 22-4. (a) Cutaneous lesion of anthrax demonstrating eschar and edema in a man, following his handling of a contaminated cow carcass in a rendering plant in Colorado. **(b)** Cutaneous lesion of anthrax with eschar (on the patient's neck), on approximately day 15 of disease. The patient had worked with air-dried goat skins from Africa. Photograph a: Courtesy of Arnold Kaufmann, Ph.D., National Center for Infectious Disease, Centers for Disease and Control and Prevention, Atlanta, Ga. Photograph b: Reprinted from Binford CH, Connor DH, eds. *Pathology of Tropical and Extraordinary Diseases*. Vol 1. Washington, DC: Armed Forces Institute of Pathology; 1976: 121. AFIP Negative 75-4203-7.

Inhalational Anthrax

Inhalational anthrax begins after an incubation period of 1 to 6 days with nonspecific symptoms of malaise, fatigue, myalgia, and fever. There may be an associated nonproductive cough and mild chest discomfort. These symptoms usually persist for 2 or 3 days, and in some cases there may be a short period of improvement. This is followed by the sudden onset of increasing respiratory distress with dyspnea, stridor, cyanosis, increased chest pain, and diaphoresis. There may be associated edema of the chest and neck. Chest X-ray examination usually shows the characteristic widening of the mediastinum and, often, pleural effusions (Figure 22-5). Pneumonia has not been a consistent finding but can occur in some patients.[5] While cases of inhalational anthrax have been rare in this century, several have occurred in patients with underlying pulmonary disease, suggesting that this condition may increase susceptibility to the disease.[13] Meningitis is present in up to 50% of cases, and some patients may present with seizures. The onset of respiratory distress is followed by the rapid onset of shock and death within 24 to 36 hours. Mortality has been essentially 100% despite appropriate treatment.

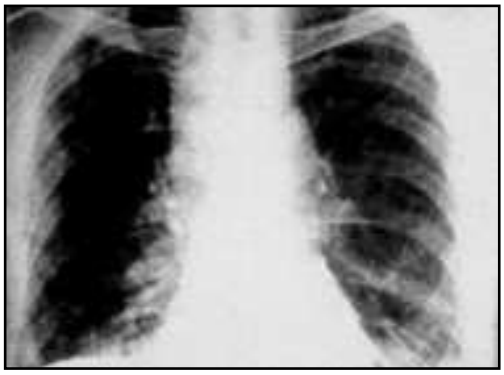


Fig. 22-5. This roentgenogram, taken on day 2 of illness, shows the lungs of a 51-year-old laborer with occupational exposure to airborne anthrax spores. Marked mediastinal widening is evident, with a small parenchymal infiltrate. Reprinted from Binford CH, Connor DH, eds. *Pathology of Tropical and Extraordinary Diseases*. Vol 1. Washington, DC: Armed Forces Institute of Pathology; 1976: 119. AFIP Negative 71-1290-2.

Oropharyngeal and Gastrointestinal Anthrax

Oropharyngeal and gastrointestinal anthrax result from the ingestion of

infected meat that has not been sufficiently cooked. After an incubation period of 2 to 5 days, patients with oropharyngeal disease present with severe sore throat or a local oral or tonsillar ulcer, usually associated with fever, toxicity, and swelling of the neck due to cervical or submandibular lymphadenitis and edema. Dysphagia and respiratory distress may also be present. Gastrointestinal anthrax begins with nonspecific symptoms of nausea, vomiting, and fever; these are followed in most cases by severe abdominal pain. The presenting sign may be an acute abdomen, which may be associated with hematemesis, massive ascites, and diarrhea. Mortality in both forms may be as high as 50%, especially in the gastrointestinal form.

Meningitis

Meningitis may occur following bacteremia, as a complication of any of the other clinical forms of the disease. Meningitis may also occur, very rarely, without a clinically apparent primary focus. It is very often hemorrhagic, which is important diagnostically, and almost invariably fatal (Figure 22-6).

Fig. 22-6. Meningitis with subarachnoid hemorrhage in a man from Thailand who died 5 days after eating undercooked carabao (water buffalo). Reprinted from Binford CH, Connor DH, eds. *Pathology of Tropical and Extraordinary Diseases*. Vol 1. Washington, DC: Armed Forces Institute of Pathology; 1976: 121. AFIP Negative 75-12374-3.

DIAGNOSIS

The most critical aspect in making a diagnosis of anthrax is a high index of suspicion associated with a compatible history of exposure. Cutaneous anthrax should be considered following the development of a painless pruritic papule, vesicle, or ulcer—often with surrounding edema—that develops into a black eschar. With extensive or massive edema, such a lesion is almost pathognomonic. Gram’s stain or culture of the lesion will usually confirm the diagnosis. The differential diagnosis should include tularemia, staphylococcal or streptococcal disease, and orf (a viral disease of sheep and goats, transmissible to humans).

The diagnosis of inhalational anthrax is extraordinarily difficult, but the disease should be suspected with a history of exposure to a *B*

anthracis–containing aerosol. The early symptoms are entirely nonspecific. However, (1) the development of respiratory distress in association with radiographic evidence of a widened mediastinum due to hemorrhagic mediastinitis, and (2) the presence of hemorrhagic pleural effusion or hemorrhagic meningitis should suggest the diagnosis. Sputum examination is not helpful in making the diagnosis, since pneumonia is not usually a feature of inhalational anthrax.

Gastrointestinal anthrax is exceedingly difficult to diagnose because of the rarity of the disease and its nonspecific symptoms. Only with a history of ingesting contaminated meat in the setting of an outbreak is diagnosis usually considered. Microbiologic cultures are not helpful in confirming the diagnosis. The diagnosis of oropharyngeal anthrax can be made from the clinical and physical findings in a patient with the appropriate epidemiological history.

Meningitis due to anthrax is clinically indistinguishable from meningitis due to other etiologies. An important distinguishing feature is that the cerebral spinal fluid is hemorrhagic in as many as 50% of cases. The diagnosis can be confirmed by identifying the organism in cerebral spinal fluid by microscopy, culture, or both.

Serology is generally only of use in making a retrospective diagnosis. Antibody to protective antigen or the capsule develops in 68% to 93%[49–52] of reported cases of cutaneous anthrax and 67% to 94%[51,52] of reported cases of oropharyngeal anthrax. A positive skin test to anthraxin (an undefined antigen derived from acid hydrolysis of the bacillus that was developed and evaluated in the former Soviet Union) has also been reported[53] to be of value in the retrospective diagnosis of anthrax. Western countries have limited experience with this test.[54]

TREATMENT

Penicillin is the drug of choice for anthrax. Cutaneous anthrax without toxicity or systemic symptoms may be treated with oral penicillin. If evidence of spreading infection or systemic symptoms is present, then intravenous therapy with high-dose penicillin (2 million units administered every 6 h) may be initiated until a clinical response is obtained. Effective therapy will reduce edema and systemic symptoms but will not change the evolution of the skin lesion itself. Treatment should be continued for 7 to 10 days.

Tetracycline, erythromycin, and chloramphenicol have also been used successfully. These drugs may be used for treatment of the rare case caused by naturally occurring penicillin-resistant organisms. Additional antibiotics shown to be active in vitro include ciprofloxacin, gentamicin, cefazolin, cephalothin, vancomycin, clindamycin, and imipenem.[55–57] These drugs should be effective in vivo, but there is no reported clinical

experience.

Inhalational, oropharyngeal, and gastrointestinal anthrax should be treated with large doses of intravenous penicillin (2 million units administered every 2 h) with appropriate vasopressors, oxygen, and other supportive therapy.

PROPHYLAXIS

Prophylactic Treatment After Exposure

Experimental evidence[58] has demonstrated that treatment with antibiotics beginning 1 day after exposure to a lethal aerosol challenge with anthrax spores can provide significant protection against death. All three drugs used in this study—ciprofloxacin, doxycycline, and penicillin—were effective. The optimal protection was afforded by combining antibiotics with active immunization.

Active Immunization

The only licensed human vaccine against anthrax is produced by the Michigan Department of Public Health. This vaccine is made from sterile filtrates of microaerophilic cultures of an attenuated, unencapsulated, nonproteolytic strain (V770-NP1-R) of *B anthracis*. The filtrate, containing predominantly protective antigen, is adsorbed to aluminum hydroxide. The final product also contains formaldehyde, in a final concentration of no more than 0.02%, and benzethonium chloride 0.0025%, as preservatives. Some vaccine lots contain very small amounts of lethal factor and lesser amounts of edema factor, as determined by antibody responses in vaccinated animals,[18,59,60] although this antibody response has not been reported in the limited observations in human vaccinees.[61] Although protective antigen by itself is an effective immunogen,[62] it is unknown whether the small amounts of lethal or edema factor that are present in some lots of the vaccine contribute to its protective efficacy. The potency of vaccine lots is determined by showing protection of parenterally challenged guinea pigs. There is no characterization of the amount and form of the protective antigen or other toxin components in the vaccine. The vaccine is stored at 2°C to 8°C. The recommended schedule for vaccination is 0.5 mL given subcutaneously at 0, 2, and 4 weeks, followed by boosters of 0.5 mL at 6, 12, and 18 months. Annual boosters are recommended if the potential for exposure continues.

The vaccine should be given to industrial workers exposed to potentially contaminated animal products imported from countries in which animal anthrax remains uncontrolled. These products include wool, goat hair, hides, and bones. People in direct contact with potentially infected animals

as well as laboratory workers should also be immunized. Vaccination is also indicated for protection against the use of anthrax in biological warfare. Approximately 150,000 service members received this licensed MDPH vaccine between 11 January and 28 February 1991 (25%–30% of the total U.S. forces deployed during the Persian Gulf War).

A live, attenuated, unencapsulated, spore vaccine is used for humans in the former USSR. The vaccine is given by scarification or subcutaneously. Its developers claim it to be reasonably well tolerated and to show some degree of protective efficacy against cutaneous anthrax in clinical field trials.[53]

In the United States, immunization with the licensed vaccine induced an immune response, measured by indirect hemagglutination, to protective antigen in 83% of vaccinees 2 weeks after the first three doses,[63] and in 91% of those tested after receiving two or more doses.[50] One hundred percent of the vaccinees develop a rise in titer in response to the yearly booster dose. When tested by an enzyme-linked immunosorbent assay, the current serological test of choice, more than 95% of vaccinees seroconvert after the initial three doses.[61,64]

A rough correlation between antibody titer to protective antigen and protection of experimental animals from infection exists after vaccination with the human vaccine. However, the exact relationship between antibody to protective antigen as measured in these assays, and immunity to infection remains obscure because the live, attenuated Sterne veterinary vaccine (made from an unencapsulated, toxin-producing strain) protects animals better than the human vaccine, yet it induces lower levels of antibody to protective antigen.[59–61]

The protective efficacy of experimental protective antigen-based vaccines produced from sterile culture filtrates of *B anthracis* was clearly demonstrated using various animal models and routes of challenge.[21,65] A placebo-controlled clinical trial was conducted with a vaccine similar to the currently licensed U.S. vaccine.[66] This field-tested vaccine was composed of the sterile, cell-free culture supernatant from an attenuated, unencapsulated strain of *B anthracis*—different from that used to produce the licensed vaccine and grown under aerobic, rather than microaerophilic, conditions.[67] It was precipitated with alum rather than adsorbed to aluminum hydroxide. The study population worked in four mills in the northeastern United States where *B anthracis*–contaminated imported goat hair was used. The vaccinated group, compared to a placebo-inoculated control group, was afforded 92.5% protection against cutaneous anthrax, with a lower 95% confidence limit of 65% effectiveness. There were insufficient cases of inhalational anthrax to determine whether the vaccine was effective against this form of the disease. This same vaccine was previously shown to protect rhesus monkeys against an aerosol exposure to

anthrax spores.[67]

There have been no controlled clinical trials in humans of the efficacy of the currently licensed U.S. vaccine. This vaccine has been extensively tested in animals and has protected guinea pigs against both an intramuscular[60,61] and an aerosol challenge.[59] The licensed vaccine has also been shown to protect rhesus monkeys against an aerosol challenge.[58,68]

Side Effects

In two different studies, the incidence of significant local and systemic reactions to the vaccine used in the placebo-controlled field trial was 2.4% to 2.8%[66] and 0.2% to 1.3%.[67] The vaccine currently licensed in the United States is reported to have a similar incidence of reactions.[64,69] Local reactions considered significant consist of induration, erythema in an area larger than 5 cm in diameter, edema, pruritus, warmth, and tenderness. These reactions peak at 1 to 2 days and usually disappear within 2 to 3 days. Very rare reactions include edema extending from the local site to the elbow or forearm, and a small, painless nodule that may persist for weeks. People who have recovered from a cutaneous infection with anthrax may have very severe local reactions.[66] Systemic reactions are characterized by mild myalgia, headache, and mild-to-moderate malaise that lasts for 1 to 2 days. There are no long-term sequelae of local or systemic reactions.

SUMMARY

Anthrax is a zoonotic disease that occurs in domesticated and wild animals. Humans become infected by contact with infected animals or contaminated products. Under natural circumstances, infection occurs by the cutaneous route and only extremely rarely by the inhalational or gastrointestinal routes.

An aerosol exposure to spores causes inhalational anthrax. This form of the disease, which is of military concern because of its potential for use as a biological warfare agent, begins with nonspecific symptoms followed in 2 to 3 days by the sudden onset of respiratory distress with dyspnea, cyanosis, and stridor. It is rapidly fatal. Radiographic examination of the chest often reveals the characteristic mediastinal widening, indicative of hemorrhagic mediastinitis. Hemorrhagic meningitis frequently coexists. Given the rarity of the disease and its rapid progression, the diagnosis of inhalational anthrax is difficult to make. Treatment consists of massive doses of antibiotics and supportive care. Postexposure antibiotic prophylaxis is effective in experimental animals and should be instituted as soon as possible after exposure. A licensed nonliving vaccine is available for human use.

REFERENCES

1. Dirckx JH. Virgil on anthrax. *Am J Dermatopathol*. 1981;3:191–195.

2. Koch R. Die Aetiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des *Bacillus anthracis* [in German]. *Beiträge zur Biologie der Pflanzen*. 1876;2:277–310.

3. Pasteur, Chamberland, Roux. Compte rendu sommaire des expériences faites à Pouilly-le-Fort, près Melun, sur la vaccination charbonneuse [in French]. *Comptes Rendus des séances De L'Académie des Sciences*. 1881;92:1378–1383.

4. LaForce FM. Woolsorters' disease in England. *Bull N Y Acad Med*. 1978;54:956–963.

5. Abramova FA, Grinberg LM, Yampolskaya OV, Walker DH. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979. *Proc Natl Acad Sci U S A*. 1993;90:2291–2294.

6. Walker DH, Yampolska L, Grinberg LM. Death at Sverdlovsk: What have we learned? *Am J Pathol*. 1994;144: 1135–1141.

7. Kaufmann AF. Observations on the occurrence of anthrax as related to soil type and rainfall. *Salisbury Med Bull Suppl*. 1990;68:16–17.

8. de Vos V. The ecology of anthrax in the Kruger National Park, South Africa. *Salisbury Med Bull Suppl*. 1990;68:19–23.

9. Wilson GS, Miles AA. *Topley and Wilson's Principles of Bacteriology and Immunity.* Vol 2. Baltimore, Md: Williams & Wilkins; 1955: 1940.

10. Davies JCA. A major epidemic of anthrax in Zimbabwe, III: Distribution of cutaneous lesions. *Cent Afr J Med*. 1983;29:8–12.

11. Glassman HN. World incidence of anthrax in man. *Public Health Rep*. 1958;73:22–24.

12. Fujikura T. Current occurrence of anthrax in man and animals. *Salisbury Med Bull Suppl*. 1990;68:1.

13. Brachman PS. Inhalation anthrax. *Ann N Y Acad Sci*. 1980;353:83–93.

14. Elkina AV. The epidemiology of a pulmonary form of anthrax. *Zh Mikrobiol Epidemiol Immunobiol*. 1971;48:112–116.

15. Plotkin SA, Brachman PS, Utell M, Bumford FH, Atchison MM. An epidemic of inhalation anthrax, the first in the twentieth century, I: Clinical features. *Am J Med*. 1960;29:992–1001.

16. Davies JC. A major epidemic of anthrax in Zimbabwe. *Cent Afr J Med (Zimbabwe)*. Part 1, 1982;28(12):291–298, Part 2, 1983;29(1):8–12, Part 3, 1985;31(9):176–180.

17. Bail O. Quoted in: Sterne M. Anthrax. In: Stableforth AW, Galloway IA, eds. *Infectious Diseases of Animals*. Vol 1. London, England: Butterworths Scientific Publications; 1959: 22.

18. Ivins BI, Ezzell JW Jr, Jemski J, Hedlund KW, Ristroph JD, Leppla SH. Immunization studies with attenuated strains of *Bacillus anthracis. Infect Immun*. 1986;52:454–458.

19. Keppie J, Harris-Smith PW, Smith H. The chemical basis of the virulence of *Bacillus anthracis*, IX: Its aggressins and their mode of action. *Br J Exp Pathol*. 1963;44:446–453.

20. Smith H, Keppie J. Observations on experimental anthrax: Demonstration of a specific lethal factor produced in vivo by *Bacillus anthracis. Nature*. 1954;173:869–870.

21. Lincoln RE, Fish DC. Anthrax toxin. In: Montie TC, Kadis S, Ajl SJ, eds. *Microbial Toxins*. Vol 3. New York, NY: Academic Press; 1970: 361–414.

22. Stephen J. Anthrax toxin. In: Dorner F, Drews J, eds. *Pharmacology of Bacterial Toxins.* Oxford, England: Pergamon Press; 1986: 381–395.

23. Leppla SH. The anthrax toxin complex. In: Alouf JE, Freer JH, eds. *Sourcebook of Bacterial Protein Toxins*. London, England: Academic Press; 1991: 277–302.

24. Bartkus JM, Leppla SH. Transcriptional regulation of the protective antigen gene of *Bacillus anthracis. Infect Immun*. 1989;57:2295–2300.

25. Uchida I, Hornung JM, Thorne CB, Klimpel KR, Leppla SH. Cloning and characterization of a gene whose product is a *trans*-activator of anthrax toxin synthesis. *J Bacteriol*. 1993;175:5329–5338.

26. Sirard J-C, Mock M, Fouet A. The three *Bacillus anthracis* toxin genes are coordinately regulated by bicarbonate and temperature. *J Bacteriol*. 1994;176:5188–5192.

27. Vietri NJ, Marrero R, Hoover T, Welkos SL. Identification and characterization of a *trans*-activator involved in the regulation of encapsulation by *Bacillus anthracis*. *Gene*. 1995;152(1):1–9.

28. Stanley JL, Smith H. Purification of factor I and recognition of a third factor of the anthrax toxin. *J Gen Microbiol*. 1961;26:49–66.

29. Beall FA, Taylor MJ, Thorne CB. Rapid lethal effects in rats of a third component found upon fractionating the toxin of *Bacillus anthracis. J Bacteriol*. 1962;83:1274–1280.

30. Klimpel KR, Arora N, Leppla SH. Anthrax toxin lethal factor contains a zinc metalloprotease consensus sequence which is required for lethal toxin activity. *Mol Microbiol*. 1994;13:1093–1100.

31. Mikesell P, Ivins BE, Ristroph JD, Dreier TM. Evidence for plasmid-mediated

toxin production in *Bacillus anthracis. Infect Immun*. 1983:39:371–376.

32. Cataldi A, Labruyere E, Mock M. Construction and characterization of a protective antigen-deficient *Bacillus anthracis* strain. *Mol Microbiol*. 1990;4:1111–1117.

33. Pezard C, Berche P, Mock M. Contribution of individual toxin components to virulence of *Bacillus anthracis. Infect Immun*. 1991;59:3472–3477.

34. Singh Y, Klimpel KR, Quinn CP, Chaudhary VK, Leppla SH. The carboxyl-terminal end of protective antigen is required for receptor binding and anthrax toxin activity. *J Biol Chem*. 1991;266:15493–15497.

35. Little SL, Lowe JR. Location of receptor-binding region of protective antigen from *Bacillus anthracis. Biochem Biophys Res Commun*. 1991;180:531–537.

36. Escuyer V, Collier RJ. Anthrax protective antigen interacts with a specific receptor on the surface of CHO-K1 cells. *Infect Immun*. 1991;59:3381–3386.

37. Bhatnagar R, Singh Y, Leppla SH, Friedlander AM. Calcium is required for the expression of anthrax lethal toxin activity in the macrophagelike cell line J774A.1. *Infect Immun*. 1989;57:2107–2114.

38. Klimpel KR, Molloy SS, Thomas G, Leppla SH. Anthrax toxin protective antigen is activated by a cell surface protease with sequence specificity and catalytic properties of furin. *Proc Natl Acad Sci USA*. 1992;89: 10277–10281.

39. Ezzell JW Jr, Abshire TG. Serum protease cleavage of *Bacillus anthracis* protective antigen. *J Gen Microbiol*. 1992;138:543–549.

40. Ross JM. The pathogenesis of anthrax following the administration of spores by the respiratory route. *J Pathol Bacteriol*. 1957;73:485–494.

41. Dutz W, Kohout E. Anthrax. *Pathol Annu*. 1971;209–248.

42. Kashiba S, Morishima T, Kato K, Shima M, Amano T. Leucotoxic substance produced by *Bacillus anthracis. Biken J*. 1959;2:97–104.

43. Bail O, Weil E. Beiträge zum Studium der Milzbrandinfektion [in German]. *Arch Hyg Bacteriol*. 1911;73:218–264.

44. O'Brien J, Friedlander A, Dreier T, Ezzell J, Leppla S. Effects of anthrax toxin components on human neutrophils. *Infect Immun*. 1985;47:306–310.

45. Wright GG, Read PW, Mandell GL. Lipopolysaccharide releases a priming substance from platelets that augments the oxidative response of polymorphonuclear neutrophils to chemotactic peptide. *J Infect Dis*. 1988;157:690–696.

46. Hoover DL, Friedlander AM, Rogers LC, Yoon I-K, Warren RL, Cross AS. Anthrax edema toxin differentially regulates lipopolysaccharide-induced monocyte production of tumor necrosis factor alpha and interleukin-6 by increasing intracellular cyclic AMP. *Infect Immun*. 1994;62:4432–4439.

47. Friedlander AM. Macrophages are sensitive to anthrax lethal toxin through an acid-dependent process. *J Biol Chem*. 1986;261:7123–7126.

48. Hanna PC, Acosta D, Collier RJ. On the role of macrophages in anthrax. *Proc Natl Acad Sci U S A*. 1993;90:10198–10291.

49. Turnbull PCB, Leppla SH, Broster MG, Quinn CP, Melling J. Antibodies to anthrax toxin in humans and guinea pigs and their relevance to protective immunity. *Med Microbiol Immunol*. 1988;177:293–303.

50. Buchanan TM, Feeley JC, Hayes PS, Brachman PS. Anthrax indirect microhemagglutination test. *J Immunol*. 1971;107:1631–1636.

51. Sirisanthana T, Nelson KE, Ezzell J, Abshire TG. Serological studies of patients with cutaneous and oral-oropharyngeal anthrax from northern Thailand. *Am J Trop Med Hyg*. 1988;9:575–581.

52. Harrison LH, Ezzell JW, Abshire TG, Kidd S, Kaufmann AF. Evaluation of serologic tests for diagnosis of anthrax after an outbreak of cutaneous anthrax in Paraguay. *J Infect Dis*. 1989;160:706–710.

53. Shlyakhov EN, Rubinstein E. Human live anthrax vaccine in the former USSR. *Vaccine*. 1994;12:727–730.

54. Pfisterer RM. Retrospective verification of the diagnosis of anthrax by means of the intracutaneous skin test with the Russian allergen “anthraxin” in a recent epidemic in Switzerland. *Salisbury Med Bull Suppl*. 1990;68:80.

55. Lightfoot NF, Scott RJD, Turnbull PCB. Antimicrobial susceptibility of *Bacillus anthracis. Salisbury Med Bull Suppl*. 1990;68:95–98.

56. Doganay M, Aydin N. Antimicrobial susceptibility of *Bacillus anthracis. Scand J Infect Dis*. 1991;23:333–335.

57. Mikesell P. Major, Medical Service, US Army. Investigator, Bacteriology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, January 1991.

58. Friedlander AM, Welkos SL, Pitt MLM, et al. Postexposure prophylaxis against experimental inhalation anthrax. *J Infect Dis*. 1993;167(5):1239–1243.

59. Ivins BE, Welkos SL. Recent advances in the development of an improved, human anthrax vaccine. *Eur J Epidemiol*. 1988;4:12–19.

60. Little SF, Knudson GB. Comparative efficacy of *Bacillus anthracis* live spore vaccine and protective antigen vaccine against anthrax in the guinea pig. *Infect Immun*. 1986;52:509–512.

61. Turnbull PCB, Broster MG, Carman JA, Manchee RJ, Melling J. Development of antibodies to protective antigen and lethal factor components of anthrax toxin in humans and guinea pigs and their relevance to protective immunity. *Infect Immun*. 1986;52:356–363.

62. Ivins BE, Welkos SL. Cloning and expression of the *Bacillus anthracis* protective antigen gene in *Bacillus subtilis. Infect Immun*. 1986;54:537–542.

63. Johnson-Winegar A. Comparison of enzyme-linked immunosorbent and hemagglutination assays for determining anthrax antibodies. *J Clin Microbiol*. 1984;20:357–361.

64. Pittman PRE. Lieutenant Colonel, Medical Corps, US Army. Chief, Clinical Investigation, Medical Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, January 1994.

65. Hambleton P, Carman JA, Melling J. Anthrax: The disease in relation to vaccines. *Vaccine*. 1984;2:125–132.

66. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. *Am J Public Health*. 1962;52:632–645.

67. Wright GG, Green TW, Kanode RG Jr. Studies on immunity in anthrax, V: Immunizing activity of alum-precipitated protective antigen. *J Immunol*. 1954;73:387–391.

68. Ivins BE, Fellows PF, Pitt MLM, et al. Efficacy of a standard human anthrax vaccine against *Bacillus anthracis* aerosol challenge in rhesus monkeys. *Salisbury Med Bull Suppl*. 1996;87(suppl):125–126.

69. Puziss M, Wright GG. Studies on immunity in anthrax, X: Gel-adsorbed protective antigen for immunization of man. *J Bacteriol*. 1963;85:230–236.

Chapter 23

PLAGUE

THOMAS W. McGOVERN, M.D., FAAD*; AND ARTHUR M. FRIEDLANDER, M.D.†

INTRODUCTION

HISTORY
- The First Pandemic
- The Black Death (The Second Pandemic)
- The Third Pandemic

PLAGUE AND WARFARE
- Endemic Disease
- Plague as a Biological Warfare Agent

THE INFECTIOUS AGENT

EPIDEMIOLOGY

INCIDENCE

PATHOGENESIS

CLINICAL MANIFESTATIONS
- Bubonic Plague
- Septicemic Plague
- Pneumonic Plague
- Plague Meningitis
- Pharyngeal Plague
- Cutaneous Manifestations

DIAGNOSIS
- Signs and Symptoms
- Laboratory Confirmation

TREATMENT
- Isolation
- Antibiotics

PREVENTION
Postexposure Prophylaxis
Immunization

SUMMARY

* *Major, Medical Corps, U.S. Army; Chief, Dermatology Service, Irwin Army Community Hospital, Fort Riley, Kansas 66442*
† *Colonel, Medical Corps, U.S. Army; Chief, Bacteriology Division, U.S. Army Medical Research Institute of Infections Diseases, Fort Detrick, Frederick, Maryland 21702-5011; and Clinical Associate Professor of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799*

INTRODUCTION

Plague is a zoonotic infection caused by *Yersinia pestis*, a Gram-negative bacillus, which has been the cause of three great pandemics of human disease in the common era: in the 6th, 14th, and 20th centuries. The naturally occurring disease in humans is transmitted from rodents and is characterized by the abrupt onset of high fever, painful local lymphadenopathy draining the exposure site (ie, a *bubo*, the inflammatory swelling of one or more lymph nodes, usually in the groin; the confluent mass of nodes, if untreated, may suppurate and drain pus), and bacteremia. Septicemic plague can sometimes ensue from untreated bubonic plague or, de novo, after a flea bite. Patients with the bubonic form of the disease may develop secondary pneumonic plague (also called plague pneumonia); this complication can lead to human-to-human spread by the respiratory route and cause primary pneumonic plague, the most severe and frequently fatal form of the disease.

During the last four millennia, plague has played a role in many military campaigns. During the Vietnam War, plague was endemic among the native population, but U.S. soldiers escaped relatively unaffected. This excellent protection of troops was largely due to our understanding of the rodent reservoirs and flea vectors of disease, the pathophysiology of the various clinical forms of plague, the widespread use throughout the war of a plague vaccine, and prompt treatment of plague victims with effective antibiotics. Mortality from endemic plague continues at low rates throughout the world despite the availability of effective antibiotics. People continue to die of plague, not because the bacilli have become resistant but, most often, because physicians do not include plague in their differential diagnosis (in the United States) or because treatment is absent or delayed (in underdeveloped countries).

To be best prepared to treat soldiers who are plague victims of endemic or biological agent attack by an enemy, military physicians must understand the

natural mechanisms by which plague spreads between species, the pathophysiology of disease in fleas and humans, the minimal diagnostic information necessary to begin treatment with effective antibiotics, and the proper use and capabilities of the presently available plague vaccine.

The United States military's concern with plague is both as an endemic disease and as a biological warfare threat. A better understanding of the preventive medicine aspects of the disease will aid in the prompt diagnosis and effective treatment necessary to survive an enemy attack of plague.

Key terms in this chapter include *enzootic* and *epizootic*. These refer, respectively, to plague that is normally present in an animal community at all times but that occurs in only a small number of animals and in a mildly virulent form, and to widespread plague infections leading to death within an animal community (ie, equivalent to an *epidemic* in a human population). The death of a rodent pressures the living fleas to leave that host and seek other mammals, including humans. Understanding these two simple concepts will help us to understand how and when humans may be attacked, both in endemic and biological warfare scenarios.

HISTORY

The biblical book of I Samuel records what may be the oldest reference to bubonic plague. In approximately 1320 BC, the Philistines stole the Ark of the Covenant from the Israelites and returned home. Then, I Samuel continues,

> [t]he Lord's hand was heavy upon the people of Ashdod and its vicinity; he brought devastation upon them and afflicted them with tumors. And rats appeared in their land, and death and destruction were throughout the city... [T]he Lord's hand was against that city, throwing it into a great panic. He afflicted the people of the city, both young and old, with an outbreak of tumors in the groin.[1]

After this time, plague became established in the countries bordering the eastern Mediterranean Sea.[2] In 430 BC, Sparta won the Peloponnesian War partly because of the plague of Athens.[3] Some scholars believe that this was the bubonic plague, but others suggest that it may have been due to other bacterial or viral diseases.[4]

The First Pandemic

Procopius gave us the first identifiable description of epidemic plague in his account of the plague of the Byzantine empire during the reign of Justinian I (AD 541–542),[5] which we now consider to be the first great pandemic of the common era. As many as 100 million Europeans, including 40% of the population of Constantinople, died during this epidemic.[6,7] Repeated, smaller epidemics followed this plague.[8]

The Black Death (The Second Pandemic)

The second plague pandemic, known as the Black Death, thrust this dread disease into the collective memory of western civilization.[8] Plague bacilli in fleas on the fur of marmots (a rodent of the genus *Marmota*) probably entered Europe via the trans-Asian silk road during the early 14th century. When bales of these furs were opened in Astrakhan and Saray, hungry fleas jumped from the fur seeking the first available blood meal, often a human leg.[8–10] In 1346, plague arrived in Caffa (modern Feodosiya, Ukraine), on the Black Sea. The large rat population there helped spread the disease as they stowed away on ships bound for major European ports such as Pera, a suburb of Constantinople, and Messina, in Sicily. By 1348, plague had already entered Britain at Weymouth.[5]

The Black Death took the lives of 24 million people between the years 1346 and 1352 and claimed perhaps another 20 million by the end of the 14th century.[6] However, the plague continued through 1720, with a final foray into Marseilles. Thirty percent to 60% of the populations of major cities such as Genoa, Milan, Padua, Lyons, and Venice succumbed during the 15th to the 18th centuries.[10]

Physicians of the time offered no effective treatment because they did not understand the epidemiology of plague. At the highly regarded University of Paris, physicians theorized that a conjunction of the planets Saturn, Mars, and Jupiter at 1:00 PM on March 20, 1345, caused a corruption of the surrounding atmosphere that led to the plague.[6] They recommended a simple diet; avoidance of excessive sleep, exercise, and emotion; regular enemas; and abstinence from sexual intercourse.[11] While some people killed cats and dogs, thinking them to be carriers of disease, no one ever thought to kill the rats.[6] Christians blamed the disease on Muslims, Muslims on Christians, and both Christians and Muslims on Jews or on witches.[8]

In 1666, a church rector in Eyam, Derbyshire, England, persuaded the whole community to quarantine itself when plague erupted there. This was the worst possible solution, since the people then stayed in close proximity to the infected rats. The city experienced virtually a 100% attack rate with 72% mortality (the average mortality for the Black Death was consistently 70%–80%).[8,12]

Accurate clinical descriptions of the Black Death were written by contemporary observers such as Boccaccio, who wrote in his *Decameron*:

> The symptoms were not the same as in the East, where a gush of blood from the nose was a plain sign of inevitable death, but it began both in men and women with certain swellings [buboes] in the groin or under the armpit. They grew to the size of a small apple or an egg, more or less, and were vulgarly called tumours. In a short space of time these tumours spread from the two parts named all over the body. Soon after this, the symptoms changed and black or purple spots appeared on the arms or thighs or any

> other part of the body, sometimes a few large ones, sometimes many little ones.[13(p646)]

Guy de Chauliac in Avignon added his own commentary, describing pneumonic plague and the axillary and groin forms of bubonic plague:

> Doctors dared not visit the sick for fear of infection; or, when they did, they helped little and gained nothing.[14(p646)]
>
> . . . .
>
> The disease is three fold in its infection; that is to say, firstly, men suffer in their lungs and breathing and whoever have these corrupted, or even slightly attacked, cannot by any means escape nor live beyond two days...and it is found that all those who have died thus suddenly have had their lungs infected and have spat blood. There is another form of the sickness, however, at present running its course concurrently with the first; that is, certain aposthumes appear under both arms and by these also people quickly die. A third form of the disease—like the two former, running its course at the same time with them—is that from which people of both sexes suffer from aposthumes in the groin. This is likewise quickly fatal.[15(p646)]

Some writers described bizarre neurological disorders, which gave rise to the term “Dance of Death,” followed by anxiety and terror, resignation, blackening of the skin, and death. The sick gave off a terrible stench: “Their sweat, excrement, spittle, breath, [were] so foetid as to be overpowering”[; in addition, their urine was] “turbid, thick, black, or red.”[6(p70)] The second great pandemic slowly died out in Europe by 1720. Many reasons, including the following, have been suggested to explain its decline:

- The oriental rat flea, *Xenopsylla cheopis*, the main vector of the plague bacillus, could no longer exist in the cool European climate.[5]
- The black rat, *Rattus rattus*, was replaced by the brown rat, *Rattus norvegicus*, which was less likely to live in close proximity to man.[5,8]
- A new and less virulent species of *Y pestis*, or a related *Yersinia* species such as *Y pseudotuberculosis*, may have developed, causing natural immunization of infected rats and humans.[8]
- The European population was generally iron deficient, and iron is an essential factor for the bacterium’s virulence.[12]
- Flea density on humans decreased as the use of soap became more widespread.[5]

The Third Pandemic

The third, or modern, plague pandemic arose in 1894 in China and spread throughout the world via modern transportation.[12,16] It was also in 1894 that Alexandre J. E. Yersin discovered that *Yersinia pestis* satisfied Koch's postulates for bubonic plague.[17] The reservoir of plague bacilli in the fleas of the Siberian marmot was likely responsible for the Manchurian pneumonic plague epidemic of 1910–1911, which caused 50,000 deaths.[2] The modern pandemic arrived in Bombay in 1898, and during the next 50 years, more than 13 million Indians died of plague.[2,18]

The disease officially arrived in the United States in March 1900, when the lifeless body of a Chinese laborer was discovered in a hotel basement in San Francisco, California[19]; the disease appeared in New York City and Washington state the same year.[20] New Orleans, Louisiana, was infected in 1924 and 1926.[20] Rodents throughout the western United States were probably infected from the San Francisco focus, leading to more infected rodents in the western United States than existed in Europe at the time of the Black Death.[12] Therefore, human plague was initially a result of urban rat epizootics until 1925. After general rat control and hygiene measures were instituted in various port cities, urban plague vanished—only to spread into rural areas, where virtually all cases in the United States have been acquired since 1925.[21]

PLAGUE AND WARFARE

It is an axiom of warfare that battle casualties are far fewer than casualties caused by disease and nonbattle injuries.[3] *Y pestis* can cause disease both through endemic exposure and as a biological warfare agent. Medical officers need to be able to distinguish likely from unlikely cases of endemic disease, and to keep the possible biological warfare threat in mind.

Endemic Disease

Just as plague befell armies of antiquity, so the disease has also afflicted armies in more recent times. Frederick the Great's troops were devastated by plague in 1745, as were Catherine the Great's in 1769–1771 when they returned from the Balkans with plague. In 1798, French military operations in Egypt were significantly impeded by plague, which even caused them to abandon their attack on Alexandria. The modern pandemic began in China, when Chinese troops were deployed in an epidemic plague area to suppress a Muslim rebellion. Military traffic is responsible for the rapid spread of disease to nearly every country in Asia.[2]

For the U.S. military since the mid 20th century, endemic plague has not been a source of disease and nonbattle injuries. During World War II and the Vietnam War, U.S. forces were almost entirely free of plague. However, the disease remains on and near our military bases because local mammal

populations maintain reservoirs of infection.

World War II

Endemic plague has been established in Hawaii (on the islands of Hawaii and Maui) since December 1899. No evidence of the disease, however, in either rodents or humans, has been found on the islands of Oahu or Kauai since the first decade of this century. A “small outbreak”[22(p667)] occurred during World War II on the island of Hawaii (in 1943) but was contained by means of very strenuous rat control measures [that] were carried out in each of the endemic plague areas.… [T]hese measures were of sufficient thoroughness to prevent any spread of plague to military personnel during the war in the Pacific.[22(p667)]

Official policy during World War II was to vaccinate U.S. troops with a killed plague vaccine. No U.S. troops contracted plague, although they served in known endemic areas.[22,23]

Vietnam War

Plague entered Vietnam in Nha Trang in 1898[16] and several pneumonic epidemics have occurred since (in 1911, 1915, 1925, and 1941).[2,24] Cases have been reported from Vietnam every year since 1898 except during the Japanese occupation during World War II.[2] When French forces departed Vietnam after the Indochina War, public health conditions deteriorated and plague flourished. The reported plague incidence increased from 8 cases in 1961, to 110 cases in 1963, to an average of 4,500 cases from 1965 through 1969.[21,25–28] The mortality in clinically diagnosed cases was between 1% and 5%. In untreated individuals, it was much higher (60%–90%).[2,26] Only 8 American troops were affected (1 case per 1 million man-years) during the Vietnam War.[28] American success was attributed to

- the use of flea insecticide (*Xenopsylla cheopis* became resistant to the insecticide dichloro-diphenyltrichloroethane [DDT] during the war, but others were employed)[26];
- immunization of virtually all American troops with plague vaccine[2]; and
- a thorough understanding of the epidemiology of disease, which led to the use of insect repellents, protective clothing, and rat-proofed dwellings.[2]

It was during the 1960s that our knowledge of plague grew dramatically. This is due in great part to the work of two officers of the Medical Service Corps, U.S. Army, Lieutenant Colonel Dan C. Cavanaugh and Lieutenant Colonel John D. Marshall. These scientists studied plague ecology, related plague epidemics to weather as a function of flea physiology (epidemics virtually disappeared when the temperature rose above 28°C),[2] developed serologic tests for plague infection, and developed the data to demonstrate the efficacy of the whole-cell

killed plague vaccine.[29]

Disease Threat on U.S. Military Installations

Human exposure to plague on military installations may occur when pets bring home infected rodents, their fleas, or both; at recreation areas with sick or dead rodents and their infected fleas; or at field training and bivouac sites. The consequences of plague at a military installation include human illness, death, or both; pet or other animal illness, death, or both; lost use of training and bivouac sites; large expenditures of money, manpower, and equipment to eliminate the plague risk; and the lost use of recreation areas.[21] Plague risk has been identified on and near several U.S. military installations (Exhibit 23-1).

EXHIBIT 23-1
U.S. MILITARY INSTALLATIONS WITH IDENTIFIED PLAGUE RISKS*

Plague-infected animals on the installation; human case reported on post:

- Fort Hunter Liggett, California
- United States Air Force Academy, Colorado†

Human case reported in the same county:

- Edwards Air Force Base, Colorado‡
- F. E. Warren Air Force Base, Wyoming
- Kirtland Air Force Base, New Mexico§
- Peterson Air Force Base, Colorado

Plague-infected animals on the installation:

- Dugway Proving Ground, Utah
- Fort Carson, Colorado
- Fort Ord, California
- Fort Wingate Army Depot Activity, New Mexico
- Marine Corps Mountain Warfare Training Center, Bridgeport, California
- Navajo Army Depot Activity, Arizona
- Pueblo Army Depot Activity, Colorado
- Rocky Mountain Arsenal, Colorado
- Vandenberg Air Force Base, California
- White Sands Missile Range, New Mexico

Plague-infected animals or fleas are not on the installation but are in the same county:

- Bridgeport Naval Facility, California
- Camp Roberts, California
- Dyess Air Force Base, Texas
- Fort Bliss, Texas
- Fort Lewis, Washington
- Sierra Army Depot, California
- Tooele Army Depot, Utah
- Umatilla Army Depot Activity, Oregon
- Nellis Air Force Base, Nevada

Plague-infected animals or fleas are not on the installation or in the county, but susceptible animals are present:

- Fort Huachuca, Arizona

---

*Does not include military installations near Los Angeles and San Francisco, California, where urban plague cases and deaths were not uncommon in the first quarter of the 20th century; no plague cases have occurred in these urban areas since the mid-1920s.
† Fatality: 18-mo-old child died of pneumonic plague; rock squirrels and their fleas had taken up residence in the ducts of the on-base house.
‡ Two human cases in the same county in 1995; animal surveillance on base began in 1996.
§ Plague-infected animals in the county in 1995; last human case in the county in 1993; no animal surveillance on base since 1986.
Sources: (1) Harrison FJ. *Prevention and Control of Plague.* Aurora, Colo: United States Army Center for Health Promotion and Preventive Medicine, Fitzsimons Army Medical Center; September 1995: 3–8. Technical Guide 103. (2) Data collected from Preventive Medicine Officers on 30 military bases in the United States, March 1996.

Plague as a Biological Warfare Agent

The first attempt at what we now call “biological warfare” is purported to have occurred at the Crimean port city of Caffa on the Black Sea during the years 1346–1347.[2,6] During the conflict between Christian Genoese sailors and Muslim Tatars, the Tatar army was struck with plague. The Tatar leader catapulted corpses of Tatar plague victims at the Genoese sailors. The Genoese became infected with plague and fled to Italy. However, the disease was most likely spread by the local population of infected rats, not by the corpses, since an infected flea leaves its host as soon as the corpse cools.[6]

The 20th-century use of plague as a potential biological warfare weapon is the immediate concern of this chapter. Medical officers need to keep this use of plague in mind, particularly when the disease appears in an unlikely setting.

World War II

During World War II, the Japanese army established a secret biological

warfare research unit (Unit 731) in Manchuria, where epidemics of pneumonic plague had occurred in 1910–1911, 1920–1921, and 1927, and a cholera epidemic had spread in 1919. General Shiro Ishii, the physician leader of Unit 731, was fascinated by plague because it could create casualties out of proportion to the number of bacteria disseminated, the most dangerous strains could be used to make a very dangerous weapon, and its origins could be concealed to appear as a natural occurrence. Early experiments, however, demonstrated that dropping bacteria out of aerial bombs had little effect because air pressure and high temperatures that were created by the exploding bombs killed nearly 100% of the bacteria.[30]

One of Ishii's greatest achievements was his use of the human flea, *Pulex irritans*, as a stratagem to simultaneously protect the bacteria and target humans. This flea is resistant to air drag, naturally targets humans, and could also infect a local rat population to prolong an epidemic. Infected fleas may regurgitate up to 24,000 organisms in a single feeding. Spraying fleas out of compressed-air containers was not successful since aircraft had to fly too low for safety. High flying meant too much dispersion. Clay bombs solved these problems and resulted in an 80% survival rate of fleas.[30]

The Japanese apparently used plague as a biological warfare agent in China several times during World War II. At 0500 hours on a November morning in 1941, a lone Japanese plane made three low passes over the business center of Changteh, a city in the Hunan province. Although no bombs were dropped, a strange mixture of wheat and rice grains, pieces of paper, cotton wadding, and other unidentified particles were. Within 2 weeks, individuals in Changteh started dying of plague. This miniepidemic was thought to be of human origin for the following reasons[30]:

- Changteh and the whole surrounding area of China had never been afflicted by plague.
- Plague usually spreads with rice (because rats infest the grain) along shipping routes, but the nearest epidemic center was 2,000 km away by land or river. Changteh exported, not imported, rice. No individual who contracted plague had recently traveled outside the city.
- All reported instances of human plague occurred in the area of the city where the strange particles were dropped.
- No evidence of excessive rat mortality occurred until 2 months *after* the people began dying.
- The first six human cases occurred within 15 days of the aerial incident.

Applying the concepts implicit in these five points will help medical officers differentiate endemic plague from plague used as a biological warfare agent. In fact, these concepts are important in making a diagnosis of most forms of biological warfare.

In another incident, on October 4, 1940, a Japanese plane dropped rice and wheat grains mixed with fleas over the city of Chuhsien, in Chekiang province. A

month later, bubonic plague appeared for the first time there, in the area where the particles had been dropped. There were 21 plague deaths in 24 days. Again, on October 27, 1940, a Japanese plane was seen releasing similar particles over the city of Ningpo, in Chekiang province. Two days later, bubonic plague occurred for the first time in that city, producing 99 deaths in 34 days. No epizootic or excessive mortality was found in the rat population.[30]

Since World War II

An article[31] published in the popular press in 1993 stated that in the 1970s and 1980s the Soviet Union created lethal diseases that defied cures. This included a genetically engineered, dry, antibiotic-resistant form of plague. In this article, a defecting Soviet microbiologist was quoted as saying that producing this form of plague had been a top priority of the Soviets in the 5-year plan that started in 1984.

During the Korean War, allied forces were accused of dropping on North Korea insects that were capable of spreading plague, typhus, malaria, Japanese B encephalitis, and other diseases. No evidence exists to support such claims.[32]

THE INFECTIOUS AGENT

Y pestis is a Gram-negative, nonacid-fast, nonmotile, nonsporulating, nonlactose-fermenting, bipolar coccobacillus measuring 0.5–0.8 × 1.5–2.0 µm. The Yersinieae comprise Genus XI of the family Enterobacteriaceae, which includes the related enteropathogenic bacteria *Y enterocolitica* and *Y pseudotuberculosis*. Its bipolar appearance is best appreciated when Wright-Giemsa, Wayson's, and Gram's stains are used (Figure 23-1). *Y pestis* grows optimally at 28°C, producing tiny, 1- to 3-mm "beaten-copper" colonies after 48 hours on blood or MacConkey's agar. After 24 hours' growth in standard peptone broth, moderate growth with little or no turbidity is observed. Biochemically, the plague bacillus produces no hemolysins; is positive for catalase; and is negative for hydrogen sulfide, oxidase, urease, and fermentation of lactose, sucrose, rhamnose, and melibiose.[2]

The known virulence factors of *Y pestis* are encoded on the chromosome and its three plasmids. A chromosomal locus responsible for pigmentation phenotype, iron-inducible proteins, and iron uptake is necessary for virulence from a peripheral route of inoculation.[33] The pH 6 antigen (also encoded on the

chromosome), a protein located on the surface of the bacterium, is necessary for complete virulence.[34] It is induced in vitro at low pH, perhaps in vivo at sites of inflammation and cellular necrosis, and within phagocytic cells.

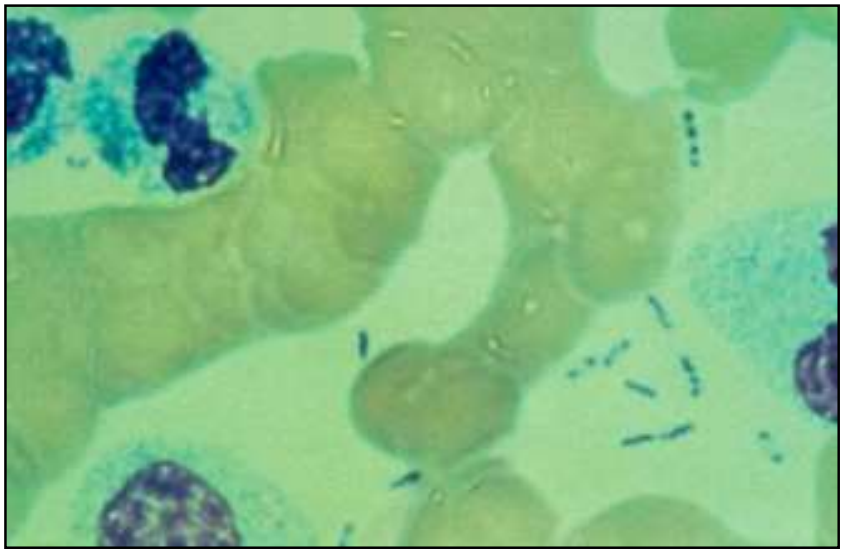


Fig. 23-1. This Wright-Giemsa stain of a peripheral blood smear from a patient with septicemic plague demonstrates the bipolar, safety-pin staining of Yersinia pestis. Gram's and Wayson's stains can also demonstrate this pattern. Photomicrograph: Courtesy of Ken Gage, Ph.D., Centers for Disease Control and Prevention, Fort Collins, Colo.

The low calcium response (Lcr) plasmid of approximately 75 kilobase (kb), which is homologous in *Y pestis* and the other two *Yersinia* pathogens, *Y pseudotuberculosis* and *Y enterocolitica*, encodes for several secreted proteins, including *Yersinia* outer-membrane proteins (Yops), necessary for virulence.[35] These proteins are produced in vitro in a low-calcium environment and, in some instances, by attachment to eucaryotic cells.[36] They include

- the V antigen, which is involved in regulation of growth and other plasmid-encoded secreted virulence proteins,[37] and which may also have a more direct role in virulence[33];
- Yop M, which binds thrombin, inhibits platelet aggregation, and may prevent an effective inflammatory response[38];
- Yops K and L, of unknown function[39]; and
- several proteins that interfere with phagocytic cell function, including Yop H, a tyrosine phosphatase,[40] and Yop E.[41]

Although additional virulence factors encoded on the Lcr plasmid have been described[35] for the other *Yersinia* species, confirmation of their importance in plague is not yet established.

Y pestis also possesses two additional plasmids not present in the other *Yersinia* species. First, a 9.5-kb plasmid encodes for a plasminogen activator protease, which is most active at temperatures higher than 30°C.[33] This proteolytic enzyme is necessary for systemic spread of infection from a peripheral subcutaneous site, perhaps by causing degradation of fibrin and extracellular matrix proteins, and by impairing the inflammatory response.[42] This same protease has predominantly coagulase activity at temperatures lower than 30°C.[33]

The second unique plasmid, of approximately 100 kb, codes for the protein capsule (fraction 1 antigen) of *Y pestis*. The capsule is antiphagocytic and necessary for full

virulence in some animal species.[43] The 100-kb plasmid also encodes for an exotoxin that is active in the mouse and rat but not in primates.[33]

EPIDEMIOLOGY

During the modern pandemic, W. G. Liston, a member of the Indian Plague Commission (1898–1914), made the association of plague with rats and incriminated the rat flea as a vector.[2] Subsequently, more than 200 species of animals and 80 species of fleas have been implicated in maintaining *Y pestis* endemic foci throughout the world.[21]

Throughout history, the oriental rat flea (*Xenopsylla cheopis*) has been largely responsible for spreading bubonic plague.[5] After the flea ingests a blood meal on a bacteremic animal, bacilli can multiply and eventually block the flea's foregut, or proventriculus, with a fibrinoid mass of bacteria (Figure 23-2).[2] When an infected flea with a blocked foregut attempts to feed again, it regurgitates clotted blood and bacteria into the victim's blood-stream, and so passes the infection on to the next mammal—whether rat or human. As many as 24,000 organisms may be inoculated into the mammalian host.[2] This flea desiccates rapidly in very hot and dry weather when away from its hosts, but flourishes at humidity just above 65% and temperatures between 20°C and 26°C,[2] and can survive 6 months without a feeding.[21]

Fig. 23-2. The oriental rat flea *(Xenopsylla cheopis)* has historically been most responsible for the spread of plague to humans. This flea has a blocked proventriculus, equivalent to a human's gastroesophageal region. In nature, this flea would develop a ravenous hunger because of its inability to digest the fibrinoid mass of blood and bacteria. The ensuing biting of the nearest mammal will clear the proventriculus through regurgitation of thousands of bacteria into the bite wound, thereby inoculating the mammal with the plague bacillus. Photomicrograph: Courtesy of Ken Gage, Ph.D., Centers for Disease Control and Prevention, Fort Collins, Colo.

Although the largest outbreaks of plague have been associated with *X cheopis*, all fleas should be considered dangerous in plague endemic areas.[2] During the Black Death, the human flea, *Pulex irritans*, may have aided in human-to-human spread of plague; and during other epidemics, bedbugs (*Cimex lectularius*), lice, and flies have been found to contain *Y pestis*.[5] The presence of plague bacilli in these latter insects is associated with ingestion of contaminated blood from plague victims, however, and plays little or no role as a vector for the disease. The most important vector of human plague in the United States is *Diamanus montanus*, the most common flea on rock squirrels and California ground squirrels.[21]

Throughout history, the black rat, *Rattus rattus*, has been most responsible worldwide for the persistence and spread of plague in urban epidemics. *R rattus* is a nocturnal, climbing animal that does not burrow. Instead, it nests overhead and lives in close proximity to humans.[5] In the United Kingdom and much of Europe, the brown rat, *R*

norvegicus, has replaced *R rattus* as the dominant city rat.[44] Unlike *R rattus*, *R norvegicus* is essentially a burrowing animal that lives under farm buildings and in ditches. However, *R norvegicus* may be involved in both rural and urban outbreaks of plague.[5]

Most carnivores, except cats, are resistant to plague infection, but animals such as domestic dogs, all rodents, and even burrowing owls may mechanically transmit fleas. Mammals that are partially resistant to plague infection serve as continuous reservoirs of plague. In the United States, deer mice (*Peromyscus* species) and ground squirrels (*Spermophilus* species) are thought to serve as the main reservoirs. Some susceptible mammals are only occasionally infected: chipmunks, tree squirrels, cottontail rabbits, and domestic cats (Figure 23-3).

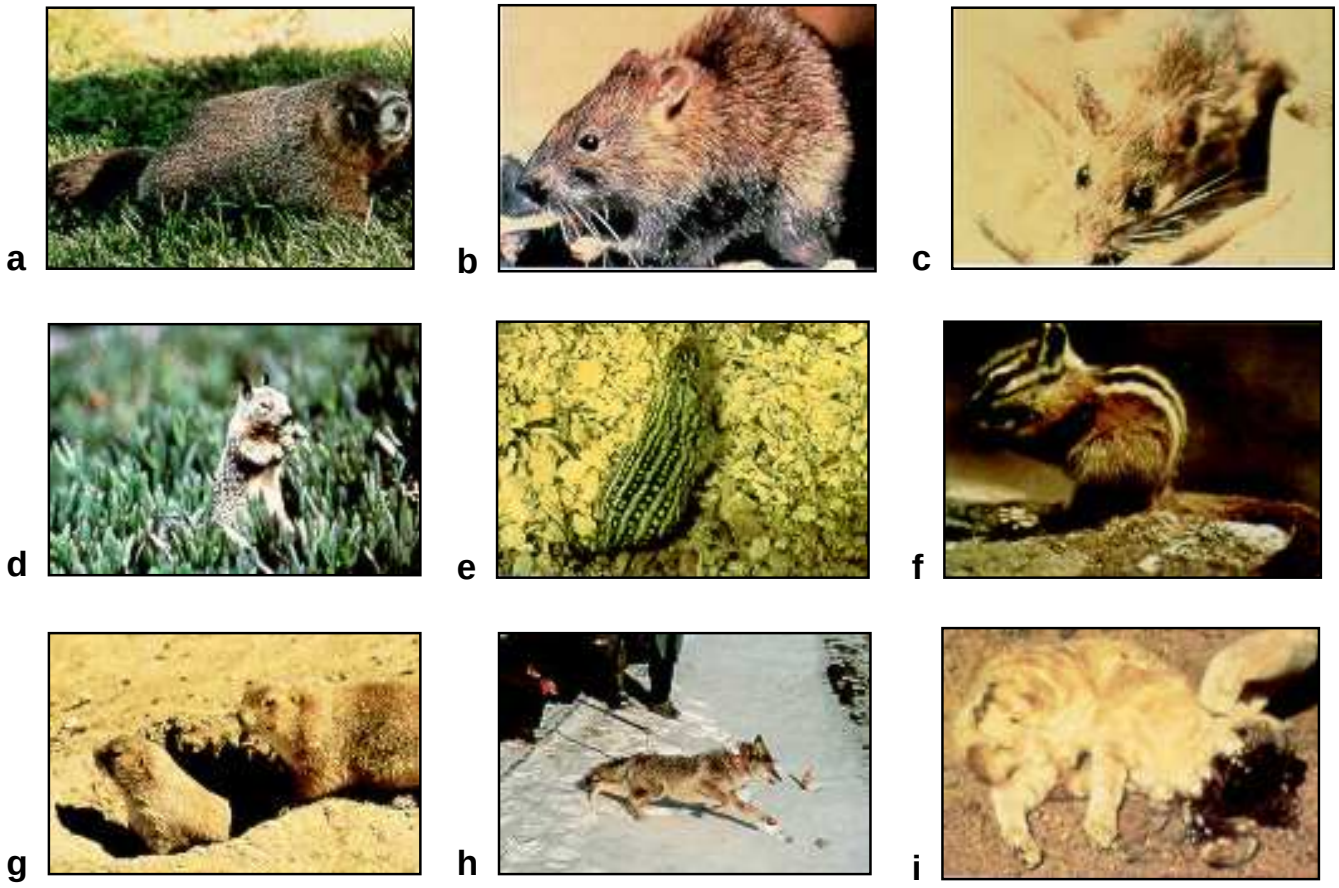


Fig. 23-3. Known mammalian reservoirs of plague in the United States (noninclusive). The common North American marmot **(a)** and the brown rat (Rattus norvegicus) **(b)**, which has largely replaced the black rat, are considered to be reservoirs of plague (ie, hosts to infected fleas). Other reservoirs of plague during enzootics are thought to include the deer mouse **(c)**, the California ground squirrel **(d)**, and the 13-lined ground squirrel **(e)**. Other infective mammals that can spread plague to humans include the chipmunk **(f)**, prairie dogs **(g)**, and the coyote **(h)**. Domestic and nondomestic cats are also reservoirs of plague. This cat **(i)**, which died of pneumonic plague, demonstrates a necrotic head. Photographs a, h: Courtesy of Denver Zoological Society, Denver, Colo. Photographs b–g, i: Courtesy of Centers for Disease Control and Prevention, Fort Collins, Colo.

Highly susceptible animals amplify both fleas and bacilli. Such epizootics occur in chipmunks, ground squirrels, and wood rats, but especially in prairie dogs, rock squirrels (*Spermophilus variegatus*), and California ground squirrels (*Spermophilus beechyi*). Although prairie dog fleas rarely bite humans, the infectious rodents can transmit plague to humans via direct contact (eg, handling a live or dead animal; stumbling into a nest while walking; or dissecting specimens [primarily laboratory personnel]). Rock squirrels and California ground squirrels both infect humans via direct contact and fleas.[5,21,45,46]

Many mammals in the United States harbor plague (Exhibit 23-2). Knowledge of this wide-spread harborage is important, because certain mammal–flea complexes found in the United States are dangerous: they contain both a susceptible mammal and a flea known to bite humans. These pairings include the following[21]:

- the rock squirrel (*S variegatus*) or California ground squirrel (*S beechyi*) and the fleas *Diamanus montanus* or *Hoplopsyllus anomalus*;
- the prairie dog (*Cynomys* species) and the flea *Opisochrostis hirsutus*; and
- Richardson's ground squirrel (*Spermophilus richardsoni*) or the golden-mantled ground squirrel (*S lateralis*) and the fleas *Oropsylla labis, O idahoensis*, or *Thrassus bacchi*.

EXHIBIT 23-2
MAMMALS KNOWN TO HARBOR PLAGUE IN THE UNITED STATES

Carnivores
- Black bears, cats (including bobcats and mountain lions), coyotes, dogs, foxes, martens, raccoons, skunks, weasels, wolverines, wolves

Rodents
- Chipmunks, gophers, marmots, mice, prairie dogs, rats, squirrels, voles

Lagomorphs
- Hares, rabbits

Hooved Stock
- Pigs, mule deer, pronghorn antelope

Adapted from Harrison FJ. *Prevention and Control of Plague.* Aurora, Colo: US Army Center for Health Promotion and Preventive Medicine, Fitzsimons Army Medical Center; September 1995: 25–28.Technical Guide 103.

Plague exists in one of two states in nature, enzootic or epizootic. An enzootic is the state of a stable rodent–flea infection cycle in a relatively resistant host population, without excessive rodent mortality. Importantly for humans, when the disease is in an enzootic state, the fleas have no need to seek less desirable hosts—such as ourselves. During an epizootic, on the other hand, plague bacilli have been introduced into moderately or highly susceptible mammals. High mortality occurs, most conspicuously in

larger colonial rodents such as prairie dogs.[47]

Man is an accidental host in the plague cycle (Figure 23-4) and is not necessary for the persistence of the organism in nature. Humans usually acquire plague from

- fleas whose usual host is another mammal (eg, from flea bites, flea feces inoculated into skin with bites, and by directly biting the fleas [during the grooming behavior practiced in some cultures]);
- fleas whose usual host is a human;
- infected animals (eg, from aerosols, draining abscesses, eating infected tissue, and handling infected pelts); and
- other humans, via aerosol or direct contact with infected body substances.

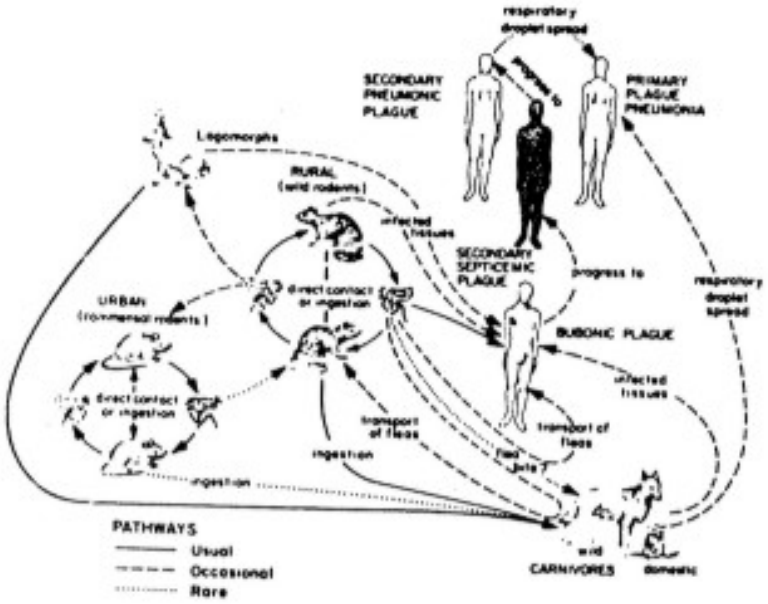


Fig. 23-4. Plague cycles in the United States. This drawing shows the usual, occasional, and rare routes by which plague is known to have spread between various mammals and humans. Reprinted with permission from Poland JD. Plague. In: Hoeprich PD, Jordan MC, eds. *Infectious Diseases: A Modern Treatise of Infectious Processes*. Philadelphia, Pa: Lippincott; 1989: 1297.

The greatest risk to humans occurs when large concentrations of people live under unsanitary conditions in close proximity to large commensal or wild rodent populations that are infested with fleas that bite both humans and rodents.[2]

Human-to-human transmission of plague can occur from patients with pulmonary infection. However, understanding of the epidemiology of pneumonic plague is incomplete. Most epidemics have occurred in cool climates with moderate humidity and close contact between susceptible individuals. Outbreaks of pneumonic plague have been rare in tropical climates even during epidemics of bubonic disease. Respiratory transmission may occur more efficiently via larger droplets or fomites rather than via small-particle aerosols.[48]

INCIDENCE

Worldwide cases of plague and mortalities are shown in Figure 23-5, and the known foci of plague in Figure 23-6. In 1992, most of the reported 1,582 cases occurred in Myanmar and Vietnam in Asia, and Zaire and Madagascar in Africa. Worldwide mortality was 8.7%. The outbreaks in 1994 of pneumonic and bubonic plague in India, and bubonic plague in Tanzania and Peru, highlight the potential for epidemics to arise from these foci.[49]

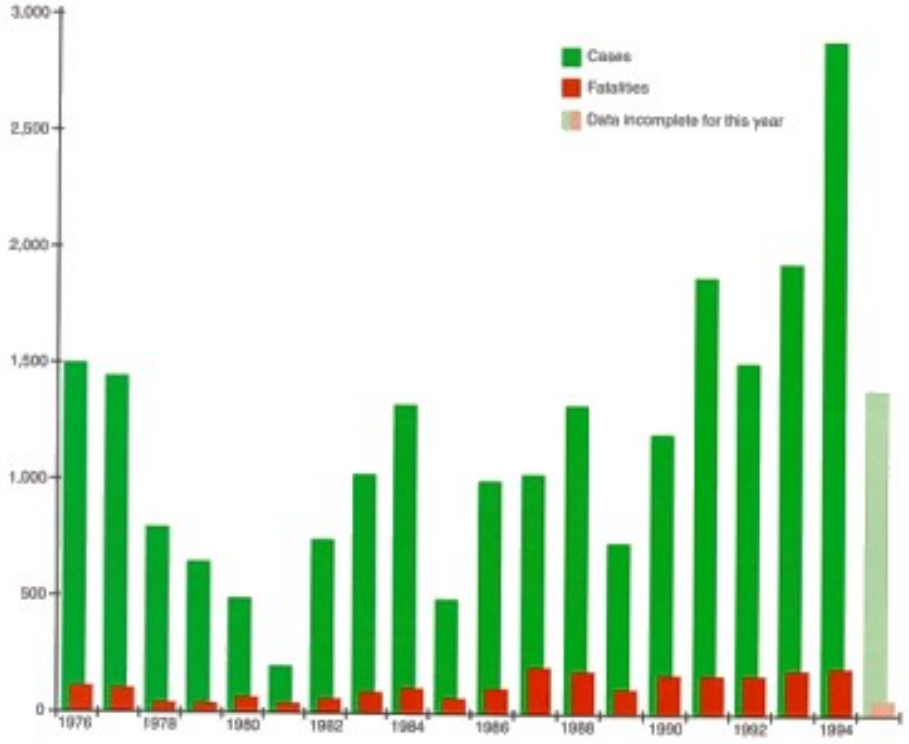


Fig. 23-5. Absolute numbers of annual worldwide plague cases, 1976–1994. Fatalities (a subset of the total cases) are shown in red, total cases in green. Data are as of 7 February 1996; reports for 1995 were not complete at that time. Note that the mortality rate continues between 5% and 12% despite the availability of effective antibiotics. Data sources: (1) Human plague in 1990. *WHO Weekly Epidemiological Record*. 1 Nov 1991;44:321–324. (2) Human plague in 1993. *WHO Weekly Epidemiological Record*. 17 Feb 1995;7:45–48. (3) Barkway J. World Health Organization, Geneva, Switzerland. Personal communication, 7 February 1996.

Fig. 23-6. Known worldwide foci of human plague infection. Data sources: (1) Human plague in 1990. *WHO Weekly Epidemiological Record*. 1 Nov 1991;44:321–324. (2) Human plague in 1993. *WHO Weekly Epidemiological Record*. 17 Feb 1995;7:45–48. (3) Barkway J. World Health Organization, Geneva, Switzerland. Personal communication, February 1996. (4) Ken Gage, Ph.D., Centers for Disease Control and Prevention, Fort Collins, Colorado. Personal communication, March 1996.

Plague has been endemic in the continental United States since at least 1900[20] and now is permanently established from the eastern slope of the Rocky Mountains westward—especially in pine-oak or piñon–juniper woodland habitats at altitudes of 5,000 to 9,000 ft, or on lower, dry grassland or desert scrub areas.[21] Between 1970 and 1990, 56% of all cases occurred in New Mexico, 14% in Arizona, and 10% in Colorado.[45]

In the first quarter of this century, virtually all 432 cases and 284 deaths (65.7% mortality) in the United States occurred in urban port cities. Epidemics occurred in San Francisco, California, during the years 1900–1904 (118 deaths) and 1907–1908 (78 deaths). The last time plague was transmitted between humans in the United States was during the 1924–1925 pneumonic plague epidemic in Los Angeles, California. Eighty percent of cases since 1925 have been sylvatic, involving contact with wild-rodent habitats.[20] Most cases (58%) are in men and occur within a 1-mile radius of home,[21] and half the victims in the United States have been younger than 20 years old.[20]

Between 1926 and 1960, the United States averaged only 1 case of plague per year. This number steadily rose to 3 per year during the 1960s, 11 during the 1970s, 18 during the 1980s, and then decreased to 9 per year since 1990.[45] The number of states reporting human plague cases has steadily increased over the last 5 decades, most likely because increasing encroachment of humans on previously wild areas brings people closer to infected animals and their fleas.[21]

In the United States, 93% of cases have occurred between April and November, peaking in July. During the last 25 years, pneumonic plague accounted for 11% of cases, and bubonic or septicemic plague, or both, for 89%. One case of meningitic plague also occurred.[45]

Epizootic cycles occur approximately every 5 years. The last large epizootic with a large die-off of rodents (1982–1984) was accompanied by the highest number of humans infected with plague since the urban epidemics of the first quarter of the century. The numbers of rodents slowly recovered to their characteristic levels by 1991, and the stage is now set for another epizootic, with the potential for increased human plague infections.[45,46]

PATHOGENESIS

As few as 1 to 10 *Y pestis* organisms are sufficient to infect rodents and primates via the oral, intradermal, subcutaneous, and intravenous routes.[33] Estimates of infectivity by the respiratory route for nonhuman primates vary from 100 to 20,000 organisms.[50,51]

After being introduced into the mammalian host by a flea, where it had been at ambient temperature, the organism is thought to be initially susceptible to phagocytosis and killing by neutrophils. However, some of the bacteria may grow and proliferate within tissue macrophages.[52] Within the human host, several new environmental signals (including elevated temperature of 37°C, contact with eucaryotic cells, and perhaps the location within cells or in necrotic foci at low pH) are thought to induce the synthesis and activity of a multitude of factors contributing to virulence. These include the antiphagocytic fraction 1 capsule, pH 6 antigen, the antiphagocytic Yops H and E, V antigen, Yop M, and plasminogen activator. The bacteria in this state are now resistant to phagocytosis and they proliferate unimpeded extracellularly.

During the incubation phase, the bacilli most commonly spread to regional lymph nodes, where suppurative lymphadenitis develops, producing the characteristic bubo. Dissemination from the local site is thought to be related to the action of both plasminogen activator and Yop M. Infection will progress if untreated; septicemia will develop and the infection will spread to other organs. The endotoxin of *Y pestis* probably contributes to the development of septic shock, which is similar to the shock state seen in other causes of Gram-negative sepsis. The endotoxin also contributes to the resistance of the organism to the bactericidal activity of serum.[33] The acral cyanosis and necrosis seen in some cases of septicemic plague may also be related to the coagulase activity of the plasminogen activator, which occurs at temperatures lower than

37°C.[2]

Tissues most commonly infected include the spleen, liver, lungs, skin, and mucous membranes. Late infection of the meninges also occurs, especially if suboptimal antibiotic therapy has been given.

Primary pneumonic plague, the most severe form of disease, arises from inhalation of an infectious aerosol. Primary pneumonic plague is more rapidly fatal than secondary, because the inhaled droplets already contain phagocytosis-resistant bacilli, which have arisen from their growth at 37°C in the vertebrate host.[47]

Primary septicemic plague can occur from direct inoculation of bacilli into the bloodstream, bypassing initial multiplication in the lymph nodes. Asymptomatic pharyngeal carriage of plague has been reported to occur in contacts of patients with either bubonic or pneumonic plague.[53,54]

CLINICAL MANIFESTATIONS

In the United States, most patients (85%–90%) with human plague present clinically with the bubonic form, 10% to 15% with the primary septicemic form, and 1% with the pneumonic form. Secondary septicemic plague occurs in 23% of patients who present with bubonic plague, and secondary pneumonic plague occurs in 9%.[46] If *Y pestis* were used as a biological warfare agent, the clinical manifestations of plague would be *(a)* epidemic pneumonia with blood-tinged sputum if aerosolized bacteria were used or *(b)* bubonic or septicemic plague, or both, if fleas were used as carriers.

Bubonic Plague

Buboes manifest after a 1- to 8-day incubation period, with the regular onset of symptoms of sudden fever, chills, and headache often followed several hours later by nausea and vomiting. Presenting symptoms include prostration or severe malaise (75%), headache (20%–85%), vomiting (25%–49%), chills (40%), altered mentation (26%–38%), cough (25%), abdominal pain (18%), and chest pain (13%).[2] Six to 8 hours after onset of symptoms, buboes, heralded by severe pain, occur in the groin (90%, with femoral more frequent than inguinal), axillary, or cervical lymph nodes—depending on the site of bacterial inoculation (Figure 23-7). Buboes become visible within 24 hours; they are so intensely painful that even nearly comatose patients will attempt to shield them from trauma and will abduct their extremities to decrease pressure. Other manifestations of bubonic plague include bladder distention, apathy, confusion, fright, anxiety, oliguria, and anuria. Tachycardia, hypotension, leukocytosis, and fever are frequently encountered. Untreated, septicemia will develop in 2 to 6 days.[55] Approximately 5% to 15% of bubonic plague patients will develop secondary pneumonic plague and, as a result, the potential for airborne transmission.[56]

a

b

Fig. 23-7. A femoral bubo **(a)**, the most common site of an erythematous, tender, swollen, lymph node in patients with plague. This painful lesion may be aspirated in a sterile fashion to relieve pain and pressure; it should not be incised and drained. The next most common lymph node regions involved are the inguinal, axillary **(b)**, and cervical areas. Bubo location is a function of the region of the body in which an infected flea inoculates the plague bacilli. Photographs: Courtesy of Ken Gage, Ph.D., Centers for Disease Control and Prevention, Fort Collins, Colo.

Septicemic Plague

Septicemic plague may occur primarily, or secondarily as a complication of hematogenous dissemination of bubonic plague. Presenting signs and symptoms of primary septicemic plague are essentially the same as those for any Gram-negative septicemia: fever, chills, nausea, vomiting, and diarrhea. Later, purpura (Figure 23-8), disseminated intravascular coagulation (DIC), and acral cyanosis and necrosis (Figure 23-9) may be seen.

Fig. 23-8. Purpuric lesions can be seen on the upper chest of this girl with plague. The bandage on her neck indicates that a bubo

has been aspirated. Photograph: Courtesy Ken Gage, Ph.D., Centers of Disease Control and Prevention, Fort Collins, Colo.

a

b

Fig. 23-9. This patient is recovering from bubonic plague that disseminated to the blood (septicemic form) and the lungs (pneumonic form). Note the dressing over the tracheostomy site. At one point, the patient's entire body was purpuric. Note the acral necrosis of **(a)** the patient's nose and fingers and **(b)** the toes. Photographs: Courtesy Ken Gage, Ph.D., Centers of Disease Control and Prevention, Fort Collins, Colo.

In New Mexico between 1980 and 1984, plague was suspected in 69% of patients who had bubonic plague, but in only 17% of patients who had the septicemic form. The mortality was 33.3% for septicemic plague versus 11.5% for bubonic, thus highlighting the difficulty of diagnosing septicemic plague. Diagnosis of septicemic plague took longer (5 vs 4 d) after onset, although patients sought physicians earlier (1.7 vs 2.1 d) and were hospitalized sooner (5.3 vs 6.0 d) than patients with bubonic plague. The only symptom present significantly more frequently in septicemic than in bubonic plague was abdominal pain (40% vs < 10%), probably due to hepatosplenomegaly.[57]

The risk of *developing* septicemic plague is higher for individuals older than 40 years of age, although the risk of *dying* from septicemic plague is higher for those younger than 30 years. This difference is most likely due to older undiagnosed patients being treated empirically with antibiotics that kill *Y pestis*, and younger undiagnosed patients being treated with antibiotics (such as penicillin) that do not affect *Y pestis*. Earlier diagnosis and appropriate therapy, not newer antibiotics, will have the greatest effect on reducing mortality from septicemic plague.[57]

Pneumonic Plague

Pneumonic plague may occur primarily, from inhalation of aerosols, or secondarily, from hematogenous dissemination. Patients typically have a productive cough with blood-tinged sputum within 24 hours after onset of symptoms.[2] The findings on chest roentgenography may be variable, but bilateral alveolar infiltrates appear to be the most common finding in pneumonic plague (Figure 23-10).[58,59]

Fig. 23-10. This chest roentgenogram shows right middle-and lower-lobe involvement in a patient with pneumonic plague. Photograph: Courtesy Ken Gage, Ph.D., Centers for Disease Control and Prevention, Fort Collins, Colo.

Plague Meningitis

Plague meningitis is seen in 6% to 7% of cases. The condition manifests itself most often in children after 9 to 14 days of ineffective treatment. Symptoms are similar to those of other forms of

acute bacterial meningitis.[60]

Pharyngeal Plague

Asymptomatic pharyngeal carriage has been reported to occur in contacts of plague patients.[53,54] Rarely, pharyngitis—resembling tonsillitis and associated with cervical lymphadenopathy—has been reported.[17,55] A plague syndrome of cervical buboes, peritonsillar abscesses, and fulminant pneumonia has also been reported to occur among Indians of Ecuador, who are known to catch and kill fleas and lice with their teeth. It is thought, although not proven, that endobronchial aspiration from peritonsillar abscesses leads to fulminant pneumonia. A similar syndrome may have occurred in Vietnam.[55]

Cutaneous Manifestations

Approximately 4% to 10% of plague patients are said to have an ulcer or pustule at the inoculation site (Figure 23-11).[59,61] The flea typically bites the lower extremities; therefore, femoral and inguinal buboes are the most common. Infection arising from the skinning of infected animals typically produces axillary buboes. Buboes may point and drain spontaneously or, rarely, they may require incision and drainage because of pronounced necrosis.

Fig. 23-11. This child has left axillary bubonic plague. The erythematous, eroded, crusting, necrotic ulcer on the child's left upper quadrant is located at the presumed primary inoculation site. Photograph: Courtesy of Ken Gage, Ph.D., Centers for Disease Control and Prevention, Fort Collins, Colo.

Petechiae and ecchymoses may occur during hematogenous spread to such an extent that the signs mimic severe meningococcemia, and the microscopic lesions are almost indistinguishable. The pathogenesis of these lesions is probably that of a generalized Shwartzman reaction (DIC secondary to the *Y pestis* endotoxin). Purpura and acral gangrene may also be due to the activities of the plasminogen activator/coagulase enzyme, and prognosis is poor when these signs occur.[2,62] Patients in the terminal stages of pneumonic and septicemic plague often develop large ecchymoses on the back. Lesions like these are likely to have given rise to the medieval epithet "the Black Death."

Ecthyma gangrenosum has been reported in several patients.[53,62] The only case cultured grew *Y pestis*, which suggests that the skin lesions were the result of septicemic seeding of the organism.[62]

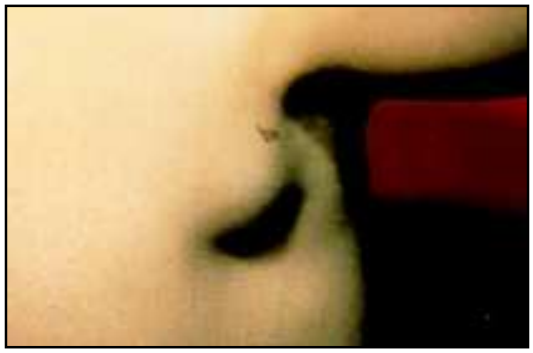
DIAGNOSIS

Signs and Symptoms

A patient with a typical presentation of bubonic plague (eg, with a painful bubo in the setting of fever, prostration, and possible exposure to rodents or fleas in an endemic area) should readily suggest the diagnosis of plague. However, if the medical officer is not familiar with the disease or if the patient presents in a nonendemic area or without a bubo, then the diagnosis can be most difficult. When a bubo is present, the differential diagnosis should include tularemia, cat scratch disease, lymphogranuloma venereum, chancroid, tuberculosis, streptococcal adenitis, and scrub typhus (Figure 23-12). In both tularemia and cat scratch disease, the inoculation site will usually be more evident and the patient will usually not be septic. In chancroid and scrofula, the patient has less local pain, the course is more indolent, and there is no sepsis. Patients with chancroid and lymphogranuloma venereum will have a recent history of sexual contact and genital lesions. Those with the latter disease may be as sick as patients with plague. Streptococcal adenitis may be difficult to distinguish initially, but the patient is usually not septic, and the node is more tender when plague is present.

a

c

b 

d

Fig. 23-12. (a) Small femoral bubo and presumed inoculation site (on the inferior thigh) in a patient with tularemia. This Gram-negative bacterial infection (with *Francisella tularensis*) may closely mimic bubonic plague and is successfully treated with the same antibiotics. **(b)** Axillary bubo seen in child with cat scratch disease. **(c)** Greenblatt's sign of ipsilateral femoral and inguinal buboes with intervening depression over the inguinal ligament, seen in a patient with lymphogranuloma venereum caused by *Chlamydia trachomatis*. **(d)** Large inguinal bubo seen in a patient with chancroid caused by *Haemophilus ducreyi*. Photographs: Courtesy of Dermatology Service, Fitzsimons Army Medical Center, Aurora, Colo.

The implications of the absence of a bubo were clearly demonstrated in a review of 27 cases of plague seen in New Mexico.[59] There were no deaths among 10 patients with typical bubonic plague. However, 3 of 5 patients died who presented with an upper respiratory infection syndrome of fever, sore throat, and headache. Similarly, 3 of 5 patients died who presented with fever, chills, and anorexia. The other 7 patients presented with nonspecific gastrointestinal and urinary tract symptoms without a bubo. Thus, other causes of lymphadenitis, upper respiratory tract infection, gastrointestinal disease including appendicitis, and nonspecific febrile illnesses, must all be considered.

The differential diagnosis of septicemic plague also includes meningococcemia, Gram-negative sepsis, and the rickettsioses. The patient with pneumonic plague who presents with systemic toxicity, a productive cough, and bloody sputum suggests a large differential diagnosis. However, demonstration of Gram-negative rods in the sputum should readily suggest the correct diagnosis, because *Y pestis* is perhaps the only Gram-negative bacterium that can cause extensive, fulminant pneumonia with bloody sputum in an otherwise healthy, immunocompetent host.

Laboratory Confirmation

In patients with lymphadenopathy, a bubo aspirate should be obtained by inserting a 20-gauge needle attached to a 10-mL syringe containing 1 mL of sterile saline. Saline is injected and withdrawn several times until it is tinged with blood. Repeated, sterile bubo aspiration may also be done to decompress buboes and relieve pain. Drops of the aspirate should be air-dried on a slide for one of the following stains: Gram's, Wright-Giemsa, or Wayson's. If available, a direct fluorescent antibody (DFA) stain of bubo aspirate for the presence of *Y*

pestis capsular antigen should be performed; a positive DFA result is more specific for *Y pestis* than are the other listed stains (Figure 23-13).[63,64]

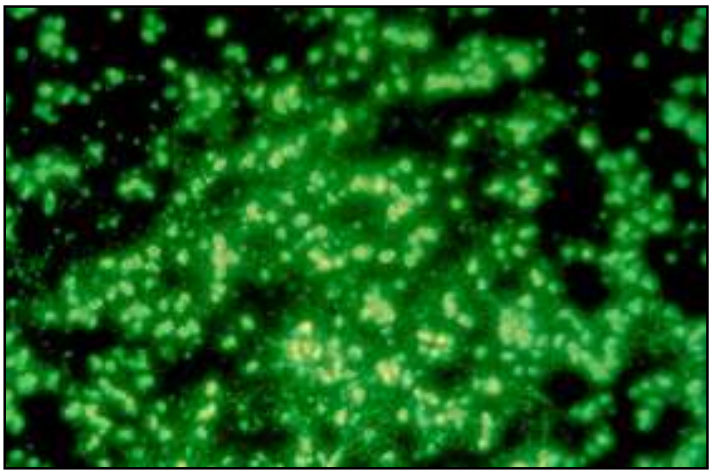


Fig. 23-13. These *Yersinia pestis* fluorescent cells are from infected mouse spleen. Notice how the outlines of the coccobacilli "light up" in this direct fluorescent antibody (DFA) test. The DFA test is specific and therefore better than the other stains discussed in this chapter (original magnification × 1,000). Photograph: Courtesy of M. C. Chu, Centers for Disease Control and Prevention, Fort Collins, Colo.

Both Wright-Giemsa stain and DFA stain for *Y pestis* should also be performed on peripheral blood smears and sputum specimens, when applicable. Although a bipolar, safety-pin staining morphology has been reported to be specific for *Y pestis*, it is not. Other bacteria such as *Pasteurella* species, *Escherichia coli*, *Klebsiella* species, and diplococci (*Streptococcus*) may also exhibit this morphology. None of the listed stains is better than any other for demonstrating the bipolar, safety-pin morphology. In fact, even *Y pestis* will sometimes not exhibit this morphology.[64]

Cultures of blood, bubo aspirate, sputum, and cerebrospinal fluid (if indicated) should be performed. Tiny, 1- to 3-mm "beaten-copper" colonies will appear on blood agar by 48 hours, but it is important to remember that cultures may be negative at 24 hours. In a recent study, 24 (96%) of 25 blood cultures of patients with bubonic plague were positive on standard supplemented peptone broth.[59]

Complete blood counts often reveal leukocytosis with a left shift. Leukemoid reactions with up to 100,000 white blood cells per microliter may be seen, especially in children. Platelet counts may be normal or low, and partial thromboplastin times are often increased. When DIC is present, fibrin degradation products will be elevated. Because of liver involvement, alanine aminotransferase, aspartate aminotransferase, and bilirubin levels are often increased.

Serologic assays measuring the immune response to plague infection are mainly of value retrospectively, since patients present clinically before they develop a significant antibody response. Enzyme-linked immunosorbent assay (ELISA) tests and the older, less-sensitive passive hemagglutination assay

(PHA) both measure antibodies to the fraction 1 capsule. They are available from the Centers for Disease Control and Prevention, Fort Collins, Colorado, and the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland. Rapid diagnostic tests are available on an investigative basis.

An immunological assay to detect circulating fraction 1 antigen in the serum of acutely infected patients can detect levels as low as 0.4 ng/mL serum.[65] During plague infection, fraction 1 antigenemia may reach levels of 4 to 8 µg/mL serum. During a plague outbreak in Namibia, 38 cases of plague were confirmed: 50% by culture, 34% by antibody response, and 16% by antigenemia.[66] Because fraction 1 antigen and antibody do not occur simultaneously in serum, and because neither may be present early in infection, titers for both should be performed on several sequential blood specimens.

A polymerase chain reaction (PCR) test, using primers for the plasminogen activator gene, can detect as few as 10 *Y pestis* organisms, even in the presence of flea tissue. This test may be useful in surveillance of rats and could be adapted to aid in the diagnosis of human infection.[67]

TREATMENT

Isolation

All patients with plague should be isolated for the first 48 hours after the initiation of treatment. Special care must be taken in handling blood and bubo discharge. If pneumonic plague is present, then strict, rigidly enforced respiratory isolation procedures must be followed, including the use of gowns, gloves, and eye protection. Patients with pneumonia must be isolated until they have completed at least 4 days of antibiotic therapy. If patients have no pneumonia or draining lesions at 48 hours, they may be taken out of strict isolation.

Antibiotics

Since 1948, streptomycin has remained the treatment of choice for bubonic, septicemic, and pneumonic plague. It should be given intramuscularly in a dose of 30 mg/kg/d in two divided doses. In cases of suspected meningitis or in patients who are hemodynamically unstable, intravenous chloramphenicol (50–75 mg/kg/d in four divided doses) should be added. Gentamicin has had much less clinical usage but can be used as an alternative to streptomycin or given together with chloramphenicol. Treatment should be continued for a minimum of 10 days or 3 to 4 days after clinical recovery. If clinically indicated, oral tetracycline can be used to complete a 10-day course of treatment after at least 5 days of systemic therapy. In patients with very mild bubonic plague who are not septic, tetracycline can be used orally at a dose of 2 g/d in 4 divided doses for 10 days. Doxycycline should be an acceptable alternative, although there are no published data on its efficacy in humans. Doxycycline, ofloxacin,

and ceftriaxone have all been shown to be effective in experimental animal models of septicemic plague.[68]

In pregnant women, streptomycin or gentamicin should be used unless chloramphenicol is specifically indicated. Streptomycin is also the treatment of choice in newborns.

If treated with antibiotics, buboes typically recede in 10 to 14 days and do not require drainage. Patients are unlikely to survive primary pneumonic plague if antibiotic therapy is not initiated within 18 hours of the onset of symptoms. Without treatment, mortality is 60% for bubonic plague and 100% for the pneumonic and septicemic forms.[53]

PREVENTION

All plague-control measures must include insecticide use, public health education, and reduction of rodent populations with chemicals such as cholecalciferol.[2,25] Fleas must *always* be targeted before rodents, because killing rodents may release massive amounts of infected fleas.[56] Use of insecticides in rodent areas is effective because rodents pick up dust on their feet and carry it back to their nests, where they distribute it over their bodies via constant preening.[2] Plague must be reported to the World Health Organization as an internationally quarantinable disease for which travelers may be detained up to 6 days.

Postexposure Prophylaxis

Not only contacts of patients with pneumonic plague but also individuals who have been exposed to aerosols (eg, in a biological warfare attack) should be treated with tetracycline 15 to 30 mg/kg/d (1–2 g/d) administered in four divided doses for 7 days. Doxycycline 100 mg administered twice daily is probably an effective alternative if tetracycline is not available. Pregnant women and children under 8 years of age should receive trimethoprim/sulfamethoxazole (40 mg sulfa/kg/d) administered orally in two divided doses for 7 days.

Hospital personnel who are observing recommended isolation procedures do not require prophylactic therapy, nor do contacts of patients with bubonic plague. However, people who were in the same environment and who were potentially exposed to the same source of infection as the contact case should be given prophylactic antibiotics. In addition, previously vaccinated individuals should receive prophylactic antibiotics if they have been exposed to a plague aerosol.

Immunization

The first plague vaccine, consisting of killed whole cells, was developed by Russian physician Waldemar M. W. Haffkine, working in India in 1897. In 1942, Karl F. Meyer, D.V.M., began developing an immunogenic and less-reactogenic vaccine for the U.S. Army from an agar-grown, formalin-killed, suspension of

virulent plague bacilli. With minor modifications, this is the same procedure used to prepare the licensed vaccine we have available today. Live-attenuated vaccines have been unsuccessful, since they are much more reactogenic than the present killed vaccine.[23]

Only individuals at high risk for plague should be immunized—such as military troops and other field personnel working in plague endemic areas in which exposure to rats and fleas cannot be controlled. Laboratory personnel working with *Y pestis*, people who reside in enzootic or epidemic plague areas, and those whose vocations bring them into regular contact with wild animals, particularly rodents and rabbits, should also be vaccinated.[69]

The dose schedule for adults is 1.0 mL initially, with 0.2 mL at 1 to 3 months, followed by a third dose 5 to 6 months later. Booster doses of 0.2 mL are given every 6 months for 1.5 years, and then every 1 to 2 years thereafter if risk for exposure continues. If an accelerated schedule is essential, then 0.5 mL at 0, 7, and 14 days has been recommended, although no supporting data exist.[69]

Approximately 92% to 93% of vaccinees will produce antibody titers after the initial series of three injections.[69–71] Local side effects include erythema, soreness, or swelling, in any combination, in 11% of vaccinees and 6% of injections. Systemic side effects include headache, malaise, and myalgias in 4% of vaccinees and 1% of injections. Rarely, sterile abscesses, necrotic lesions, or anaphylaxis may occur.[72]

Data from animal and human investigations suggest that the killed plague vaccine is effective for preventing or ameliorating bubonic but not pneumonic plague.[50,51,73–75] A recombinant vaccine candidate that protects laboratory animals from inhalational challenge is being studied.

SUMMARY

Plague is a zoonotic infection caused by the Gram-negative bacillus *Yersinia pestis*. Three great human pandemics have been responsible for more deaths than any other infectious agent in history. Plague is maintained in nature, predominantly in urban and sylvatic rodents, by a flea vector. Humans are not necessary for persistence of the organism, and we acquire the disease from animal fleas, contact with infected animals, or, rarely, from other humans, via aerosol or direct contact with infected secretions.

To be able to differentiate endemic disease from plague used in biological warfare, medical officers must understand the typical way in which humans contract plague in nature. First, a die-off of animals in the mammalian reservoir that harbors bacteria-infected fleas will occur. Second, troops who have been in close proximity to such infected mammals will become infected. By contrast, in the most likely biological warfare scenario, plague would be spread via aerosol. A rapid, person-to-person spread of fulminant pneumonia, characterized by blood-tinged sputum, would then ensue. If, on the other hand, an enemy force were to release fleas infected with *Y pestis*, then soldiers would present with classic bubonic plague before a die-off in the local mammalian reservoir occurred.

The most common form of the disease is bubonic plague, characterized by painful lymphadenopathy and severe constitutional symptoms of fever, chills, and headache. Septicemic plague without localized lymphadenopathy occurs less commonly and is difficult to diagnose. Secondary pneumonia may follow either the bubonic or the septicemic form. Primary pneumonic plague is spread by airborne transmission, when aerosols from an infected human or animal are inhaled.

Diagnosis is established by isolating the organism from blood or other tissues. Rapid diagnosis may be made with fluorescent antibody stains of sputum or tissue specimens. Patients should be isolated and treated with aminoglycosides, preferably streptomycin, plus chloramphenicol when meningitis is suspected or shock is present. A licensed, killed, whole-cell vaccine is available to protect humans against bubonic, but not against primary pneumonic, plague.

REFERENCES

1. I Samuel 5:6, 9 (NIV).
2. Cavanaugh DC, Cadigan FC, Williams JE, Marshall JD. Plague. In: Ognibene AJ, Barrett O'N. *General Medicine and Infectious Diseases*. Vol 2. In: Ognibene AJ, Barrett O'N. *Internal Medicine in Vietnam*. Washington, DC: Office of The Surgeon General and Center of Military History; 1982: Chap 8, Sec 1.
3. Doyle RJ, Lee NC. Microbes, warfare, religion, and human institutions. *Can J Microbiol*. 1985;32:193–200.
4. Langmuir DA, Worthen TD, Solomon J, et al. The Thucydides syndrome: A new hypothesis for the cause of the plague at Athens. *N Engl J Med*. 1985;313:1027–1030.
5. Bayliss JH. The extinction of bubonic plague in Britain. *Endeavour*. 1980;4(2):58–66.
6. Mee C. How a mysterious disease laid low Europe's masses. *Smithsonian*. 1990;20(Feb):66–79.
7. Gibbon E. *The History of the Decline and Fall of the Roman Empire*. London, England: W Allason; 1781; Chap 43.
8. McEvedy C. The bubonic plague. *Sci Am*. 1988;Feb:118–123.
9. Lederberg J. Biological warfare: A global threat. *American Scientist*. 1971;59(2):195–197.
10. Slack P. The black death past and present, II: Some historical problems. *Trans Roy Soc Trop Med Hyg*. 1989;83:461–463.
11. Sloan AW. The black death in England. *SA Mediese Tydskrif*. 1981;59:646–650.
12. Ampel NM. Plagues—What's past is present: Thoughts on the origin and history of new infectious diseases. *Rev Infect Dis*. 1991;13(Jul-Aug):658–665.

13. Boccaccio G (ca 1350); Aldington C, trans. *The Decameron*. London, England: Folio Society; 1954: 24–28. Quoted by: Sloan AW. The black death in England. *SA Mediese Tydskrif*. 1981;59:646–650.

14. Coulton GG. *The Black Death*. London, England: Benn; 1929: 37. Quoted by: Sloan AW. The black death in England. *SA Mediese Tydskrif*. 1981;59:646–650.

15. Gasquet FA. *The Great Pestilence*. London, England: Simpson, Marshall, Hamilton, Kent; 1893. Quoted by: Sloan AW. The black death in England. *SA Mediese Tydskrif*. 1981;59:646–650.

16. Plague in Vietnam. *Lancet*. 1968;13 Apr:799–800.

17. Butler T. *Plague and Other Yersinia Infections*. New York, NY: Plenum Press; 1983.

18. Cavanaugh DC. KF Meyer's work on plague. *J Infect Dis*. 1974;129(suppl):S10–S12.

19. Risse GB. A long pull, a strong pull and all together: San Francisco and bubonic plague, 1907–1908. *Bull Hist Med*. 1992;66(2):260–286.

20. Caten JL, Kartman L. Human plague in the United States: 1900–1966. *JAMA*. 1968;205(6):81–84.

21. Harrison FJ. *Prevention and Control of Plague*. Aurora, Colo: US Army Center for Health Promotion and Preventive Medicine, Fitzsimons Army Medical Center; September 1995. Technical Guide 103.

22. Mason VR. Central pacific area. In: Coates JB, ed. *Activities of Medical Consultants*. Vol 1. In: Havens WP. *Internal Medicine in World War II*. Washington, DC: US Department of the Army, Medical Department, Office of The Surgeon General; 1961: Chap 7: 647, 667.

23. Meyer KF, Cavanaugh DC, Bartelloni PJ, Marshall JD Jr. Plague immunization, I: Past and present trends. *J Infect Dis*. 1974;129(suppl):S13–S18.

24. Trong P, Nhu TQ, Marshall JD. A mixed pneumonic bubonic plague outbreak in Vietnam. *Milit Med*. 1967;Feb:93–97.

25. Butler T. The black death past and present, I: Plague in the 1980s. *Trans Roy Soc Trop Med Hyg*. 1989;83:458–460.

26. Marshall JD, Joy RJT, AI NV, Quy DV, Stockard JL, Gibson FL. Plague in Vietnam 1965–1966. *Am J Epidemiol*. 1967;86(2):603–616.

27. Meyer KF. Effectiveness of live or killed plague vaccines in man. *Bull WHO*. 1970;42:653–666.

28. Reiley CG, Kates ED. The clinical spectrum of plague in Vietnam. *Arch Intern Med*. 1970;126(12):990–994.

29. Engelman RC, Joy RJT. Two hundred years of military medicine. Fort Detrick, Frederick, Md: US Army Medical Department, Historical Unit; 1975.

30. Williams P, Wallace D. Unit 731: *Japan's Secret Biological Warfare in World War II*. New York, NY: The Free Press; 1989.

31. Barry J. Planning a plague? *Newsweek*. 1993;(Feb 1):40–41.

32. Cowdrey AE. “Germ warfare” and public health in the Korean conflict. *J Hist Med All Sci*. 1984;39:153–172.

33. Brubaker RR. Factors promoting acute and chronic diseases caused by Yersiniae. *Clin Microbiol Rev*. 1991;4(3): 309–324.

34. Lindler LE, Klempner MS, Straley SC. *Yersinia pestis* pH 6 antigen: Genetic, biochemical, and virulence characterization of a protein involved in the pathogenesis of bubonic plague. *Infect Immun*. 1990;58:2569–2577.

35. Straley SC, Skrzypek E, Plano GV, Bliska JB. Yops of *Yersinia* spp pathogenic for humans. *Infect Immun*. 1993;61:3105–3110.

36. Rosqvist R, Magnusson K-E, Wolf-Watz H. Target cell contact triggers expression and polarized transfer of *Yersinia* Yop E cytotoxin into mammalian cells. *EMBO J*. 1994;13:964–972.

37. Price SB, Leung KY, Barve SS, Straley SC. The *Yersinia pestis* V antigen is a regulatory protein necessary for Ca^{2+}-dependent growth and maximal expression of low Ca^{2+} response virulence genes. *J Bacteriol*. 1991;173:2649–2657.

38. Reisner BS, Straley SC. *Yersinia pestis* Yop M: Thrombin binding and overexpression. *Infect Immun*. 1992;60: 5242–5252.

39. Straley SC. The plasmid-encoded outer-membrane proteins of *Yersinia pestis. Rev Infect Dis*. 1988;10:S323–S326.

40. Guan K, Dixon JE. Protein tyrosine phosphatase activity of an essential virulence determinant in *Yersinia. Science*. 1990;249:553–556.

41. Rosqvist R, Forsberg A, Wolf-Watz H. Intracellular targeting of the *Yersinia* Yop E cytotoxin in mammalian cells induces actin microfilament disruption. *Infect Immun*. 1991;59:4562–4569.

42. Sodeinde OA, Subrahmanyam YVBK, Stark K, Quan T, Bao Y, Goguen JD. A surface protease and the invasive character of plague. *Science*. 1992;258:1004–1007.

43. Burrows TW. Virulence of *Pasteurella pestis* and immunity to plague. *Ergebn Mikrobiol*. 1963;37:59–113.

44. Hirst LF. *The Conquest of Plague: A Study of the Evolution of Epidemiology.* Oxford, England: Clarendon Press; 1953.

45. Craven RB, Maupin GO, Beard ML, Quan TJ, Barnes AM. Reported cases of human plague infections in the United States, 1970–1991. *J Med Entomol*. 1993;30(4):758–761.

46. Gage KL, Lance SE, Dennis DT, Montenieri JA. Human plague in the United States: A review of cases from 1988–1992 with comments on the likelihood of increased plague activity. *Border Epidemiological Bulletin*. 1992;19(6):1–10.

47. Poland JD. Plague. In: Hoeprich PD, Jordan MC, eds. *Infectious Diseases: A Modern Treatise of Infectious Processes*. Philadelphia, Pa: JB Lippincott; 1989: Chap 151.

48. Cavanaugh DC, Williams JE. Plague: Some ecological interrelationships. In: Traub R,

Starcke H, eds. *Fleas*. Rotterdam, Netherlands: A A Balkema; 1980: 245–256.

49. Centers for Disease Control and Prevention. Update: Human Plague—India, 1994. *MMWR*. 1994;43(41): 761–762.

50. Ehrenkranz NF, Meyer KF. Studies on immunization against plague, VIII: Study of three immunizing preparations in protecting primates against pneumonic plague. *J Infect Dis*. 1955;96:138–144.

51. Speck RS, Wolochow H. Studies on the experimental epidemiology of respiratory infections, VIII: Experimental pneumonic plague in *Macacus rhesus. J Infect Dis*. 1957;100:58–68.

52. Cavanaugh DC, Randall R. The role of multiplication of *P pestis* in mononuclear phagocytes in the pathogenesis of flea-borne plague. *J Immunol*. 1959;83:348–363.

53. Legters LJ, Cottingham AJ Jr, Hunter DH. Clinical and epidemiologic notes on a defined outbreak of plague in Vietnam. *Am J Trop Med Hyg.* 1970;19(4):639–652.

54. Marshall JD, Quy DV, Gibson FL. Asymptomatic pharyngeal plague infection in Vietnam. *Am J Trop Med Hyg*. 1967;16(2):175–177.

55. Conrad FG, LeCocq FR, Krain R. A recent epidemic of plague in Vietnam. *Arch Intern Med*. 1968;122(3):193–198.

56. Poland JD. Plague. In: Hoeprich PD, ed. *Infectious Diseases: A Guide to the Understanding and Management of Infectious Processes*. New York, NY: Harper & Row; 1972.

57. Hull HF, Montes JM, Mann JM. Septicemic plague in New Mexico. *J Infect Dis*. 1987;155(1):113–118.

58. Alsofrom DJ, Mettler FA Jr, Mann JM. Radiographic manifestations of plague in New Mexico, 1975–1980: A review of 42 proved cases. *Radiology*. 1981;139:561–565.

59. Crook LD, Tempest B. Plague: A clinical review of 27 cases. *Arch Intern Med*. 1992;152(June):1253–1256.

60. Becker TM, Poland JD, Quan TJ, White ME, Mann JM, Barnes AM. Plague meningitis—A retrospective analysis of cases reported in the United States, 1970–1979. *West J Med*. 1987;147:554–557.

61. Welty TK. Plague. *Am Fam Phys*. 1986;33(6):159–164.

62. Welty TK, Grabman J, Kompare E, et al. Nineteen cases of plague in Arizona: A spectrum including ecthyma gangrenosum due to plague and plague in pregnancy. *West J Med*. 1985;142(May):641–646.

63. Tikomirov EV, Gratz NA, eds. *WHO Plague Manual*. Rev 3rd ed. Fort Collins, Colo: Centers for Disease Control and Prevention; 1996.

64. Chu MC. Acting Chief, Diagnostic and Reference Section, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, Colo. Personal communication, 7 February 1996.

65. Williams JE, Gentry MK, Braden CA, Leister F, Yolken RH. Use of an enzyme-linked immunosorbent assay to measure antigenaemia during acute plague. *Bull WHO*. 1984;62(3):463–466.

66. Williams JE, Arntzen L, Tyndal GL, Isaacson M. Application of enzyme immunoassays for the confirmation of clinically suspect plague in Namibia, 1982. *Bull WHO*. 1986;64(5):745–752.

67. Hinnebusch J, Schwan TG. New method for plague surveillance using polymerase chain reaction to detect *Yersinia pestis* in fleas. *J Clin Microbiol*. 1993;31(6):1511–1514.

68. Bonacorsi SP, Scavizzi MR, Guiyoule A, Amouroux JH, Carniel E. Assessment of a fluoroquinolone, three betalactams, two aminoglycosides, and a tetracycline in treatment of murine *Yersinia pestis* infection. *Antimicrob Agents Chemother*. 1994;38(3):481–486.

69. Centers for Disease Control, Immunization Practices Advisory Committee. Plague vaccine. *MMWR*. 1982;31(22):301–304.

70. Bartelloni PJ, Marshall JD, Cavanaugh DC. Clinical and serological responses to plague vaccine USP. *Milit Med*. 1973;(11):720-722.

71. Marshall JD Jr, Cavanaugh DC, Bartelloni PJ, Meyer KF. Plague immunization, III: Serologic response to multiple inoculations of vaccine. *J Infect Dis*. 1974;129(suppl):S26-S29.

72. Marshall JD Jr, Baratelloni PJ, Cavanaugh DC, Kadull PJ, Meyer KF. Plague immunization, II: Relation of adverse clinical reactions to multiple immunizations with killed vaccine. *J Infect Dis*. 1974;129(suppl):S19–S25.

73. Williams JE, Cavanaugh DC. Measuring the efficacy of vaccination in affording protection against plague. *Bull WHO*. 1979;57(2):309–313.

74. Cavanaugh DC, Elisberg BL, Llewellyn CH, et al. Plague immunization, V: Indirect evidence for the efficacy of plague vaccine. *J Infect Dis*. 1974;129(suppl):S37-S40.

75. Pitt MLM, Estep JE, Welkos SL, Friedlander AM. Efficacy of killed whole-cell vaccine against a lethal aerosol challenge of plague in rodents. Annual meeting, American Society for Microbiology; 1994; Las Vegas, Nev. Abstract E-45.

Chapter 24

TULAREMIA

MARTIN E. EVANS, M.D.* AND ARTHUR M. FRIEDLANDER, M.D.†

INTRODUCTION

THE INFECTIOUS AGENT

THE DISEASE
- Epidemiology
- Pathogenesis
- Clinical Manifestations
- Diagnosis
- Treatment

PROPHYLAXIS

SUMMARY

Lieutenant Colonel (Ret), Medical Corps, U.S. Air Force; Assistant Professor of Internal Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799; currently, Associate Professor, Division of Infectious Diseases, Department of Internal Medicine, University of Kentucky School of Medicine, Lexington, Kentucky 40536-0084

† Colonel, Medical Corps, U.S. Army; Chief, Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011; and Clinical Associate Professor of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799

INTRODUCTION

Tularemia is a zoonosis caused by the Gramnegative, facultative intracellular bacterium, *Francisella tularensis.* The disease is characterized by fever, localized skin or mucous membrane ulceration, regional lymphadenopathy, and, occasionally, pneumonia.

In 1911, G. W. McCoy discovered the disease in Tulare County, California, as a cause of a plague-like illness in ground squirrels.[1] An organism was isolated and named *Bacterium tularense.*[2,3] The first bacteriologically confirmed case of human disease was reported in 1914.[4] Edward Francis subsequently described transmission by deer flies via infected blood and coined the term *tularemia* in 1921.[5] Transovarian transmission in ticks was reported in 1926.[6] In 1959, the Soviets proposed changing the genus name to *Francisella* in recognition of the contributions of Edward Francis to the understanding of this disease.[7] *F tularensis* has been considered an important biological warfare threat because of its very high infectivity after aerosolization.

THE INFECTIOUS AGENT

F tularensis is a nonmotile, obligately aerobic, Gram-negative coccobacillus. There are two biovars[8]:

- *F tularensis* biovar *tularensis* is the most common isolate in the United States. It is recovered from rodents and ticks, and is highly virulent for rabbits and humans. It produces acid from glycerol and has citrulline ureidase activity.
- *F tularensis* biovar *palearctica* is more common outside the United States. It is recovered from water, mosquitoes, and aquatic mammals, and is relatively avirulent for rabbits and humans. It does not produce acid from glycerol, and does not have citrulline ureidase activity.

The subspecies are indistinguishable serologically, although they may be distinguished by 16S ribosomal ribonucleic acid (rRNA) analysis.[9] A capsule has been reported that may contribute to virulence.[10,11] *F tularensis* may have a lipopolysaccharide (LPS),[12] but the biological activity of the LPS has not been well characterized and its role in pathogenicity is unclear. No known toxins are produced.

THE DISEASE

Epidemiology

Tularemia occurs in North America, Europe, the Middle East, Russia, and Japan, but is rare in the United Kingdom, Africa, and Central and South America. In the United States, the disease is most prevalent in Arkansas, Illinois, Missouri, Texas, Virginia, and Tennessee, although cases have been reported from all states except Hawaii.[13,14]

The principal reservoir of tularemia in North America is the tick; more than 10 species have been implicated.[13,14] *F tularensis* is maintained in tick populations by transovarial passage, and is probably transmitted to humans via feces since the bacterium has not been found in the tick salivary glands. The bacterium has been isolated from 55 other arthropods and more than 100 nonarthropods.[13] In North America, the rabbit is the most common vertebrate associated with transmission of tularemia. In other areas of the world, such as the former Soviet Union, tularemia is maintained in water rats and other aquatic mammals. Pharyngitis, abdominal pain, and fever may result from the ingestion of contaminated water in these areas.[15] With the disruption of normal sanitation during World War II, hundreds of thousands of civilians and large numbers of Russian troops contracted tularemia.[16]

The reported incidence in the United States since 1967 has been fewer than 200 cases per year. This compares with 2,291 cases reported in 1939 and more than 1,100 cases per year during the 1940s.[17,18] The decline in incidence may be due to a declining interest in rabbit hunting, less recognition of the disease by physicians, or inadvertent cure of the disease by physicians who treat febrile patients with aminoglycoside antibiotics.[13]

Pathogenesis

F tularensis is usually introduced into the host through breaks in the skin, or through the mucous membranes of the eye, respiratory tract, or gastrointestinal tract.[19–23] Ten virulent organisms injected subcutaneously, and 10 to 50 organisms given by aerosol can cause infection in humans.[23–25]

After inoculation, *F tularensis* is ingested by and multiplies within macrophages.[26] The host defense against *F tularensis* is mediated primarily by (*a*) T-cell–independent mechanisms, which appear early (< 3 days after infection), and (*b*) T-cell–dependent mechanisms, which appear later (> 3 days after infection). In the T-cell–independent mechanisms, macrophages, which have ingested bacteria, secrete tumor necrosis

factor–alpha (TNF-alpha). TNF-alpha stimulates natural killer (NK) cells to produce interferon-gamma (IFN-γ), which, in turn, feeds back on macrophages and stimulates the cells to kill intracellular bacteria through the production of nitric oxide.[26–28] In the T-cell–dependent mechanism, macrophages present bacterial antigen in the context of the major histocompatibility complex (MHC-II) to cluster of differentiation 4+ (CD4+) T lymphocytes. These cells respond by proliferating and secreting TNF-alpha, IL-2, and IFN-γ, which stimulate the macrophages to kill intracellular bacteria.[27–32] Cell-mediated immunity constitutes the major protective mechanism.[33]

The role of humoral immunity and neutrophils in the host defense against *F tularensis* is unclear. Specific immunoglobulins (IgG, IgA, and IgM) appear within 1 week of infection, and passive transfer of immune serum protects naive mice against challenge with attenuated vaccine strains.[34–37] This protection, however, is not evident when mice are challenged with virulent wild-type strains.[38] Although some of the antibodies produced are opsonic and facilitate phagocytosis by neutrophils, neutrophils are not efficient in killing ingested bacteria.[39,40] Recent studies using animals depleted of neutrophils suggest that neutrophils are important in resistance to infection with attenuated strains, but the relevance of these findings to virulent wild-type tularemia is unknown.[41] Overall, these data suggest that the humoral immune response plays a limited role in the host defense against naturally acquired infection.[33]

Clinical Manifestations

Tularemia can be divided into the ulceroglandular (75% of patients) and the typhoidal (25% of patients) forms, based on the clinical signs. Patients with ulceroglandular tularemia have lesions on the skin or mucous membranes (including the conjunctiva), lymph nodes larger than 1 cm in diameter, or both. Patients with typhoidal tularemia, on the other hand, present with lymph nodes smaller than 1 cm in diameter and without skin or mucous membrane lesions. This simplified scheme is suggested instead of the more-complicated, previous classification (ie, ulceroglandular, glandular, oculoglandular, typhoidal), because it is more in keeping with the clinical, pathophysiological, and prognostic aspects of the disease.[13]

After an incubation period of 3 to 6 days,[42–45] patients with the ulceroglandular form of the disease develop a constellation of symptoms consisting of fever (85%), chills (52%), headache (45%), cough (38%), and myalgias (31%). The fever is often accompanied by a

pulse–temperature disassociation (ie, the pulse increases less than 10 beats per min per 1°F increase in temperature above normal[46]). Patients may also complain of chest pain, vomiting, arthralgia, sore throat, abdominal pain, diarrhea, dysuria, back pain, or stiff neck.[13]

A cutaneous ulcer occurs in approximately 60% of patients and is the most common sign of tularemia. Ulcers are generally single lesions of 0.4 to 3.0 cm in diameter, with heaped-up edges (Figure 24-1). Lesions associated with infection acquired from mammalian vectors are usually located on the upper extremities, whereas lesions associated with infection acquired from arthropod vectors are usually located on the lower extremities. Ulcerative lesions are almost always accompanied by regional lymphadenopathy.[13]

Fig. 24-1. Cutaneous ulcer of tularemia. Photograph:Courtesy of William Beisel, M.D., Colonel, Medical Corps, US Army (Ret).

Enlarged lymph nodes are seen in approximately 85% of patients, and may be the initial, or the only, sign of infection. Nodes are usually tender and 0.5 to 10 cm in diameter (mean 2.0 cm). Although enlarged nodes usually occur as single lesions, they may appear in groups or in a sporotrichoid distribution. The appearance of enlarged nodes in upper or lower extremities and the correlation with the vector is the same as for ulcerative lesions.[19] Enlarged lymph nodes may become fluctuant, drain spontaneously, or persist for as long as 3 years.[22]

Pharyngitis may occur in up to 25% of patients with tularemia.[13,22,47,48] The posterior pharynx may not be inflamed; however, there may be erythema, exudate, petechiae, hemorrhage, or ulcers. On occasion, patients with pharyngitis may also develop a retropharyngeal abscess or suppuration of regional lymph nodes.[47,49–51] Pneumonia commonly accompanies pharyngitis, perhaps reflecting acquisition of the disease by the aerosol route.

The lower respiratory tract is involved in 47% to 94% of patients.[13,52,53] The variability in these figures is probably due to the variable use of chest radiographs during patient evaluations. Patients present with nonproductive or productive cough, and less commonly with pleuritic chest pain, shortness of breath, or hemoptysis. Examination of the sputum is not helpful for making the diagnosis of tularemia pneumonia. Chest radiographs show that approximately 50% of patients have pneumonia, and 1% or fewer have hilar adenopathy without parenchymal involvement.

Pleural effusions are seen in 15% of patients with pneumonia. Interstitial patterns, cavitary lesions, bronchopleural fistulae, and calcifications have been reported in patients with tularemia pneumonia (Figure 24-2).[53–62]

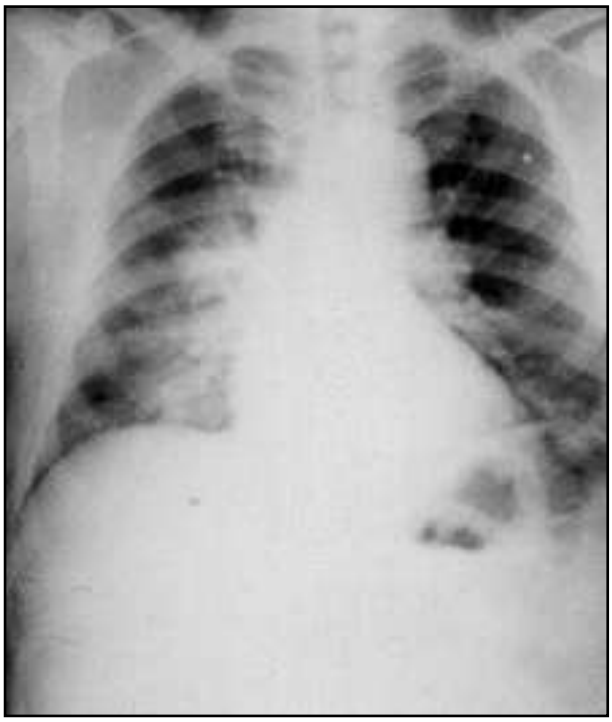


Fig. 24-2. Chest roentgenogram of tularemia pneumonia showing bilateral infiltrates. Photograph: Courtesy of William Beisel, M.D., Colonel, Medical Corps, US Army (Ret).

Approximately 30% of patients with ulceroglandular tularemia and 80% of patients with typhoidal tularemia have pneumonia. The higher incidence of pneumonia in patients with typhoidal tularemia probably accounts for the higher mortality associated with this form of the disease.[13] Other, infrequent clinical syndromes associated with tularemia include pericarditis, enteritis, appendicitis, peritonitis, erythema nodosum, and meningitis.[13,22,63–66]

Patients usually do not have abnormalities in the hematocrit, hemoglobin, or platelet levels. The peripheral white blood cell count may range between 5,000 and 22,000 cells per microliter, but it is usually only mildly elevated. Differential blood cell counts are usually normal, although patients may have a lymphocytosis late in the disease.[13,67] Patients may have microscopic pyuria, which may lead to the erroneous diagnosis of a urinary tract infection.[13,68] Mild elevations in lactic dehydrogenase, serum transaminases, and alkaline phosphatase are commonly seen. Some patients may experience rhabdomyolysis associated with elevations in the serum creatine kinase and urinary myoglobin levels.[69] The cerebrospinal fluid is usually normal, although mild abnormalities in protein, glucose, and blood cell count have been reported.[13]

Diagnosis

Tularemia can be diagnosed by recovery of *F tularensis* in culture, or from serologic evidence of infection in a patient with a compatible clinical syndrome. Although the organism is difficult to culture,[24,25,53,70] it can be recovered from blood, ulcers, conjunctival exudates, sputum, gastric washings, and pharyngeal exudates.[23,70] Recovery may be possible even after the institution of appropriate antibiotic therapy.[23] The organism grows poorly on standard media. On media containing cysteine or other sulfhydryl compounds (eg, glucose cysteine blood agar, thioglycollate broth), *F tularensis* appears as small, smooth, opaque colonies after 24 to 48 hours of incubation at 37°C. The bacterium has occasionally been recovered on charcoal yeast extract (CYE),[71] or Thayer-Martin agar,[72] or from radiometric detection systems if the media are subcultured onto chocolate agar.[73,74] The organism can readily be recovered from animals inoculated with infectious materials, but this is rarely done because of the likelihood of epizootics within the animal colony and the risk of infection to laboratory workers.[75,76] Identification of the organism is made on the basis of its growth characteristics and bacterial agglutination or fluorescent staining using antisera specific for *F tularensis.*

Most diagnoses of tularemia are made serologically using bacterial agglutination or enzyme-linked immunosorbent assay (ELISA). Measurable levels of antibodies that agglutinate *F tularensis* appear within 1 week of infection,[35,77] but levels high enough to allow confidence in the specificity of the serologic diagnosis (an agglutination titer > 1:160, for example) do not appear until more than 2 weeks after infection.[13,34–36] The serologic response may be blunted by prior administration of antibiotics.[78] Because antibodies to *F tularensis* may cross-react with *Brucella*,[79–81] *Proteus* OX19,[30,82] and *Yersinia* organisms,[30,36,82,83] and because detectable antibody levels may persist for many years after a bout of tularemia,[13,36] the serologic diagnosis of an acute infection should, ideally, be made only if a 4-fold or greater increase in serologic response is seen during the course of the patient’s illness.

Treatment

Patients with tularemia who do not receive appropriate antibiotic treatment may have a prolonged illness characterized by malaise, weakness, weight loss, and other symptoms that last for months.[22,82,84,85] Before the availability of effective antibiotics, ulceroglandular and typhoidal tularemia had mortalities of approximately 4% and 35%, respectively.[44,57] With appropriate treatment, tularemia has an overall mortality of

approximately 1% to 2.5%.[13,86,87]

Streptomycin is the drug of choice for the treatment of tularemia. The drug is bactericidal, and patients treated with streptomycin usually respond within 48 hours of its administration.[19,42,58,88] Relapses are uncommon, and resistance has not been reported.[70,89] Other aminoglycosides such as gentamicin have been used with some success and are probably reasonable alternatives.[13] Bacteriostatic drugs such as chloramphenicol and tetracycline are often efficacious, but relapses occur if the drug is given too early in the course of the disease or if is not continued long enough.[13,23,70] To date, there is only limited clinical experience with erythromycin and the fluoroquinolones.[31,71,90–92]

PROPHYLAXIS

Antibiotic prophylaxis after exposure to tularemia is difficult. The optimal bactericidal antibiotics such as streptomycin are impractical because they must be given parenterally. Limited studies[93] carried out in small numbers of human volunteers showed that treatment with tetracycline begun 24 hours after exposure to an aerosol of tularemia protected subjects from disease. An oral dose of 2 g/d for 14 days was necessary.

Vaccines to prevent tularemia have included those made from killed, whole cells and live, attenuated strains. A whole-cell, killed vaccine was developed by L. Foshay and associates[94] in the 1930s, but proved to be of limited efficacy. Experimental studies[24] done with human volunteers showed that this vaccine reduced the frequency of systemic symptoms but did not prevent the local lesion after intracutaneous challenge. Additional studies[25] with aerosol challenge in humans showed that the killed vaccine neither prevented nor modified the disease.

A live, attenuated vaccine had been developed and used in humans in the Soviet Union in the 1940s and 1950s.[95] The vaccine proved to be a mixture of variants of varying virulence. At the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, in 1961, H. T. Eigelsbach and C. M. Downs[96] further purified and characterized a strain from this vaccine. This derivative was called live vaccine strain (LVS). Extensive evaluations[16,25,97,98] have demonstrated that the LVS vaccine protected human volunteers against an aerosol challenge with virulent *F tularensis.*

Evidence based on an analysis of laboratory-acquired infections[99] indicates that immunization with the live, attenuated LVS vaccine prevents the typhoidal and ameliorates the ulceroglandular forms of tularemia. The LVS vaccine is currently available as an Investigational New Drug from the

U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, Maryland 21702-5011.

SUMMARY

Tularemia is a zoonotic disease caused by infection with the Gram-negative, facultative intracellular bacterium, *Francisella tularensis*. The organism is highly infectious by both the cutaneous and aerosol routes. Naturally occurring tularemia occurs most commonly in its ulceroglandular form; patients present with a cutaneous ulcer or mucous-membrane lesion and regional tender lymphadenopathy. The typhoidal form occurs in 25% of naturally occurring cases; patients present with systemic symptoms without local lesions or marked lymphadenopathy. Pneumonia occurs in up to 80% of patients with typhoidal tularemia.

A biological warfare attack with aerosolized *F tularensis* would probably produce pneumonia with or without accompanying mucous membrane lesions. Diagnosis is usually established by serology, as the organism is difficult to culture. The treatment of choice is streptomycin, with other aminoglycoside drugs being reasonable alternatives. Immediate postexposure antibiotic prophylaxis with tetracycline prevents disease. A live, attenuated vaccine, available as an Investigational New Drug, is effective against aerosol infection.

REFERENCES

1. McCoy GW. Plague-like disease in rodents. *Public Health Bull.* 1911;43:53–71.
2. McCoy GW, Chapin CW. Further observations on a plague-like disease of rodents with a preliminary note on the causative agent, *Bacterium tularense. J Infect Dis.* 1912;10:61–72.
3. McCoy GW, Chapin CW. Studies of plague, a plague-like disease, and tuberculosis among rodents in California. *Public Health Bull.* 1912;53:3–23.
4. Wherry WB, Lamb BH. Infection of man with *Bacterium tularense. J Infect Dis.* 1914;15:331–340.
5. Francis E. Tularemia (Francis 1921), I: The occurrence of tularemia in nature as a disease of man. *Public Health Reports.* 1921;36:1731–1751.
6. Parker RR, Spencer RR. Hereditary transmission of tularemia infection by the wood tick *Dermacentor andersoni Stiles. Public Health Reports.* 1926;41:1403–1407.
7. Rockwood SW. Tularemia: What’s in a name? *ASM News.* 1983;49:63–65.

8. Eigelsbach HT, McGann VG. Gram-negative aerobic rods and cocci. Genus *Francisella Dorofe'ev* 1947, 176 AL. In: Krieg NR, Hold JG, eds. Vol 1. *Bergey's Manual of Systematic Bacteriology.* Baltimore, Md: Williams & Wilkins; 1986: 394–399.

9. Forsman M, Sandström G, Jaurin B. Identification of *Francisella* species and discrimination of type A and type B strains of *F tularensis* by 16S rRNA analysis. *Appl Environmental Microbiol.* 1990;56:949–955.

10. Pavlova IB, Meshcheryakova IS, Emelyanova OS. Study of the microstructure of the two races of the tularemia bacterium in strains with different degrees of virulence. *J Hyg Epidemiol Microbiol Immunol.* 1967;11:320–327.

11. Cherwonogrodzky JW, Knodel MH, Spence MR. Increased encapsulation and virulence of *Francisella tularensis* live vaccine strain (LVS) by subculturing on synthetic medium. *Vaccine*. 1994;12:773–775.

12. Fulop MJ, Webber T, Manchee RJ, Kelly DC. Production and characterization of monoclonal antibodies directed against the lipopolysaccharide of *Francisella tularensis. J Clin Microbiol.* 1991;29:1407–1412.

13. Evans ME, Gregory DW, Schaffner W, McGee ZA. Tularemia: A 30-year experience with 88 cases. *Medicine*. 1985;64:251–269.

14. Jellison WL. *Tularemia in North America, 1930–1974.* Missoula, Mont: University of Montana; 1974: 276.

15. Karpoff SP, Antonoff NI. The spread of tularemia through water, as a new factor in its epidemiology. *J Bacteriol.* 1936;32:243–258.

16. McCrumb FR Jr. Aerosol infection of man with *Pasteurella tularensis. Bacteriol Rev.* 1961;25:262–267.

17. Brooks GF, Buchanan TM. Tularemia in the United States: Epidemiologic aspects in the 1960's and follow-up of the outbreak of tularemia in Vermont. *J Infect Dis.* 1970;121:357–359.

18. Olsen PF. Tularemia. In: Hubbert WT, McCulloch WF, Schnurrenberger PR, eds. *Diseases Transmitted From Animals to Man*. Springfield, Ill: Charles C Thomas; 1975: 191–222.

19. Evans ME, McGee ZA. Tularemia. In: Kass EH, Platt R, eds. Current therapy in infectious diseases. Philadelphia, Pa: BC Decker; 1983: 18–19.

20. Francis E. Tularemia. *Atlantic Med J.* 1927;30:337–344.

21. Quan SF, McManus AG, von Fintel H. Infectivity of tularemia applied to intact skin and ingestion in drinking water. *Science*. 1956;123:942–943.

22. Foshay L. Tularemia: A summary of certain aspects of the disease including methods for early diagnosis and the results of serum treatment in 600 patients. *Medicine*. 1940;19:1–83.

23. McCrumb FRJ, Snyder MJ, Woodward TE. Studies on human infection with *Pasteurella tularensis:* Comparison of streptomycin and chloramphenicol in the prophylaxis of clinical disease. *Trans Assoc Am Phys.* 1957;70:74–80.

24. Saslaw S, Eigelsbach HT, Wilson HE, Prior JA, Carhart S. Tularemia vaccine study, I: Intracutaneous challenge. *Arch Intern Med.* 1961;107:121–133.

25. Saslaw S, Eigelsbach HT, Prior JA, Wilson HE, Carhart S. Tularemia vaccine study, II: Respiratory challenge. *Arch Intern Med.* 1961;107:134–146.

26. Fortier AH, Polsinelli T, Green SJ, Nacy CA. Activation of macrophages for destruction of *Francisella tularensis:* Identification of cytokines, effector cells, and effector molecules. *Infect Immun.* 1992;60:817–825.

27. Anthony LSD, Ghadirian E, Nestel FP, Kongshavn PAL. The requirement for gamma interferon in resistance of mice to experimental tularemia. *Microb Pathog.* 1989;7:421–428.

28. Leiby DA, Fortier AH, Crawford RM, Schreiber RD, Nacy CA. In vivo modulation of murine immune response to *Francisella tularensis* LVS by administration of anticytokine antibodies. *Infect Immun.* 1992;60:84–89.

29. Anthony LSD, Kongshavn PAL. H-2 restriction in acquired cell-mediated immunity to infection with *Francisella tularensis* LVS. *Infect Immun.* 1988;56:452–456.

30. Surcel H, Ilonen J, Poikonen K, Herva E. *Francisella tularensis*-specific T-cell clones are human leukocyte antigen class II restricted, secrete interleukin-2 and gamma interferon, and induce immunoglobulin production. *Infect Immun.* 1989;57:2906–2908.

31. Surcel H, Syrjälä H, Karttunen R, Tapaninaho S, Herva E. Development of *Francisella tularensis* antigen responses measured as T-lymphocyte proliferation and cytokine production (tumor necrosis factor alpha, gamma interferon, and interleukin-2 and -4) during human tularemia. *Infect Immun.* 1991;59:1948–1953.

32. Karttunen R, Surcel H-M, Andersson G, Ekre H-PT, Herva E. *Francisella tularensis*–induced in vitro gamma interferon, tumor necrosis factor alpha, and interleukin 2 responses appear within 2 weeks of tularemia vaccination in human beings. *J Clin Microbiol.* 1991;29:753–756.

33. Tärnvik A. Nature of protective immunity to *Francisella tularensis. Rev Infect Dis.* 1989;11:440–451.

34. Syrjälä H, Koskela P, Ripatti T, Salminen A, Herva E. Agglutination and ELISA methods in the diagnosis of tularemia in different clinical forms and severities of the disease. *J Infect Dis.* 1986;153:142–145.

35. Sato T, Fujita H, Ohara Y, Homma M. Microagglutination test for early and specific serodiagnosis of tularemia. *J Clin Microbiol.* 1990;28:2372–2374.

36. Koskela P, Salminen A. Humoral immunity against *Francisella tularensis* after natural infection. *J Clin Microbiol.* 1985;22:973–979.

37. Fortier AH, Slayter MV, Ziemba R, Meltzer MS, Nacy CA. Live vaccine strain of *Francisella tularensis:* Infection and immunity in mice. *Infect Immun.* 1991;59:2922–2928.

38. Thorpe BD, Marcus S. Phagocytosis and intracellular fate of *Pasteurella tularensis*, III: In vivo studies with passively transferred cells and sera. *J Immunol.* 1965;94:578–585.

39. Hood AM. Virulence factors of *Francisella tularensis. J Hyg (Camb).* 1977;79:49–60.

40. Proctor RA, White JD, Ayala E, Canonico PG. Phagocytosis of *Francisella tularensis* by rhesus monkey peripheral leukocytes. *Infect Immun.* 1975;11:146–151.

41. Sjostedt A, Conlan JW, North RJ. Neutrophils are critical for host defense against primary infection with the facultative intracellular bacterium *Francisella tularensis* in mice and participate in defense against reinfection. *Infect Immun.* 1994;62:2779–2783.

42. Francis E. Tularemia. In: Christian HA, ed. *Industrial Diseases and Infectious Diseases.* Vol 1. Oxford, England: Oxford University Press; 1948: 955–989.

43. Giddens WR, Wilson JWJ, Dienst FT, Hargrove MD. Tularemia, an analysis of one-hundred forty-seven cases. *J La State Med Soc.* 1957;109:93–98.

44. Pullen RL, Stuart BM. Tularemia: Analysis of 225 cases. *JAMA*. 1945;129:495–500.

45. Young LS, Bicknell DS, Archer BG. Tularemia epidemic: Vermont, 1968: Forty-seven cases linked to contact with muskrats. *N Engl J Med.* 1969;280:1253–1260.

46. Bazett HC. Studies on the effects of baths on man, I: Relationship between effects produced and the temperature of the bath. *Am J Physiol*. 1924;70:412–429.

47. Hughes WT, Etteldorf JN. Oropharyngeal tularemia. *J Pediatr*. 1957;51:363–372.

48. Butler T. Plague and tularemia. *Pediatr Clin North Am.* 1979;26:355–366.

49. Levy HB, Webb CH, Wilkinson JD. Tularemia as a pediatric problem. *Pediatrics*. 1950;6:113–122.

50. Everett DE, Templer JW. Oropharyngeal tularemia. *Arch Otolaryngol*. 1980;106:237–238.

51. Anscheutz RR. Tularemia with extensive pharyngitis. *Am J Dis Child*.

1941;62:150–153.

52. Blackford SD, Casey CJ. Pleuropulmonary tularemia. *Arch Intern Med*. 1941;67:43–71.

53. Overholt EL, Tigertt WD. Roentgenographic manifestations of pulmonary tularemia. *Radiology*. 1960;74:758–764.

54. Archer VW, Blackford SD, Wissler JE. Pleuropulmonary manifestations in tularemia: A roentgenographic study based on thirty-four unselected cases. *JAMA*. 1935;104:897–898.

55. Avery FW, Barnett TB. Pulmonary tularemia: A report of five cases and consideration of pathogenesis and terminology. *Am Rev Respir Dis*. 1967;95:584–591.

56. Dennis JM, Bourdreau RP. Pleuropulmonary tularemia: Its roentgen manifestations. *Radiology*. 1957;68:25–30.

57. Dienst FTJ. Tularemia, a perusal of three-hundred thirty-nine cases. *J La State Med Soc*. 1963;115:114–127.

58. Hunt JS. Pleuropulmonary tularemia: Observations on 12 cases treated with streptomycin. *Ann Intern Med.* 1947;26:263–276.

59. Ivie JM. Roentgenological observations on pleuropulmonary tularemia. *Am J Roentgenol*. 1955;74:466–471.

60. Kennedy JA. Pulmonary tularemia: A discussion of the disease as a clinical entity, with a report of three cases. *JAMA*. 1942;118:781–787.

61. Miller RP, Bates JH. Pleuropulmonary tularemia: A review of 29 patients. *Am Rev Respir Dis*. 1969;99:31–41.

62. Rubin SA. Radiographic spectrum of pleuropulmonary tularemia. *Am J Roentgenol*. 1978;131:277–281.

63. Lovell VM, Cho CT, Lindsey NJ, Nelson PL. *Francisella tularensis* meningitis: A rare clinical entity. *J Infect Dis*. 1986;154:916–918.

64. Adams CW. Tularemic pericarditis. *Dis Chest*. 1958;34:1–8.

65. Jager BV, Ransmeier JC. Constrictive pericarditis due to *Bacterium tularense:* Report of a case and review of reported cases of pericarditis occurring with tularemia. *Bull Johns Hopkins Hosp*. 1943;72:166–178.

66. Kleibl K. Les tularémides Érythèmes polymorphes et érythèmes noueux tularémiques [in French]. *Med Mal Infect.* 1971;11:447–451.

67. Gelfand MS, Mehra N, Simmons BP. Tularemia and atypical lymphocytosis. *J Tenn Med Assoc.* 1989;8:417–418.

68. Penn RL, Kinasewitz GT. Factors associated with a poor outcome in tularemia.

Arch Intern Med. 1987;147:265–268.

69. Kaiser AB, Reives D, Price AH, et al. Tularemia and rhabdomyolysis. *JAMA*. 1985;253:241–243.

70. Overholt EL, Tigertt WD, Kadull PJ, et al. An analysis of forty-two cases of laboratory-acquired tularemia: Treatment with broad-spectrum antibiotics. *Am J Med.* 1961;30:785–806.

71. Westerman EL, McDonald J. Tularemia pneumonia mimicking Legionnaire's disease: Isolation of organisms on CYE agar and successful treatment with erythromycin. *South Med J*. 1983;76:1169–1171.

72. Berdal BP, Soderlund E. Cultivation and isolation of *Francisella tularensis* on selective chocolate agar as used routinely for the isolation of gonococci. *Acta Pathol Microbiol Immunol Scand [B].* 1977;85:108–109.

73. Fowler J, Taylor R, Gelfand M. Tularemia pneumonia—Tennessee. *MMWR*. 1983;32:262–263.

74. Provenza MJ, Klotz SA, Penn RL. Isolation of *Francisella tularensis* from blood. *J Clin Microbiol.* 1986;24:453–455.

75. Pike RM. Laboratory associated infections: Summary and analysis of 3,921 cases. *Health Lab Sci.* 1976;13:105–108.

76. Francis E. Immunity in tularemia. *Trans Assoc Am Physicians*. 1936;51:394–398.

77. Syrjälä H, Herva E, Honen J, Saukkonen K, Salminen A. A whole-blood lymphocyte stimulation test for the diagnosis of human tularemia. *J Infect Dis*. 1984;150:912–915.

78. Saslaw S, Carhart S. Studies with tularemia vaccines in volunteers, III: Serological aspects following intracutaneous or respiratory challenge in both vaccinated and nonvaccinated volunteers. *Am J Med Sci.* 1961;241:689–699.

79. Francis E, Evans AC. Agglutination, cross-agglutination and agglutination absorption in tularemia. *Public Health Rep.* 1926;41:1273–1295.

80. Ransmeier JC, Ewing CL. The agglutination reaction in tularemia. *J Infect Dis*. 1941;69:193–205.

81. Saslaw S, Carlisle HN. Studies with tularemia vaccines in volunteers challenged with *Pasteurella tularensis. Am J Med Sci.* 1961;242:166–172.

82. Warring WB, Ruffin JS. A tick-borne epidemic of tularemia. *N Engl J Med*. 1946;234:137–140.

83. Koskela P, Herva E. Cell-mediated immunity against *Francisella tularensis* after natural infection. *Scand J Infect Dis.* 1980;12:281–287.

84. Taylor RR. Report on tularemia with its diagnosis and treatment. *JAMA*.

1950;47:47–50.

85. Van Metre TEJ, Kadull PJ. Laboratory acquired tularemia in vaccinated individuals: A report of 62 cases. *Ann Intern Med.*1959;50:621–632.

86. Rohrbach BW, Westerman E, Istre GR. Epidemiology and clinical characteristics of tularemia in Oklahoma, 1979 to 1985. *South Med J.* 1991;84:1091–1096.

87. Taylor JP, Istre GR, McChesney TC, Satalowich FT, Parker RL, McFarland LM. Epidemiologic characteristics of human tularemia in the southwest-central states, 1981–1987. *Am J Epidemiol.* 1991;133:1032–1038.

88. Woodward TE. Chemosuppression of specific infections with antibiotics. *J Chronic Dis.* 1962;15:611–622.

89. Woodward TE. Tularemia. In: Beeson PB, McDermott W, Wyngaarden JB, eds. *Textbook of Medicine.* Philadelphia, Pa: WB Saunders; 1979: 465–468.

90. Halstead CC, Kulasinghe HP. Tularemia pneumonia in urban children. *Pediatrics*. 1978;61:660–662.

91. Kudelina RI, Olsufiev NG. Sensitivity to macrolide antibiotics and lincomycin in *Francisella tularensis holarctica. J Hyg Epidemiol Microbiol Immunol*. 1980;24:84–88.

92. Syrjälä H, Schildt R, Raisainen S. In vitro susceptibility of *Francisella tularensis* to fluoroquinolones and treatment of tularemia with norfloxacin and ciprofloxacin. *Eur J Clin Microbiol Infect Dis.* 1991;10:68–70.

93. Sawyer WD, Dangerfield HG, Hogge AL, Crozier D. Antibiotic prophylaxis and therapy of airborne tularemia. *Bacteriol Rev*. 1966;30:542–548.

94. Foshay L, Hesselbrock WH, Wittenberg MJ, Rodenberg AH. Vaccine prophylaxis against tularemia in man. *Am J Pub Health.* 1942;32:1131–1145.

95. Tigertt WD. Soviet viable *Pasteurella tularensis* vaccines: A review of selected articles. *Bacteriol Rev.* 1962;26: 354–373.

96. Eigelsbach HT, Downs CM. Prophylactic effectiveness of live and killed tularemia vaccines, I: Production of vaccine and evaluation in the white mouse and guinea pig. *J Immunol.* 1961;87:415–425.

97. McCrumb FR Jr. Commission on Epidemiological Survey. Review of tularemia: Studies on tularemia vaccine 1960–62. In: *Annual Report*. Washington, DC: Armed Forces Epidemiological Board; 1962: 81–86.

98. Hornick RB, Eigelsbach HT. Aerogenic immunization of man with live tularemia vaccine. *Bacteriol Rev.* 1966;30:532–538.

99. Burke DS. Immunization against tularemia: Analysis of the effectiveness of live *Francisella tularensis* vaccine in the prevention of laboratory-acquired tularemia. *J Infect Dis*. 1977;135:55–60.

Chapter 25

BRUCELLOSIS

DAVID L. HOOVER, M.D.*; AND ARTHUR M. FRIEDLANDER, M.D.†

INTRODUCTION

THE INFECTIOUS AGENT

THE DISEASE
- Epidemiology
- Pathogenesis
- Clinical Manifestations
- Diagnosis
- Treatment

PROPHYLAXIS

SUMMARY

* *Colonel, Medical Corps, U.S. Army; Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D. C. 20307-5100; and Associate Professor of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799*

† *Colonel, Medical Corps, U.S. Army; Chief, Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011; and Clinical Associate Professor of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799*

INTRODUCTION

Brucellosis is a zoonotic infection of domesticated and wild animals, caused by organisms of the genus Brucella. Humans become infected by ingestion of animal food products, direct contact with infected animals, or inhalation of infectious aerosols.

Brucellosis in humans has a strong association with military medicine.[1] In 1751, Cleghorn, a British army surgeon stationed on the Mediterranean island of Minorca, described cases of chronic, relapsing febrile illness and cited Hippocrates's description of a similar disease more than 2,000 years earlier.[2] Three additional British army surgeons working on the island of Malta during the 1800s were responsible for important observations of the disease. J. A. Marston described clinical characteristics of his own infection in 1861.[3] In 1887, David Bruce, for whom the genus *Brucella* is named, isolated the causative organism from the spleens of five fatal cases and placed it within the genus *Micrococcus*.[4] Ten years later, M. L. Hughes, who had coined the name "undulant fever," published a monograph that detailed clinical and pathological findings in 844 patients.[5]

In that same year, B. Bang, a Danish investigator, identified an organism, which he called the "Bacillus of abortion," in placentas and fetuses of cattle suffering from contagious abortion.[6] In 1917, A. C. Evans recognized that Bang's organism was identical to that described by Bruce as the causative agent of human brucellosis. The organism infects mainly cattle, sheep, goats, and other ruminants, in which it causes abortion, fetal death, and genital infection.[7,8] Humans, who are usually infected incidentally by contact with infected animals or ingestion of dairy foods, may develop numerous symptoms in addition to the usual ones of fever, malaise, and muscle pain. Disease frequently becomes chronic and may relapse, even with treatment.

The ease of transmission by aerosol suggests that *Brucella* organisms might be a candidate for use as a biological warfare agent. Indeed, the United States began development of *B suis* as a biological weapon in 1942. The agent was formulated to maintain longterm viability, placed into bombs, and tested in field trials during 1944–1945 using animal targets. By 1967, the United States terminated its offensive program for development and deployment of *Brucella* as a biological weapon. Although the munitions developed were never used in combat, the studies reinforced the concern that *Brucella* organisms might be used against U.S. troops as a biological warfare agent.[9]

THE INFECTIOUS AGENT

Brucellae are small, nonmotile, nonsporulating, nontoxigenic,

nonfermenting, aerobic, Gram-negative coccobacilli that may, based on DNA homology, represent a single species.[10] Conventionally, however, they are classified into six species, each comprising several biovars. Each species has a characteristic, but not an absolute, predilection to infect certain animal species (Table 25-1). Only *Brucella melitensis, B suis, B abortus, and B canis* cause disease in man. Infection of humans with *B ovis* and *B neotomae* has not been described.

Brucellae grow best on trypticase, soybased, or other enriched media with a typical doubling time of 2 hours. Most biovars of *B abortus* require incubation in an atmosphere of 5% to 10% carbon dioxide for growth. Brucellae may produce urease, oxidize nitrite to nitrate, and are oxidase and catalase positive. Species and biovars are differentiated by their carbon dioxide requirements; ability to use glutamic acid, ornithine, lysine, and ribose; hydrogen sulfide production; growth in the presence of thionine or basic fuchsin dyes; agglutination by antisera directed against certain lipopolysaccharide epitopes; and by susceptibility to lysis by bacteriophage. Recently, analysis of fragment lengths of deoxyribonucleic acid (DNA) cut by various restriction enzymes has also been used to differentiate brucellae groupings.[10]

TABLE 25-1
TYPICAL HOST SPECIFICITY OF *BRUCELLA* SPECIES

***Brucella* Species**	**Animal Host**	**Human Pathogenicity**
B suis	Swine	High
B melitensis	Sheep, goats	High
B abortus	Cattle, bison	Intermediate
B canis	Dogs	Intermediate
B ovis	Sheep	None
B neotomae	Rodents	None

The lipopolysaccharide (LPS) component of the outer cell membranes of brucellae is quite different—both structurally and functionally—from that of other Gram-negative organisms.[11,12] The lipid A portion of a *Brucella* organism LPS contains fatty acids 16 carbons long, and lacks the 14-carbon myristic acid typical of lipid A of Enterobacteriaceae. This unique structural feature may underlie the remarkably reduced pyrogenicity (less than 1/100th) of *Brucella* LPS, compared with the pyrogenicity of *Escherichia coli* LPS.[13] In addition, the O-polysaccharide portion of LPS from smooth organisms contains an unusual sugar, 4,6-dideoxy-4-formamido-alpha-D-mannopyranoside, which is expressed either as a homopolymer of alpha-1,2- linked sugars (A type), or as 3 alpha-1,2 and 2

alpha- 1,3-linked sugars (M type). These variations in O-polysaccharide linkages lead to specific, taxonomically useful differences in immunoreactivity between A and M sugar types.[14]

THE DISEASE

Epidemiology

Animals may transmit *Brucella* organisms during septic abortion, at the time of slaughter, and in their milk. Brucellosis is rarely, if ever, transmitted from person to person. The incidence of human disease is thus closely tied to the prevalence of infection in sheep, goats, and cattle, and to practices that allow exposure of humans to potentially infected animals or their products. In the United States, where most states are free of infected animals and where dairy products are routinely pasteurized, illness occurs primarily in individuals such as veterinarians, shepherds, cattlemen, and slaughterhouse workers who have occupational exposure to infected animals. In many other countries, humans more commonly acquire infection by ingestion of unpasteurized dairy products, especially cheese.

Less obvious exposures can also lead to infection. In Kuwait, for example, disease with a relatively high proportion of respiratory complaints has occurred in individuals who have camped in the desert during the spring lambing season[15]. In Australia, an outbreak of *B suis* infection was noted in hunters of infected feral pigs.[16] *B canis*, a naturally rough strain that typically causes genital infection in dogs, can rarely infect man.

Brucellae are also highly infectious in laboratory settings; numerous laboratory workers who culture the organism become infected. Fewer than 200 total cases per year (0.04 cases per 100,000 population) are reported in the United States. The incidence is much higher in other regions such as the Middle East; countries bordering the Mediterranean Sea; and China, India, Mexico, and Peru; for example, 33 cases per 100,000 population in Jordan (1987) and 88 cases per 100,000 population in Kuwait (1985), respectively.[17,18]

Pathogenesis

Brucellae can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract.[19] In the gastrointestinal tract, the organisms are phagocytosed by lymphoepithelial cells of gut-associated lymphoid tissue, from which they gain access to the submucosa.[20] Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them,[21,22] and are also phagocytosed by macrophages (Figure 25-1). Bacteria transported in

macrophages, which traffic to lymphoid tissue draining the infection site, may eventually localize in lymph nodes, liver, spleen, mammary gland, joints, kidneys, and bone marrow.

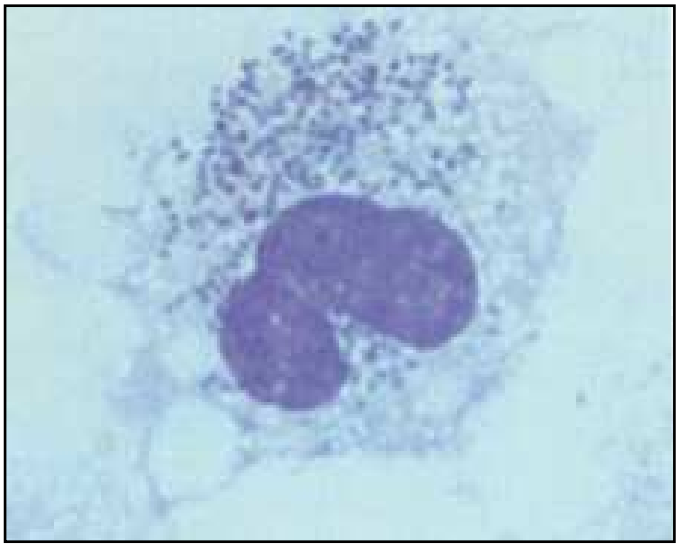


Fig. 25-1. Cultured human monocyte-derived macrophage infected with *Brucella melitensis*. The bacteria, which replicate in phagolysosomes, have a coccobacillary appearance (eosin Y–methylene blue–azure A, original magnification x 1,000). Photograph: Courtesy of Robert Crawford, Ph.D., Senior Scientist, American Registry of Pathology, Washington, DC.

In macrophages, brucellae may inhibit fusion of phagosomes and lysosomes, and replicate in the phagosome.[23] If unchecked by macrophage microbicidal mechanisms, the bacteria destroy their host cells and infect additional cells. Brucellae can also replicate extracellularly in host tissues. Histopathologically, the host cellular response may range from abscess formation to lymphocytic infiltration to granuloma formation with caseous necrosis.

Studies in experimental models have provided important insights into host defenses that eventually control infection with *Brucella* organisms. Serum complement effectively lyses some rough strains (ie, those that lack O-polysaccharide side chains on their LPS), but has little effect on smooth strains (ie, bacteria with a long O-polysaccharide side chain); *B melitensis* may be less susceptible than *B abortus* to complement-mediated killing.[24,25] Administration of antibody to mice prior to challenge with rough or smooth strains of brucellae reduces the number of organisms that appear in liver and spleen. This effect is due mainly to antibodies directed against LPS, with little or no contribution of antibody directed against other cellular components.[26]

Reduction in intensity of infection in mice can be transferred from immune to nonimmune animals by both cluster of differentiation 4+ (CD4+) and CD8+ T cells[27] or by immunoglobulin (Ig) fractions of serum. Administration of antibody to interferon gamma (IFN-γ) worsens experimental infection.[28] Moreover, macrophages treated with IFN-γ in vitro inhibit intracellular bacterial replication. [29] In ruminants, vaccination with killed bacteria provides some protection against challenge, but live

vaccines are much more effective.

These observations suggest that brucellae, like other facultative or obligate intramacrophage pathogens, are primarily controlled by macrophages activated to enhanced microbicidal activity by IFN-γ and other cytokines produced by immune T lymphocytes. It is likely that antibody, complement, and macrophage-activating cytokines produced by natural killer (NK) cells play supportive roles in early infection or in controlling growth of extracellular bacteria.

In ruminants, *Brucella* organisms bypass the most effective host defenses by targeting embryonic and trophoblastic tissue. In cells of these tissues, the bacteria grow not only in the phagosome but also in the cytoplasm and the rough endoplasmic reticulum.[30] In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to fetal death and abortion. In ruminants, the presence in the placenta of erythritol may further enhance growth of brucellae. Products of conception at the time of abortion may contain up to 10^{10} bacteria per gram of tissue.[31] When septic abortion occurs, the intense concentration of bacteria and aerosolization of infected body fluids during parturition often result in infection of other animals and people.

Clinical Manifestations

Clinical manifestations of brucellosis are diverse and the course of the disease is variable.[32] Patients with brucellosis may present with an acute, systemic febrile illness; an insidious chronic infection; or a localized inflammatory process. Disease may be abrupt or insidious in onset, with an incubation period of 3 days to several weeks. Patients usually complain of nonspecific symptoms such as fever, sweats, fatigue, anorexia, and muscle or joint aches (Table 25-2). Neuropsychiatric symptoms, notably depression, headache, and irritability, occur frequently. In addition, focal infection of bone, joints, or genitourinary tract may cause local pain. Cough, pleuritic chest pain, and dyspepsia may also be noted. Symptoms of patients infected by aerosol are indistinguishable from those of patients infected by other routes. Chronically infected patients frequently lose weight. Symptoms often last for 3 to 6 months and occasionally for a year or more. Physical examination is usually normal, although hepatomegaly, splenomegaly, or lymphadenopathy may occur. Brucellosis does not usually cause leukocytosis, and some patients may be moderately neutropenic.[33] Although disease manifestations cannot be strictly related to the infecting species, *B melitensis* tends to cause more severe, systemic illness than the other brucellae; *B suis* is more likely to cause

localized, suppurative disease.

Infection with *B melitensis* leads to bone or joint disease in about 30% of patients; sacroiliitis develops in 6% to 15%, particularly in young adults.[34–36] Arthritis of large joints occurs with about the same frequency as sacroiliitis. In contrast to septic arthritis caused by pyogenic organisms, joint inflammation seen in patients with *B melitensis* is mild, and erythema of overlying skin is uncommon. Synovial fluid is exudative, but cell counts are in the low thousands with predominantly mononuclear cells. In both sacroiliitis and peripheral joint infections, destruction of bone is unusual. Organisms can be cultured from fluid in about 20% of cases; culture of the synovium may increase the yield. Spondylitis, another important osteoarticular manifestation of brucellosis, tends to affect middle-aged or elderly patients, causing back (usually lumbar) pain, local tenderness, and occasionally radicular symptoms.[37]

TABLE 25-2
SYMPTOMS AND SIGNS OF BRUCELLOSIS

Symptom or Sign	Patients Affected (%)
Fever	90–95
Malaise	80–95
Body Aches	40–70
Sweats	40–90
Arthralgia	20–40
Splenomegaly	10–30
Hepatomegaly	10–70

Data sources: (1) Mousa AR, Elhag KM, Khogali M, Marafie AA. The nature of human brucellosis in Kuwait: Study of 379 cases. *Rev Infect Dis.* 1988;10(1):211–217. (2) Buchanan TM, Faber LC, Feldman RA. Brucellosis in the United States, 1960–1972: An abattoir-associated disease, I: Clinical features and therapy. *Medicine (Baltimore).* 1974;53(6):403–413. (3) Gotuzzo E, Alarcon GS, Bocanegra TS, et al. Articular involvement in human brucellosis: A retrospective analysis of 304 cases. *Semin Arthritis Rheum.* 1982;12(2):245–255.

Radiographic findings, similar to those of tuberculous infection, typically include disk space narrowing and epiphysitis, particularly of the anterosuperior quadrant of the vertebrae, and presence of bridging syndesmophytes as repair occurs. Bone scan of spondylitic areas is often negative or only weakly positive. Paravertebral abscess occurs rarely. In contrast with frequent infection of the axial skeleton, osteomyelitis of long bones is rare.[38]

Infection of the genitourinary tract, an important target in ruminant animals, also may lead to signs and symptoms of disease in man.[39,40] Pyelonephritis and cystitis and, in males, epididymoorchitis, may occur. Both diseases may mimic their tuberculous counterparts, with “sterile” pyuria on routine bacteriologic culture. With bladder and kidney infection, *Brucella* organisms can be cultured from the urine. Brucellosis in

pregnancy can lead to placental and fetal infection.[41] Whether abortion is more common in brucellosis than in other severe bacterial infections, however, is unknown.

Lung infections have also been described, particularly before the advent of effective antibiotics. Although up to one quarter of patients may complain of respiratory symptoms, mostly cough, dyspnea, or pleuritic pain, chest X-ray examinations are usually normal.[42] Diffuse or focal infiltrates, pleural effusion, abscess, and granulomas may be noted.

Hepatitis and, rarely, liver abscess also occur. Mild elevations of serum lactate dehydrogenase and alkaline phosphatase are common. Biopsy may show well-formed granulomas or nonspecific hepatitis with collections of mononuclear cells.[32]

Other sites of infection include the heart, central nervous system, and skin. Brucella endocarditis, a rare, but most feared complication, accounts for 80% of deaths from brucellosis.[43] Central nervous system infection usually manifests itself as chronic meningoencephalitis, but subarachnoid hemorrhage and myelitis also occur. A few cases of skin abscesses have been reported.

Diagnosis

A thorough history that elicits details of appropriate exposure (eg, laboratories, animals, animal products, or environmental exposure to locations inhabited by potentially infected animals) is the most important diagnostic tool. Brucellosis should also be strongly considered in differential diagnosis of febrile illness if troops have been exposed to a presumed biological attack. Polymerase chain reaction and antibody-based antigen detection systems may demonstrate the presence of the organism in environmental samples collected from the attack area.

When the disease is considered, diagnosis is usually made by serology. Although a number of serologic techniques have been developed and tested, the tube agglutination test remains the standard method.[44] This test, which measures the ability of serum to agglutinate killed organisms, reflects the presence of anti–O-polysaccharide antibody. Use of the tube agglutination test after treatment of serum with 2-mercaptoethanol or dithiothreitol to dissociate IgM into monomers detects IgG antibody. A titer of 1:160 or higher is considered diagnostic. Most patients already have high titers at the time of clinical presentation, so a 4-fold rise in titer may not occur. IgM rises early in disease and may persist at low levels (eg, 1:20) for months or years after successful treatment. Persistence or increase of 2-mercaptoethanol–resistant titers has been associated with persistent disease or relapse.[45] Serum testing should always include dilution to at least 1:320, since inhibition of agglutination at

lower dilutions may occur. The tube agglutination test does not detect antibodies to *B canis* because this rough organism does not have O-polysaccharide on its surface. Immunoenzymatic assays (eg, enzyme-linked immunosorbent assays [ELISAs]) have been developed for use with *B canis*, but are not well standardized. ELISAs developed for other brucellae similarly suffer from lack of standardization.

In addition to serologic testing, diagnosis should be pursued by microbiologic culture of blood or body fluid samples. Cultures should be held for at least 2 months, with weekly subcultures onto solid medium. Because it is extremely infectious for laboratory workers, the organism should be subcultured only in a biohazard hood. The reported frequency of isolation from blood varies widely, from less than 10% to 90%; *B melitensis* is said to be more readily cultured than *B abortus*. Culture of bone marrow may increase the yield.[46]

Treatment

Brucellae are sensitive in vitro to a number of oral antibiotics and to aminoglycosides. Therapy with a single drug has resulted in a high relapse rate, so combined regimens should be used whenever possible.[47] A 6-week regimen of doxycycline 200 mg/d administered orally, with the addition of streptomycin 1 g/d administered intramuscularly for the first 2 to 3 weeks is effective therapy for adults with most forms of brucellosis.[48] Patients with spondylitis may require longer treatment. A 6-week oral regimen of both rifampin 900 mg/d and doxycycline 200 mg/d is also effective, and should result in nearly 100% response and a relapse rate lower than 10%.[49] Several studies,[48,50,51] however, suggest that treatment with a combination of streptomycin and doxycycline may result in less frequent relapse than treatment with the combination of rifampin and doxycycline. Notable failures have occurred when spondylitis was treated with the latter combination.[50]

Endocarditis may best be treated with rifampin, streptomycin, and doxycycline for 6 weeks; infected valves should be replaced early in therapy.[52] Central nervous system disease responds to a combination of rifampin and trimethoprim/sulfamethoxazole, but may need prolonged therapy. The latter antibiotic combination is also effective for children under 8 years of age.[53] The Joint Food and Agriculture Organization–World Health Organization Expert Committee recommends treatment of pregnant women with rifampin.[49]

Organisms used in a biological attack may be resistant to these first-line antimicrobial agents. Medical officers should make every effort to obtain tissue and environmental samples for bacteriological culture, so

that the antibiotic susceptibility profile of the infecting brucellae may be determined and the therapy adjusted accordingly.

PROPHYLAXIS

To prevent brucellosis, animal handlers should wear appropriate protective clothing when working with infected animals. Meat should be well-cooked; milk should be pasteurized. Laboratory workers should culture the organism only with appropriate Biosafety Level 2 or 3 containment (see Chapter 19, The U.S. Biological Warfare and Biological Defense Programs, for a discussion of the biosafety levels that are used at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland).

In the event of a biological attack, the standard gas mask should adequately protect personnel from airborne brucellae, since the organisms are probably unable to penetrate intact skin. After personnel have been evacuated from the attack area, clothing, skin, and other surfaces can be decontaminated with standard disinfectants to minimize risk of infection by accidental ingestion, or by conjunctival inoculation of viable organisms. There is no commercially available vaccine for humans.

SUMMARY

Brucellosis is a zoonosis of large animals, especially cattle, camels, sheep, and goats. Although humans usually acquire *Brucella* organisms by ingestion of contaminated foods (oral route) or slaughter of animals (percutaneous route), the organism is highly infectious by the airborne route; this is the presumed route of infection of the military threat. Laboratory workers commonly become infected when cultures are handled outside a biosafety cabinet. Individuals presumably infected by aerosol have symptoms indistinguishable from patients infected by other routes: fever, chills, and myalgia are most common, occurring in more than 90% of cases.

Since the bacterium disseminates throughout the reticuloendothelial system, it may cause disease in virtually any organ system. Large joints and the axial skeleton are favored targets; arthritis appears in approximately one third of patients. Fatalities occur rarely, usually in association with central nervous system or endocardial infection.

Serologic diagnosis uses an agglutination test that detects antibodies to lipopolysaccharide. This test, however, is not useful to diagnose infection caused by *B canis*, a naturally O-polysaccharide deficient strain. Infection can be most reliably confirmed by culture of blood, bone marrow, or other infected body fluids, but the sensitivity of culture varies widely.

Nearly all patients respond to a 6-week course of oral therapy with a combination of rifampin and doxycycline; fewer than 10% of patients relapse. Six weeks of doxycycline with addition of streptomycin for the first 3 weeks is also effective therapy. No vaccine is available for humans.

REFERENCES

1. Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis*. Baltimore, Md: Waverly Press; 1950: 1–8.

2. Cleghorn G. *Observations of the Epidemical Diseases of Minorca (From the Years 1744 to 1749).* London, England; 1751. Cited in: Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis*. Baltimore, Md: Waverly Press; 1950: 1–8.

3. Marston JA. Report on fever (Malta). *Army Medical Rept*. 1861;3:486–521. Cited in: Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

4. Bruce D. Note on the discovery of a micro-organism in Malta fever. *Practitioner* (London). 1887;39:161–170. Cited in: Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

5. Hughes ML. *Mediterranean, Malta or Undulant Fever*. London, England: Macmillan and Co; 1897. Cited in: Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

6. Bang B. Die Aetiologie des seuchenhaften (“infectiösen”) Verwerfens. *Z Thiermed* (Jena). 1897;1:241–278. Cited in: Evans AC. Comments on the early history of human brucellosis. In: Larson CH, Soule MH, eds. *Brucellosis.* Baltimore, Md: Waverly Press; 1950: 1–8.

7. Meador VP, Hagemoser WA, Deyoe BL. Histopathologic findings in *Brucella abortus*–infected, pregnant goats. *Am J Vet Res*. 1988;49(2):274–280.

8. Nicoletti P. The epidemiology of bovine brucellosis. *Adv Vet Sci Comp Med*. 1980;24(69):69–98.

9. Department of the Army. *US Army Activity in the US Biological Warfare Programs,* Vols 1 and 2. Washington, DC: HQ, DA; 24 February 1977. Unclassified.

10. Grimont F, Verger JM, Cornelis P, et al. Molecular typing of *Brucella* with cloned DNA probes. *Res Microbiol*. 1992;143(1):55–65.

11. Bundle DR, Cherwonogrodzky JW, Caroff M, Perry MB. The

lipopolysaccharides of *Brucella abortus* and *B melitensis. Ann Inst Pasteur Microbiol.* 1987;138(1):92–98.

12. Moreno E, Borowiak D, Mayer H. *Brucella* lipopolysaccharides and polysaccharides. *Ann Inst Pasteur Microbiol*. 1987;138(1):102–105.

13. Goldstein J, Hoffman T, Frasch C, et al. Lipopolysaccharide (LPS) from *Brucella abortus* is less toxic than that from *Escherichia coli*, suggesting the possible use of *B abortus* or LPS from *B abortus* as a carrier in vaccines. *Infect Immun*. 1992;60(4):1385–1389.

14. Cherwonogrodzky JW, Perry MB, Bundle DR. Identification of the A and M antigens of *Brucella* as the O-polysaccharides of smooth lipopolysaccharides. *Can J Microbiol*. 1987;33(11):979–981.

15. Mousa AR, Elhag KM, Khogali M, Marafie AA. The nature of human brucellosis in Kuwait: Study of 379 cases. *Rev Infect Dis*. 1988;10(1):211–217.

16. Robson JM, Harrison MW, Wood RN, Tilse MH, McKay AB, Brodribb TR. Brucellosis: Re-emergence and changing epidemiology in Queensland. *Med J Aust.* 1993;159(3):153–158.

17. Dajani YF, Masoud AA, Barakat HF. Epidemiology and diagnosis of human brucellosis in Jordan. *J Trop Med Hyg*. 1989;92(3):209–214.

18. Mousa AM, Elhag KM, Khogali M, Sugathan TN. Brucellosis in Kuwait: A clinico-epidemiological study. *Trans R Soc Trop Med Hyg*. 1987;81(6):1020–1021.

19. Buchanan TM, Hendricks SL, Patton CM, Feldman RA. Brucellosis in the United States, 1960–1972:
An abattoir-associated disease, III: Epidemiology and evidence for acquired immunity. *Medicine (Baltimore)*. 1974;53(6):427–439.

20. Ackermann MR, Cheville NF, Deyoe BL. Bovine ileal dome lymphoepithelial cells: Endocytosis and transport of *Brucella abortus* strain 19. *Vet Pathol*. 1988;25(1):28–35.

21. Elsbach P. Degradation of microorganisms by phagocytic cells. *Rev Infect Dis*. 1980;2:106–128.

22. Braude AI. Studies in the pathology and pathogenesis of experimental brucellosis, II: The formation of the hepatic granulomas and its evolution. *J Infect Dis.* 1951;89:87–94.

23. Harmon BG, Adams LG, Frey M. Survival of rough and smooth strains of *Brucella abortus* in bovine mammary gland macrophages. *Am J Vet Res*. 1988;49(7):1092–1097.

24. Young EJ, Borchert M, Kretzer FL, Musher DM. Phagocytosis and killing of *Brucella* by human polymorpho-nuclear leukocytes. *J Infect Dis.* 1985;151(4):682–690.

25. Corbeil LB, Blau K, Inzana TJ, et al. Killing of *Brucella abortus* by bovine serum. *Infect Immun.* 1988;56(12): 3251–3261.

26. Montaraz JA, Winter AJ, Hunter DM, Sowa BA, Wu AM, Adams LG. Protection against *Brucella abortus* in mice with O-polysaccharide-specific monoclonal antibodies. *Infect Immun*. 1986;51(3):961–963.

27. Araya LN, Elzer PH, Rowe GE, Enright FM, Winter AJ. Temporal development of protective cell-mediated and humoral immunity in BALB/c mice infected with *Brucella abortus. J Immunol*. 1989;143(10):3330–3337.

28. Zhan Y, Cheers C. Endogenous gamma interferon mediates resistance to *Brucella abortus* infection. *Infect Immun.* 1993;61(11):4899–4901.

29. Jiang X, Baldwin CL. Effects of cytokines on intracellular growth of *Brucella abortus. Infect Immun.* 1993;61(1): 124–134.

30. Anderson TD, Cheville NF. Ultrastructural morphometric analysis of *Brucella abortus*–infected trophoblasts in experimental placentitis: Bacterial replication occurs in rough endoplasmic reticulum. *Am J Pathol*. 1986;124(2):226–237.

31. Anderson TD, Cheville NF, Meador VP. Pathogenesis of placentitis in the goat inoculated with *Brucella abortus*, II: Ultrastructural studies. *Vet Pathol*. 1986;23(3):227–239.

32. Young EJ. Human brucellosis. *Rev Infect Dis*. 1983;5(5):821–842.

33. Crosby E, Llosa L, Miro QM, Carrillo C, Gotuzzo E. Hematologic changes in brucellosis. *J Infect Dis*. 1984;150(3):419–424.

34. Gotuzzo E, Alarcon GS, Bocanegra TS, et al. Articular involvement in human brucellosis: A retrospective analysis of 304 cases. *Semin Arthritis Rheum.* 1982;12(2):245–255.

35. Alarcon GS, Bocanegra TS, Gotuzzo E, Espinoza LR. The arthritis of brucellosis: A perspective one hundred years after Bruce's discovery. *J Rheumatol*. 1987;14(6):1083–1085.

36. Mousa AR, Muhtaseb SA, Almudallal DS, Khodeir SM, Marafie AA. Osteoarticular complications of brucellosis: A study of 169 cases. *Rev Infect Dis*. 1987;9(3):531–543.

37. Howard CB, Alkrinawi S, Gadalia A, Mozes M. Bone infection resembling phalangeal microgeodic syndrome in children: A case report. *J Hand Surg [Br]*. 1993;18(4):491–493.

38. Rotes-Querol J. Osteo-articular sites of brucellosis. *Ann Rheum Dis*. 1957;16:63–68.

39. Ibrahim AIA, Shetty SD, Saad M, Bilal NE. Genito-urinary complications of brucellosis. *Br J Urol*. 1988;61: 294–298.

40. Kelalis PP, Greene LF, Weed LA. Brucellosis of the urogenital tract: A mimic of

tuberculosis. *J Urol*. 1962;88: 347–353.

41. Lubani MM, Dudin KI, Sharda DC, et al. Neonatal brucellosis. *Eur J Pediatr*. 1988;147(5):520–522.

42. Buchanan TM, Faber LC, Feldman RA. Brucellosis in the United States, 1960–1972: An abattoir-associated disease, I: Clinical features and therapy. *Medicine (Baltimore)*. 1974;53(6):403–413.

43. Peery TM, Belter LF. Brucellosis and heart disease, II: Fatal brucellosis. *Am J Pathol*. 1960;36:673–697.

44. Young EJ. Serologic diagnosis of human brucellosis: Analysis of 214 cases by agglutination tests and review of the literature. *Rev Infect Dis*. 1991;13(3):359–372.

45. Buchanan TM, Faber LC. 2-mercaptoethanol *Brucella* agglutination test: Usefulness for predicting recovery from brucellosis. J *Clin Microbiol.* 1980;11(6):691–693.

46. Gotuzzo E, Carrillo C, Guerra J, Llosa L. An evaluation of diagnostic methods for brucellosis—The value of bone marrow culture. *J Infect Dis*. 1986;153(1):122–125.

47. Hall WH. Modern chemotherapy for brucellosis in humans. *Rev Infect Dis*. 1990;12(6):1060–1099.

48. Luzzi GA, Brindle R, Sockett PN, Solera J, Klenerman P, Warrell DA. Brucellosis: Imported and laboratory-acquired cases, and an overview of treatment trials. *Trans R Soc Trop Med Hyg*. 1993;87(2):138–141.

49. Joint FAO/WHO expert committee on brucellosis. *World Health Organ Tech Rep Ser.* 1986;740(1):1–132.

50. Ariza J, Gudiol F, Pallares R, et al. Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus streptomycin: A randomized, double-blind study. *Ann Intern Med.* 1992;117(1):25–30.

51. Montejo JM, Alberola I, Glez ZP, et al. Open, randomized therapeutic trial of six antimicrobial regimens in the treatment of human brucellosis. *Clin Infect Dis*. 1993;16(5):671–676.

52. Chan R, Hardiman RP. Endocarditis caused by *Brucella melitensis. Med J Aust.* 1993;158(9):631–632.

53. Lubani MM, Dudin KI, Sharda DC, et al. A multicenter therapeutic study of 1,100 children with brucellosis. *Pediatr Infect Dis J*. 1989;8(2):75–78.

Chapter 26

Q FEVER

WILLIAM R. BYRNE, M.D., FACP*

INTRODUCTION

MILITARY RELEVANCE

HISTORY

THE INFECTIOUS AGENT

THE DISEASE
- Epidemiology
- Pathogenesis
- Clinical Disease in Domestic Animals
- Clinical Disease in Humans
- Diagnosis

TREATMENT

PROPHYLAXIS

SUMMARY

Colonel, Medical Corps, U.S. Army; Chief, Genetics and Physiology Branch, Bacteriology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011

INTRODUCTION

Q fever is a zoonotic disease caused by *Coxiella burnetii*, a rickettsia-like organism of low virulence but remarkable infectivity. A single organism may initiate infection. In addition, despite the fact that *C burnetii* is unable to grow or replicate outside host cells, there is a sporelike form of the organism that is extremely resistant to heat, pressure, desiccation, and many standard antiseptic compounds; this allows *C burnetii* to persist in the environment for long periods (weeks or months) under harsh conditions. This persistence, coupled with a primary mode of transmission by inhalation of infected aerosols, allows for the development of acute infection following only indirect exposure to an infected source. In contrast to this high degree of inherent resilience and transmissibility, the acute clinical disease associated with Q fever infection is usually a benign, although a temporarily incapacitating, illness in humans. Even without treatment, the vast majority of patients recover. Chronic disease as a result of Q fever is rare, although it is frequently fatal.

The primary reservoir for natural human infection is livestock, particularly parturient females, and the distribution is worldwide. Outbreaks of Q fever are infrequently reported, however, and the disease may be endemic in areas where cases are rarely or never reported. Humans who work in animal husbandry, especially those who assist during parturition (eg, calving or lambing) are at risk for acquiring Q fever. However, a definite risk also exists for persons who live in close proximity to, or who pass through, an area where animal birthing is occurring, even if this occurred months previously.

MILITARY RELEVANCE

Since the disease was described in 1937, thousands of cases involving military personnel of many countries have been reported (an excellent review was published in 1978,[1]) and infection with *Cburnetii* should be considered a possibility whenever troops are present in an area with infected animals.

American soldiers in Italy during World War II were affected, with 5 confirmed outbreaks of Q fever during the winter of 1944 and spring of 1945,[2] usually in troops occupying farm buildings recently or concurrently inhabited by farm animals.[3] This degree of close contact with farm animals was not an absolute requirement for infection, however: approximately 1,700 cases occurred in late spring, 1945, at an airbase in southern Italy as a result of sheep and goats herded in pastures nearby.[4] During World War II, cases of acute Q fever were also identified in soldiers in Virginia shortly after debarking from a 9-day voyage from Naples, Italy,[4,5] and a

single case was identified in a soldier stationed in Panama.[6]

Hundreds of cases consistent with Q fever were observed in German soldiers in Serbia and southern Yugoslavia during World War II. Outbreaks occurred in the apparent absence of disease in the indigenous population. The disease was most commonly referred to as “Balkengrippe”; infection with *C burnetii* was not confirmed by laboratory testing, but the clinical and epidemiological features of the illness described were most consistent with Q fever. Similar cases were observed in German troops during World War II in Italy, Crimea, Greece, Ukraine, and Corsica.[1]

An outbreak of acute Q fever associated with an epidemic of spontaneous abortion in sheep and goats occurred in 78 British troops stationed in Cyprus, from December 1974 to June 1975[7]; Swedish troops were also affected.[8] Q fever outbreaks have also been described[1] among Swiss soldiers in 1948, Greek soldiers from 1946 to 1956, and Royal Air Force airmen on the Isle of Man in 1958. These outbreaks occurred in the soldiers’ home countries when the troops were stationed or training in close proximity to sheep or goats, particularly parturient animals. Outbreaks attributed to sheep or goat exposure in deployed soldiers have been described[1] in American airmen in Libya in 1951 and French soldiers in Algeria in 1955.

Among American military personnel in the Persian Gulf War, one case of meningoencephalitis associated with acute Q fever was reported, with the onset of symptoms 2 weeks after return from the Persian Gulf.[9] One other soldier, with acute Q fever pneumonia, was diagnosed in Saudi Arabia in March 1991.[10] This occurred in a first sergeant in an engineering battalion. Subsequent epidemiological evaluation and serologic testing of the unit identified three additional acute seroconversions among soldiers of the same battalion.[11] Exposure to sheep, goats, or camels was identified in all of these infections acquired in Saudi Arabia.

Q fever is probably endemic in Somalia,[12] and serologic evidence of acute Q fever was identified in two American soldiers evaluated in Somalia for fever of unknown origin.[11,13]

These reports all underscore the importance of considering the diagnosis of Q fever in a febrile soldier in or recently returned from an area where the disease may be present. This is particularly true if the soldier has been in close proximity to or in an area previously occupied by animals which may harbor *C burnetii*.

The potential of *C burnetii* as a biological warfare threat is directly related to its infectivity. It has been estimated that 50 kg of dried, powdered *C burnetii* would produce casualties a rate equal to that of similar amounts of anthrax or tularemia organisms.[14] Q fever has been evaluated as a potential biological warfare agent by the United States,[15]

but munitions and stocks (except that required for vaccine research) were publicly destroyed by executive order of President Richard M. Nixon between May 1971 and May 1972.[16]

HISTORY

Q fever was first described in 1937 by Edward Derrick,[17] while he was the Director of Microbiology and Pathology for the Queensland (Australia) Health Department at Brisbane. In 1935, he was contacted about a febrile illness that had been occurring among abattoir workers in Brisbane. When routine blood cultures and serologic testing did not reveal a diagnosis, Derrick suspected that he was dealing with a new illness. He thoroughly described the clinical characteristics and designated the disease Q (for query) fever. Derrick's laboratory investigation demonstrated that it was possible to transmit the disease to animals by inoculating guinea pigs and mice with the blood of humans suffering from acute Q fever. Although Derrick had initially concluded that the infectious agent was a virus, studies of a guinea pig liver emulsion sent to MacFarlane Burnet in Melbourne subsequently indicated that the causative organism was a rickettsia,[18] according to the terminology used at that time.

Interestingly, Derrick may not have been the first to transfer the disease to laboratory animals. Hideyo Noguchi, working at the Rockefeller Institute in New York City in 1925, may have passed *C burnetii* to guinea pigs from ticks that had been collected at Saw Tooth Canyon by Ralph Parker at the Rocky Mountain Laboratory in Hamilton, Montana.[19] This agent, however, was ultimately lost in animal passage.

About the same time that the investigations were being done in Australia, Gordon Davis was studying Rocky Mountain Spotted Fever at the Rocky Mountain Laboratory. He observed that a febrile illness resulted when ticks collected from the area around the nearby Nine Mile Creek were allowed to feed on guinea pigs.[20] The disease produced in guinea pigs did not, however, resemble Rocky Mountain Spotted Fever. Herald Cox was subsequently able to characterize the organism (then called the "Nine Mile Agent") as similar to rickettsia and to cultivate this organism in the yolk sac membrane of embryonated hen eggs.[21] The relation of Q fever to the Nine Mile Agent was established by Rolla Dyer, director of the National Institutes of Health at the time, after the spleens of infected mice were sent to him by Burnet. In an event that presaged the problems of transmission of Q fever in laboratory workers, Dyer himself acquired acute Q fever during a visit to Hamilton in 1938.[22]

The work of Ralph Parker, also at the Rocky Mountain Laboratory, indicated that ticks are the reservoir of the "Nine Mile Agent." Derrick had

also suspected tick transmission from a primary reservoir, and from a secondary reservoir of domestic animals. The significance of exposure to parturient animals was not, however, recognized until 1950.

The causative agent of Q fever was ultimately designated *Coxiella burnetii* to recognize the outstanding contributions of both Cox and Burnet to the isolation and characterization of this new pathogen.[23] The disease, following clinical description and microbiological characterization of the etiologic agent, has been identified in at least 51 countries on 5 continents.[24]

THE INFECTIOUS AGENT

Coxiella burnetii is classified in the family Rickettsiaceae, but is not included in the genus *Rickettsia* and therefore is not a true rickettsia. It is not closely related to any other bacterial species when comparative 16s ribosomal ribonucleic acid (RNA) analysis is performed,[25] thus the genus *Coxiella* has only one species. The closest relative according to 16s ribosomal RNA analysis is *Legionella,*[25,26] but *Legionella* has different growth characteristics *(Legionella,* being a facultative intracellular parasite, is able to survive and multiply extracellularly) and causes a different clinical syndrome.

C burnetii must occupy an intracellular environment in order to grow or reproduce, similar to true rickettsia, although, as previously stated, the organism has a sporelike form that is very resistant to heat and desiccation.[27] This sporelike form may be observed in human tissue.[28] The particular cytological niche occupied by *C burnetii* is the usually very destructive environment of the phagolysosome of eukaryotic cells (Figure 26-1), which has a strongly acidic milieu (normal pH is 4.5) and numerous digestive enzymes. While inhabiting the phagolysosome, *C burnetii* usually lives in relatively peaceful coexistence with the host, causing little direct damage to the cell, at least initially.

Fig. 26-1 is not shown due to copyright restrictions, please refer to the textbook.

Fig. 26-1. Electron micrograph of *Coxiella burnetii* in the phagolysosome of an infected yolk sac cell, demonstrating both large (LCV) and small (SCV) cell variants. The bar in the lower right corner represents 0.6 µm. After Renografin (manufactured by Squibb Diagnostics, Princeton, NJ) purification, the cells were fixed with primary fixative and stained with potassium permanganate. The phagolysosome contains many pleomorphic *C burnetii* organisms. Multiplication by binary transverse fission with septa formation (arrows) is seen.

The LCVs resemble Gram-negative bacteria, with outer and cytoplasmic membranes separated by a periplasmic space. The LCV is more metabolically active than the SCV, has less peptidoglycan in the cell wall, and is capable of production of the sporelike form. The loose outer membrane, increased periplasmic space, and bleb formation of some of the LCVs probably indicate that they are undergoing deterioration or have been damaged during preparation. The SCVs appear as extremely dense organisms and are heat-resistant, relatively dormant structures which have the ability to survive in an adverse environment.

Replication occurs by binary fission within the host cell; the dormant, sporelike form, is produced under certain circumstances.[29] Dormant *C burnetii* can be stimulated to a brief period of growth by exposure to an acidic environment.[30] Sustained growth and replication of *C burnetii* outside a host cell is not possible.

Phase variation has been described with *C burnetii* maintained in the laboratory.[31] The virulent organism, which is associated with natural infection and a smooth lipopolysaccharide (LPS), is designated as Phase I. This phase is resistant to complement and is a potent immunogen. Serial passage of *C burnetii* in eggs eventually results in the bacterium's conversion to Phase II, which has a rough LPS and is much less virulent than Phase I. This phase is sensitive to complement and is a poor immunogen. The conversion from Phase I to Phase II is irreversible[32] and is the result of a mutation caused by a chromosomal deletion.

Coxiella burnetii also contains several plasmids, and dissimilar plasmid types may be associated with different manifestations of disease.[33] The cell wall of a Phase I *C burnetii* organism contains, in association with lipopolysaccharide, an immunomodulatory complex,[34] which produces toxic reactions in mice (eg, hepatomegaly, splenomegaly, liver necrosis)

and lymphocyte hyporesponsiveness in vitro.

THE DISEASE

Epidemiology

Coxiella burnetii is extremely infectious. Under experimental conditions, *a single organism* is capable of producing infection and disease in humans.[35]

The host range of *C burnetii* is very diverse and includes a large number of mammalian species and arthropods. Among these, however, man is the only host identified that normally experiences an illness as a result of infection. A number of different strains of *C burnetii* have been identified worldwide, and different clinical manifestations and complications may be associated with the various strains.

Humans have been infected most commonly by contact with domestic livestock, particularly goats, cattle, and sheep. The risk of infection is substantially increased if humans are exposed to these animals at parturition. During gestation, the proliferation of *C burnetii* in the placenta facilitates aerosolization of large numbers of the pathogen during parturition. *Coxiella* organisms thus produced may persist in the local environment, and produce infection, for weeks or months afterwards.

C burnetii is also shed in the urine and feces of infected animals, in addition to being present in the blood and tissues. Survival of the organism on inanimate surfaces, such as straw, hay, or clothing, allows for transmission to individuals who are not in direct contact with infected animals; for example,

- soldiers sleeping in barns previously occupied by infected animals,[3]
- laundry workers handling infected clothing,[36]
- coworkers of an individual with an infected cat in the home,[37] and
- residents of an urban community living along a road utilized by farm vehicles.[38]

Investigation of outbreaks of Q fever frequently report a significant proportion of patients who have no identifiable risk factor. Human-to-human transmission has been reported,[39] but it is a very rare event.

As mentioned previously, the distribution of *C burnetii* is worldwide.[24] With the exception of a few countries (New Zealand is an example), Q fever cases have been identified practically everywhere that an attempt has been made to identify evidence of infection, either in man or in animals.

In the United states, the epidemiology of Q fever is variable. Sporadic but regularly occurring cases have been observed[40] in areas with endemic foci in cattle, and clusters of cases have been described[41] in areas with infected dairy herds. Livestock is not the only source of Q fever infections in this country: a small outbreak in Maine associated with exposure to a parturient cat has been described,[42] similar to an outbreak in Nova Scotia, Canada.[43] Since 1985, outbreaks of Q fever in the United States have been reported in five states among differing groups of individuals:

- slaughterhouse workers in California[44];
- faculty, laboratory workers, and staff exposed to sheep at a medical school in Colorado[45];
- individuals exposed to sheep at a sheep research station in Idaho[46];
- laboratory animal personnel in Arkansas working with parturient sheep[47]; and
- workers in an animal research laboratory in South Carolina who handled and performed surgery on sheep.[48]

Although reported outbreaks[49,50] of Q fever in the United States have been relatively uncommon in recent years, underreporting undoubtedly occurs. For example, although the first 2 cases of Q fever from 2 adjacent rural counties in Michigan were reported in 1984, a study[51] published just 4 years later showed that 15% of the general population surveyed in those 2 counties and 43% of goat owners were seropositive.

Pathogenesis

Human infection with *C burnetii* is usually the result of inhalation of infected aerosols. Following this, the organisms are phagocytized by host cells, predominately unstimulated macrophages. This uptake of *C burnetii* by host phagocytic cells is not energy dependent, but is probably the result of contact by the pathogen with an existing receptor. After phagocytosis by host cells, conditions within the phagolysosome trigger growth and multiplication of *C burnetii*, with little initial damage to the host cell. Eventually the cytoplasm becomes engorged with *C burnetii* organisms and lysis of the host cell occurs. Dissemination of the pathogen occurs as a result of circulation of organisms free in the plasma, on the surface of cells, and carried by circulating macrophages.

In animals, infection frequently lasts for the life of the animal, in a more-or-less dormant state, with periodic increases in organism numbers during periods of relative immunosuppression, particularly parturition,[52] but also in laboratory animals treated with adrenocorticosteroids[53] or irradiation.[54]

C burnetii causes little overt disease in animals (and no apparent disease in ticks), except that luxuriant growth in the placenta may increase the rate of spontaneous abortion in some species. Edema and thrombohemorrhagic lesions may be identified in the placentas of infected animals.

There is little host reaction at the initial portal of entry, either in the lung following inhalation of aerosol or in the skin following a tick bite. Q fever develops without formation of a primary infectious focus in the area of the tick bite, and the organism does not infect the vascular endothelium as do true rickettsial pathogens.

In man, polyclonal production of antibody represents the initial immune response to *C burnetii,* but humoral immunity alone is ineffective for control of the organism, although the presence of antibody does contribute significantly to antibody-dependent cellular cytotoxicity later in the course of the infection. Passive transfer of immune serum to laboratory animals does not improve clearance of organisms from the spleen[55]. Pretreatment of laboratory animals with cyclophosphamide, an antineoplastic agent that severely inhibits production of antibody, does not adversely affect the course of infection.[56] *C burnetii* organisms that have been opsonized, however, are much more efficiently destroyed by host phagocytic cells than are unopsonized organisms. Control of the infection by the host eventually results from the development of specific cell-mediated immunity, with killing by activated macrophage and natural killer cells. This process may result in a granulomatous reaction without the scarring and tissue reaction observed with true granulomata.

The host immune response in man appears to be modified by the *C burnetii* organism itself in chronic infection, in that the lymphocytes of patients with Q fever endocarditis exhibit profound hyporesponsiveness to *C burnetii* antigen, although they retain their reactivity to other antigens.[57]

The presence of LPS on the cell surface of *C burnetii* protects the pathogen from host microbicidal activities. The phase variation previously described is the result of alteration of the LPS, with the virulent Phase I organism having a smooth LPS. The Phase II organism, the result of serial passage of *C burnetii* in eggs, has a rough LPS, is much less immunogenic than the Phase I, and is less virulent.[58] Phase I organisms are resistant to the lytic action of complement, while Phase II organisms are sensitive to the alternate pathway of complement.[59]

Clinical Disease in Domestic Animals

Except for spontaneous abortion, illness in domestic animals as a result of *C burnetii* infection is unusual, although the organism has a propensity for proliferation in the female reproductive system—particularly

the uterus and the mammary glands. Differences between the manifestations in domestic animals, however, are worthy of comment.

In sheep, the infection tends to be transient, followed by spontaneous remission. Infected sheep will usually cease shedding the pathogen after a few months and no longer be infectious to other animals in the flock, except during parturition. Although *C burnetii* has frequently been recovered from the placentas of sheep and has been associated with epidemic abortions, shedding in the milk is rare.

By contrast, chronic shedding—over months or years—of *Coxiella* in the milk of lactating cows can be expected. This aspect of the infection can facilitate maintenance of *Coxiella* in a herd, particularly a dairy herd. Infection in cows is also associated with an increased incidence of spontaneous abortion and may be associated with infertility.

Goats also show an increased disposition for abortion during epizootics of Q fever, and infection in a herd may be maintained by chronic shedding.

Clinical Disease in Humans

Man is the only host susceptible to infection by *C burnetii* that commonly develops an illness as a result of the infection. The incubation period varies from 10 to 40 days, with the duration of the incubation period being inversely correlated with the magnitude of the inoculum.[35] A higher inoculum also increases the severity of the disease. Q fever in humans may be manifested by asymptomatic seroconversion, acute illness, or chronic disease. The frequency of these manifestations parallels this order in decreasing magnitude. In epidemiological surveys, most seropositive individuals do not recall having the illness. The frequency of chronic disease (usually endocarditis) compared with acute disease is difficult to determine precisely due to underreporting of acute infection but is probably less than 1% of the total infected population.

The tendency for *C burnetii* to produce asymptomatic seroconversion has been documented in several publications. In one study,[35] experimental infection in humans showed that in 2 of 4 volunteers infected with a single organism by aerosol, a diagnosis could be established by serologic conversion without clinical illness. Asymptomatic seroconversion did not occur with higher infecting doses (5–1,500 organisms). In an outbreak in Canada attributed to indirect exposure to contaminated clothing, 6 (37.5%) of 16 individuals diagnosed by seroconversion did not have an associated illness.[37] In Switzerland in 1983, during the course of a serosurvey to investigate a large outbreak of Q fever, more than half of the 415 serologically confirmed patients were asymptomatic or minimally ill.[60] These reports underscore the value of an epidemiological investigation when even a single case of acute Q fever is recognized.

Infection with *C burnetii* has been reported[61] to persist in humans, as it does in animals, in an asymptomatic state. Phase I *C burnetii* has been recovered from the placentas of asymptomatic women infected from 1 to 6 months,[62] to 3 years[63] previously. Infection with Q fever may rarely affect the outcome of pregnancy adversely.[64]

Acute Q Fever

There is no characteristic illness for acute Q fever, and manifestations may vary considerably between locations where the disease is acquired.

When symptomatic, the onset of Q fever may be abrupt or insidious, with fever, chills (including frank rigors), and headache being the most common signs and symptoms (Table 26-1). The headache is usually described as severe, throbbing, and frontal or retro-orbital in location. Diaphoresis, malaise, fatigue, and anorexia are also very common. Weight loss of 7 kg or more during the course of acute illness has been reported with surprising frequency, particularly when other general symptoms lasted more than 2 weeks.[2,65] Myalgias are also a frequent complaint, while arthralgias are relatively unusual. Cough tends to appear later in the illness than some of the other more common symptoms, such as fever, chills, and headache, and may not be a prominent complaint. Chest pain occurs in a minority of patients and may be pleuritic or a vague substernal discomfort.

Relatively infrequent symptoms include sore throat, gastrointestinal upset, and neck stiffness, although this last symptom has been severe enough in reported cases of acute Q fever to warrant a lumbar puncture to exclude bacterial meningitis. Although nonspecific evanescent skin eruptions have been reported,[66,67] there is no characteristic rash.

Most patients appear mildly to moderately ill—when the onset is abrupt, Q fever has been mistaken for influenza. The temperature tends to fluctuate, with peaks of 39°C to 40°C, and

TABLE 26-1
SIGNS AND SYMPTOMS IN ACUTE Q FEVER

Signs and Symptoms	Frequency (%)
Onset*	
Gradual	30–70
Abrupt	30–70
Fever	80–100
Chills, rigors	75–100
Headache, retro-orbital pain	50–100
Diaphoresis	40–100
Malaise	50–100
Weakness, fatigue	40–85

in approximately one fourth of the cases is biphasic; in two thirds of patients with acute disease, the febrile period lasts 13 days or less. The duration of fever is usually longer in older patients.[68]

Neurological symptoms are not uncommon and in one study[65] were observed in up to 23% of acute cases. Encephalopathic symptoms, hallucinations (visual and auditory), expressive dysphasia, hemifacial pain resembling trigeminal neuralgia, diplopia, and dysarthria were also reported. Other manifestations involving the central nervous system, such as encephalitis, encephalomyelitis, optic neuritis, or myelopathy may also occur,[9,69,70] particularly late in the acute illness.

Physical findings in acute Q fever are as nonspecific as the clinical symptomatology. Rales are probably the most commonly observed physical finding; evidence of pleural effusion (including friction rub) and consolidation may also be noted, but not in the majority of infections. Although hepatomegaly, splenomegaly, jaundice, pharyngeal injection, and hepatic and splenic tenderness have all been reported, they are relatively unusual in acute infection.

Reports of abnormalities on chest X-ray examination vary with locale, but abnormalities are

Anorexia	35–45
Weight loss (≥7 kg)	50–80
Myalgias	45–85
Arthralgias	10–20
Chest pain	40–50
Cough	50–60
Sore throat	5–35
Nausea, vomiting	15–20
Diarrhea	5–20
Neck stiffness	5–7
Neurological signs	10–35

*Some report gradual onset; others, abrupt onset; coincidentally, the frequency is the same.

Data sources: (1) Robbins FC, Ragan CA. Q fever in the Mediterranean area: Report of its occurrence in Allied troops, I: Clinical features of the disease. *Am J Hyg.* 1946;44:6–22. (2) Feinstein M, Yesner R, Marks JL. Epidemics of Q fever among troops returning from Italy in the spring of 1945, I: Clinical aspects of the epidemic at Camp Patrick Henry, Virginia. *Am J Hyg.* 1946;44:72– 87. (3) Marrie TJ, Langille D, Papukna V, Yates L. Truckin' pneumonia—An outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with a newborn kitten. *Epidem Inf.* 1989;102:119–127. (4) Langley JM, Marrie TJ, Covert A, et al. Poker players pneumonia: An urban outbreak of Q fever following exposure to a parturient cat. *N Engl J Med.* 1988;319:354–356. (5) Raoult D, Marrie TJ. State-of-the-art clinical lecture: Q fever. *Clin Inf Dis.* 1995;20:489–496. (6) Clark WH, Lennette EH, Railsback OC, Romer MS. Q fever in California. *Arch Intern Med.* 1951;88:155–161. (7) Dupont HT, Raoult D, Brouqui P, et al. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *Am J Med.* 1992;93:427–434. (8) Tselentis Y, Gikas A, Kofteridis D, et al. Q fever in the

Greek island of Crete: Epidemiologic, clinical, and therapeutic data from 98 cases. *Clin Inf Dis .* 1995;20:1311–1316.

probably seen in 50% to 60% of patients.[71] An abnormal chest radiograph may be seen in the absence of pulmonary symptoms, while a normal chest radiograph may be observed in a patient with pulmonary symptoms. The most common abnormality observed in a recent report from England was a unilateral, homogenous infiltrate involving one or two lobes,[71] although lobar consolidation and pleural effusions[72] may also be seen. Rounded opacities and hilar adenopathy are not uncommon,[43] at least in Canada, and the diagnosis of Q fever should be at least be considered when these abnormalities are observed in the setting of acute pneumonia.

Laboratory abnormalities of routine tests most commonly involve tests of liver function, and patients with acute Q fever may present with a clinical picture of acute hepatitis. Depending on the locale, reported elevations of aspartate aminotransferase, alanine transferase, or both, in the range of 2- to 3-fold higher than the upper limit of normal, are observed in 50% to 75% of patients, while elevation of the alkaline phosphatase is observed in 10% to 15% of patients. The total bilirubin can be expected to be elevated in 10% to 15% of patients with acute Q fever. The white blood cell count is usually normal; the erythrocyte sedimentation rate is elevated in one third of patients.[65] Mild anemia or thrombocytopenia may also be observed.

Complications recorded in a recent outbreak involving 147 symptomatic cases of Q fever included 2 of acute endocarditis, 2 of renal failure, and 1 of reactive polyarthropathy.[65] Persistent nonspecific symptoms, such as fatigue and malaise, were reported in 32% of the patients in this series, while weight loss (defined as ≥ 7 kg) was identified in 71%, although none developed serologic evidence suggestive of chronic Q fever. An interesting epidemiological feature identified in the study was a significantly higher percentage of smokers in the affected group than in the general population of the area surveyed.

Chronic Q Fever

Chronic infection with *C burnetii* is usually manifested by infective endocarditis, which is also the most severe complication of Q fever. In addition, a report[73] from France of 92 cases published in 1993 also listed hepatitis, infected vascular prostheses and aneurysms, osteomyelitis, pulmonary infection, cutaneous infection, and an asymptomatic form. In addition, 7 of the 92 patients described in this report experienced fever only. Also noted was the observation that although 81% of patients had an identifiable risk factor, only 31% lived in a rural area. In addition, some form of immunodeficiency was observed in 20% of the patients, raising the possibility that chronic Q fever occurs as a result of reactivation of latent infection.[73] Inflammatory pseudotumor of the lung as a chronic complication of Q fever has also been reported.[74,75]

In Q fever endocarditis, fever has been recorded in 85% of patients, along with other systemic symptoms, such as chills, headache, myalgias, and weight loss, in a recent study[73] of 84 cases. Fever was not as prominent, however, in chronic compared to acute Q fever. Other frequently reported clinical features of Q fever endocarditis in this very large series included congestive heart failure (76%), splenomegaly (42%), hepatomegaly (41%), clubbing (21%), and cutaneous signs, often the result of a leukocytoclastic vasculitis (22%). Approximately 90% of patients in this study had preexisting valvular heart disease; more than half had a vascular prosthesis.

Routine blood cultures in Q fever endocarditis are negative, and Q fever should be considered when culture-negative endocarditis is encountered. The diagnosis of infective endocarditis secondary to Q fever is confirmed by serologic testing: antibody to Phase I organisms is usually higher than that for Phase II, and, more significantly, immunoglobulin A (IgA) antibody to *C burnetii* is also present.[76]

Diagnosis

Diagnosis of Q fever is usually accomplished by serologic testing because culture of *C burnetii* is potentially hazardous to laboratory personnel and requires animal inoculation or cell culture.

A number of serologic methods are used, including complement fixation (CF), indirect fluorescent antibody (IFA), macroagglutination and microagglutination, and enzyme-linked immunosorbent assay (ELISA). Significant antibody titers are usually not identifiable until 2 to 3 weeks into the illness. In 1987, the sensitivities of the different antibody assay methods were reported[77] as 94% for ELISA, 91% for IFA, and 78% for CF. Following infection, significant antibody titers may be present for years,

particularly with more sensitive assays, such as the ELISA.

Of the methods currently utilized for the diagnosis of Q fever, the ELISA is the most sensitive and the easiest to perform. The utility of the ELISA for epidemiological screening and diagnosis of Q fever has recently been confirmed.[78] This assay, performed at the United States Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, can establish a diagnosis of acute Q fever from a single serum specimen, with a sensitivity of 80% to 84% in early convalescence and 100% in intermediate and late convalescence.[79] In general, antibodies to the rough Phase II organism are identified earlier in the illness, during the first few months following infection, followed by a decline in antibody to Phase II organisms and a rise in antibody to the smooth, virulent Phase I organism. Antibodies of the IgM type are usually observed within the first 6 to 12 months following infection, with persistence of IgG antibodies afterward. Polymerase chain reaction (PCR) may also be useful in the future for the diagnosis of Q fever,[80–83] but remains to be validated in acute clinical cases.

TREATMENT

The treatment of Q fever was the subject of an excellent review that was published in 1993.[84] Tetracyclines have been the mainstay of therapy since the 1950s. When initiated within the first few days of illness, treatment with a tetracycline shortens the course of the disease. Attempted prophylaxis with a tetracycline (20 g of oxytetracycline administered over 5–6 d), however, has produced mixed results.[35] Initiation of the antibiotic early in the incubation period (24 h after exposure) merely prolonged the incubation period, while initiation of therapy late in the incubation period prevented the development of disease.

Macrolide antibiotics, such as erythromycin, are also effective for the treatment of acute Q fever.[72,85] A new macrolide, azithromycin, has also demonstrated efficacy in a few cases, but experience is very limited. [86]

When chronic Q fever infection is manifested by infective endocarditis, treatment is very difficult; the mortality is 24% even when patients receive appropriate treatment.[73] At least 2 years of therapy are required, usually with a tetracycline combined with rifampin or a quinolone, although trimethoprim-sulfamethoxazole has also been used.[84] Quinolones alone or in combination have also been effective. Most recently, the addition of hydroxychloroquine to tetracycline has shown promising results both in vitro[87] and in a small number of patients.[88]

PROPHYLAXIS

Q fever can be prevented by immunization. Vaccine prophylaxis for Q fever has been studied and used almost since the discovery that the responsible organism could be propagated in the yolk sac of eggs. Immunization with formalin-killed *C burnetii* confers protection against Q fever in laboratory personnel,[89] abattoir workers,[90,91] and human volunteers experimentally exposed to aerosolized *C burnetii.*[92] In Australian abattoir workers, the results of efficacy studies were impressive: a single injection of 30 µg of vaccine antigen (Q-Vax, manufactured by CSL Ltd., Parkville, Victoria, Australia) conferred protective immunity that began 2 weeks after immunization and persisted for at least 5 years.[90] Protection depends primarily on cell-mediated immunity, the presence of which may be detected by positive skin test reactions and in vitro lymphocyte transformation in response to *C burnetii* antigen,[93] although these tests are not positive in all individuals previously infected with *C burnetii.*

These long-lasting indicators of cell-mediated immunity develop in most individuals after natural infection, but are also seen after immunization,[90,93] although to a lesser extent. Conversion from a negative lymphocyte proliferative response to a positive was observed in 11 (85%) of 13 of the individuals vaccinated.[93] In the same study, only 5 (38%) of 13 of vaccinated subjects seroconverted, and 31 (60%) of 52 developed a positive skin test following vaccination. Therefore, although the whole cell Q fever vaccine used in the Australian abattoirs confers protection, there does not appear to be a measurable response reliably associated with protective immunity.

C burnetii formalin-killed whole cell vaccines are generally well tolerated after subcutaneous injection, although some individuals develop severe local reactions at the site of injection. These reactions can involve formation of sterile abscesses that may drain spontaneously or may require surgical incision.[94] The incidence of severe, persistent local reactions is immunologically mediated by a delayed hypersensitivity response, resulting from previous natural infection with *C burnetii* or repeated immunization[92]; the risk of severe local reaction increases with the number of Q fever immunizations. This observation led to the development of an intradermal skin test using 0.02 µg of specific formalin-killed whole cell vaccine to detect presensitized or immune individuals.[95]

Severe local reactions to the vaccine were found to be associated with induration of 5 mm or larger at the skin test site by 7 days after inoculation. Although cumbersome, inconvenient, and costly, this prior screening procedure proved to be very effective in reducing the number of severe

local reactions to Q fever vaccine. Subsequent experience with this skin test at the Rocky Mountain Laboratory in Montana showed that there were no severe local reactions in 80 individuals whose skin tests were negative when they were immunized with one or two doses of vaccine. Prior to the availability of skin test screening, severe local reactions occurred in 42 (45%) of 94 vaccinated individuals.[95] Additionally, in Australian abattoirs, more than 4,000 individuals whose skin tests were negative received the formalin-killed vaccine during the course of vaccine efficacy studies, and of these, only 1 developed a significant chronic reaction.[90] The advisability of prior skin testing was further reinforced when severe local reactions were observed in 3 of 10 individuals with a positive skin test to *C burnetii* antigen who mistakenly received a single dose of vaccine.[96]

Although an effective Q fever vaccine is licensed in Australia, all Q fever vaccines used in the United States are currently investigational. Certain groups of individuals should be considered for vaccine prophylaxis, including the following:

- veterinarians, veterinary technicians, and animal care workers who may come into contact with *C burnetii*–infected animals, particularly pregnant animals;
- laboratory investigators, technicians, and other personnel who perform research on live *C burnetii* organisms; and
- abattoir workers who have contact with cattle, sheep, or goats (particularly pregnant animals) that may be infected with *C burnetii.*

Research efforts are currently underway to develop a Q fever vaccine that is safe to administer to anyone, including Q fever–immune individuals. The residue of *C burnetii* organisms following chloroform-methanol extraction (CMR vaccine) has been tested for safety in nonimmune volunteers[97] and is currently being tested for safety in Q fever–immune individuals. Antibiotic prophylaxis of Q fever has been tested with a tetracycline, as was discussed in the treatment section of this chapter.

SUMMARY

Q fever, a zoonotic disease caused by the rickettsia-like organism *Coxiella burnetii,* is important to military medicine primarily because of its exceptional infectivity. The disease is transmitted mainly by inhalation of infected aerosols, and a single organism may cause infection in humans. The disease is world-wide in distribution; the primary reservoir for human infection is livestock, particularly goats, sheep, and cattle. Contact with parturient animals or products of conception poses especially high risk, since the organism is present in very high numbers in this setting. The organism is also very resistant to pressure and desiccation, and may persist in a sporelike form in the environment for months after the source has left the area.

Diagnosis of Q fever is performed by serologic testing. Treatment with tetracyclines is effective. Prevention is possible with a formalin-killed, whole-cell vaccine, but prior skin testing to exclude immune individuals is necessary to avoid severe local reactions to the vaccine. A Q fever vaccine is licensed in Australia, but not in the United States, where all Q fever vaccines are investigational.

REFERENCES

1. Spicer AJ. Military significance of Q fever: A review. *J R Soc of Med.* 1978;71:762–767.

2. Robbins FC, Ragan CA. Q fever in the Mediterranean area: Report of its occurrence in Allied troops, I: Clinical features of the disease. *Am J Hyg.* 1946;44:6–22.

3. Robbins FC, Gauld RL, Warner FB. Q fever in the Mediterranean area: Report of its occurrence in Allied troops, II: Epidemiology. *Am J Hyg*. 1946;44:23–50.

4. The Commission of Acute Respiratory Diseases, Fort Bragg, North Carolina. Epidemics of Q fever among troops returning from Italy in the spring of 1945, III: Etiological studies. *Am J Hyg.* 1946;44:88–102.

5. Feinstein M, Yesner R, Marks JL. Epidemics of Q fever among troops returning from Italy in the spring of 1945, I: Clinical aspects of the epidemic at Camp Patrick Henry, Virginia. *Am J Hyg.* 1946;44:72–87.

6. Cheney G, Geib WA. The identification of Q fever in Panama. *Am J Hyg.* 1946;44:158–172.

7. Spicer AJ, Crowther RW, Vella EE, Bengtsson E, Miles R, Pitzolis G. Q fever and animal abortion in Cyprus. *Trans R Soc Trop Med Hyg.* 1977;71(1):16–20.

8. Rombo L, Grandien M. Serum Q fever antibodies in Swedish UN soldiers in Cyprus. *Scand J Inf Dis*. 1978;10:157–158.

9. Ferrante MA, Dolan MJ. Q fever meningoencephalitis in a soldier returning from the Persian Gulf War. *Clin Infect Dis.* 1993;16:489–496.

10. Lennox J. Assistant Professor of Medicine, Emory University School of Medicine, and Acting Medical Director, Infectious Disease Program, Grady Memorial Hospital, Atlanta, Ga. Personal communication, May 1993.

11. Waag D. US Army Medical Research Institute of Infectious Diseases, Pathogenesis and Immunology Branch, Bacteriology Division, Fort Detrick, Frederick, Md. Personal communication, September 1994.

12. Gray GC, Rodier GR, Matras-Maslin VC, et al. Serologic evidence of respiratory and rickettsial infections among Somali refugees. *Am J Trop Med Hyg*. 1995;52(4):349–353.

13. Magill A. Major, Medical Corps, US Army. Infectious Diseases Officer, Department of Immunology, Division of Communicable Diseases and Immunology, Walter Reed Institute of Research, Walter Reed Army Medical Center, Washington, DC. Personal communication, September 1994.

14. World Health Organization. *Health Aspects of Chemical and Biological Weapons: Report of a WHO Group of Consultants.* Geneva, Switzerland: WHO; 1970.

15. Department of the Army. *US Army Activity in the US Biological Warfare Program.* Vol 2. Washington, DC: HQ, DA; 24 Feb 1977: D 1–2; Appendix 4, pp E4-1, 2. Unclassified.

16. Department of the Army. *US Army Activity in the US Biological Warfare Program.* Vol 2. Washington, DC: HQ, DA; 24 Feb 1977: L 1–6. Unclassified.

17. Derrick EH. “Q” fever, a new fever entity: Clinical features, diagnosis and laboratory investigation. *Med J Aust.* 1937;2:281–299.

18. Burnet FM, Freeman M. Experimental studies on the virus of “Q” fever. *Med J Aust.* 1937;2:299–305.

19. Noguchi H. A filter-passing infectious agent obtained from *Dermacentor andersoni*. *J Exp Med*. 1926;44:1–10.

20. Davis GE, Cox HR. A filter-passing infectious agent isolated from ticks, I: Isolation from *Dermacentor andersoni,* reactions in animals, and filtration experiments. *Public Health Rep.* 1938;53:2259–2267.

21. Cox HR. Studies of a filter-passing infectious agent isolated from ticks, V: Further attempts to cultivate in cell-free media. Suggested classification. *Public Health Rep*. 1939;54:2171–2178.

22. Dyer RE. A filter-passing infectious agent isolated from ticks, IV: Human

infection. *Public Health Rep.* 1938;53:2277–2283.

23. Philip CB. Comments on the name of the Q fever organism. *Public Health Rep.* 1948;63:58.

24. Kaplan MM, Bertagna P. The geographical distribution of Q fever. *Bull WHO.* 1955;13:829–860.

25. Weisburg WG, Dobson ME, Samuel JE, et al. Phylogenetic diversity of the rickettsias. *J Bacteriol.* 1989;171:4202– 4206.

26. Tzianabos T, Moss CW, McDade JE. Fatty acid composition of rickettsiae. *J Clin Microbiol*. 1981;13:603–605.

27. Williams JC. Infectivity, virulence, and pathogenicity of *Coxiella burnetii* for various hosts. In: Williams JC, Thompson HA, eds. *Q Fever: The Biology of* Coxiella burnetii. Boca Raton, Fla: CRC Press; 1991: Chap 2, p 25, Table 2.

28. McCaul TF, Dare AJ, Gannon JP, Galbraith AJ. *In vivo* endogenous spore formation by *Coxiella burnetii* in Q fever endocarditis. *J Clin Pathol*. 1994;47:978–981.

29. McCaul TF, Williams JC. Developmental cycle of *Coxiella burnetii*: Structure and morphogenesis of vegetative and sporogenic differentiations. *J Bacteriol*. 1981;147:1063–1076.

30. Hackstadt T, Williams JC. Biochemical strategem for obligate parasitism of eukaryotic cells by *Coxiella burnetii.* Proc Natl Acad Sci U S A. 1981;78:3240–3244.

31. Stoker MGP, Fiset P. Phase variation of the Nine Mile and other strains of *Rickettsia burnetii. Can J Microbiol*. 1956;2:310–321.

32. Baca OG, Paretsky D. Q fever and *Coxiella burnetii*: A model for host parasite interactions. *Microbiol Rev.* 1983;47:127–149.

33. Samuel JE, Frazier ME, Mallavia LP. Correlation of plasmid type and disease caused by *Coxiella burnetii. Infect Immun.* 1985;49:775–777.

34. Waag DM, Williams JC. Immune modulation by Coxiella burnetii: Characterization of a Phase I immunosuppressive complex expressed among strains. *Immunopharmacol Immunotoxicol.* 1988;10:231–260.

35. Tigertt WD, Benenson AS. Studies on Q fever in man. *Trans Assoc Am Phys*. 1956;69:98–104.

36. Oliphant JW, Gordon DA, Meis A, et al. Q fever in laundry workers presumably transmitted from contaminated clothing. *Am J Hyg.* 1949;49:76–82.

37. Marrie TJ, Langille D, Papukna V, Yates L. Truckin' pneumonia—An outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with a newborn kitten. *Epidem Inf.* 1989;102: 119–127.

38. Salmon MM, Howells B, Glencross EJG, et al. Q fever in an urban area. *Lancet.* 1982;1:1002–1004.

39. Mann JS, Douglas JG, Inglis JM, et al. Q fever: Person to person transmission within a family. *Thorax*. 1986;41: 974–975.

40. Wisniewski HJ, Piraino FF. Review of virus infections in the Milwaukee area, 1955–1965. *Public Health Reports.* 1969;84:175–181.

41. Epidemiology of a Q fever outbreak in Los Angeles County, 1966. *Health Services and Mental Health Administration Health Reports.* 1972;87:71–74.

42. Pinsky RL, Fishbein DB, Greene CR, Gensheimer KF. An outbreak of cat-associated Q fever in the United States. *J Inf Dis.* 1991;164(July):202–204.

43. Langley JM, Marrie TJ, Covert A, et al. Poker players pneumonia: An urban outbreak of Q fever following exposure to a parturient cat. *N Engl J Med.* 1988;319:354–356.

44. Centers for Disease Control. Q fever among slaughterhouse workers—California. *MMWR*. 1986;35:223–226.

45. Meiklejohn G, Reimer LG, Graves PS, Helmick C. Cryptic epidemic of Q fever in a medical school. *J Inf Dis.* 1981;144(2):107–113.

46. Rauch AM, Tanner M, Pacer RE, Barrett MJ, Brokopp CD, Schonberger LB. Sheep-associated outbreak of Q fever, Idaho. Arch *Intern Med.* 1987;147:341–344.

47. Graham CJ, Yamauchi T, Rountree P. Q fever in animal laboratory workers: An outbreak and its investigation. *Am J Inf Con*. 1989;17:345–348.

48. Hamedeh GN, Turner BW, Trible W, et al. Laboratory outbreak of Q fever. *J Fam Pract*. 1992;35:683–685.

49. D'Angelo LJ, Baker EF, Schlosser W. Q fever in the United States, 1948–1977. *J Infect Dis*. 1979;139:613–615.

50. Sawyer LA, Fishbein DB, McDade JE. Q fever in patients with hepatitis and pneumonia: Results of a laboratory-based surveillance in the United States. *J Infect Dis.* 1988;158:497–498.

51. Sienko DG, Bartlett PC, McGee HB, Wentworth BB, Herndon JL, Hall WN. Q fever: A call to heighten our index of suspicion. *Arch Intern Med.* 1988;148:609–612.

52. Sidwell RW, Gebhardt LP. Studies of latent Q fever infection, III: Effects of parturition upon latently infected guinea pigs and white mice. *Am J Epidemiol.* 1967;84:132–137.

53. Sidwell RW, Thorpe BD, Gebhardt LP. Studies of latent Q fever infections, II: Effects of multiple cortisone injections. *Am J Hyg.* 1964;79:320–327.

54. Sidwell RW, Thorpe BD, Gebhardt LP. Studies of latent Q fever infections, I: Effects of whole body X-irradiation upon latently infected guinea pigs, white mice, and deer mice. *Am J Hyg.* 1964;79:113–124.

55. Humphres RC, Hinrichs DJ. Role of antibody in *Coxiella burnetii* infection. *Infect Immun.* 1981;31:641–645.

56. Kazar J, Rajcani J, Schramek S. Differential effects of cyclophosphamide on *Coxiella burnetii* infection in mice. *Acta Virol.* 1982;26:174–182.

57. Koster FT, Williams JC, Goodwin JS. Cellular immunity in Q fever: Modulation of responsiveness by a suppressor T-cell monocyte circuit. *J Immunol.* 1985;135:1067–1072.

58. Hackstadt T, Peacock MG, Hitchcock PJ, Cole RL. Lipopolysaccharide variations in *Coxiella burnetii:* Intrastrain heterogenicity in structure and antigenicity. *Infect Immun.* 1985;48:359–365.

59. Vishwanath S, Hackstadt T. Lipopolysaccharide phase variation determines the complement-mediated serum susceptibility of *Coxiella burnetii. Infect Immun*. 1988;56:40–44.

60. Dupuis G, Petite J, Olivier P, Vouilloz. *Int J Epidemiol.* 1987;16:282–287.

61. Fergusson RJ, Shaw TRD, Kitchin AH, et al. Subclinical chronic Q fever. *Q J Med.* 1985;57:669–676.

62. Marrie TJ. Q fever in pregnancy: Report of two cases. *Inf Dis Clin Pract.* 1993;2:207–209.

63. Syrucek L, Sobeslavsky O, Gutvirth I. Isolation *of Coxiella burnetii* from human placentas. *J Hyg Epidemiol Microbiol Immunobiol*. 1958;2:29–35.

64. Friedland JS, Jeffrey I, Griffin GE, Booker M, Courtney-Evans R. Q fever and intrauterine death. *Lancet*. 1994;343:288.

65. Smith DL, Ayres JG, Blair I, et al. A large Q fever outbreak in the West Midlands: Clinical aspects. *Respir Med.* 1993;87:509–516.

66. Clark WH, Lennette EH, Railsback OC, Romer MS. Q fever in California. *Arch Intern Med.* 1951;88:155–161.

67. Dupont HT, Raoult D, Brouqui P, et al. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *Am J Med.* 1992;93:427–434.

68. Derrick EH. The course of infection with *Coxiella burnetii. Med J Aust.* 1973;1:1051–1057.

69. Hwang YM, Lee MC, Suh DC, Lee WY. *Coxiella* (Q fever)-associated myelopathy. *Neurology*. 1993;43:338–342.

70. Sempere AP, Elizaga J, Duarte J, et al. Q fever mimicking herpetic encephalitis. *Neurology*. 1993;43:2713–2714.

71. Smith DL, Wellings R, Walker C, et al. The chest x-ray report in Q fever: A report on 69 cases from the 1989 West Midlands outbreak. *Br J Radiol*. 1991;64:1101–1108.

72. Tselentis Y, Gikas A, Kofteridis D, et al. Q fever in the Greek island of Crete: Epidemiologic, clinical, and therapeutic data from 98 cases. *Clin Inf Dis*. 1995;20:1311–1316.

73. Brouqui P, Dupont HT, Drancourt M, et al. Chronic Q fever: Ninety-two cases from France, including 27 cases without endocarditis. *Arch Intern Med.* 1993;153:642–648.

74. Janigan DT, Marrie TJ. An inflammatory pseudotumor of the lung in Q fever pneumonia. *N Engl J Med.* 1983;308:86–88.

75. Lipton JH, Fong TC, Gill MJ, et al. Q fever inflammatory pseudotumor of the lung. *Chest.* 1987;92:756–757.

76. Peacock MG, Philip RN, Williams JC, et al. Serological evaluation of Q fever in humans: Enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. *Infect Immun.* 1983;41:1089–1098.

77. Peter O, Dupuis D, Bee R, et al. Comparison of enzyme-linked immunosorbent assay for diagnosis of Q fever. *J Clin Microbiol*. 1987;25:1063–1067.

78. Uhaa IJ, Fishbein DB, Olson JG, et al. Evaluation of specificity of indirect enzyme-linked immunosorbent assay for diagnosis of human Q fever. *J Clin Microbiol.* 1994;32:1560–1565.

79. Waag D, Chulay J, Marrie T, England M, Williams J. Validation of an enzyme immunoassay for serodiagnosis of acute Q fever. *Eur J Clin Microbiol Infect Dis.* 1995;14(5):421–427.

80. Hoover TA, Vodkin MH, Williams JC. A *Coxiella burnetii* repeated DNA element resembling a bacterial insertion sequence. *J Bacteriol*. 1992;174:5540–5548.

81. Stein A, Raoult D. Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. *J Clin Microbiol*. 1992;30:2462–2466.

82. Willems H, Thiele D, Krauss H. Plasmid based differentiation and detection of *Coxiella burnetii* in clinical samples. *Eur J Epidemiol.* 1993;9:411–418.

83. Fritz E, Thiele D, Willems H, Wittenbrink M-M. Quantitation of *Coxiella burnetii* by polymerase chain reaction and a colorimetric microtiter plate hybridization assay. *Eur J Epidemiol.* 1995;11:549–557.

84. Raoult D. Treatment of Q fever. *Antimicrob Agents Chemother*. 1993;37:1733–1736.

85. Sobradillo V, Zalacain R, Capelastegui A, Uresandi F, Corral J. Antibiotic

treatment in pneumonia due to Q fever. *Thorax*. 1992;47:276–278.

86. Schonwald S, Skerk V, Petricevic I, Car V, Majerus-Misic L, Gunjaca M. Comparison of three-day and five-day courses of azithromycin in the treatment of atypical pneumonia. *Eur J Clin Microbiol Infect Dis*. 1991;10(10):877–880.

87. Maurin M, Benoliel AM, Bongard P, Raoult D. Phagolysosomal alkalinization and the bactericidal effect of antibiotics: The *Coxiella burnetii* paradigm. *J Infect Dis.* 1992;166:1097–1102.

88. Raoult D, Marrie TJ. State-of-the-art clinical lecture: Q fever. *Clin Inf Dis.*1995;20:489–496.

89. Marmion BP. Development of Q fever vaccines, 1937–1967. *Med J Aust.* 1967;2:1074–1078.

90. Marmion BP, Ormsbee RA, Kyrkou M, et al. Vaccine prophylaxis of abattoir-associated Q fever: Eight years' experience in Australian abattoirs. *Epidemiol Infect*. 1990:104:275–287.

91. Shapiro RA, Siskind V, Schofield FD, Stallman N, Worswick DA, Marmion BP. A randomized, controlled, double-blind, cross-over, clinical trial of Q fever vaccine in selected Queensland abattoirs. *Epidemiol Infect.* 1990;104: 267–273.

92. Benenson AS. Q fever vaccine: Efficacy and present status. In: Smadel JE, ed. *Symposium on Q fever by the Committee on Rickettsial Diseases*. Washington, DC: Armed Forces Epidemiology Board; 1959: 47–60.

93. Izzo AA, Marmion BP, Worswick DA. Markers of cell-mediated immunity after vaccination with an inactivated, whole-cell Q fever vaccine. *J Infect Dis.* 1988;157:781–789.

94. Bell JF, Lackman DB, Meis A, Hadlow WJ. Recurrent reaction at site of Q fever vaccination in a sensitized person. *Milit Med.* 1964;124:591–595.

95. Lackman DB, Bell EJ, Bell JF, Pickens EG. Intradermal sensitivity testing in man with a purified vaccine for Q fever. *Am J Publ Health*. 1962;52:87–93.

96. Luoto L, Bell JF, Casey M, Lackman D. Q fever vaccination of human volunteers, I: The serologic and skin-test response following subcutaneous injections. *Am J Hyg*. 1963;78:1–15.

97. Fries LF, Waag DM, Williams JC. Safety and immunogenicity in human volunteers of a chloroform-methanol residue vaccine for Q fever. *Infect Immun*. 1993;61:1251–1258.

RECOMMENDED READING

Interested readers will find thorough reviews of both organism and disease in the following outstanding monographs:

Marrie TJ, ed. *Q Fever: The Disease.* Vol 1. Boca Raton, Fla: CRC Press; 1990.

Williams TC, Thompson HA, eds. *Q Fever: The Biology of* Coxiella burnetii. Boca Raton, Fla: CRC Press; 1991.

Chapter 27

SMALLPOX

DAVID J. MCCLAIN, M.D.*

INTRODUCTION

SMALLPOX AND BIOLOGICAL WARFARE

STRUCTURE AND BIOLOGY OF POXVIRUSES

PATHOGENESIS AND CLINICAL MANIFESTATIONS

DIAGNOSIS

MEDICAL MANAGEMENT
- Active Immunoprophylaxis
- Passive Immunoprophylaxis
- Chemoprophylaxis and Chemotherapy

SUMMARY

* *Research Medical Officer, Division of Virology, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

INTRODUCTION

The poxviruses (of the family *Poxviridae*) are a family of large, enveloped deoxyribonucleic acid (DNA) viruses.[1] The most notorious poxvirus is variola, the causative agent of smallpox. Smallpox was an important cause of morbidity and mortality in the developing world until recent times. Since the host range of the variola virus is confined to humans, aggressive case identification and contact vaccination were ultimately successful in controlling the disease. The last occurrence of endemic smallpox was in Somalia in 1977, and the last human cases were laboratory-acquired infections in 1978.[2] By 1980, the World Health Organization (WHO) General Assembly ratified the declaration of success made by the Global Commission for the Certification of Smallpox Eradication.

SMALLPOX AND BIOLOGICAL WARFARE

The concept of using variola virus in warfare is an old one. British colonial commanders considered distributing blankets from smallpox victims among Native Americans as a biological weapon.[3–5] During the American Civil War, allegations were made about the use of smallpox as a biological weapon, although there subsequently proved to be no definite evidence for such.[6,7] In the years leading up to and during World War II, the Japanese military explored

weaponization of smallpox during the operations of Unit 731 in Mongolia and China.[8,9]

Nevertheless, the actual potential of variola virus as a biological weapon remains controversial. Given the ease of administration and the availability of the vaccinia virus as a vaccine against smallpox,[10] some have argued that smallpox would have limited biological warfare potential.[11] The Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, presently maintains over 12 million doses of vaccinia vaccine in storage, and WHO has in storage enough vaccine to protect 200 to 300 million people.[12] On the other hand, the potency of stock-piled vaccine will gradually decline. The discontinuation of routine vaccination has rendered civilian and military populations more susceptible to a disease that is not only infectious by aerosol but also infamous for its devastating morbidity and mortality. WHO voiced concerns that smallpox "can easily be produced in large quantities in the laboratory and...freeze-dried and its virulence thus preserved for months or years."[13(p69)]

Since 1983, there have existed two WHO-approved and -inspected repositories of variola virus: the CDC in the United States and Vector Laboratories in Russia. WHO continues to debate whether, given the completion of sequencing of several reference strains, all stocks of variola virus should be destroyed. Proponents of retaining these smallpox stocks argue that military or terrorist use of variola virus as a weapon would readily be countered by rigorous case contact evaluation and vaccination. Furthermore, they reason that even if the repositories are eliminated, other potential sources of smallpox exist:

- Given the fact that viable variola virus could be recovered from scabs up to 13 years after collection,[14] it is conceivable that cadavers preserved in permafrost[15] or dry crypts[16] could release the virus.
- Virus specimens from the smallpox-eradication campaign may remain unrecognized or unreported.
- Using the published sequence of variola and its significant homology with other *Orthopoxviruses*, a malevolent laboratory could theoretically engineer a recombinant virus exhibiting variola virus's virulence by starting with monkeypox virus.

Additionally, in the event that smallpox should re-emerge under one of the above scenarios, destroying the legitimate repositories of variola virus would hinder investigation into both *(a)* the mechanisms of viral pathogenesis, so as to effect countermeasures, and *(b)* detailed molecular epidemiology, so as to establish the precise phylogenetic relationship of an isolate to other known strains. Lastly, extinction of smallpox would disallow study of its unique proteins that interfere with host immune and regulatory functions (eg, homologues of epidermal growth factor, transforming growth factor, interleukin-1β, interferon-γ, and zinc-finger protein).[17,18]

Those who advocate eradication of the official variola virus repositories cite the possibility of accidental or intentional release of the virus as a threat to international public health; political instability in Russia has reemphasized this concern. In addition, retention of these repositories might legitimize clandestine stockpiling or offensive biological warfare research on variola virus. Even if a virulent variola-like poxvirus could be engineered by an offensive biological warfare power, the lack of an animal model that accurately reflects human pathology[19] would severely limit how it could be selected and tested. From the standpoint of understanding *Orthopoxvirus* biology, published sequence information on variola would be adequate to confirm the identity of any smallpoxlike virus that might emerge in the future. Finally, further studies on poxvirus pathogenesis would be much more safely and successfully pursued using a poxvirus that does possess a good animal model, such as ectromelia (ie, mouse pox).[20] Despite the promise of variola virus's extinction as a biological entity, the prospect of surreptitious weaponization of smallpox remains vexing, and vaccination of military personnel could be seen as a defensive posture implying willingness to use variola virus as a weapon.[21]

STRUCTURE AND BIOLOGY OF POXVIRUSES

Possessing one of the largest genomes of any virus, an *Orthopoxvirus* consists of one piece of double-stranded DNA, which is cross-linked at each end.[22] With their brick-shaped morphology, poxviruses have a biconcave core containing the DNA genome. The virus-encoded enzymes in the core are critical to transcription of the viral DNA. Genes encoding the nonessential functions important for virus virulence are arrayed near the ends of the genome; as would be expected, the greatest heterogeneity between poxviruses is at these genomic ends.[23,24] There are 187 putative proteins identified from the sequencing of variola virus, of which 150 bear marked similarity to those of vaccinia virus. Encoded sequences include one for hemagglutinin, an envelope protein, and proteins that enhance growth in human cells. The other 37 proteins represent either variola-specific sequences, or *open reading frame* (ie, DNA sequences that are transcribed into ribonucleic acid [RNA], and hence are translated, via reading of the genetic code, into amino acid sequences) divergences from vaccinia counterparts.[25] These relatively small differences in vaccinia and variola virus proteins suggest that the variola-unique proteins act synergistically in bringing about the local and systemic manifestations for which smallpox is noted.

Most poxvirus virions appear to enter cells by pinocytosis, and then to uncoat within cytoplasmic vesicles. Poxvirus replication occurs in these discrete cytoplasmic inclusions, independent of the cell nucleus. Host biosynthetic processes are inhibited soon after poxvirus infection occurs. Virus transcription initiates almost immediately after entry, as DNA transcription is initiated by core enzymes while the genome is still in the core. Early gene products have to do with DNA synthesis, ensuring adequate levels of precursors for DNA synthesis and inhibition of host defense mechanisms.[26] This is followed by release of DNA and subsequent synthesis of RNA and proteins.[27] It has been postulated[28] that viral DNA is transcribed and replicated from viral cores, or “deoxyribonucleoproteids.” Although the virus encodes a DNA-dependent RNA polymerase, there is evidence[29] that subunits of cellular RNA polymerase II from the nucleus are somehow used in replication. Recombination events occur with high frequency during the replication process[30]; these may also occur among different species of *Orthopoxviruses*.[31]

Viral DNA synthesis and intermediate regulatory genes are required for late gene expression.[32] Proteins that are translated late include most of the structural proteins, as well as enzymes required for assembly of virion progeny. Maturation of virions is a complicated process entailing sequential assembly of poxvirus-specified macromolecules into particles. Unlike membranes of other enveloped viruses that are contiguous with host membranes, poxviruses assemble in the viroplasm into uniform, spherical, immature particles. This immature particle subsequently undergoes extensive conformational and biochemical changes before release, although most virions remain within the cell at the end of the growth cycle.[33] On release from the cell, the virion’s outer (second) membrane fuses with the cell membrane; thus the released virion presents viral antigens not displayed in intracellular forms.[34] The sequence of events leading to release and dissemination of virions varies widely among different poxviruses.[35]

Compared with other genera of vertebrate poxviruses (Table 27-1), members of the *Orthopoxvirus* genus *(a)* possess similar morphology and host range and *(b)* are antigenically related. Cross-reacting and species-specific neutralizing antigens have been identified by serum absorption and monoclonal antibody studies.[36,37] Up to six antigens have been identified as neutralizing epitopes of Orthopoxviruses, one of these existing on naturally released but not on

TABLE 27-1 CLASSIFICATION OF *CHORDOPOXVIRIDAE* (VERTEBRATE POXVIRUSES)

Genera	Representative Species
Orthopoxvirus	Variola, vaccinia, monkeypox, cowpox, rabbitpox, raccoon pox, tatera pox, buffalopox, camelpox
Avipoxvirus	Fowlpox, canary pox

artificially released virions.[38]

Undoubtedly, both cellular and humoral immune responses are important to recovery from smallpox. The inability of poxviruses to persist stably within the host cell accounts for their infections being relatively short-lived, without establishment of a latent infection. The importance of cellular immunity in recovery from infection has been demonstrated with other poxviruses,[39] and the same is generally assumed with variola. Vaccination experiences demonstrated the rare but terrible consequence of vaccinia necrosum in persons with defects of cellular immunity. Early presentation on the host cell membrane of virus-encoded proteins provides means for immune recognition.[40] It has been demonstrated that both antibody-dependent cellular cytotoxicity[41] and heterogeneous cluster of differentiation (CD) 4+ cytotoxic T-lymphocyte clones[42] are induced in response to vaccinia infection, and some immunodominant B-cell epitopes have been defined in both mice and vaccinated humans.[43] The relatively large size of poxvirus polypeptides facilitates their recognition and phagocytosis by the reticuloendothelial system. Viral antigens contained in the viral envelope are of preeminent importance with regard to protective antibody responses: envelope antigens were absent from virion suspensions used for inactivated smallpox vaccines, which proved to be ineffective.[44–47]

Capripoxvirus	Goatpox, sheeppox, lumpy skin disease
Leporipoxvirus	Myxoma, hare fibroma
Parapoxvirus	Orf (milker's nodule), pseudo-cowpox
Suipoxvirus	Swinepox
Molluscipoxvirus	Molluscum contagiosum
Yatapoxvirus	Tanapox, Yaba

A naturally occurring relative of variola, monkey-pox virus, is found in Africa, and the disease it causes, monkeypox, is clinically indistinguishable from smallpox, with the exception of notable enlargement of cervical and inguinal lymph nodes. The disease occurs mostly in monkeys from the tropical rain forests of central Africa, with sporadic transmission to humans. Some evidence supports the role of squirrels as the principal animal reservoir of the virus.[48] Over a span of 3 years, 331 cases of monkeypox disease in a population of 5 million have been reported.[49] Under natural conditions, the virus is transmitted by direct contact with an infected individual, fomites, and, occasionally by aerosol.

Concern has been raised whether the monkeypox virus could be weaponized and, if so, whether it would constitute a threat similar to that posed by variola virus. However, epidemiological evidence indicates that monkeypox virus has limited potential for person-to-person transmission, accounting for about 30% of the observed cases.[50] There is one report of its spread though four human generations.[51] A stochastic model for interhuman spread of monkeypox indicates that it is very unlikely that the virus could sustain itself indefinitely in a community by interhuman transmission.[52] The finite transmission potential of monkeypox prompted WHO to maintain active surveillance rather than a vaccination program in the endemic areas. Successful vaccinia virus immunization, as judged by the presence of a preexisting vaccination scar, affords approximately 85% protection against monkeypox.[53] Nevertheless, *(a)* the pathogenicity of monkeypox for humans, *(b)* the potential morbidity of an aerosolized monkeypox virus attack, and *(c)* the theoretical potential that genetic recombination could produce a modified animal poxvirus with enhanced virulence for humans have raised the specter that another poxvirus besides variola might constitute either a serious biowarfare threat or a reemergent public health problem.

PATHOGENESIS AND CLINICAL MANIFESTATIONS

Variola virus is highly stable and retains its infectivity for long periods outside the host.[54] It is infectious by aerosol,[55] but natural airborne spread to other than close contacts is controversial.[56,57] Approximately 30% of susceptible contacts became infected during the era of endemic smallpox,[58] and the WHO eradication campaign was predicated on close person-to-person proximity being required for transmission to occur reliably. Nevertheless, variola virus's potential in low relative humidity for airborne dissemination was alarming in two hospital outbreaks.[59] Patients with smallpox were infectious from the time of onset of their eruptive exanthem, most commonly from days 3 through 6 after onset of fever. Infectivity was markedly enhanced if the patient manifested a cough. Indirect transmission via contaminated bedding or other fomites was infrequent.[60] Some close contacts harbored virus in their throats without developing disease, and hence might have served as a means of secondary transmission.[61] There is no animal reservoir for variola virus, although monkeys are susceptible to infection.[62]

On natural exposure to aerosolized virus, variola travels from the upper or the lower respiratory tract to regional lymph nodes, where it replicates and gives rise to viremia, which is followed soon thereafter by a rash.[63] The incubation period of smallpox averages 12 days, and contacts are quarantined for a minimum of 16 to 17 days following exposure. Following infection via the respiratory route and replication in local lymph nodes, variola virus disseminates systemically to other lymphoid tissues, spleen, liver, bone marrow, and lung. During this prodromal period, variola virus can be recovered from the blood, but the yield is much lower later in the illness. Clinical manifestations begin acutely with malaise, fever, rigors, vomiting, headache, and backache; 15% of patients develop delirium. Approximately 10% of light-skinned patients exhibit an erythematous rash during this phase.[64] Two to 3 days later, an enanthem appears concomitantly with a discrete rash about the face, hands, and forearms. Owing to the lack of a keratin layer on mucous membranes, lesions there shed infected epithelial cells and give rise to infectious oropharyngeal secretions in the first few days of the eruptive illness.[65] These respiratory secretions are the most important but not the sole means of virus transmission to contacts.

a. Third Day of Rash

b. Fifth Day of Rash

c. Seventh Day of Rash

Fig. 27-1. This series of photographs illustrates the evolution of skin lesions in an unvaccinated infant with the classic form of variola major. **(a)** The third day of rash shows synchronous eruption of skin lesions; some are becoming vesiculated. **(b)** On the fifth day of rash, almost all papules are vesicular or pustular. **(c)** On the seventh day of rash, many lesions are umbilicated, and all lesions are in the same general stage of development. Photographs: Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 10–14. Photographs by I. Arita.

Following subsequent eruptions on the lower extremities, the rash spreads centrally during the next week to the trunk. Lesions quickly progress from macules to papules and eventually to pustular vesicles (Figure 27-1). Lesions are more abundant on the extremities and face, and this centrifugal distribution is an important diagnostic feature. In distinct contrast to the lesions seen in varicella, smallpox lesions on various segments of the body remain generally synchronous in their stage of development. From 8 to 14 days after onset, the pustules form scabs, which leave depressed depigmented scars on healing. Although variola titers in the throat, conjunctiva, and urine diminish with time,[66] virus can readily be recovered from scabs throughout convalescence.[67] Therefore, patients should be isolated and considered infectious until all scabs separate.

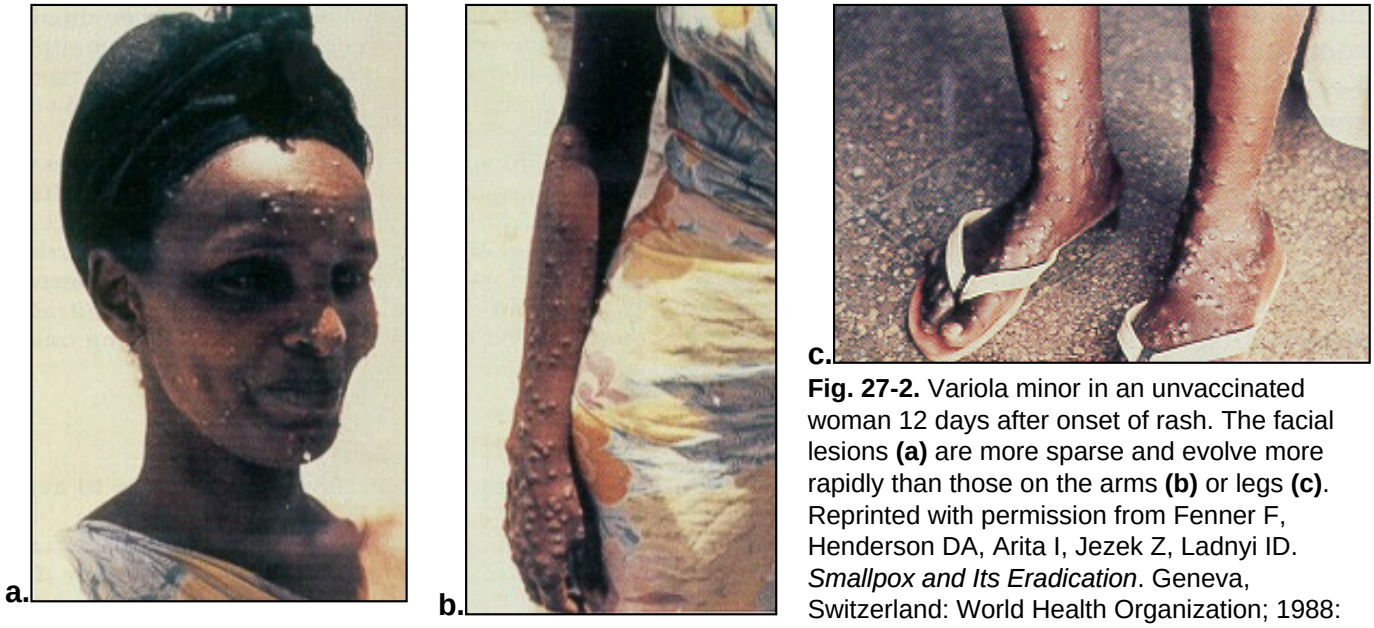


Fig. 27-2. Variola minor in an unvaccinated woman 12 days after onset of rash. The facial lesions **(a)** are more sparse and evolve more rapidly than those on the arms **(b)** or legs **(c)**. Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 36. Photographs by I. Arita.

For the past century, two distinct types of small-pox have been recognized. Variola major, the prototypical disease, was prevalent in Asia and parts of Africa. Variola minor, or alastrim, was distinguished by milder systemic toxicity and more diminutive pox lesions (Figure 27-2). Variola minor was found in Africa, South America, and Europe before the eradication of endemic disease, and caused 1% mortality in unvaccinated victims.

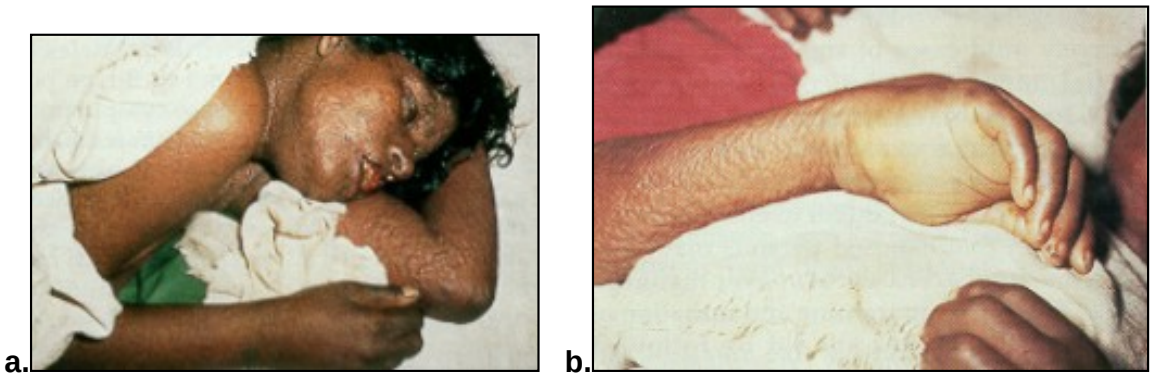


Fig. 27-3. Flat-type smallpox in an unvaccinated woman on the sixth day of rash. Extensive flat lesions (a and b) and systemic toxicity with fatal outcome were typical. Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 33. Photographs by F. Dekking.

Three quarters of endemic cases of variola major fell into the classic, or ordinary, variety (see Figure 27-1). The fatality rate was 3% in vaccinated patients and 30% in unvaccinated. Other clinical forms of smallpox were associated with variola major, and it is likely that differences in both strain virulence and host response were responsible for these variations in clinical manifestations.[68] Flat-type smallpox, noted in 2% to 5% of patients, was typified by *(a)* severe systemic toxicity and *(b)* the slow evolution of flat, soft, focal skin lesions (Figure 27-3).

This syndrome caused 66% mortality in vaccinated patients and 95% mortality in unvaccinated. Hemorrhagic-type smallpox, seen in fewer than 3% of patients, was heralded by the appearance of extensive petechiae (Figure 27-4), mucosal hemorrhage, and intense toxemia; death usually intervened before the development of typical pox lesions.[69]

Bacterial superinfection of pox lesions was relatively uncommon except in the absence of proper hygiene and medical care. Arthritis and osteomyelitis developed late in the course of disease in about 1% to 2% of patients, more frequently occurred in children, and was often manifested as bilateral joint

involvement, particularly of the elbows.[70] Viral inclusion bodies could be demonstrated in the joint effusion and bone marrow of the involved extremity. This complication reflected infection and inflammation of a joint followed by spread to contiguous bone metaphyses, and sometimes resulted in permanent joint deformity.[71] Cough and bronchitis were occasionally reported as prominent manifestations of smallpox, with attendant implications for spread of contagion; however, pneumonia was unusual.[72] Pulmonary edema occurred frequently in hemorrhagic- and flat-type smallpox. Orchitis was noted in approximately 0.1% of patients. Encephalitis developed in 1 in 500 cases of variola major, compared with 1 in 2,000 cases of variola minor.[73] Keratitis and corneal ulcers were important complications of smallpox, progressing to blindness in slightly fewer than 1% of cases.[74] Disease during pregnancy precipitated high perinatal mortality, and congenital infection was also recognized.[75,76]

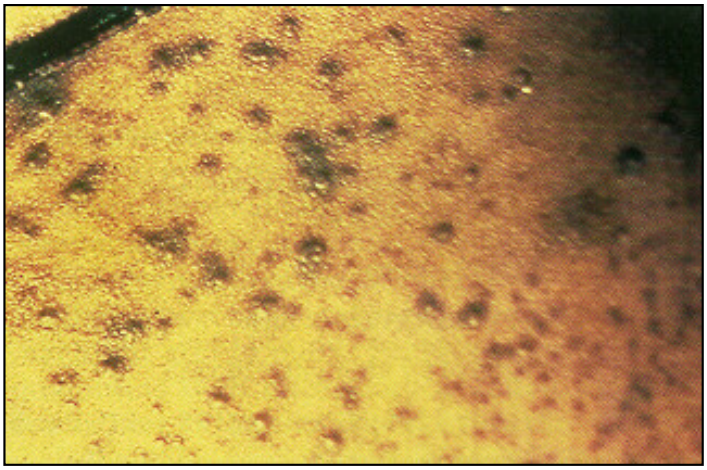


Fig. 27-4. Early hemorrhagic-type smallpox with cutaneous signs of hemorrhagic diathesis. Death usually intervened before the complete evolution of pox lesions. Reprinted with permission from Herrlich A, Mayr A, Munz E, Rodenwaldt E. *Die pocken; Erreger, Epidemiologie und klinisches Bild*. 2nd ed. Stuttgart, Germany: Thieme; 1967. In: Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: 35.

Partial immunity from vaccination resulted in modified-type smallpox, in which sparse skin lesions evolved variably, often without pustules, and quickly, with crusting occurring as early as the seventh day of illness. Some fully immune individuals would develop fever, sore throat, and conjunctivitis (called contact fever), on exposure to smallpox. This lasted several days, but did not give rise to the toxicity or minor skin lesions that signify variola sine eruptione.

Persons who recovered from smallpox possessed long-lasting immunity, although a second attack could occur in 1 in 1,000 persons after an intervening period of 15 to 20 years.[77] As discussed earlier, both humoral and cellular responses are important components of recovery from infection. Neutralizing antibodies peak 2 to 3 weeks following onset, and last longer than 5 years.[78]

DIAGNOSIS

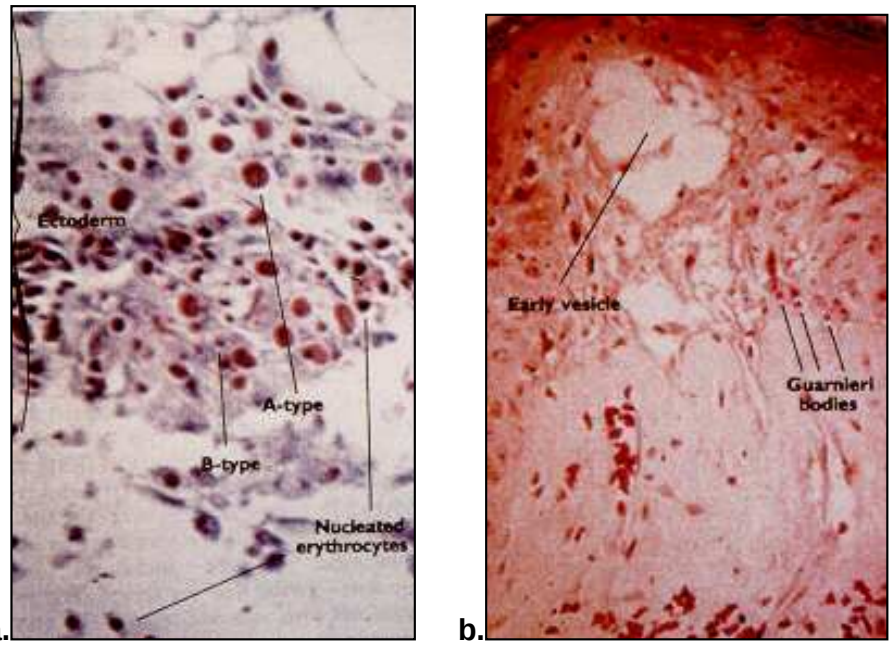
Given modern clinicians' lack of experience with smallpox, greater perspicacity is required to distinguish the forme fruste of this disease from other vesicular exanthems, such as those of chickenpox, erythema multiforme with bullae, or allergic contact dermatitis. Table 27-2 delineates some of the key features that differentiate variola from varicella (chickenpox virus). The failure to recognize relatively mild cases of smallpox in persons with partial immunity, who could unwittingly contribute to secondary spread by ambulating with their contagious exanthems undiagnosed, would present a particular problem to infection control. An additional threat to effective quarantine is the fact that exposed persons may shed virus from the oropharynx without ever manifesting disease. Therefore, quarantine and initiation of medical countermeasures should be followed promptly by an accurate diagnosis so as to avert panic.

TABLE 27-2
DIFFERENTIATION BETWEEN SMALLPOX AND CHICKENPOX

	Variola	Varicella
Incubation Period	7–17 d	14–21 d
Prodrome	Fever and malaise for 2–4 d before onset of rash	Minimal to none
Pock Distribution	Centrifugal; usually on palms and soles	Centripetal; seldom on palms and soles
Pock Appearance	Vesicular—> pustular—> umbilicated—>scab	Vesicular on erythematous base—>pustular—>scab
Evolution of Pocks	Synchronous	Asynchronous
Scab Formation	10–14 d after onset of rash	4–7 d after onset of rash
Scab Separation	14–28 d after onset of rash	Within 14 d after onset of rash
Infectivity	From onset of enanthem until all scabs separate	From 1 d before rash until all vesicles scab

Rapid diagnostic tests may play an important role in discriminating smallpox from other diseases.[79,80] The usual method of diagnosis is demonstration of characteristic virions on electron microscopy of vesicular scrapings. Under light microscopy, aggregations of variola virus particles, called Guarnieri bodies, correspond to B-type poxvirus inclusions (Figure 27-5). These cytoplasmic inclusions are hematoxylinophilic, stain reddish purple with Giemsa stain, and contain Feulgen-positive material.[81] Another rapid but relatively insensitive test for Guarnieri bodies in vesicular scrapings is Gispen's modified silver stain, in which cytoplasmic inclusions appear black. The gel diffusion test, in which vesicular fluid from a pox lesion was incubated with vaccinia hyperimmune serum, constitutes a rapid and inexpensive method when microscopy is not available.[82]

Fig. 27-5. Cytoplasmic inclusion bodies in cells infected with *Orthopoxviruses.* **(a)** B-type (pale-red, irregular) inclusion, or Guarnieri, bodies, and A-type (large eosinophilic, with halo) inclusion bodies in ectodermal cells of the chorioallantoic membrane, in a pock produced by cowpox virus. A number of nucleated erythrocytes are in the ectoderm and free in the mesoderm, and the surface of the pock is ulcerated. Hematoxylin-eosin stain. **(b)** This section of the



skin of a patient with hemorrhagic-type smallpox shows Guarnieri bodies and free erythrocytes below an early vesicle. Hematoxylin-eosin stain. Photographs: Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication.* Geneva, Switzerland: World Health Organization; 1988: 85.

None of the above laboratory tests are capable of discriminating smallpox from vaccinia, monkeypox, or cowpox. Classically, this differentiation required isolation of the virus and characterization of its growth on chorioallantoic membrane. Pocks produced by smallpox are small and grayish white, whereas those for vaccinia are larger and sometimes hemorrhagic. Variola minor exhibited greater temperature sensitivity on chorioallantoic membranes than did variola major.[83] The development of polymerase chain reaction diagnostic techniques promises a more accurate and less cumbersome method of discriminating among variola virus and other *Orthopoxviruses.*

MEDICAL MANAGEMENT

Medical personnel must be prepared to recognize a vesicular exanthem in possible biowarfare theaters as potentially smallpox, and to initiate appropriate countermeasures. Any confirmed case should be considered an international emergency, with an immediate report made not only to the chain of command but also to public health authorities. Strict quarantine with respiratory isolation should be applied for 17 days to *all* persons in direct contact with the index case or cases, especially the unvaccinated. Immediate vaccination should also be undertaken for all personnel exposed to either weaponized variola or monkeypox virus or a clinical case of smallpox. Medical personnel should have a history of vaccination and should undergo immediate revaccination to ensure solid immunity.

Vaccination with a verified clinical *take* (ie, the local response to vaccination whereby a vesicle forms with surrounding erythema and induration) within the past 3 years is considered to render a person immune to naturally occurring smallpox. However, given the difficulties and uncertainties under wartime conditions of verifying the adequacy of troops' prior vaccination, routine revaccination of all potentially exposed personnel would seem prudent if a significant prospect of smallpox exposure is believed to exist.

The key to control and eventual eradication of endemic smallpox was vigorous case identification, followed by quarantine and immunization of contacts. The fact that heterologous immunity provided by vaccination wanes with time is not fully appreciated: two thirds of smallpox victims in the 1960s had preexisting vaccination scars. Passive immunoprophylaxis using immune globulin was also examined, but, owing to the accepted efficacy of active immunization, the two methods were never compared against each other. No chemotherapy demonstrated efficacy against smallpox disease, but modest efficacy was shown in chemoprophylaxis. Present speculation regarding smallpox chemotherapy relies on in vitro data only.

Active Immunoprophylaxis

Vaccinia Vaccine

Early attempts to control smallpox included inoculation with material from smallpox lesions. This practice, known as variolization, caused severe cases of smallpox in about 1 in 200 inoculations.[84] In 1796, Jenner noted that milkmaids were free of the facial scars that marked most of the population during the smallpox epidemics of that time. The observation that they “cannot take smallpox” was attributed to the localized pox lesions that they developed on their hands. Jenner reasoned that infectious material (which he dubbed a “virus”) from cowpox lesions provided protection from smallpox, and used it to vaccinate an 8-year-old boy. The boy later resisted variolation, demonstrating that an animal poxvirus that is not virulent for humans could be used as a potent vaccine against smallpox.[85]

Vaccinia virus is another member of the orthopox genus of the Poxvirus family that possesses little pathogenicity for immunocompetent humans. Although the exact origin of vaccinia virus remains obscure,[86] it is related to cowpox, and strains of vaccinia virus became the vaccines of choice for the prevention of smallpox. The smallpox vaccines used in the eradication effort were prepared on a large scale by inoculating the shaved abdomens of calves, sheep, or water buffalo with seed stocks of vaccinia virus, harvesting the infected exudative lymph from the inoculation sites, and bottling the product with phenol or brilliant green as a bacteriostatic agent.

Smallpox vaccines were most often administered by intradermal inoculation with a bifurcated needle, a process that became known as *scarification* because of the permanent scar that resulted. This method proved effective and successful when applied in the worldwide campaign to eradicate endemic smallpox, which primarily employed the New York City Board of Health (NYCBOH), EM-63, Lister, and Temple of Heaven strains. Although the Lister and Temple of Heaven vaccines putatively derived from transformation of variola into an attenuated virus, both animal studies[87] and restriction endonuclease analyses[88] indicate that they actually resulted from contamination with vaccinia during animal passages.

The human dose for vaccinia immunization is approximately 5 $\log_{10}$ plaque-forming units given percutaneously. A vesicle typically appears at the vaccination site 5 to 7 days after the inoculation, with surrounding erythema and induration. The lesion forms a scab and gradually heals over the next 1 to 2 weeks.

Vaccination Complications

Side effects arising from vaccination are relatively uncommon but nevertheless finite. Low-grade fever and axillary lymphadenopathy may coincide with the culmination of the cutaneous pox lesion. The attendant erythema and induration of the vaccination vesicle is frequently misdiagnosed as bacterial superinfection. Formation of a scar on healing of the vesicle occurs routinely, and constitutes a permanent record of a *take,* or a successful primary vaccination.

TABLE 27-3
RATES * OF REPORTED COMPLICATIONS ASSOCIATED WITH VACCINIA VACCINATIONS

Age	Total	Inadvertent Inoculation	Generalized Vaccinia	Eczema Vaccinatum	Progressive Vaccinia	Postvaccinial Encephalitis	Erythema Multiforme	Other†
Primary vaccination								
<5 y	564.4	262.9	38.7	2.6	15.5	594.3	154.9	1,633.3
5-19 y	371.2	139.7	34.9	—‡	8.7	87.3	214.0	855.8
>19 y	606.1	212.1	30.3	—‡	—‡	30.3	636.4	1,515.2
Overall rates§								
	529.2	241.5	38.5	1.5	12.3	164.6	266.2	1,253.8
Revaccination								
<5 y	109.1	—‡	—‡	—‡	—‡	72.7	18.2	200.0

5-19 y	47.7	9.9	2.0	—‡	—‡	2.0	23.9	85.5
>19 y	25.0	9.1	4.5	6.8	4.5	9.1	54.5	113.5
Overall rates								
	42.1	9.0	3.0	3.0	2.0	10.0	39.1	108.2

* Cases per million vaccinations
† Includes patients with lesions that had bacterial superinfection, or that made the patient uncomfortable enough to consult a physician. Unusual complications included were a patient with fetal vaccinia, a patient with a melanoma developing in the vaccine scar, and a patient with monoarticular arthritis following vaccination.
‡ No instances of this complication were identified during the 1968 survey of 10 states
§ Overall rates for each complication include persons of unknown age
Adapted with permission from Lane JM, Ruben, FL, Neff, JM, Millar, JD. Complications of smallpox vaccination, 1968: Results of ten statewide surveys. *J Infect Dis.* 1970:122(4):307.

One of the most thorough surveys of adverse reactions associated with vaccinia vaccines was conducted in the United States and published by the CDC in 1968,[89] and is summarized in Table 27-3.

As a consequence of percutaneous inoculation, infectious vaccine virus is present in the local lesion. Consequently, inadvertent inoculation to other skin and mucous membrane sites (autoinoculation) or to other persons (secondary inoculation) is the most frequent complication of vaccinia intradermal vaccination.[90–92] Ocular vaccinia is a particularly troublesome problem resulting from secondary inoculation (Figure 27-6). Erythematous or urticarial rashes may occur approximately 10 days after primary vaccination and, rarely, Stevens-Johnson syndrome occurs. Generalized vaccinia is characterized by a vesicular rash of varying extent occurring 6 to 9 days after primary vaccination; this complication is usually self-limited.

Fig. 27-6. Ocular vaccinia following inadvertent autoinoculation with vaccine. This complication can cause corneal scarring and hence visual impairment. Ocular vaccinia should be treated aggressively with a topical antiviral drug under close ophthalmological supervision. Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication.* Geneva, Switzerland: World Health Organization; 1988: 298. Photograph by C. H. Kempe.

More severe complications of vaccinia vaccination include eczema vaccinatum, progressive vaccinia, and postvaccinial encephalitis. These complications are rare, but occur at least 10 times more often among primary vaccinees than among revaccinees. Eczema vaccinatum is a localized or systemic dissemination of vaccinia virus among persons who have eczema (Figure 27-7). Although usually mild and self-limited, this complication could be severe or even fatal in up to 10% of cases.

Progressive vaccinia (vaccinia necrosum) is a progressive necrosis in the area of vaccination, often with metastatic lesions (Figure 27-8). This complication occurred almost exclusively in persons with cellular immunodeficiency,[93] with a case fatality rate of higher than 75%.[94]

Fig. 27-7. Eczema vaccinatum. The widespread pox lesions on this patient's eczematous skin illustrate the severe illness that can occur in up to 10% of cases. Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication.* Geneva, Switzerland: World Health Organization; 1988: 298. Photograph by I. D. Ladyni.

Fig. 27-8. Progressive vaccinia or vaccinia necrosum. As seen in this child, progressive viral replication at the inoculation site in an immunocompromised individual leads to inexorable local tissue destruction. Reprinted with permission from Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. *Smallpox and Its Eradication.* Geneva, Switzerland: World Health Organization; 1988: 298. Photograph by C. H. Kempe.

Postvaccinial encephalitis most frequently affects primary vaccinees, with attendant mortality and serious neurologic morbidity of 25%.[95] Although the U.S. survey indicated the opposite, most countries reported a greater incidence of postvaccinial encephalitis from primary vaccination of older children and adults than of infants.[96]

Vaccinia-immune globulin (VIG) is of value in the treatment of progressive vaccinia, eczema vaccinatum,[97] and perhaps ocular vaccinia (VIG is discussed below in the section titled Passive Immunoprophylaxis). Topical idoxuridine may benefit vaccinia keratitis.[98,99]

Vaccination is contraindicated in the following conditions:

1. Immunosuppression, such as occurs with agammaglobulinemia, leukemia, lymphoma, generalized malignancy, or therapy with alkylating agents, antimetabolites, radiation, or large doses of corticosteroids. Such persons are at increased risk of progressive vaccinia.
2. Human immunodeficiency virus (HIV) infection. Severe vaccinia infections have been reported in persons immunosuppressed as a consequence of HIV disease.[100,101]
3. Either history or evidence of eczema. Other exfoliative or extensive skin lesions (eg, atopic dermatitis, psoriasis, burns) may also place a person at increased risk for vaccination.
4. Current household, sexual, or other close physical contact with a person or persons possessing the condition or conditions listed in exclusion criteria 1 through 3, above.
5. Pregnancy. There have been rare instances of fetal vaccinia, usually following primary vaccination of pregnant women.[102,103] This complication usually resulted in fetal demise

or early postnatal mortality.

Despite the caveats listed above, most authorities state that, with the exception of significant impairment of systemic immunity, there are no absolute contraindications to *postexposure* vaccination of a person who experiences *bona fide* exposure to variola virus. However, in such circumstances, concomitant administration of VIG is recommended for pregnant women and individuals with eczema.

Indicators of Immunity

Vaccination with live vaccinia virus remains the principal defensive measure against smallpox, and against monkeypox, as well. Immunity to smallpox is gauged by the local response to vaccination (the *take*). However, not all vaccinations giving clinical takes will boost neutralizing antibody responses.[104] Evidence indicates that vaccinia provides protection against smallpox for at least 3 years after the vaccination.[105] With longer intervals between exposure and primary vaccination (or revaccination), protection is reduced. If vaccination is accomplished within a few days after exposure, then protection is also demonstrable,[106] approaching complete protection in those who have had their primary vaccination previously.[107] Postexposure vaccine failures were reported during trials in the smallpox era. Combined active immunization and passive immunization with VIG may provide improved postexposure prophylaxis. However, concomitant administration of VIG may attenuate the immune response to vaccination.[108]

Protection following vaccinia immunization, like immunity following recovery from smallpox, has both a humoral and a cellular basis. One prospective study[109] of 146 contacts of patients with smallpox demonstrated that no contacts with significant titers of neutralizing antibody titer contracted the disease. However, 2 contacts with detectable titers still contracted the disease, and some persons with no neutralizing antibody were spared. In another investigation[110] of 57 contacts of smallpox cases, all 6 who subsequently developed smallpox had neither a vaccination scar nor detectable neutralizing antibody. Therefore, data indicate that adequate serum titers of vaccinia-neutralizing antibody are usually sufficient, but not always necessary, for protection against variola virus. Neutralizing antibody decreases during the first 3 years after vaccination, but titers remain sustained for several years following a second booster (ie, primary immunization and two revaccinations).[111,112]

Contact Precautions

Vaccinia virus may be cultured from the site of primary vaccination for a time that begins at the development of a papule (2–5 d postvaccination) and lasts until the lesion has fully scabbed with no remaining vesicle (10–17 d postvaccination). During this period, infection-control measures are paramount in preventing secondary inoculation of the virus to other body sites or other persons. The lesion should be covered at all times with a dry dressing, and strict handwashing should be practiced after changing the dressing or touching the vaccination site. Semipermeable dressings predispose to accumulation of exudate beneath the dressing, with maceration and local secondary inoculation. Medical personnel should be excluded from caring for persons with active vaccinia lesions if the former are immunocompromised or possess chronic exfoliative skin lesions. Medical personnel who come in contact with contaminated materials (eg, dressings) from vaccinees are at relatively low risk of inadvertent inoculation, provided that they use appropriate infection control measures.[113] The CDC has recommended that laboratory personnel who work with vaccinia or recombinant vaccinia viruses be vaccinated; this recommendation is controversial,[114] however, and the risks of deliberate vaccination versus those of accidental inoculation must be weighed.

Vaccine Availability

The remaining vaccinia vaccine licensed in the United States (Dryvax, manufactured by Wyeth, Philadelphia, Pa.) is a live, infectious virus prepared from calf lymph. Like all smallpox vaccines that were marketed in the United States, it derived from the NYCBOH strain and contains 10^8 plaque-forming units per milliliter. Current vaccinia vaccine stocks (> 12 million doses) are held by the CDC. It must be noted that the potency of several lots of this lyophilized vaccine has fallen. Pharmaceutical companies in the United States lack interest in manufacturing new lots of vaccine, owing to the absence of a profitable retail market, antiquation of calf-lymph production techniques and facilities, and the manufacturer's legal liability for vaccination complications.

Because of the declining potency of the existing smallpox vaccines and continued concerns about the prospect of the use of variola virus in biological warfare, a new vaccinia vaccine is in clinical testing by the U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, Maryland. This vaccine was derived from a NYCBOH strain of vaccinia and then produced in human diploid lung fibroblast cell cultures. Unlike calf-lymph vaccines, this cell culture–derived vaccinia vaccine contains no adventitious agents.

Injectable Smallpox Vaccines

It has long been desired that vaccinia vaccine could be administered by injection; this route would preclude the complication of inadvertent inoculation of virus that was so prominent with the scarified calf-lymph vaccine. A potential disadvantage to subcutaneous or intramuscular inoculation would be the preclusion of a visible vesicle and scar as a means of assessing the take of a vaccination, which historically has been correlated to protection against smallpox. Plaque reduction neutralization antibody responses necessarily serve as the surrogate marker for an immune response that would protect against smallpox. Since the 1930s, at least eight strains of vaccinia virus were developed for parenteral administration. None were shown to be as immunogenic as standard calf-lymph strains administered by scarification, since they produced lower neutralizing antibody levels after primary and booster inoculations.[115] Therefore, evidence indicates that subcutaneous, intramuscular, and intradermal vaccination without concomitant formation of a cutaneous pox lesion does not elicit as strong a serologic response as that obtained through scarification.[116,117]

Recombinant Vaccinia Vaccines

Vaccinia virus has been proposed as a vaccine vector for foreign genes. This approach has been employed for expression of HIV proteins[118] as well as other antigens. Vaccinianaive individuals inoculated with this vaccine developed antibodies not only to HIV-1[119] but also to vaccinia. Therefore, it is possible that a future recombinant vaccinia vaccine containing a variety of viral or bacterial genes could serve multiple purposes, including immunity against smallpox. However, those vaccinia vectors engineered to be more attenuated [120] should be considered questionable in their ability to protect against variola virus. Neither would protection against variola be expected from immunization with other vertebrate poxviruses, especially those of another genus such as *Avipox.*

Passive Immunoprophylaxis

Evidence indicates that vaccinia-immune globulin is of value in postexposure prophylaxis of smallpox when given *(a)* within the first week following exposure and *(b)* concurrently with vaccination.[121] However, the prophylactic use of VIG should be carefully weighed vis-à-vis the risk of attenuating the immune response to booster vaccination.

VIG is available in the United States from the Drug Service of the CDC; the U.S. Army maintains a supply for its own use. The dose for prophylaxis or treatment is 0.6 mL/kg, administered intramuscularly. Administration immediately after or within the first 24 hours of exposure would provide the highest level of protection, especially in unvaccinated persons.

VIG is prepared from the plasma of repeatedly vaccinated persons. Development of humanized monoclonal antibodies against neutralizing epitopes that are conserved between vaccinia and variola viruses is a promising alternative to this older, cumbersome, and expensive method of VIG production.

Chemoprophylaxis and Chemotherapy

During the 1960s, methisazone (Marboran, no longer available; then manufactured by Burroughs Wellcome, Research Triangle Park, N. C.; also called 1-methylisatin 3 ' -β-thiosemicarbazone) received extensive attention as an antiviral chemotherapy for variola virus infections. Although some clinical studies found only a trend toward decreased attack rates,[122,123] most evidence suggested that methisazone decreased both morbidity and mortality when administered *prophylactically* to susceptible contacts of patients with smallpox.[124–127] Although a variety of dosages and schedules were tested, most experience was with 3 g administered orally, at least two doses given 8 hours apart. Gastrointestinal intolerance (nausea and vomiting) significantly hampered both medication administration and patient compliance.

Although clinical trials[128,129] showed no *therapeutic* efficacy of thiosemicarbazones against smallpox, anecdotal reports[130,131] suggest some usefulness of methisazone in treating progressive vaccinia.

Other antiviral compounds, such as rifampin[132] and S-adenosylhomocysteine hydrolase inhibitors,[133–135]have activity against vaccinia, and hence may be useful in the prophylaxis or treatment of smallpox. However, it should be noted that despite their in vitro efficacy against vaccinia, neither cytosine arabinoside (Ara-C)[136] nor adenine arabinoside (Ara-A)[137] demonstrated benefit in treatment of smallpox in small clinical studies.

SUMMARY

Despite the eradication of naturally occurring smallpox and the availability of a vaccine, the potential weaponization of variola virus continues to pose a military threat. This threat can be attributed to the aerosol infectivity of the virus, the relative ease of large-scale production, and an increasingly *Orthopoxvirus*-naive human populace.

Although the fully developed cutaneous eruption of smallpox is unique, earlier stages of the rash could be mistaken for varicella. Secondary spread of infection constitutes a nosocomial hazard from the time of onset of a smallpox patient’s enanthem until scabs have separated. Quarantine with respiratory isolation should be applied to secondary contacts for 17 days after the exposure.

Vaccinia vaccine remains the preeminent countermeasure for preexposure prophylaxis against smallpox. Vaccinia vaccination, vaccinia immune globulin, and methisazone each possess some efficacy in postexposure prophylaxis.

REFERENCES

1. Fenner F, Wittek R, Dumbell KR. *The Orthopoxviruses*. Orlando, Fla: Academic Press; 1989.
2. Her Majesty’s Stationery Office. *Report of the Investigations Into the Cause of the 1978 Birmingham Smallpox Occurrence*. London, England: HMSO; 1980.
3. Heagerty JJ. *Four Centuries of Medical History in Canada*. Vol 1. Toronto, Ont, Canada: MacMillan; 1928.
4. Parkman F. *The Conspiracy of Pontiac*. Vol 2. Boston, Mass: Little, Brown; 1969: 44–46.
5. Stearn EW, Stearn AE. *The Effects of Smallpox on the Destiny of the Amerindians.* Boston, Mass: Bruce Humphries; 1945: 44–45.

6. Kean RGH. *Inside the Confederate Government*. New York, NY: Oxford University Press; 1957: 89.

7. Steiner PE. *Disease in the Civil War: Natural Biological Warfare in 1861–1865*. Springfield, Ill: Charles C Thomas; 1968: 42–43.

8. Harris R, Paxman J. *A Higher Form of Killing*. New York, NY: Hill and Wang; 1982: 76–79, 153.

9. Williams P, Wallace D. *Unit 731: Japan's Secret Biological Warfare in World War II*. New York, NY: Free Press; 1989: 28, 213.

10. Henderson DA. The eradication of smallpox. In: Last JM, ed. *Maxcy-Rosenau Public Health and Preventive Medicine*. 12th ed. Norwalk, Conn: Appleton-Century-Crofts; 1986: 129–138.

11. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Potential sources for a return of smallpox. In: *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: Chap 30: 1341.

12. Capps L, Vermund SH, Johnsen C. Smallpox and biological warfare: The case for abandoning vaccination of military personnel. *Am J Public Health*. 1986;76(10):1229–1231.

13. World Health Organization. Health aspects of chemical and biological weapons: Report of a WHO group of consultants. Geneva, Switzerland: WHO; 1970: 69–70.

14. Wolff HL, Croon JAB. Survival of smallpox virus (variola minor) in natural circumstances. *Bull WHO*. 1968;38:492–493.

15. Zuckerman AJ. Paleontology of smallpox. *Lancet*. 1984;2:1454.

16. Baxter PJ, Brazier AM, Young SEJ. Is smallpox a hazard in church crypts? *Br J Industr Med*. 1988;45:359–360.

17. Joklik WK, Moss B, Fields BN, Bishop DHL, Sandakhchiev LS. Why the smallpox virus stocks should not be destroyed. *Science*. 1993;262:1225–1226.

18. Buller RM, Palumbo GJ. Poxvirus pathogenesis. *Microbiol Rev*. 1991;55:80.

19. Hahon N, Wilson BJ. Pathogenesis of variola in *Macaca irus* monkeys. *Am J Hyg*. 1960;71:69–80.

20. Mahy BWJ, Almond JW, Berns KI, et al. The remaining stocks of smallpox virus should be destroyed. *Science*. 1993;262:1223–1224.

21. Capps L, Vermund SH, Johnsen C. Smallpox and biological warfare: The case for abandoning vaccination of military personnel. *Am J Publ Health*. 1986;76:1229–1231.

22. Geshelin P, Berns KI. Characterization and localization of the naturally occurring cross-links in vaccinia virus DNA. *J Mol Biol*. 1974;88:785–796.

23. Müller HK, Wittek KR, Schaffner W, Schümperli D, Menna A, Wyler R. Comparison of five poxvirus genomes by analysis with restriction endonucleases *Hin*dIII, *Bam*I and *Eco*RI. *J Gen Virol*. 1977;38:135–147.

24. Mackett M, Archard LC. Conservation and variation in *Orthopoxvirus* genome . *J Gen Virol*. 1979;45: 683–701.

25. Massung RF, Esposito JJ, Liu LI, et al. Potential virulence determinants in terminal regions of variola smallpox virus genome. *Nature*. 1993;366:748–750.

26. Moss B. Regulation of vaccinia virus transcription. *Annu Rev Biochem*. 1990;59:661–668.

27. Kates JR, McAuslan BR. Messenger RNA synthesis by a "coated" viral genome. *Proc Natl Acad Sci USA*. 1967;57:314–320.

28. Zaslavsky V. Uncoating of vaccinia virus. *J Virol*. 1985;55:352–356.

29. Moyer RW. The role of the host cell nucleus in vaccinia virus morphogenesis. *Virus Res*. 1987;8:173–191.

30. Merchilinsky M. Intramolecular homologous recombination in cells infected with temperature-sensitive

mutants of vaccinia virus. *J Virol*. 1989;63:2030–2035.

31. Bedson HS, Dumbell KR. Hybrids derived from the viruses of variola major and cowpox. *J Hygiene*. 1964;62:147–148.

32. Keck JC, Baldick CJ, Moss B. Role of DNA replication in vaccinia virus gene expression: A naked template is required for transcription of three late *trans*-activator genes. *Cell*. 1990;61:80–89.

33. Moss B. Poxviridae and their replication. In: Fields BN, Knipe DM, eds. *Virology*. 2nd ed. New York, NY: Raven Press; 1990: 2079–2111.

34. Dales S, Pogo BGT. Biology of poxviruses. *Virol Monogr.* 1981;18:1.

35. Ichihashi Y, Matsumoto S, Dales S. Biogenesis of poxviruses: Role of A-type inclusions and host cell membranes in virus dissemination. *Virology*. 1977;101:50–60.

36. Baxby D. The surface antigens of *Orthopoxviruses* detected by cross-neutralization tests on cross-absorbed antisera. *J Gen Virol*. 1982;58:251–262.

37. Kitamoto N, Tanimoto S, Hiroi K, et al. Monoclonal antibodies to cowpox virus: Polypeptide analysis of several major antigens. *J Gen Viro*l. 1987;68:239–246.

38. Boulter EA, Appleyard G. Differences between extracellular and intracellular forms of poxvirus and other implications. *Prog Med Virol*. 1973;16:86–108.

39. Cole GA, Blanden RV. Immunology of poxviruses. In: Nahmias AJ, O'Reilly RJ, eds. *Immunology of Human Infection*. Part 2: *Viruses and Parasites; Immunodiagnosis in Prevention of Infectious Diseases*. Vol 9. In: *Comprehensive Immunology*. New York, NY: Plenum Press; 1982: 1–19.

40. Mallon VR, Holowczak JA. Vaccinia virus antigens on the plasma membrane of infected cells, I: Viral antigens transferred from infecting virus particles and synthesized after infection. *Virology*. 1985;141:201–220.

41. Perrin LH, Zinkernagel RM, Oldstone MB. Immune response in humans after vaccination with vaccinia virus: Generation of a virus-specific cytotoxic activity by human peripheral lymphocytes. *J Exp Med*. 1977;146:949–969.

42. Littaua RA, Takeda A, Cruz J, Ennis FA. Vaccinia virus-specific human CD4+ cytotoxic T-lymphocyte clones. *J Virol*. 1992;66(4):2274–2280.

43. Demkowicz WE, Maa JS, Esteban M. Identification and characterization of vaccinia virus genes encoding proteins that are highly antigenic in animals and are immunodominant in vaccinated humans. *J Virol*. 1992;66(1):386–398.

44. Payne L. Significance of extracellular enveloped virus in the in vitro and in vivo dissemination of vaccinia. *J Gen Virol* . 1980;50:89–100.

45. RamanaRao AV. The immunogenicity of inactivated vaccinia virus. *J Path Bact*. 1962;84:367–377.

46. Kaplan C, McLean D, Vallet L. A note on the immunogenicity of ultraviolet irradiated vaccinia virus in man. *J Hygiene* 1962;60:79–83.

47. Kaplan C, Benson PF, Butler NR. Immunogenicity of ultraviolet-irradiated, non-infectious, vaccinia-virus vaccine in infants and young children. *Lancet*. 1965;1:573–574.

48. Khodakevich L, Szczeniowski M, Manbu-ma-Disu, et al. The role of squirrels in sustaining monkeypox virus transmission. *Trop Geogr Med*. 1987;39:115–122.

49. Baxby D. Human poxvirus infection after the eradication of smallpox. *Epidemiol Infect*. 1988;100:321–334.

50. Jezek Z, Fenner F. Human monkeypox. *Virol Monogr*. 1988;17:93–95.

51. Jezek Z, Arita I, Mutombo M, Dunn C, Nakano JH, Szczeniowski M. Four generations of probable person-to-person transmission of human monkeypox. *Am J Epidemiol*. 1986;123:1004–1012.

52. Jezek Z, Grab B, Dixon H. Stochastic model for the interhuman spread of monkeypox. *Am J Epidemiol*. 1987;126:1082–1089.

53. Fine PEM, Jezek Z, Grab B, Dixon J. The transmission potential of monkeypox virus in human populations. *Int J Epidemiol*. 1988;17(3):643–650.

54. Huq F. Effect of temperature and relative humidity on variola virus in crusts. *Bull WHO.* 1976;54:710–712.

55. Noble J, Rich JA. Transmission of smallpox by contact and by aerosol routes in *Macaca irus*. *Bull WHO*. 1969;40:279–286.

56. Meikeljohn G, Kempe CH, Downie AW, Berge TO, St. Vincent L, Rao AR. Air sampling to recover variola virus in the environment of a smallpox hospital. *Bull WHO*. 1961;25:63–67.

57. Downie AW, Meiklejohn M, St Vincent L, Rao AR, Sundara Babu BV, Kempe CH. The recovery of smallpox from patients and their environment in a smallpox hospital. *Bull WHO*. 1965;33:615–622.

58. Foege WH, Millar JD, Henderson DA. Smallpox eradication in West and Central Africa. *Bull WHO*. 1975;52: 209–222.

59. Wehrle PF, Posch J, Richter KH, Henderson DA. An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe. *Bull WHO*. 1970;43:669–679.

60. MacCallum FO, McDonald JR. Survival of variola virus in raw cotton. *Bull WHO*. 1957;16:247–254.

61. Sarkar JK, Mitra AC, Mukherjee MK, De SK. Virus excretion in smallpox, II: Excretion in the throats of household contacts. *Bull WHO* 1973;48:523–527.

62. Horgan ES, Ali Haseeb M. Cross immunity experiments in monkey between variola, alastrim and vaccinia. *J Hygiene*. 1939;39:615–637.

63. Hahon N. Smallpox and related poxvirus infections in the simian host. *Bact Rev*. 1961;25:459–476.

64. Ricketts TF. *The Diagnosis of Smallpox*. London, England: Cassell; 1908.

65. Downie AW, St. Vincent L, Meiklejohn G, et al. Studies on the virus content of mouth washings in the acute phase of smallpox. *Bull WHO*. 1961;25:49–53.

66. Sarkar JK, Mitra AC, Mukherjee MK, De SK, Mazumdar DG. Virus excretion in smallpox. *Bull WHO*. 1973;48: 517–522.

67. Mitra AC, Sarkar JK, Mukherjee MK. Virus content of smallpox scabs. *Bull WHO*. 1974;51:106–107.

68. Sarkar JK, Mitra AC. Virulence of variola virus isolated from smallpox cases of varying severity. *Indian J Med Res*. 1967;55(1):13–20.

69. Downie AW, Fedson DS, St. Vincent L, Rao AR, Kempe CH. Haemorrhagic smallpox. *J Hyg*. 1969;67:619–629.

70. Gupta SK, Srivasta TP. Roentgen features of skeletal involvement in smallpox. *Aust Radiol*. 1973;17:205–211.

71. Cockshott P, MacGregor M. Osteomyelitis variolosa. *Q J Med*. 1958;27:369–387.

72. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. The clinical features of smallpox. In: *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: Chap 1: 48.

73. Marsden JP. Variola minor: A personal analysis of 13,686 cases. *Bulletin of Hygiene*. 1948;23:735–746.

74. Hughes K. *Fatality, Facial Scarring and Blindness From Smallpox in Bangladesh*. Geneva, Switzerland: 1978. WHO/SE/78.101.

75. Marsden JP, Greenfield CRM. Inherited smallpox. *Arch Dis Child*. 1934;9:309–314.

76. Ribeiro AM, de Salles-Gomes LF, Schmidt BJ, Kopelman BI, Pimenta de Campos E, Carvalho AA. Fetal variola: Report of two cases. *Helvetica Paediatrica Acta*. 1965;20:369–373.

77. Rao AR. *Smallpox*. Bombay, India: Kothari Book Depot; 1972.

78. Downie AW, St. Vincent L, Goldstein L, Rao AR, Kempe CH. Antibody response in non-haemorrhagic smallpox patients. *J Hygiene*. 1969;67:609–618.

79. World Health Organization. *Guide to the Laboratory Diagnosis of Smallpox for Smallpox Eradication Programs*. Geneva, Switzerland: World Health Organization; 1969.

80. Nakano JH. Evaluation of virological laboratory methods in smallpox diagnosis. *Bull WHO*. 1973;48:529–534.

81. Kato S, Takahashi, Kameyama, Kamahora J. A study on the morphological and cyto-immunological relationship between the inclusions of variola, cowpox, rabbitpox, vaccinia (variola origin) and vaccinia IHD and consideration of the term “Guarnieri body.” *Biken J*. 1959;2:353–363.

82. Mitra AC, Sarkar SK, Mukherjee MK, Chakravarty MS. Evaluation of the precipitation-in-gel reaction in the diagnosis of smallpox. *Bull WHO*. 1973;49:555–558.

83. Dumbell KR, Bedson HS, Rossier E. The laboratory differentiation between variola major and variola minor. *Bull WHO*. 1961;25:73–78.

84. Baxby D. *Jenner’s Smallpox Vaccine*. London, England: Heinemann Educational Books; 1981.

85. Bloch H. Edward Jenner: The history and effects of smallpox, inoculation, and vaccination. *Am J Dis Child*. 1993;147:772–774.

86. Baxby D. Poxviruses. In: Parker MT, Collier LH, eds. *Topley and Wilson’s Principles of Bacteriology, Virology and Immunity*. 8th ed, vol 4. London, England: Arnold; 1990: 559–571.

87. Herrlich A, Mayr A, Mahnel H, Munz E. Experimental studies on transformation of the variola virus into the vaccinia virus. *Archiv für die gesamte Virusforschung*. 1963;12:579–599.

88. Baxby D. Vaccinia virus. In: Quinnan GV, ed. *Vaccinia Viruses as Vectors for Vaccine Antigens*. New York, NY: Elsevier; 1985: 11–23.

89. Lane JM, Ruben FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: Results of ten statewide surveys. *J Infect Dis*. 1970;122:303–309.

90. Centers for Disease Control. Contact spread of vaccinia from a recently vaccinated Marine—Louisiana. *MMWR*. 1984;33(3):37–38.

91. Centers for Disease Control. Contact spread of vaccinia from a National Guard vaccinee. *MMWR*. 1985;34(13):182–183.

92. Lane JM, Reuben FL, Neff JM, et al. Complications of smallpox vaccination, 1968: National surveillance in the United States. *New Engl J Med*. 1969;281:1201–1207.

93. Fulginiti VA, Kempe CH, Hathaway WE, et al. Progressive vaccinia in immunologically deficient individuals. *Birth Defects*. 1968;4:129–145.

94. Freed ER, Richard JD, Escobar MR. Vaccinia necrosum and its relationship to impaired immunologic responsiveness. *Am J Med*. 1972;52:411–420.

95. Goldstein JA, Neff JM, Lane JM, Koplan JP. Smallpox vaccination reactions, prophylaxis and therapy of complications. *Pediatrics*. 1975;55:342–347.

96. Fenner F, Henderson DA, Arita I, Jezek Z, Ladnyi ID. Developments in vaccination and control between 1900 and 1966. In: *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988: Chap 7: 302–307.

97. Kempe CH. Studies on smallpox and complications of smallpox vaccination. *Pediatrics*. 1960;26:176–189.

98. Fulginiti VA, Winograd LA, Jackson M, Ellis P. Therapy of experimental vaccinal keratitis. *Arch Ophthalmol.* 1965;74:539–544.

99. Jack MD, Sorenson RW. Vaccinal keratitis treated with IDU. *Arch Ophthalmol*. 1963;69:730.

100. Redfield RR, Wright DC, James WD, Jones TS, Brown C, Burke DS. Disseminated vaccinia in a military recruit with human immunodeficiency virus (HIV) disease. *N Engl J Med*. 1987;316(11):673–676.

101. Guillaume JC, Saiag P, Wechsler J, Lesces MC, Roujeau JC. Vaccinia from recombinant virus expressing HIV genes. *Lancet*. 1991;377:1034–1035. Letter.

102. Goldstein JA, Neff JM, Lane JM, Koplan JP. Smallpox vaccination reactions, prophylaxis, and therapy of complications. *J Pediatr*. 1975; 55(3):342–347.

103. Centers for Disease Control. Adverse reactions to smallpox vaccination—1978. *MMWR*. 1978;28:265–267.

104. Lublin-Tannenbaum T, Katzenelson E, El-ad B, Katz E. Correlation between cutaneous reaction in vaccinees immunized against smallpox and antibody titer determined by plaque neutralization test and ELISA. *Viral Immunology*. 1990;3(1):19–25.

105. Hanna W. Studies in smallpox and vaccination. Bristol, England: Wright; 1913.

106. Dixon CW. *Smallpox*. London, England: Churchill; 1962.

107. Henderson DA. Smallpox. In: Sartwell PE, ed. *Preventive Medicine and Public Health by Maxcy-Rosenau*, 10th ed. New York: Appleton, Century-Crofts; 1973: 104–116.

108. Heering SL, Shohat T, Vonsover A, Mendelson E, Lerman Y. Neutralizing antibody titers following revaccination with vaccinia virus. *Public Health Rev*. 1990;18:94–97.

109. Mack TM, Noble J, Thomas DB. A prospective study of serum antibody and protection against smallpox. *Am J Trop Med Hyg*. 1972;21(2):214–218.

110. Sarkar JK, Mitra AC, Mukherjee MK. The minimum protective level of antibodies in smallpox. *Bull WHO*. 1975;52:307–311.

111. El-Ad B, Roth Y, Winder A, et al. The persistence of neutralizing antibodies after revaccination against smallpox. *J Infect Dis*. 1990;161:446–448.

112. Nyerges G, Hollós I, Losonczy G, Erdós L, Petrás G. Development of vaccination immunity in hospital personnel revaccinated at three-year intervals. *Acta Microbiol Acad Sci Hung*. 1972;19:63–68.

113. Centers for Disease Control. Vaccinia (smallpox) vaccine: Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR. 1991;40:1–10. Published erratum appears in *MMWR*. 1992;41(2):31.

114. Williams NR, Cooper BM. Counselling of workers handling vaccinia virus. *Occup Med*. 1993;43:125–127.

115. McIntosh K. Experience with the CV1 strain. In: Quinnan GV, ed. *Vaccinia Viruses as Vectors for Vaccine Antigens*. New York, NY: Elsevier; 1985: 91–100.

116. Cherry JD, Connor JD, McIntosh K, et al. Clinical and serologic study of four smallpox vaccines comparing variations of dose and routes of administration: Standard percutaneous revaccination of children who received primary subcutaneous vaccination. *J Infect Dis.* 1977;135:176–182.

117. Graham JS. Captain, Medical Corps, US Army. Personal communication, May 1995.

118. Guillaume JC, Saiag P, Wechsler L, Lescs MC, Roujeau RC. Vaccinia from recombinant virus expressing HIV Genes. *Lancet*. 1991;337:1034–1035.

119. Graham BS, Belshe RB, Clements ML, et al. Vaccination of vaccinia-naive adults with human immunodeficiency virus type 1 gp160 recombinant vaccinia virus in a blinded, controlled, randomized clinical trial. *J Infect Dis*. 1992;166:244–252.

120. Tartaglia J, Perkus ME, Taylor J, et al. NYVAC: A highly attenuated strain of vaccinia virus. *Virology* . 1992;188: 217–232.

121. Kempe CH, Bowles C, Meiklejohn G, et al. The use of vaccinia hyperimmune gamma-globulin in the prophylaxis of smallpox. *Bull WHO*. 1961;25:41–48.

122. Heiner GG, Fatima N, Russell PK, et al. Field trials of methisazone as a prophylactic agent against smallpox. *Am J Epidemiol*. 1971;94(5):435–449.

123. Rao AR, Jacobs ES, Kamalakshi S, Bradbury, Swamy A. Chemoprophylaxis and chemotherapy in variola major, I: An assessment of CG662 and Marboran in prophylaxis of contacts of variola major. *Indian J Med Res*. 1969;57(3):477–483.

124. Bauer DJ, St Vincent L, Kempe CH, Downie AW. Prophylacitc treatment of smallpox contacts with N-methylisatin β-thiosemicarbazone (compound 33T57, Marboran). *Lancet*. 1963;2:494–496.

125. do Valle LAR, de Melo PR, de Salles Gomes LF, Proença LM. Methisazone in prevention of variola minor among contacts. *Lancet*. 1965;2:976–978.

126. Bauer DJ, St Vincent L, Kempe CH, Young PA, Downie AW. Prophylaxis of smallpox with methisazone. *Am J Epidemiol*. 1969;90:130–145.

127. Rao AR, McKendrick GDW, Velayudhan L, Kamalakshi K. Assessment of an isothiazole thiosemicarbazone in the prophylaxis of contacts of variola major. *Lancet*. 1966;1:1072–1074.

128. Rao AR, McFadzean JA, Kamalakshi K. An isothiazole thiosemicarbazone in the treatment of variola major in man. *Lancet*. 1966;1:1068–1072.

129. Bauer DJ. Thiosemicarbazones. In: Bauer DJ, ed. *Chemotherapy of Virus Diseases*. In: *International Encyclopaedia of Pharmacology and Therapeutics*. Vol 1, § 61. Oxford, England: Pergamon Press; 1972: 101–110.

130. Brainerd HD, Hanna L, Jawetz E. Methisazone in progressive vaccinia. *N Engl J Med*. 1967;276:620–622.

131. McLean DM. Methisazone therapy in pediatric vaccinia complications. *Ann N Y Acad Sci*. 1977;284:118–121.

132. Sodeik B, Griffiths G, Ericsson M, Moss B, Doms RW. Assembly of vaccinia virus: Effects of rifampin on the intracellular distribution of viral protein p65. *J Virol*. 1994;68(2):1103–1114.

133. Tseng CK, Marquez VE, Fuller RW, et al. Synthesis of 3-deazaneplanocin A, a powerful inhibitor of S-adenosylhomocysteine hydrolase with potent and selective in vitro and in vivo antiviral activities. *J Med Chem*. 1989;32(7):1442–1446.

134. Shuto S, Obara T, Toriya M, et al. New neplanocin analogues, I: Synthesis of 6'-modified neplanocin A derivatives as broad-spectrum antiviral agents. *J Med Chem*. 1992;35(2):324–331.

135. de Clercq E, Holy A, Rosenberg I. Efficacy of phosphonylmethoxyalkyl derivatives of adenine in experimental herpes simplex virus and vaccinia virus infections in vivo. *Antimicrob Agents Chemother*. 1989;33(2):185–191.

136. Monsur KA, Hossain MS, Huq F, Rahaman MM, Haque MQ. Treatment of variola major with cytosine arabino-side. *J Infect Dis*. 1975;131(1):40–43.

137. Koplan JP, Monsur KA, Foster SO, et al. Treatment of variola major with adenine arabinoside. *J Infect Dis*. 1975;131(1):34–39.

Chapter 28

VIRAL ENCEPHALITIDES

JONATHAN F. SMITH, PH.D.[*]; KELLY DAVIS, D.V.M.[†]; MARY KATE HART, PH.D.[‡]; GEORGE V. LUDWIG, PH.D.[§]; DAVID J. MCCLAIN, M.D.[¥]; MICHAEL D. PARKER, PH.D.[¶]; AND WILLIAM D. PRATT, D.V.M., PH.D.[**]

INTRODUCTION

HISTORY AND SIGNIFICANCE

ANTIGENICITY AND EPIDEMIOLOGY
- Antigenic Relationships
- Epidemiology

STRUCTURE AND REPLICATION OF ALPHAVIRUSES
- Virion Structure
- Replication

PATHOGENESIS

CLINICAL DISEASE AND DIAGNOSIS
- Venezuelan Equine Encephalitis
- Eastern Equine Encephalitis
- Western Equine Encephalitis
- Differential Diagnosis of Alphavirus Encephalitis
- Medical Management and Prevention

IMMUNOPROPHYLAXIS
- Relevant Immune Effector Mechanisms
- Passive Immunization
- Active Immunization

SUMMARY

[*] *Chief, Department of Viral Biology, Virology Division*
[†] *Lieutenant Colonel, Veterinary Corps, U.S. Army; Chief, Experimental Pathogenesis, Pathology Division*

‡ *Department of Viral Pathogenesis and Immunology, Virology Division*
§ *Chief, Department of Cell Culture/Hybridoma, Virology Division*
¥ *Department of Viral Biology, Virology Division*
¶ *Department of Viral Biology, Virology Division*
** *Lieutenant Colonel, Veterinary Corps, U.S. Army; Chief, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

INTRODUCTION

During the 1930s, three distinct but antigenically related viruses were recovered from moribund horses and were shown to be previously unrecognized agents of severe equine encephalitis. Western equine encephalitis (WEE) virus was isolated in the San Joaquin Valley in California in 1930,[1] eastern equine encephalitis (EEE) virus in Virginia and New Jersey in 1933,[2,3] and Venezuelan equine encephalitis (VEE) virus in the Guajira peninsula of Venezuela in 1938.[4] By 1938, it was clear that EEE and WEE viruses were also natural causes of encephalitis in humans,[5–7] and naturally acquired human infections with VEE virus were documented in Colombia in 1952 in association with an equine epizootic.[8] Although these viruses cause similar clinical syndromes in horses, the consequences of the infections they cause in humans differ. EEE is the most severe of the arboviral encephalitides, with case fatality rates of 50% to 70% and with neurological sequelae common in survivors. WEE virus appears to be less neuroinvasive but has a pathology similar to that of EEE in patients with encephalitis. In contrast, severe encephalitis is rare in humans infected with VEE virus—except in children. In adults, the usual VEE syndrome is an acute, febrile, incapacitating disease with prolonged convalescence.

The three viruses under discussion in this chapter are all members of the *Alphavirus* genus of the family *Togaviridae*. As with all the alphavirus group, VEE, EEE, and WEE are transmitted in nature by mosquitoes and are maintained in cycles with various vertebrate hosts. Thus, the natural epidemiology of these viruses is controlled by environmental factors that affect the relevant mosquito and reservoir host populations and their interactions. Of the 28 viruses currently classified within this group, VEE, EEE, and WEE are the only viruses regularly associated with encephalitis. Although these encephalitic strains are restricted to the Americas, as a group, alphaviruses have worldwide distribution and include other epidemic human pathogens such as chikungunya virus (Asia and Africa), Mayaro virus (South America), O’nyong-nyong virus (Africa), Ross River virus (Australia), and Sindbis virus (Africa, Europe, and Asia). These viruses cause an acute febrile syndrome often associated with debilitating polyarthritic syndromes.

Although natural infections with the encephalitic alphaviruses are acquired by mosquito bite, the viruses are also highly infectious by aerosol. VEE virus has caused more laboratory-acquired disease than any other arbovirus. Since its initial isolation, at least 150 laboratory infections that have resulted in disease have been reported; most were known or thought to be aerosol infections.[9] Before vaccines were developed, most laboratories working with VEE virus

reported disease among their personnel. In one incident at the Ivanovskii Institute in Moscow, USSR, which was reported in 1959, at least 20 individuals developed disease, most within 28 to 33 hours, after an accident in which a small number of vials containing a minute amount of lyophilized virus were dropped and broken in a stairwell.[10,11]

Perhaps as a consequence of their adaptation to dissimilar hosts in nature, the alphaviruses replicate readily, and generally to very high titers, in a wide range of cell types and culture conditions in vitro. Virus titers of 1 billion infectious units per milliliter are not unusual, and the viruses are stable in storage and in a variety of laboratory procedures. Because of the relative ease with which these viruses can be manipulated in the laboratory, they have long served as model systems by which to study various aspects of virus replication, pathogenesis, induction of immune responses, and virus–vector relationships. As a result, the alphaviruses are well described and their characteristics well defined.[12,13]

Therefore, the collective in vitro and in vivo characteristics of alphaviruses, especially the equine encephalomyelitis viruses, lend themselves very well to weaponization. This fact was recognized by the designers of offensive biological warfare programs that were initiated before or during World War II.[14] Although other encephalitic viruses could be considered as potential weapons (eg, the tick-borne encephalitis viruses), few possess as many of the required characteristics for strategic or tactical weapons development as the alphaviruses:

- These viruses can be produced in large amounts in inexpensive and unsophisticated systems.
- They are relatively stable and highly infectious for humans as aerosols.
- Strains are available that produce either incapacitating or lethal infections.
- The existence of multiple serotypes of VEE and EEE viruses, as well as the inherent difficulties of inducing efficient mucosal immunity, confound defensive vaccine development.

The equine encephalomyelitis viruses remain as highly credible threats today, and intentional release as a small-particle aerosol, from a single airplane, could be expected to infect a high percentage of individuals within an area of at least 10,000 km^2. As a further complication, these viruses are readily amenable to genetic manipulation by modern reombinant deoxyribonucleic acid (DNA) technology. This capability is being used to develop safer and more effective vaccines,[15,16] but, in theory, could also be used to increase the weaponization potential of these viruses.

HISTORY AND SIGNIFICANCE

Descriptions of epizootics in horses, characterized by encephalitis and death and likely to have been caused by EEE virus, have been recorded as early as 1831 in Massachusetts.[17] However, it was not until the outbreaks of EEE in Delaware, Maryland, and Virginia in 1933 and 1934 that the virus was isolated, and not until a similar outbreak in North Carolina in 1935 that birds were suspected as the natural reservoir.[18] The initial isolation of EEE virus from a bird[19] and from *Culiseta melanura* mosquitoes,[20] the two major components of the EEE natural cycle, were both reported in 1951. Outbreaks of EEE virus have occurred in most eastern states and in southeastern Canada but have been concentrated along the eastern and Gulf coasts. Although only 211 cases of EEE in humans were reported[21] between 1938 (the first documented human cases[5]) and 1985, the social and economic impact of this disease has been larger than might be expected because of the high fatality rate, equine losses, extreme concern among individuals living in endemic areas during outbreaks, and the surveillance and mosquito-control measures required. Isolation of EEE virus from *Aedes albopictus* mosquitoes, which have recently been introduced into EEE endemic areas in the United States, has heightened concern because of the opportunistic feeding behavior of these mosquitoes as well as their apparent high vector competence for EEE virus.[22]

The initial isolation in 1930 of WEE virus from the brain tissues of a horse with encephalitis was made in the midst of a large and apparently unprecedented epizootic in California, which involved at least 6,000 horses and with approximate mortality of 50%.[1] Cases of human encephalitis in California were not linked to WEE until 1938, when the virus was isolated from the brain of a child. During the 1930s and 1940s, several other very extensive epizootics occurred in western and north-central states, as well as Saskatchewan and Manitoba, Canada, which affected large numbers of equines and humans. For example, it has been estimated that during 1937 and 1938, more than 300,000 equines were infected in the United States, and in Saskatchewan, 52,500 horse infections resulted in 15,000 deaths.[23,24] In 1941, unusually high numbers of human cases were reported: 1,094 in Canada and 2,242 in the United States. The attack rate in these epidemics ranged from 22.9 to 171.5 per 100,000, with case fatality rates of 8% to 15%.[24]

In the early 1940s, workers isolated WEE virus from *Culex tarsalis* mosquitoes[25] and demonstrated the presence of specific antibody to WEE virus in birds,[26] suggesting that birds are the reservoirs of the virus in nature. The annual incidence of disease in both equines and humans continues to vary widely, as would be expected of an arthropod-borne disease, and significant epidemics occurred in 1952, 1958, 1965, and 1975.[24]

The initial isolation of VEE virus was made during investigations of an epizootic occurring in horses in Venezuela in 1936, and the isolate was shown to be antigenically different from the EEE and WEE viruses isolated previously in the United States.[4,27] Over the next 30 years, many VEE outbreaks were

reported among horses, and it soon became apparent that humans were also infected in large numbers in association with these epizootics.[28] Most of those infected recovered after suffering an acute, febrile episode, but severe disease with encephalitis and death also occurred, mostly in children and older individuals. In the 1960s, major epizootics occurred in Venezuela, Colombia, Peru, and Ecuador, and apparently spread to Central America in 1969.[29] These and previous epizootics were associated with immeasurable human suffering, especially among rural people, who suffered not only from disease but also from the loss of their equines, which were essential for transportation and agriculture. Between 1969 and 1971, epizootics were reported in essentially all of Central America and subsequently continued north to Mexico and into Texas. The most recent major epizootic occurred in Venezuela and Colombia in 1995.[30]

Between active epizootics it was not possible to isolate the equine virulent viruses. During the 1950s and 1960s, however, several other antigenically different VEE strains were isolated from different geographical areas; these were attenuated in equines and persisted indefinitely in endemic areas. These enzootic strains could be differentiated antigenically not only among themselves but also from the epizootic strains,[31] they utilized different mosquito vectors than the epizootic strains,[32] and they utilized rodents as reservoir hosts.[33] Many of the enzootic strains, however, proved equally pathogenic for humans.

Therefore, within 30 years of the initial isolation of the EEE, WEE, and VEE viruses, an essentially accurate picture had emerged with respect to their endemic and epidemic behavior, arthropod vectors, reservoir hosts, and the diseases produced. Although not yet understood at the molecular level (this would come with the techniques of molecular biology that were developed during the 1970s and 1980s), these three viruses were reasonably well described as agents of disease, and the basic assays for their manipulation and production were known. The development of this body of knowledge occurred during the same period of war and political instability that fostered the establishment of biological warfare programs in the United States[34] and elsewhere, and it was evident that the equine encephalomyelitis viruses were preeminent candidates for weaponization. The viruses were incorporated into these programs for both potential offensive and defensive reasons. In 1969, the offensive biological warfare program in the United States was completely disestablished and all stockpiles destroyed[14] by executive order, which stated:

> The United States shall renounce the use of lethal biological agents and weapons and all other methods of biological warfare. The United States shall confine its biological research to defensive measures such as immunization and safety measures.[35]

However, defensive concerns remained, and efforts within the defensive program in the 1960s and 1970s produced four vaccines for the encephalomyelitis viruses: live-attenuated (TC-83) and formalin-inactivated (C84) vaccines for VEE, and formalin-inactivated vaccines for EEE and WEE. These

vaccines are used under Investigational New Drug (IND) status for at-risk individuals, are distributed freely under the provisions of the IND, and are recommended for use by any laboratory working with these viruses.[9] Although these vaccines have proven quite useful, they have certain disadvantages (which are discussed later in this chapter), and second-generation vaccines are under development.[16]

ANTIGENICITY AND EPIDEMIOLOGY

Antigenic Relationships

The American equine encephalitides comprise three virus complexes, VEE, EEE, and WEE, which, based on their serologic cross-reactivity, have been grouped with four additional virus complexes into the *Alphavirus* genus (Table 28-1).[13]

Table 28-1 is not shown due to copyright restrictions, please refer to the textbook.

Venezuelan Equine Encephalitis Virus Complex

The VEE virus complex consists of six closely related subtypes that manifest different characteristics with respect to ecology, epidemiology, and virulence for humans and equines (Table 28-2). The IA/B and C varieties are commonly referred to as *epizootic* strains. These strains have been responsible for extensive epidemics in North, Central, and South America and are highly pathogenic for humans and equines. All of the epizootic strains are exotic to the United States and have been isolated from natural foci in the world only once since 1973.[36] Subtypes II, III, IV, V, and VI and varieties ID, IE, and IF are referred to as the *enzootic* strains.[37–42]

Like the epizootic strains, the enzootic strains may cause disease in humans, but they differ from the epizootic strains in their lack of virulence for equines. The enzootic viruses are commonly isolated in specific ecological habitats, where they circulate in transmission cycles primarily involving rodents and *Culex* mosquitoes of the *Melanoconion* subgenus.[43–45] Infection of equines with some enzootic subtypes leads to an immune response capable of protecting the animals from challenge with epizootic strains.[46] Limited data, acquired following laboratory exposures, suggest that cross-protection between epizootic and enzootic strains may be much less pronounced in humans.[47–49]

TABLE 28-2
THE VENEZUELAN EQUINE ENCEPHALOMYELITIS COMPLEX

Subtype	Variety	Prototype Strain	Origin	Cycle	Disease in Horse	Disease in Man
I	A/B	Trinidad donkey	Donkey (Trinidad)[1]	Epizootic	+	+
	C	P-676	Horse (Venezuela)[2]	Epizootic	+	+
	D	3880	Human (Panama)[3]	Enzootic	-	+
	E	Mena II	Human (Panama)[1]	Enzootic	-	+
	F	78V-3531	Mosquito (Brazil)[4]	Enzootic	-	?
II (Everglades)		Fe3-7c	Mosquito (Florida)[5]	Enzootic	-	+
III (Mucambo)	A	Mucambo (BeAn8)	Monkey (Brazil)[6]	Enzootic	-	+
	B	Tonate (CaAn410-D)	Bird (French Guiana)[7]	Enzootic	-	+
	C	71D-1252	Mosquitoes (Peru)[8]	Enzootic	-	?
IV (Pixuna)		Pixuna (BeAn356445)	Mosquito (Brazil)[6]	Enzootic	-	?
V (Cabassou)		Cabassou	Mosquito (French Guiana)[7]	Enzootic	-	?
VI		Ag80-663	Mosquito (Argentina)[9]	Enzootic	-	+

Sources that contain original descriptions of or additional information about this strain: (1) Young NA, Johnson KM. Antigenic variants of Venezuelan equine encephalitis virus: Their geographic distribution and epidemiologic significance. *Am J Epidemiol.*1969;89:286. (2) Walton TE. Virulence properties of Venezuelan equine encephalitis virus serotypes in horses. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus, Washington, DC, 14–17 Sep 1971. Washington, DC: Pan American Health Organization; 1972:134. PAHO Scientific Publication 243. (3) Johnson KM, Shelokov A, Peralta PH, Dammin GJ, Young NA. Recovery of Venezuelan equine encephalomyelitis virus in Panama: A fatal case in man. *Am J Trop Med Hyg.*1968;17:432–440. (4) Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 4. Boca Raton, Fla: CRC Press; 1988: 203–231. (5) Chamberlain RW, Sudia WD, Coleman PH, Work TH. Venezuelan equine encephalitis virus from South Florida. *Science*. 1964;145:272. (6) Shope RE, Causey OR, de Andrade AHP, Theiler M. The Venezuelan equine encephalitis complex of group A arthopodborne viruses, including *Mucambo* and *Pixuna* from the Amazon region of Brazil. *Am J Trop Med Hyg*. 1964;13:723. (7) Karabastos N. *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*. 3rd. ed. San Antonio, Tex: American Society for Tropical Medicine and Hygiene; 1985. (8) Scherer WF, Anderson K. Antigenic and biological characteristics of Venezuelan encephalitis virus strains including a possible new subtype isolated from the Amazon region of Peru in 1971. *Am J Epidemiol*. 1975; 101:356. (9) Contigiani MS, De Basualdo M, Camara A, et al. Presencia de anticuerpos contra el virus de la encefalitis equina Venezolana subtipo VI en pacientes con enfermedad aguda febril [in Spanish]. *Revista Argentina de Microbiologia.* 1993;25:212-220.
Adapted with permission from Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology.* Vol 4. Boca Raton, Fla: CRC Press; 1989:206.

Eastern Equine Encephalitis Virus Complex

The EEE virus complex consists of viruses in essentially two antigenically distinct forms: the North American and the South American variants.[50] The two forms can be distinguished readily by hemagglutination-inhibition (HI) tests. All North American and Caribbean isolates show a high degree of genetic and antigenic homogeneity; they are distinct from the South American and Central American isolates, which tend to be more heterogeneous.[51,52]

EEE is endemic to focal habitats ranging from southern Canada to northern South America. The virus has been isolated as far west as Michigan but is most common along the eastern coast of the United States between New England and Florida. Enzootic transmission of EEE virus occurs almost exclusively between passerine birds (ie, the perching songbirds) and the mosquito *Culiseta melanura*. Because of the strict ornithophilic feeding behavior of this mosquito, human and equine disease requires the involvement of more general feeders, known as bridging vectors, such as members of the genera *Aedes* and *Coquilletdia*. Mosquito vectors belonging to *Culex* species are thought to play a role in maintaining and transmitting South American EEE strains.[53]

Western Equine Encephalitis Virus Complex

Six viruses—WEE, Sindbis, Y 62-63, Aura, Fort Morgan, and Highlands

J—make up the WEE complex. Several antigenic subtypes of WEE virus have been identified, but their geographical distributions overlap.[36] Most of the members of the WEE complex are distributed throughout the Americas, but subtypes of Sindbis virus and Y 62-33 have strictly Old World distributions.[13] The New World WEE complex viruses can be distinguished readily by neutralization tests. In addition, WEE complex viruses isolated in the western United States (ie, WEE) are antigenically and genetically distinct from those commonly found in the eastern United States (ie, Highlands J).[52,54]

The best studied member of the WEE virus complex—in terms of its epidemiology—is WEE virus itself. The virus is maintained in cycles involving passerine birds and the mosquito *Culex tarsalis*. Humans (and equines) become involved only tangentially and are considered to be dead-end hosts,[55] indicating that they do not normally contribute to further spread of the virus in nature. Recent studies have isolated WEE virus from male *Aedes dorsalis* mosquitoes reared in the laboratory from larvae collected in salt marsh habitats.[56] These data indicate that vertical transmission (ie, direct transmission from one generation to the next) in mosquitoes may be an important mechanism for persistence and overwintering in endemic areas.

Epidemiology

The epidemiology of the equine encephalitides in humans is closely tied to the ecology of these viruses in naturally occurring endemic foci. Most commonly, human involvement occurs following intrusion into geographical regions where natural transmission cycles are in progress, or following perturbation of those cycles by environmental changes[57] or the addition of other vectors.[22] The dramatic exception to this is epizootic VEE, in which the spreading waves of the epizootic among equines can move rapidly over large distances, and humans become infected by mosquitoes that have fed on viremic equines. The high levels of viremia in equines infected with epizootic VEE make them efficient amplifying hosts, with the result that equine infections normally precede human infections by days to weeks.[58] Medical officers should view with some suspicion evidence of widespread human VEE infections outside of endemic areas, in the absence of mosquito vectors, or in the absence of equine disease, as this combination of circumstances could indicate an unnatural release of virus into the environment.

Enzootic VEE virus subtypes, as described above, are maintained quite efficiently in transmission cycles involving mainly mosquitoes belonging to the subgenus *Melanoconion*. These mosquitoes often occur in very humid localities with abundant open spaces—such as sunny, swampy pastures cut by slowly flowing streams. The mosquitoes are ground feeders, seldom found higher than 8 m above ground, and prefer feeding on mammals rather than birds.[59] In part because their ecologies are similar to that of the mosquito vector's, ground-dwelling rodents serve as the primary vertebrate hosts for the enzootic forms of VEE virus. Following infection, these animals develop viremias of sufficient magnitude and duration to infect mosquitoes during their acquisition of a blood meal.[60] Other animals such as bats and certain birds may play a secondary

role.[61]

Seroprevalence rates among human populations living in or near endemic VEE areas vary but can approach 100%, suggesting that continuous transmission occurs.[58] However, virus activity within endemic zones can also be highly focal. In one incident at the Fort Sherman Jungle Operations Training Center in the Panama Canal Zone in December 1967, 7 of 12 U.S. soldiers camped in one area developed VEE disease within a 2-day period, but another group camped only a few yards away showed no disease.[62,63]

The incidence of disease during epizootics also varies, but it is often very high. During an outbreak in Venezuela, attack rates of 119 per 1,000 inhabitants per month were reported.[64] Following an epizootic in Guatemala and El Salvador, overall seroprevalence was estimated at 20%.[65]

Unlike the enzootic strains, the fate of the epizootic strains during interepidemic periods is unclear. Of several theories on how epizootic strains arise, the most appealing suggests that they evolve by genetic drift from enzootic strains. Results from oligonucleotide fingerprinting and sequence analysis of I-D isolates from Colombia and Venezuela reveal a close similarity to the epizootic strains, suggesting that the equine virulent epizootic strains arise naturally from variants present in populations of I-D virus.[66,67]

While the genetic evidence suggests that genetic drift of enzootic strains may lead to the development of epizootic strains, ecological data suggest that there is also a strong selective pressure to maintain the enzootic genotype in certain habitats. The enzootic VEE vector *Culex (Melanoconion) taeniopus* is fully susceptible to both I-AB and I-E strains following intrathoracic inoculation. Orally exposed mosquitoes, however, are fully competent vectors of the enzootic strain, but they fail to develop disseminated infection and transmit epizootic virus.[32,68] This observation suggests that genetic drift of enzootic strains may be selected against with this combination of vector and virus. Mosquito resistance to epizootic strains of VEE virus is rare; epizootic strains have been isolated from a large number of mosquito species, and many have been shown to be efficient vectors.[69] Thus, host switching from enzootic to epizootic vectors may be an important factor in the evolution of epizootic VEE strains. The introduction of mosquito species into previously unoccupied geographical ranges (eg, *Aedes albopictus* into North America) may, therefore, offer the opportunity for epizootic strains to reemerge.

A major outbreak of epizootic VEE occurred in the late 1960s and early 1970s. Epizootic virus first reached North America in 1966 but did not reach the United States until 1971. Studies of this epizootic demonstrated that the virus easily invaded territories in which it was formerly unknown,[64] presumably as a result of (1) the availability of large numbers of susceptible equine amplifying hosts and (2) the presence of competent mosquito vectors. The initial outbreak in North America, and the first recorded such epizootic, occurred in 1966 in Tampico, Mexico, involving approximately 1,000 equines. By the end of 1969 and the beginning of 1970, the outbreak had expanded to such an extent that theMexican government requested the TC-83 vaccine from the U.S. Army through the U.S. Department of Agriculture.[70] Despite the immunization of nearly 1 million

equines, the epizootic continued to spread and reached the United States in June 1971. The nature of the virus and the number of human and equine cases prompted the secretary of agriculture to declare a national emergency on July 16, 1971.[71] Subsequent immunization of over 2 million horses and unprecedented mosquito abatement efforts eventually stopped the epizootic before it was able to spread from Texas. Epizootic VEE has not been isolated in the United States since the 1971 outbreak.

The first large outbreak since the 1969–1971 epizootic occurred in 1995 (Figures 28-1 and 28-2). The epizootic began in northwestern Venezuela and spread across the Guajira peninsula into northeastern Colombia. An estimated 75,000 to 100,000 humans were infected, with more than 20 deaths reported. This outbreak was caused by an IC strain of VEE virus. By sequence analysis, this strain proved to be essentially identical to a virus that caused an outbreak in Venezuela in 1963.[30]

Fig. 28-1. This photograph was taken in 1995 near Buena Vista, Colombia. During large Venezuelan equine encephalitis (VEE) epizootics, typical morbidity rates among unvaccinated equines are 40% to 60%, with at least half of the affected animals progressing to lethal encephalitis. Note the disruption of the ground surface, which is caused by the characteristic flailing or swimming syndromes of moribund animals. Although clinically indistinguishable from the syndromes produced by eastern equine encephalitis (EEE) and western equine encephalitis (WEE) viruses, the capability of VEE to initiate explosive and rapidly expanding epizootics makes reliable diagnostic tests essential for the initiation of appropriate veterinary and public health measures.

Fig. 28-2. This photograph was taken in 1995 near Maicao, Colombia. Equine vaccination is the most effective means available to prevent Venezuelan equine encephalitis (VEE) epizootics as well as to control emerging outbreaks. Equines are the major amplifying hosts, and maintaining a high rate of immunity in the equine population will largely prevent human infection with the epizootic strains of VEE. Both inactivated and live-attenuated vaccines are available for veterinary use, but the ability of the live-attenuated vaccine to induce immunity in 7 to 10 days with a single inoculation makes it the only practical vaccination

strategy in the face of an outbreak. Other measures used to control outbreaks include using insecticides to reduce mosquito populations and prohibiting the transportation of equines from affected areas.

STRUCTURE AND REPLICATION OF ALPHAVIRUSES

Virion Structure

The alphavirus virion, a spherical particle approximately 60 to 65 nm in diameter, is typically composed of three different structural proteins enclosing a single molecule of single-stranded RNA. The RNA genome is packaged within an icosahedral nucleocapsid, which is constructed from multiple copies of a single species of capsid (C) protein (Figure 28-3). The nucleocapsid is, in turn, surrounded by a lipid envelope that is derived from areas of the host cell plasma membrane that had previously been modified by the insertion of two viral glycoproteins. These envelope glycoproteins, E1 and E2, form heterodimers that associate further into trimers[72,73] to form the short spikes on the surface of the virion. The glycoproteins are the primary targets of the neutralizing antibody response[74] and are the determinants of tropism and virulence.[75] Semliki Forest virus contains a third glycoprotein, E3, which is associated with the E1–E2 dimers on the virion surface. With other alphaviruses, the E3 protein is shed from the infected cell and does not appear in the mature virion.

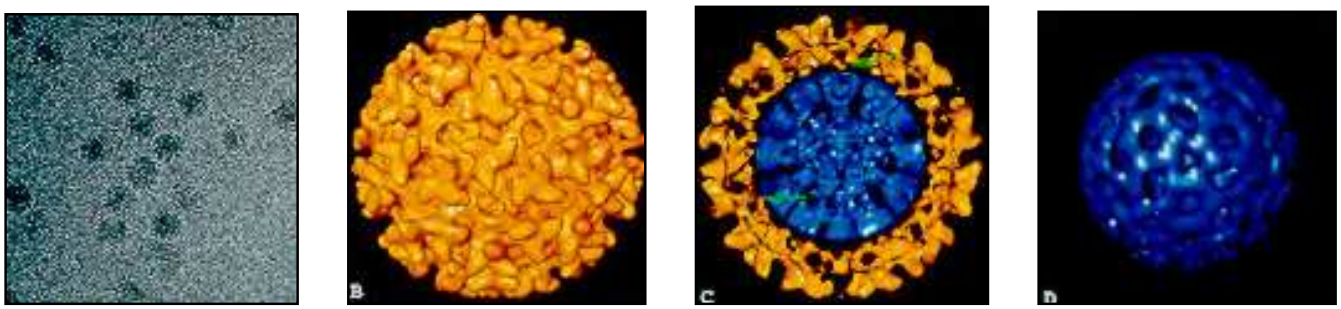


Fig. 28-3. Structure of an alphavirus. Shown is the three-dimensional reconstruction of Sindbis virus at 28 Å resolution from computer-processed images taken by electron cryomicroscopy. **(a)** The original electron micrograph shows virus particles in vitreous ice. **(b)** The surface view of the virus shows details of the 80 trimeric spikes, which are arranged in a T=4 icosahedron. Each spike protrudes 50 Å from the virion surface and is believed to be composed of three E1–E2 glycoprotein heterodimers. **(c)** The cross-sectional view shows the outer surface spikes (yellow) and the internal nucleocapsid (blue), composed of the capsid and viral RNA. The space between the spikes and the nucleocapsid would be occupied by the lipid envelope. The green arrows mark visible points of interaction between the nucleocapsid and transmembranal tails of the glycoprotein spikes. **(d)** The reconstructed capsid also exhibits a T=4 icosahedral symmetry. Computer models: Courtesy of Angel M. Paredes, Cell Research Institute and Department of Microbiology, The University of Texas at Austin, Austin, Tex. Similar but not identical versions of these computer models were published in Paredes AM, Brown DT, Rothnagel R, et al. Three-dimensional structure of a membrane-containing virus. *Proc Natl Acad Sci USA*. 1993; 90:9095–9099.

Replication

Viral Infection. The infection cycle is initiated when the glycoprotein spikes on the virion bind to receptors on the cell surface. The virus is localized initially to coated pits, where it is engulfed in a coated vesicle and transported to the endosomal compartment within the interior of the cell. A decrease in the pH in the interior of the vesicle induces a conformational change in the glycoprotein spikes, and rearrangement of the E1 glycoprotein mediates fusion of the virion envelope with the endosomal membrane.[76] This fusion results in the release of the nucleocapsid into the cytoplasm, where disassembly of the nucleocapsid releases the viral RNA genome to the synthetic apparatus of the cell.

Genomic RNA. The viral genome is a positive-stranded RNA of approximately 11,700 nucleotides and has the structural features of messenger RNA (ie, mRNA, a 5′ methylated cap [m7GpppA] and a poly-A tract at the 3′ end).[77] As a complete and functional mRNA, genomic RNA purified from virions is fully infectious when artificially introduced (ie, transfected) into susceptible cells. Similarly, RNA transcribed from a full-length complementary DNA (cDNA) clone of an alphavirus is also infectious, and it is this property that allows genetic manipulation of these viruses. Mutations introduced into a cDNA clone by site-directed mutagenesis will be reflected in the RNA transcribed from the altered clone and in the virus obtained from transfected cells. These procedures are being utilized to develop improved vaccines,[16] but conceivably could be used also to enhance specific characteristics required for weaponization.

Glycoprotein Synthesis. The alphavirus genome contains two protein coding regions. The 5′ proximal 7,500 nucleotides encode a 220,000-dalton precursor polypeptide, which is proteolytically processed to produce four components of the viral RNA polymerase. The polymerase genes are followed by a second coding region of approximately 3,800 nucleotides, which contains the information that directs the synthesis of the viral structural proteins. Soon after release of the viral genome from the nucleocapsid, the 5′ 7,500 nucleotides of the genome RNA are translated to produce the viral RNA polymerase. Early in infection, the incoming viral genome is also utilized as a template for the synthesis of a negative-stranded 45S RNA, identical in length to the genome RNA but of opposite polarity. The negative-stranded 45S RNA subsequently serves as a template for the synthesis of additional genomic RNA. The negative-stranded RNA is also utilized as a template for transcription of a 26S subgenomic mRNA, which is identical to the 3′ third of the genome. The 26S RNA is capped and polyadenylated, and is then translated to yield a precursor polypeptide that is proteolytically processed by cotranslational and posttranslational cleavages to produce the viral structural proteins. The order of the structural proteins within the precursor is C-E3-E2-6K-E1.

As the 26S mRNA is translated, the C protein is produced first and catalyzes its own cleavage from the nascent polypeptide soon after the ribosome transits into the sequences that encode E3. After release of the C protein, the free amino terminus of E3 is bound to the membranes of the rough endoplasmic reticulum. As the synthesis of nascent E3 and E2 continues, the polypeptide is translocated into the lumen of the endoplasmic reticulum, where oligosaccharides and fatty acids are added.[78] A domain of hydrophobic amino acids near the carboxyl terminus of E2 inhibits further transmembranal movement so that the last 30 to 40 amino acids of the E2 polypeptide remain exposed on the cytoplasmic side of the membrane. It is thought that the 6K polypeptide serves as an internal signal for membrane insertion of the second glycoprotein, E1, and is subsequently cleaved from

both E2 and E1 by the signal peptidase.[79] A hydrophobic anchor sequence present near the carboxyl terminus of E1 secures the protein in the membrane.

Budding and Release of Progeny Virus Particles. Soon after synthesis, the precursor E2 (pE2) and E1 interact to form multimeric complexes,[80] which are then transported through the Golgi apparatus, where the final modifications of the oligosaccharide are made. The precursor pE2 is cleaved to the mature E2 and E3 glycoproteins soon after the glycoproteins leave the Golgi apparatus,[81] and the mature viral spikes assume an orientation in the plasma membrane with the bulk of the E2 and E1 polypeptides exposed on the exterior surface of the cell. Final assembly, or budding, of progeny virus particles takes place exclusively at the plasma membrane in vertebrate cells,[82] whereas in arthropod cells, budding can also occur at intracellular membranes.[83]

Budding is initiated when intracellular nucleocapsids bind to the 30– to 40–amino acid cytoplasmic domain of the E2 glycoprotein,[84–86] inducing the formation of a locally ordered array of glycoprotein spikes and excluding most of the cellular membrane proteins from the region. Additional lateral associations between the individual spikes stabilize the lattice and promote additional E2–C protein interactions. The growing lattice is thought to draw the membrane around the nucleocapsid, completing the process of envelopment with the release of the spherical virus particle.

Maximal amounts of virus are typically produced from mammalian cells within 8 to 10 hours after infection, and disintegration of the infected cell is likely due to programmed cell death (apoptosis) rather than direct effects of the virus on cellular function.[87] In contrast, alphaviruses initially replicate to high titer in arthropod cells with little or no evidence of cytopathology. The surviving cells continue to produce lesser amounts of virus, often for weeks or months. The ability of the virus to replicate without causing cell death in arthropod cells may be critical for maintenance of the virus in the mosquito vector in nature.

PATHOGENESIS

In humans, the pathogenesis of VEE, EEE, and WEE infections acquired by aerosol—the route of biological defense concern—is unknown. Indeed, little is known of the pathogenesis following natural vector-borne infections of humans, mainly because of the limited availability of autopsy material. Much of the information on VEE pathogenesis in humans is based on a histological review of 21 human fatalities from the 1962–1963 VEE epidemic in Zulia, Venezuela.[88] With few exceptions, the histopathological lesions in these cases were comparable to those observed in experimentally infected animals. Tissues that were commonly affected in both humans[88] and animals[89–97] include those of the lymphoid and reticuloendothelial systems and the central nervous system (CNS). Interestingly, wide-spread hepatocellular degeneration and interstitial pneumonia, not ordinarily seen in experimental animals, were frequent histological findings in these cases of severe human disease.

The clinical and pathological responses of the host to VEE infection are highly dependent on a number of host and viral factors. These factors include

- the species, immune status, and age of the host animal;
- the route of infection; and
- the strain and dose of virus.

Most of the existing experimental data have come from studies using rodent models challenged subcutaneously with the Trinidad donkey (TrD) strain of VEE, an epizootic IA serotype virus. The lymphatic system and the CNS appear to be universal target organs in experimental animal models, as was seen in humans. However, the relative degree of injury caused by the TrD strain of VEE to these tissues varied among the species. TrD caused only mildly severe and reversible lesions to the lymphoid organs in the mouse and monkey,[89,90] but was extremely destructive and irreversible to those organs in the guinea pig[90] and hamster.[89,90,94,97] The virus causes lymphatic necrosis within the nodes associated with the gut; normal gut flora escape, leading to systemic bacterial infections. The severity of the viral infection in the lymphoreticular tissues (in particular the Peyer's patches of the distal intestine in hamsters) appears to contribute to the bacteremia and endotoxic shock syndrome that lead to early death.[97]

The effects of virus infection with the TrD strain of VEE on the CNS also demonstrated considerable species variability. Mice exhibited a severe paralytic episode prior to death from diffuse encephalomyelitis.[89,90] Monkeys, however, showed few if any clinical signs of CNS involvement following peripheral inoculation, and only modest pathological changes in the CNS (found mainly in the thalamus, hypothalamus, and olfactory areas of the brain).[90] However, the extent of neuroinvasion in animals is also a function of both the strain of VEE and the route of infection. Cynomolgus monkeys infected by the intranasal route developed immunoglobulin (Ig) M and IgG antibodies in the cerebrospinal fluid (CSF) and showed moderate areas of perivascular cuffing and nodular and diffuse gliosis, especially in the cortex and hypothalamus.[98] A Colombian epizootic strain of VEE given by the aerosol route caused severe clinical and pathological CNS signs and resulted in death in approximately 35% of rhesus monkeys.[91] Mice and cynomolgus monkeys challenged intracerebrally with TrD or a serologically related strain of VEE developed severe and lethal neurological signs with moderate to severe brain histopathology.[98]

VEE virus can infect the CNS directly through the olfactory nervous system. In rhesus monkeys intranasally inoculated with VEE virus, the virus gained access to the olfactory bulb within 24 hours after infection and before the onset of viremia, suggesting direct neuroinvasion via olfactory neurons.[99] However, in inoculated monkeys whose olfactory nerves had been surgically removed, VEE virus was nonetheless able to reach the olfactory bulb by 36 hours after infection, presumably by the vascular route. Although the olfactory bulb and tract were sites of early viral replication, viral infection did not appear to spread to the rest of the brain along the neural tracts in these monkeys. In 1991, researchers[100] concluded from studies carried out in outbred mice that aerosolized VEE virus can enter the CNS of nonimmunized mice by both the vascular and the olfactory pathways. Morphologic evidence of virus multiplication was observed in olfactory epithelial cells and in secretory cells of Bowman's glands. These researchers suggest two possible routes

for viral spreading from the nasal mucosa: (1) virus entry into the blood stream through the fenestrated capillaries of the olfactory zone, with subsequent systemic infection; and (2) axonal transport along the olfactory nerves, allowing direct virus entry into the olfactory bulbs.

In more recent studies, BALB/c mice were challenged either subcutaneously or by aerosol with the V3000 strain[16] of VEE, and brain and nasal tissues taken from animals sacrificed at daily intervals were examined immunocytochemically for viral antigen.[101] In mice challenged by aerosol, both the nasal olfactory epithelium and the olfactory nerve axon bundles in the underlying connective tissue were immunoreactive for VEE virus antigen within 24 hours after infection (Figure 28-4). Within 48 hours after infection, olfactory nerves, nasal-associated lymphoid tissue (NALT), and olfactory bulbs were immunoreactive (Figure 28-5). In a bilaterally symmetrical pattern, the prepiriform area and the piriform cortex were also immunoreactive by 48 hours after infection. Other areas of the brain were immunoreactive at 4 days after infection. In subcutaneously challenged mice, the olfactory nerves and nasal olfactory epithelium were not immunoreactive at any time, and the olfactory bulbs, prepiriform area and piriform cortex, and NALT were not positive until 3 days after infection. By day 4 after infection, subcutaneously challenged mice also had immunoreactive cells in other areas of the brain. These immunocytochemical findings are consistent with previous studies carried out in rodents and primates; they indicate not only that aerosolized VEE virus may enter the mouse brain by means of the olfactory nerves but also that this process is very rapid. The efficiency with which this process occurs with the equine encephalomyelitis viruses will put very high demands on the vaccines used for immunoprophylaxis (vaccines are discussed later in this chapter).

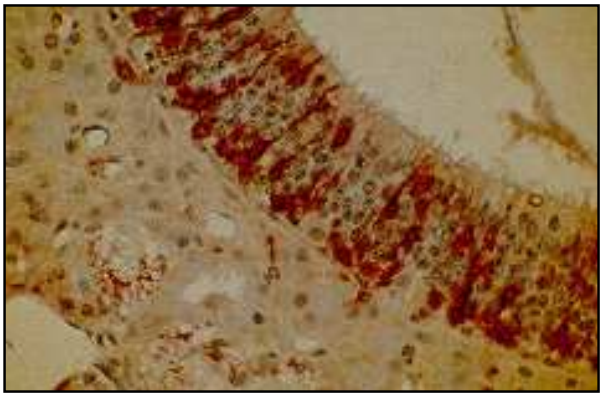


Fig. 28-4. Nasal tissue, BALB/c mouse, 2 days after exposure to aerosolized VEE virus. Note immunoreactive olfactory epithelium and olfactory nerves. Alkaline phosphatase-labeled streptavidin method using rabbit antiserum to VEE virus (Mayer's hematoxylin counterstain, original magnification × 300).

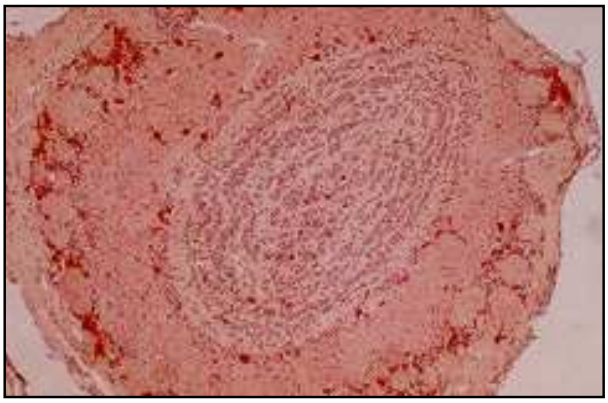


Fig. 28-5. Olfactory bulb, BALB/c mouse, 2 days after exposure to aerosolized VEE virus. Note immunoreactive cells. Alkaline phosphatase-labeled streptavidin method using rabbit antiserum to VEE virus (Mayer's hematoxylin counterstain, original magnification × 150).

CLINICAL DISEASE AND DIAGNOSIS

The three equine encephalomyelitis virus complexes within the *Alphavirus* genus—EEE, WEE, and VEE—are also recognized for their potential for neuroinvasion and encephalitis in humans, sometimes in epidemic proportions. However, many of the infections caused by these viruses are manifested as systemic viral febrile syndromes, and infections by EEE and WEE viruses may remain subclinical. Furthermore, these alphaviruses vary markedly in both their neurotropism and the severity of their neurological sequelae. Depending on the virus, patients presenting with the general syndrome of alphavirus encephalitis have a varying combination of fever, headache, confusion, obtundation, dysphasia, seizures, paresis, ataxia, myoclonus, and/or cranial nerve palsies.

Venezuelan Equine Encephalitis

The IA, IB, and IC variants of VEE virus are pathogenic for equines and have the capacity for explosive epizootics with epidemic human disease. Epidemics of VEE affecting 20,000 to 30,000 people, or more, have been documented in Venezuela and Ecuador. In contrast to the other alphavirus encephalitides, EEE and WEE, epizootic strains of VEE are mainly amplified in equines, rather than birds, so that equine disease normally occurs prior to reports of human disease. Enzootic VEE strains (variants ID, IE, and IF and subtypes II, III, IV, V, and VI) are not recognized as virulent for equines, but disease has been documented with most of these variants in humans who reside in or move into enzootic foci, or after laboratory infections (see Table 28-2). The resulting syndromes appear to be similar, if not indistinguishable, from the syndrome produced by epizootic variants, which ranges from undifferentiated febrile illness to fatal encephalitis.

Following an incubation period that can be as short as 28 hours[10] but is usually 2 to 6 days, patients typically develop a prostrating syndrome of chills, high fever (38°C–40.5°C), headache, and malaise.[102] Photophobia, sore throat, myalgias, and vomiting are also common symptoms. Frequent signs noted on physical examination include conjunctival injection, erythematous pharynx, and muscle tenderness. Although essentially all human infections with VEE virus are symptomatic,[62,63] only a small percentage manifest neurological involvement.[103] In one epidemic, it was estimated that the ratio of encephalitis to infections is less than 0.5% in adults, although possibly as high as 4% in children.[104] Mild CNS involvement is evidenced by lethargy, somnolence, or mild confusion, with or without nuchal rigidity.[105] Seizures, ataxia, paralysis, or coma herald more severe CNS involvement. In children with overt encephalitis, case fatalities range as high as 35% compared with 10% for adults.[106] However, for those who survive encephalitic involvement, neurological recovery is usually complete.[107] School-age children are believed to be more susceptible to a fulminant form of disease, in which depletion of lymphoid tissues is prominent and which follows a lethal course over 48 to 72 hours.[88,108,109]

In the first 3 days of illness, leukopenia and elevated serum glutamic-oxaloacetic transaminase (SGOT) are common. For those with CNS involvement,

a lymphocytic pleocytosis of up to 500 cells per microliter will be observed in the CSF. The CSF pleocytosis may acutely be polymorphonuclear but soon becomes predominantly lymphocytic.

Specific diagnosis of VEE can be accomplished by virus isolation, serologic testing, or both.[110] During the first 1 to 3 days of symptoms of nonspecific febrile illness, VEE virus may be recovered from either the serum or the nasopharynx.[111] Despite the theoretical possibility of person-to-person transmission of virus present in the nasopharynx, no evidence of such occurrences has been reported. Identification of the VEE subtype of an isolate involved can be accomplished by cross-neutralization tests. HI, enzyme-linked immunosorbent assay (ELISA), or plaque reduction neutralization (PRN) antibodies appear as viremia diminishes. Complement-fixing (CF) antibodies make their appearance later during convalescence. VEE IgM antibodies are present in acute phase sera,[63] and it has been reported that the VEE IgM tests do not react with sera from patients with EEE or WEE.[112] Since patients with encephalitis typically come to evaluation later in the course of clinical illness, virus is recovered less often from them,[112] and they usually have serum antibody by the time of clinical presentation.[113]

Immunity after infection is probably lifelong to the homologous serotype, but cross-immunity is weak or nonexistent to heterologous serotypes.[47–49] Thus, when viewed either as an endemic disease threat or as a potential biological warfare threat, adequate immunization will require polyvalent vaccines.

Eastern Equine Encephalitis

EEE is maintained in a natural transmission cycle between *Culiseta melanura* mosquitoes and passerine birds in swampy and forested areas. EEE outbreaks are typically recognized when severe equine or human encephalitis occurs near such areas.[114] During vector-borne EEE epidemics, the incidence of human infection is low (< 3% of the population at risk),[115] and the neurological attack rate in one outbreak was estimated as 1 in every 23 cases of human infection.[116] However, the effect on morbidity and mortality of aerosol-acquired EEE infection (which would be the expected route of infection in a biological warfare offensive) is unknown. The incubation period in humans varies from 5 to 15 days. Adults typically exhibit a febrile prodrome for up to 11 days before the onset of neurological disease[117]; however, illness in children exhibits a more sudden onset.[118] Viremia occurs during the febrile prodrome,[119] but is usually undetectable by the time clinical encephalitis develops, when HI and neutralizing antibodies become evident.[120] Despite the development of a prompt and neutralizing humoral response, virus is not eradicated from the CNS, and progressive neuronal destruction and inflammation continue.

EEE is the most severe of the arboviral encephalitides, with high mortality and severe neurological sequelae.[121] During outbreaks of EEE, the attack, morbidity, and fatality rates are highest in young children[122] and the elderly.[123] Case fatality rates are estimated to be from 50% to 75%, but asymptomatic infections and milder clinical illness are certainly underreported. The illness is characterized by

rapid onset of high fever, vomiting, stiff neck, and drowsiness. Children frequently manifest generalized, facial, or periorbital edema. Motor involvement with paresis is common during the acute phase of the illness. Major disturbances of autonomic function, such as impaired respiratory regulation or excess salivation, may dominate the clinical picture. Up to 30% of survivors are left with neurological sequelae such as seizures, spastic paralysis, and cranial neuropathies. Cognitive impairment ranges from minimal brain dysfunction to severe dementia.

Clinical laboratory findings in patients with EEE often demonstrate an early leukopenia followed by a leukocytosis. Elevated opening pressure is commonly noted on lumbar puncture, and in children, especially, the CSF lymphocytic pleocytosis may reach a cell count of thousands of mononuclear cells per microliter. Specific diagnosis of EEE depends on virus isolation or serologic testing in which rising titers of HI, CF, or neutralizing antibodies are observed. IgM antibodies are usually detectable in acute-phase sera.[112] As with other alphaviruses, neutralization tests are considered to be the most specific.

Western Equine Encephalitis

Like VEE, WEE is less virulent for adult humans than it is for equines and children, with lower rates of fatalities and neurological sequelae.[124] As with EEE, infants and the elderly are especially susceptible to severe clinical illness and neurological sequelae, with case fatality rates of about 10%. Highlands J (HJ) virus, an antigenically related member of the WEE complex that is isolated frequently in the *eastern* United States, rarely infects humans.

The incubation period is 5 to 10 days. A large percentage of patients with vector-borne infections are either asymptomatic or present with a nonspecific febrile illness or aseptic meningitis. The ratio of encephalitis cases per infection has been estimated to vary from 1:1,150 in adults, to 1:58 in children, to 1:1 in infants.[57] However, the severity of the syndrome and the incidence of inapparent infection almost certainly depend on the strain and dose of virus and the route of infection. Some unusual isolates show very high virulence in laboratory animals,[125] and in one study of laboratory-acquired infections in adults, 2 of 5 patients died.[126] Symptoms usually begin with malaise, headache, and fever, followed by nausea and vomiting.[127] Over the next few days the symptoms intensify, and somnolence or delirium may progress into coma. The severity of neurological involvement is inversely related to age, with more than 90% of children younger than 1 year old exhibiting focal or generalized seizures.[128] Physical examination typically reveals nuchal rigidity, impaired sensorium, and upper motor neuron deficits with pathologically abnormal reflexes.

Patients with the severest infections usually die within the first week of clinical illness, with over-all case fatalities averaging 10%. Other patients begin a gradual convalescence after the first week of encephalitic symptoms. Most adults recover completely, but may take months to years to recuperate from fatigability, recurrent headaches, emotional lability, and impaired concentration.[129] Some patients are left with permanent residua of motor weakness, cognitive deficits, or

a seizure disorder. Children carry a higher incidence of neurological sequelae, ranging from less than 1% in those older than 1 year old, to 10% in infants 2 to 3 months old, to more than 50% in newborns. Congenital infection in the last trimester of pregnancy has been described, with resultant encephalitis in the infants.[130]

Viremia is rarely detectable by the time patients present with encephalitic symptoms, but IgM, HI, and neutralizing antibodies can generally be found by the end of the first week of illness, and they increase in titer during the next week.[112,131,132] CF serologic responses generally appear in the second week and rise thereafter. Isolation of virus or 4-fold titer rises are diagnostic, but because of serologic cross-reactions with other alphaviruses, neutralization tests are preferred. Examination of the CSF reveals a lymphocytic pleocytosis ranging from 10 to 400 mononuclear cells per microliter. WEE virus may occasionally be isolated from the CSF or throat swabs taken within the first 2 days of illness, and is frequently recovered from brain tissue on postmortem examination.[133] Natural infection presumably confers long-term immunity.

Differential Diagnosis of Alphavirus Encephalitis

Most acute infections with VEE and WEE produce a moderately severe but nonspecific clinical illness consisting of fever, headache, and myalgias. Therefore, in a potential biological warfare scenario, alphaviruses should be considered in the differential diagnosis whenever epidemic febrile illness occurs, especially if a number of patients progress to neurological disease. Sick or dying equines in the vicinity of an epidemic febrile illness among troops should immediately suggest the possibility of large-scale alphavirus exposure. Other potential biowarfare agents that may infrequently produce or imitate a meningoencephalitic syndrome include *Brucella* species, *Yersinia pestis, Salmonella typhi, Coxiella burnetii*, and botulinum toxin. As with any diagnosis of meningoencephalitis, it is imperative to rule out any potential cause that may be specifically treatable.

TABLE 28-3
SOME IMPORTANT VIRAL CAUSES* OF ENDEMIC ENCEPHALOMYELITIS

Virus Family	Genus	Species
Togaviridae	Alphavirus	Eastern equine Western equine Venezuelan equine

Flaviviridae	St. Louis Murray Valley West Nile Japanese Dengue Tick-borne complex	
Bunyaviridae	LaCrosse Rift Valley Toscana	
Paramyxoviridae	Paramyxovirus Morbillivirus	Mumps Measles
Arenaviridae	Arenavirus	Lymphocytic choriomeningitis Machupo Junin
Picornaviridae	Enterovirus	Poliovirus Coxsackievirus Echovirus
Reoviridae	Colorado tick fever	
Rhabdoviridae	Rabies	
Herpesviridae	Herpesvirus	Herpes simplex virus types 1 and 2 Epstein-Barr virus Cytomegalovirus
Adenoviridae	Adenovirus	

*Not all-inclusive

For encephalitis cases that are more sporadic in their occurrence, other important viral etiologies that might not be readily discriminated from the alphaviruses by clinical features are listed in Table 28-3. This list is not all-inclusive but suggests other viral encephalitides that should be considered if a patient presents, a priori, with an encephalitic syndrome. Epidemiological, historical, and laboratory information remain critical to differential diagnosis. Immediate and careful consideration must be given to treatable infections that may mimic viral encephalitis (Exhibit 28-1), since prompt and appropriate intervention can be lifesaving. In addition, it should be kept in mind that vascular, autoimmune, and neoplastic diseases may imitate infectious

meningoencephalitis.

For endemic meningoencephalitic disease that occurs outside biowarfare theaters, the geographical locale and the patient's travel history are of pre-eminent importance in diagnosing an arboviral encephalitis. Risk for disease is increased relative to the patient's amount of arthropod contact near swampy or forested areas during the summer. Encephalitic illness of equines in the surrounding locale is an important indication of ongoing transmission of encephalitic alphaviruses. Examination of the CSF, to include viral cultures, is critical in differentiating bacterial from viral infections, and infectious from noninfectious etiologies. Serum and CSF tests based on polymerase chain reaction (PCR) techniques hold great promise in more-rapid diagnosis of infectious encephalitis. In some instances it will be necessary to *(a)* institute chemotherapy for possible, treatable, infecting organisms and *(b)* await definitive laboratory diagnostic tests.

EXHIBIT 28-1
NONVIRAL CAUSES OF ENCEPHALOMYELITIS

Treatable infectious conditions that can mimic viral encephalitis:

- Partially treated bacterial meningitis
- Brain abscess
- Subdural empyema
- Embolic encephalitis associated with bacterial endocarditis
- Lyme disease
- Tuberculous meningitis
- Fungal meningitis
- Rocky Mountain spotted fever
- Cat scratch disease
- Cerebral malaria
- Trypanosomiasis
- Toxoplasmosis

Vascular, autoimmune, and neoplastic diseases that can mimic infectious meningoencephalitis:

- Lupus cerebritis
- Cerebral and granulomatous arteritis
- Lymphomatous cerebritis
- Whipple's disease
- Behçet syndrome
- Carcinomatosis meningitis

Medical Management and Prevention

No specific therapy exists for the togaviral encephalitides; hence, treatment is aimed at management of specific symptoms (eg, anticonvulsant medication, protection of the airway). The extremes of high fever occasionally produced by WEE infection in humans is a special problem among the arboviral encephalitides that may require aggressive antihyperthermia measures.

The U.S. Army has extensive experience with a live-attenuated vaccine for VEE (TC-83) in humans. However, this vaccine would be expected to protect efficiently against only IA/B and IC serotypes. The TC-83 vaccine is also reactogenic, with more than 20% of vaccine recipients experiencing fever, malaise, and headache after the

vaccination. Half of these patients experience symptoms severe enough to warrant bed rest for 1 to 2 days.

Use of an effective vaccine in horses would prevent outbreaks of epizootic VEE, as equines are the major amplifying species for VEE virus. Vaccination of horses is not a useful public health tool for EEE, WEE, or enzootic VEE, however, since horses are not important as amplifying hosts for these diseases. Investigational formalin-inactivated vaccines for humans are available for WEE and EEE, but they require multiple injections and are poorly immunogenic. Insecticide measures of vector control may also have an impact on ameliorating epidemic transmission.

IMMUNOPROPHYLAXIS

Relevant Immune Effector Mechanisms

The equine encephalomyelitis viruses constitute both an endemic disease threat as well as a biological warfare threat; therefore, adequate immunoprophylaxis of military personnel will require protection against both vector-borne and aerosol-acquired infection. The requirements for protection against parenteral infection are well described, but the requirements for protection against infectious aerosols are certainly more stringent, and are largely unidentified. Within a few days of infection with an alphavirus, specific antibodies can be detected in the serum of animals or humans. Within 7 to 14 days, a virus-neutralizing antibody response develops, as measured by the ability of serum antibodies to block virus infectivity in vitro or in vivo. Protection from mosquito-vectored alphavirus disease is believed to be primarily mediated by this virus-specific neutralizing antibody response, which is largely directed against epitopes on the E2 glycoprotein. Protection mediated by nonneutralizing antibodies to alphaviruses, directed largely at epitopes on the E1 glycoprotein, has also been described.[134–136] However, it has proven more difficult to correlate protection from aerosol exposure with serum neutralization or antibody titers.[137]

Other nonspecific or immune responses that occur following alphavirus infection include the induction of secretion of interferon[138–141] and the activation of cytotoxic macrophages.[142] There have also been reports of virus-specific cytotoxic T cell responses induced against alphaviruses,[143–146] although it has proven difficult to show that these T cell responses play a significant role in protection.

Passive Immunization

Passive transfer of neutralizing antisera or monoclonal antibodies to naive recipients protects animals from subsequent parenteral challenge with homologous VEE strains.[136,140,147] Passive transfer of nonneutralizing, anti-E1 monoclonal antibodies directed against appropriate epitopes is also protective against Sindbis,[134] WEE,[135] and VEE[136] viruses. However, for the respiratory route of infection, uniform protection was not observed after passive transfer of hyperimmune serum to hamsters[137] or neutralizing monoclonal antibodies to mice,[148] suggesting that either additional immune mechanisms or the presence of protective antibodies along the respiratory tract may be

needed. The time between the administration of immune serum and virus exposure may also be relevant. Protection of mice from intracerebral inoculation with WEE virus was observed if immune serum was given no more than 3 days prior to virus exposure.[149,150] Similarly, monkeys passively immunized with horse antiserum to EEE or WEE resisted intranasal challenge from homologous virus 24 hours later, but they were unable to resist a second challenge with the same virus 7 weeks later.[151] However, as the immune serum given in both studies was xenogeneic, the loss of protective capacity was presumably related in part to active clearance of the immune serum by the recipients.

The effect of giving immune serum to animals after the establishment of intracerebral infections has also been evaluated. Several studies, employing different alphaviruses, have demonstrated at least partial protection if the immune serum was administered within 24 hours of infection.[149,150,152–154] Other studies have suggested that postinfection serum transfer may also cause a more severe pathology, or may merely delay the onset of disease symptoms.[152,155] Aggressive serotherapy following infections of two laboratory workers who developed acute WEE encephalitis resulted in the survival of one patient[156]
but was ineffective in the second patient.[157]

In an EEE outbreak in New Jersey in 1959, 22 of 32 diagnosed patients died. Most patients had demonstrable antibody during the onset or progression of encephalitis, and neutralizing antibody titers in sera from patients who died were generally similar to those observed in patients who recovered.[116] This finding, coupled with animal studies indicating that transfer of virus-neutralizing anti-sera was unable to prevent progression of disease if infection of the brain was firmly established (described above[149,150,152–154]), suggests that serotherapy would be an ineffective means of treatment for these virus infections, unless initiated very early in the course of disease.

Active Immunization

Vaccines currently available for use against the equine encephalomyelitis viruses include TC-83, which is a live attenuated vaccine for VEE, and inactivated vaccines for VEE, EEE, and WEE. All are used under IND status. The characteristics of these vaccines and the responses induced in human vaccinees are summarized in Table 28-4.

TABLE 28-4
VACCINES CURRENTLY AVAILABLE FOR VEE, EEE, AND WEE VIRUSES

Vaccine	Form/Strain	Dose (mL)/Route of Administration	Schedule	Responding %	Duration *	Booster Dose/Route
VEE (TC-83)	Attenuated TrD	0.5 mL/sc	Day 0	82%	92%	C-84/sc
VEE (C-84) †	Inactivated TC-83	0.5 mL/sc	After TC-83	76% NR‡ 100% WT§	60% 100%	0.5 mL/sc
EEE	Inactivated PE-6¥	0.5 mL/sc	Days 0, 28	58%	75%	0.1 mL/id
WEE	Inactivated CM-4884¥	0.5 mL/sc	Days 0, 7, 28	50%	20%	0.5 mL/sc

* % of responders whose virus-neutralizing titers persist for at least 1 y
† current IND protocols specify use of C-84 only as a booster vaccine
‡ TC-83 nonresponders
§ TC-83 responders given C-84 to boost waning titers
¥ laboratory designation
EEE: eastern equine encephalitis
id: intradermal
IND: investigational new drug
sc: subcutaneous
TC: tissue culture
TrD: Trinidad donkey
VEE: Venezuelan equine encephalitis
WEE: western equine encephalitis

Live Vaccines

The TC-83 VEE vaccine was developed in 1961 by serial passage of the virulent TrD strain in fetal guinea pig heart cells,[158] and is administered sub-cutaneously (0.5 mL) at 1×10^4 to 2×10^4 plaque-forming units (pfu) per dose. The vaccine was used initially in laboratory and field personnel at risk for exposure to VEE,[159] and more than 6,000 people received the vaccine between 1964 and 1972.[160] For reasons that remain unclear, approximately 20% of the people who receive TC-83 fail to make a minimum neutralizing antibody response and presumably would not be protected should they be exposed to the virus. Another 25% of vaccine recipients experience clinical reactions ranging from mild transient symptoms to fever, chills, sore throat, and malaise sufficient to require bed rest.[161] However, for recipients who respond with postvaccination titers of at least 1:20, long-term follow-up studies have shown that titers persist for several years.[162] In humans, documented vaccine-breakthrough infections have been attributed largely to exposure to heterologous, enzootic strains of VEE virus.[47–49] Although pregnant mares were not adversely affected by TC-83,[163] pregnant women are advised

not to receive the TC-83 vaccine, as wild-type VEE may have been associated with spontaneous abortions or stillbirths during an epidemic in Venezuela in 1962.[164]

In animals, TC-83 vaccination will protect hamsters from a lethal VEE subcutaneous or aerosol challenge,[137] although up to 20% of hamsters may die of reactions to the vaccine.[95,165] Subcutaneous immunization of monkeys[98] with the vaccine produces *(a)* neutralizing antibody responses in serum and *(b)* protection from virulent VEE virus delivered by peripheral or intranasal challenge. However, TC-83 provides only partial protection against aerosol challenge in outbred mice.[100] TC-83 has been extensively administered to horses, burros, and mules, in part because large numbers of equines were vaccinated during the 1969–1970 epizootic. TC-83 immunization produces febrile responses and leukopenia in some equines,[166,167] but neutralizing antibody responses to homologous (serotype IA) virus eventually develop in 90% of these animals.[166,168] Although it was difficult to accurately assess vaccine efficacy under the conditions of an ongoing epizootic, herds of animals known to have been immunized at least 2 weeks prior to any disease occurrence in the area did not sustain any VEE-related deaths, whereas unimmunized herds experienced up to 60% mortality rates.[161]

An unresolved problem with the use of TC-83, and presumably with other live-attenuated alphavirus vaccines, is the phenomenon of vaccine interference, in which prior immunity to heterologous alphaviruses inhibits vaccine virus replication and subsequent immune responses. This occurrence has been observed in horses,[169,170] in which preexisting antibodies to EEE and WEE may have interfered with TC-83 vaccination. Interference has also been observed in humans, in whom prior vaccination with Chikungunya virus has reduced the response to TC-83, and vice versa (D.J.McC., unpublished research, 1994).

Inactivated Vaccines

Against VEE (C-84). Early attempts to develop an inactivated vaccine against VEE resulted in preparations that contained residual live virus and caused disease in 4% of those who received it.[158,171] Because of the problems associated with incomplete inactivation, development of an inactivated VEE vaccine (C-84) was begun, using the TC-83 attenuated strain of virus.[172] Initial clinical trials with the C-84 inactivated vaccine were begun in 1976 in 14 volunteers previously immunized with TC-83, and subsequently in 14 naive volunteers.[173] The vaccine was found to be safe and elicited only mild tenderness at the site of injection. Although C-84 was immunogenic, three doses were required to maintain neutralizing antibody titers in recipients. A subsequent study has shown that most TC-83 non-responders and 100% of individuals with waning titers from TC-83 immunizations respond to a booster dose of C-84 and have a high probability of maintaining a titer for 3 years.[160]

The observation that hamsters given C-84 vaccine were protected from subcutaneous challenge but not from an aerosol exposure to VEE virus[137] raised concerns that C-84 vaccination may not protect at-risk laboratory workers from aerosol exposure. Therefore, C-84 is currently administered only as a booster immunogen.

Against EEE and WEE. The PE-6 strain of EEE virus was passed in primary chick-embryo cell cultures, and then was formalin-treated and lyophilized to produce an inactivated vaccine for EEE.[174] This vaccine is administered as a 0.5-mL dose

subcutaneously on days 0 and 28, with 0.1-mL intradermal booster doses given as needed to maintain neutralizing antibody titers. Mild reactions to the vaccine were observed, and immunogenicity was demonstrated in initial clinical trials.[175] The vaccine was given to 896 at-risk laboratory workers between 1976 and 1991. No significant clinical reactions have been observed. A long-term follow-up study of 573 recipients indicated a 58% response rate after the primary series, and a 25% chance of failing to maintain adequate titers for 1 year. Response rates and persistence of titers increased with the administration of additional booster doses.[176]

The WEE vaccine was similarly prepared using the B-11 or CM-4884) virus strain, and caused only mild clinical reactions when administered to WEE-naive individuals.[177] Between 1976 and 1990, 359 laboratory workers were immunized with this vaccine. Long-term follow-up studies have indicated that administration of three doses of 0.5 mL subcutaneously on days 0, 7, and 28 results in a 50% responder rate (neutralization titer > 1:40) after the primary series. Only 20% of recipients maintain a titer for 1 year, although this level can be increased to 60% to 70% with additional booster immunizations.[176]

SUMMARY

The equine encephalomyelitis viruses consist of three antigenically related viruses within the *Alphavirus* genus of the family *Togaviridae*: Venezuelan equine encephalomyelitis (VEE), western equine encephalomyelitis (WEE), and eastern equine encephalomyelitis (EEE). These viruses are vectored in nature by various species of mosquitoes and cause periodic epizootics among equines. Infection of equines with virulent strains of any these viruses produces a similar clinical course of severe encephalitis with high mortality. However, the clinical course following infection of humans differs. EEE is the most severe of the arbovirus encephalitides, with case fatality rates of 50% to 70%. WEE virus is generally less virulent for adults, but the infection commonly produces severe encephalitis in children, with case fatality rates approaching 10%. In contrast, encephalitis is rare following VEE virus infection, but essentially all infected individuals develop a prostrating syndrome of high fever, headache, malaise, and prolonged convalescence.

Although natural infections are acquired by mosquito bite, these viruses are also highly infectious in low doses as aerosols. They can be produced in large amounts in inexpensive and unsophisticated systems, are relatively stable, and are readily amenable to genetic manipulation. For these reasons, the equine encephalomyelitis viruses are classic biological warfare threats.

No specific therapy exists for infections caused by these viruses. A live-attenuated vaccine for VEE (TC-83) and inactivated vaccines for VEE, EEE, and WEE have been developed and are used under IND status. Although these vaccines are useful in protecting at-risk individuals, they have certain disadvantages, and improved vaccines are under development.

REFERENCES

1. Meyer KF, Haring CM, Howitt B. The etiology of epizootic encephalomyelitis of horses in the San Joaquin Valley, 1930. *Science.* 1931;74:227.

2. Giltner LT, Shahan MS. The 1933 outbreak of infectious equine encephalomyelitis in the eastern states. *North Am Vet*. 1933;14:25.

3. Ten Broeck C, Hurst EW, Traub E. Epidemiology of equine encephalitis in the eastern United States. *J Exp Med*. 1935;62:677.

4. Kubes V, Rios FA. The causative agent of infectious equine encephalitis in Venezuela. *Science*. 1939;90:20.

5. Webster LT, Wright FH. Recovery of eastern equine encephalomyelitis virus from brain tissue of human cases of encephalitis in Massachusetts. *Science*. 1938;88:305.

6. Howitt BE. Recovery of the virus of equine encephalomyelitis from the brain of a child. *Science*. 1938;88:455.

7. Fothergill LD, Dingle JH, Farber S, et al. Human encephalitis caused by a virus of eastern variety of equine encephalitis. *N Engl J Med*. 1983;219:411.

8. Sanmartin-Barberi C, Groot H, Osborn-Mesa E. Human epidemic in Colombia caused by the Venezuelan equine encephalitis virus. *Am J Trop Med Hyg*. 1954;3:283.

9. Richmond JY, McKinney RW. *Biosafety in Microbiological and Biomedical Laboratories*. 3rd ed. Washington, DC: US Department of Health and Human Services; May 1993. HHS Publication (CDC) 93–8395.

10. Slepushkin AN. An epidemiological study of laboratory infections with Venezuelan equine encephalomyelitis. *Vopr Virusol*. 1959;3:311–314.

11. Shubladze AK, Gaidmovich SYa, Gavrilov VI. A virological study of laboratory infections with Venezuelan equine encephalomyelitis. *Vopr Virusol*. 1959;3:305–310.

12. Strauss JH, Strauss EG. The alphaviruses: Gene expression, replication, and evolution. *Microbiological Reviews*. 1994;58(3):491–562.

13. Peters CJ, Dalrymple JM. Alphaviruses. In: Fields BM, Knipe DM, eds. *Virology.* 2nd ed, Vol 1. New York, NY: Raven Press; 1990: 713–761.

14. Huxsoll DL, Patrick WC, Parrott CD. Veterinary services in biological disasters. *J Am Vet Med Assoc*. 1987;190(6):714–722.

15. Davis NL, Willis LV, Smith JF, Johnston RE. In vitro synthesis of infectious Venezuelan equine encephalitis virus RNA from a cDNA clone: Analysis of a viable deletion mutant. *Virology*. 1991;171:189–204.

16. Davis NL, Powell N, Greenwald GF, et al. Attenuating mutations in the E2 glycoprotein gene of Venezuelan equine encephalitis virus: Construction of single and multiple mutants in a full-length cDNA clone. *Virology*. 1991;183:20–31.

17. Hanson RP. An epizootic of equine encephalomyelitis that occurred in Massachusetts in 1831. *Am J Trop Med Hyg*. 1957;6:858.

18. Ten Broeck C. Birds as possible carriers of the virus of equine encephalomyelitis. *Arch Pathol*. 1938;25:759.

19. Kissling RE, Rubin H, Chamberlain RW, Edison ME. Recovery of the virus of eastern equine encephalomyelitis from the blood of a purple grackle. *Proc Soc Exp Biol Med*. 1951;77:398.

20. Chamberlain RW, Rubin H, Kissling RE, Eidson, ME. Recovery of eastern equine encephalomyelitis from a mosquito, *Culiseta melanura (Coquillett). Proc Soc Exp Biol Med*. 1951;77:396.

21. Morris CD. Eastern equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 3. Boca Raton, Fla: CRC Press; 1988: 203–231.

22. Mitchell CJ, Niebylski ML, Smith GC, et al. Isolation of eastern equine encephalitis virus from *Aedes albopictus* in Florida. *Science*. 1992;257:526–527.

23. Davison RO. Encephalomyelitis in Saskatchewan, 1941. *Can J Public Health*. 1942;33:83.

24. Reisen WK, Monath TP. Western equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 5. Boca Raton, Fla: CRC Press; 1988: 203–231.

25. Hammon W McD, Reeves WC, Brookman B, Izumi EM. Isolation of viruses of western equine and St. Louis encephalitis from *Culex tarsalis* mosquitoes. *Science*. 1941;94:328.

26. Hammon W McD, Gray JA, Evans FC, Izumi EM. Western equine and St. Louis encephalitis antibodies in the sera of mammals and birds from an endemic area. *Science*. 1941;94:305.

27. Beck CE, Wyckoff RW. Venezuelan equine encephalomyelitis. *Science*. 1938;88:530.

28. Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 4. Boca Raton, Fla: CRC Press; 1988: 203–231.

29. Franck PT, Johnson KM. An outbreak of Venezuelan equine encephalomyelitis in Central America: Evidence for exogenous source of a virulent virus subtype. *Am J Epidemiol*. 1971;94:487.

30. Weaver SC, Salas R, Rico-Hesse R, et al. Re-emergence of epidemic Venezuelan equine encephalitis in South America. *Lancet*. 1966;348:436–440.

31. Young NA, Johnson KM. Antigenic variants of Venezuelan equine encephalitis virus: Their geographic distribution and epidemiologic significance. *Am J Epidemiol*. 1969;89:286.

32. Scherer WF, Cupp EW, Dziem GM, Breener RJ, Ordonez JV. Mesenteronal infection threshold of an epizootic strain of Venezuelan encephalitis virus in *Culex (Melanoconion) taeniopus* mosquitoes and its implication to the apparent disappearance of this virus strain from an enzootic habitat in Guatemala. *Am J Trop Med Hyg*. 1982;31:1030–1037.

33. Jonkers AH. Silent hosts of Venezuelan equine encephalitis (VEE) virus in endemic

situations: Mammals. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 263–268. PAHO Scientific Publication 243.

34. Bernstein BJ. The birth of the US Biological Warfare Program. *Sci Am*. 1987;256(6):116–121.

35. National Security Agency. *National Security Decision*. Washington, DC: NSA; Nov. 25, 1969. Memorandum 35.

36. Walton TE. Arboviral encephalomyelitides of livestock in the western hemisphere. *J Am Vet Med Assoc*. 1992;200:1385–1389.

37. Contigiani MS, De Basualdo M, Camara A, et al. Presencia de anticuerpos contra el virus de la encefalitis equina Venezolana subtipo VI en pacientes con enfermedad aguda febril [in Spanish]. *Revista Argentina de Microbiologia*. 1993;25:212–220.

38. Karabatsos N. *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*. 3rd ed. San Antonio, Tex: American Society for Tropical Medicine and Hygiene; 1985.

39. Shope RE, Causey OR, de Andrade AHP, Theiler M. The Venezuelan equine encephalitis complex of group A arthropodborne viruses, including *Mucambo* and *Pixuna* from the Amazon region of Brazil. *Am J Trop Med Hyg*. 1964;13:723.

40. Chamberlain RW, Sudia WD, Coleman PH, Work TH. Venezuelan equine encephalitis virus from South Florida. *Science*. 1964;145:272.

41. Scherer WF, Anderson K. Antigenic and biological characteristics of Venezuelan encephalitis virus strains including a possible new subtype isolated from the Amazon region of Peru in 1971. *Am J Epidemiol*. 1975;101:356.

42. Walton TE. Virulence properties of Venezuelan equine encephalitis virus serotypes in horses. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 134. PAHO Scientific Publication 243.

43. Cupp EW, Scherer WF, Ordonez JV. Transmission of Venezuelan encephalitis virus by naturally infected *Culex (Melanoconion) opisthopus. Am J Trop Med Hyg.* 1979;28:1060–1063.

44. Scherer WF, Dickerman RW, Cupp EW, Ordonez JV. Ecologic observations of Venezuelan encephalitis virus in vertebrates and isolations of Nepuyo and Patois viruses from sentinel hamsters at Pacific and Atlantic habitats in Guatemala, 1968–1980. *Am J Trop Med Hyg.* 1985;34:790–798.

45. Scherer WF, Dickerman RW, Diaz-Najera A, Ward BA, Miller MH, Schaffer PA. Ecologic studies of Venezuelan encephalitis virus in southeastern Mexico, III: Infection of mosquitoes. *Am J Trop Med Hyg*. 1971;20:969–979.

46. Walton TE. Equine arboviral encephalomyelitides: A review. *J Equine Vet Sci*. 1988;8:49–53.

47. De Mucha-Macias J, Sanchez-Spindola I. Two human cases of laboratory infection with Mucambo virus. *Am J Trop Med Hyg*. 1965;14(3):475–478.

48. Franck PT. In discussion: Round table on epidemic control. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 400. PAHO Scientific Publication 243.

49. Dietz WH, Peralta PH, Johnson KM. Ten clinical cases of human infection with Venezuelan equine encephalo-myelitis virus, subtype I-D. *Am J Trop Med Hyg*. 1979;28:329–334.

50. Casals J. Antigenic variants of eastern equine encephalitis virus. *J Exp Med*. 1964;119:547.

51. Weaver SC, Bellew LA, Gousset L, Repik PM, Scott TW, Holland JJ. Diversity within natural populations of eastern equine encephalomyelitis virus. *Virology*. 1993;195:700–709.

52. Weaver SC, Rico-Hesse R, Scott TW. Genetic diversity and slow rates of evolution in New World alphaviruses. *Curr Top Microbiol Immunol*. 1992;176:99–117.

53. Dietz WH, Galindo P, Johnson KM. Eastern equine encephalitis in Panama: The epidemiology of the 1973 epizootic. *Am J Trop Med Hyg*. 1980;29:133–140.

54. Trent DW, Grant JA. A comparison of new world alphaviruses in the western equine encephalomyelitis complex by immunochemical and oligonucleotide fingerprint techniques. *J Gen Virol*. 1980;47:261–282.

55. Hardy JL. The ecology of western equine encephalomyelitis virus in the central valley of California, 1945–1985. *Am J Trop Med Hy*g. 1987;37(suppl):18S–32S.

56. Fulhorst CF, Hardy JL, Eldridge BF, Pressor SV, Reeves WC. Natural vertical transmission of western equine encephalomyelitis virus in mosquitoes. *Science*. 1994;263:676–678.

57. Reeves WC, Hammon WM. Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943–52. *Univ Calif Pub Health*. 1962;4:257.

58. Johnson KM, Martin DH. Venezuelan equine encephalitis. *Adv Vet Sci Comp Med*. 1974;18:79.

59. Galindo P. Endemic vectors of Venezuelan encephalitis. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 249–252. PAHO Scientific Publication 243.

60. Young NA, Johnson KM, Gauld LW. Viruses of the Venezuelan equine encephalomyelitis complex. *Am J Trop Med Hyg*. 1969;18:290–296.

61. Seymour C, Dickerman RW, Martin MS. Venezuelan encephalitis virus infection in neotropical bats, II: Experimental infections. *Am J Trop Med Hyg*. 1978;27:297–306.

62. Franck PT, Johnson KM. An outbreak of Venezuelan encephalitis in man in the Panama Canal Zone. *Am J Trop Med Hyg*. 1970;19:860–863.

63. Sanchez JL, Lednar WM, Macasaet FF, et al. Venezuelan equine encephalitis: Report of an outbreak associated with jungle exposure. *Milit Med*. 1984;149:618–621.

64. Groot H. The health and economic impact of Venezuelan equine encephalitis (VEE). In:

Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 7–27. PAHO Scientific Publication 243.

65. Hinman AR, McGowan JE, Henderson BE. Venezuelan equine encephalomyelitis: Surveys of human illness during an epizootic in Guatemala and El Salvador. *Am J Epidemiol*. 1971;93:130–136.

66. Kinney RM, Tsuchiya KR, Sneider JM, Trent DW. Genetic evidence that epizootic Venezuelan equine encephalitis (VEE) viruses may have evolved from enzootic VEE subtype 1-D virus. *Virology*. 1992;191:569–580.

67. Weaver SC, Bellew LA, Rico-Hesse R. Phylogenetic analysis of alphaviruses in the Venezuelan equine encephalitis complex and identification of the source of epizootic viruses. *Virology*. 1992;191:282–290.

68. Weaver SC, Scherer WF, Cupp EW, Castello DA. Barriers to dissemination of Venezuelan encephalitis viruses in the middle American enzootic vector mosquito, *Culex (melanoconion) taeniopus. Am J Trop Med Hyg*. 1984;33:953–960.

69. Turell MJ, Ludwig GV, Beaman JR. Transmission of Venezuelan equine encephalomyelitis virus by *Aedes sollicitans* and *Aedes taeniorhynchus* (Diptera: Culicidae). *J Med Entomol*. 1992;29:62–65.

70. Reta G. Equine disease: Mexico. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 209–214. PAHO Scientific Publication 243.

71. Sharman R. Equine disease. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 221–224. PAHO Scientific Publication 243.

72. Anthony RP, Brown DT. Protein-protein interactions in an alphavirus membrane. *J Virol*. 1991;65:1187–1194.

73. Rice CM, Strauss JH. Association of Sindbis virus glycoproteins and their precursors. *J Mol Biol*. 1982;154:325–348.

74. Dalrymple JM. Antigenic characterization of two Sindbis envelope glycoproteins separated by isoelectric focusing. *Virology*. 1976;69:93–102.

75. Davis NL, Fuller FJ, Dougherty WG, Olmsted RA, Johnston RE. A single nucleotide change in the E2 glycoprotein gene of Sindbis virus affects penetration rate in cell culture and virulence in neonatal mice. *Proc Natl Acad Sci*. USA 1986;83:6771–6775.

76. Bron RJ, Wahlberg M, Garoff H, Wilschut J. Membrane fusion of Semliki Forest virus in a model system: Correlation between fusion kinetics and structural changes in the envelope glycoprotein. *EMBO J*. 1993;12:693–701.

77. Strauss EG, Strauss JH. Structure and replication of the alphavirus genome. In: Schlesinger S, Schlesinger MJ, eds. *The Togaviridae and Flaviviridae*. New York, NY: Plenum Press; 1986: 350–390.

78. Sefton B. Immediate glycosylation of Sindbis virus membrane proteins. *Cell*. 1977;10:659–668.

79. Liljestrom P, Garoff H. Internally located cleavable signal sequences direct the formation of Semliki Forest virus membrane proteins from a polyprotein precursor. *J Virol*. 1991;65:147–153.

80. Ziemiecki AH, Simons GK. Formation of the Semliki Forest virus membrane glycoprotein complexes in the infected cell. *J Gen Virol*. 1980;50:111–123.

81. de Curtis IA, Simons K. Dissection of Semliki Forest virus glycoprotein delivery from the trans-Golgi network to the cell surface in permeabilized BHK cells. *Proc Natl Acad Sci USA*. 1988;85:8052–8056.

82. Simons K, Garoff H. The budding mechanisms of enveloped viruses. *J Gen Virol*. 1980;50:1–21.

83. Gliedman JB, Smith JF, Brown DT. Morphogenesis of Sindbis virus in cultured *Aedes albopictus* cells. *J Virol*. 1975;16:913–926.

84. Metsiikko K, Garoff H. Oligomers of the cytoplasmic domains of the p62/E2 membrane protein of Semliki Forest virus bind to the nucleocapsid in vitro. *J Virol*. 1990;64:4678–4683.

85. Paredes AM, Brown DT, Rothnagel R, et al. Three-dimensional structure of a membrane-containing virus. *Proc Natl Acad Sci USA*. 1993;90:9095–9099.

86. Lopez S, Yao J-S, Kuhn RJ, Strauss EG, Strauss JH. Nucleocapsid–glycoprotein interactions required for alphavirus assembly. *J Virol*. 1994;68:1316–1323.

87. Levine V, Huang Q, Isaacs JT, Reed JC, Griffin DE, Hardwick JM. Conversion of lytic to persistent alphavirus infection by the bcl-2 cellular oncogene. *Nature*. 1993;361:739–741.

88. de la Monte S, Castro F, Bonilla NJ, Gaskin de Urdaneta A, Hutchins GM. The systemic pathology of Venezuelan equine encephalitis virus infection in humans. *Am J Trop Med Hyg*. 1985;34:194–202.

89. Jackson AC, SenGupta SK, Smith JF. Pathogenesis of Venezuelan equine encephalitis virus infection in mice and hamsters. *Vet Pathol*. 1991;28:410–418.

90. Gleiser CA, Gochenour WS, Berge TO, Tigertt WD. The comparative pathology of experimental Venezuelan equine encephalomyelitis infection in different animal hosts. *J Infect Dis*. 1962;110:80–97.

91. Gochenour WS Jr. The comparative pathology of Venezuelan encephalitis virus infection in selected animal hosts. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 113–117. PAHO Scientific Publication 243.

92. Austin FJ, Scherer WF. Studies of viral virulence, I: Growth and histopathology of virulent and attenuated strains of Venezuelan encephalitis virus in hamsters. *Am J Pathol*. 1971;62:195–210.

93. Walker DH, Harrison A, Murphy K, Flemister M, Murphy FA. Lymphoreticular and myeloid pathogenesis of Venezuelan equine encephalitis in hamsters. *Am J Pathol*. 1976;84:351–370.

94. Jahrling PB, Scherer WF. Histopathology and distribution of viral antigens in hamsters infected with virulent and benign Venezuelan encephalitis viruses. *Am J Pathol*. 1973;72:25–38.

95. Jahrling PB, Scherer WF. Growth curves and clearance rates of virulent and benign Venezuelan encephalitis viruses in hamsters. *Infect Immunol*. 1973;8:456–462.

96. Gorelkin L, Jahrling PB. Pancreatic involvement by Venezuelan equine encephalomyelitis virus in the hamster. *Am J Pathol*. 1974;75:349–362.

97. Gorelkin L, Jahrling PB. Virus-initiated septic shock: Acute death of Venezuelan encephalitis virus-infected hamsters. *Lab Invest*. 1975;32:78–85.

98. Monath TP, Cropp CB, Short WF, et al. Recombinant vaccinia-Venezuelan equine encephalomyelitis (VEE) vaccine protects nonhuman primates against parenteral and intranasal challenge with virulent VEE virus. *Vac Res*. 1992;1:55–68.

99. Danes L, Kufner J, Hruskova J, Rychterova V. The role of the olfactory route on infection of the respiratory tract with Venezuelan equine encephalomyelitis virus in normal and operated Macaca rhesus monkeys, I: Results of virological examination. *Acta Virol (Praha)*. 1973;17:50–56.

100. Ryzhikov AB, Tkacheva NV, Sergeev AN, Ryabchikova EI. Venezuelan equine encephalitis virus propagation in the olfactory tract of normal and immunized mice. *Biomed Sci*. 1991;2:607–614.

101. Vogel P, Abplanalp D, Kell W, et al. Venezuelan equine encephalitis in BALB/c mice. *Arch Pathol Lab Med*. 1996;120:164–172.

102. Sanmartin C, Mackenzie RB, Trapido H, et al. Encefalitis equina Venezolana en Colombia, 1967 [in Spanish]. *Bol Of Sanit Panam*. 1973;74:104–137.

103. Martin DH, Eddy GA, Sudia WD, Reeves WC, Newhouse VF, Johnson KM. An epidemiologic study of Venezuelan equine encephalomyelitis in Costa Rica, 1970. *Am J Epidemiol*. 1972;95:2565–2578.

104. Sanmartin C. Diseased hosts: Man. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 186–188. PAHO Scientific Publication 243.

105. Sanmartin-Barberi C, Groot H, Osborno-Mesa E. Human epidemic in Colombia caused by the Venezuelan equine encephalomyelitis virus. *Am J Trop Med Hyg*. 1954;3:283–293.

106. Bowen GS, Fashinell TR, Dean PB, Gregg MG. Clinical aspects of human Venezuelan equine encephalitis in Texas. *Bull Pan Am Health Organ*. 1976;10:46–57.

107. Leon CA, Jaramillo R, Martinez S, et al. Sequelae of Venezuelan equine encephalitis in humans: A four year follow-up. *Int J Epidemiol*. 1975;4:131–140.

108. Avilán Rovira J. In discussion: Sanmartin C. Diseased hosts: Man. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 189–195. PAHO Scientific Publication 243.

109. Johnson KM, Shelokov A, Peralta PH, Dammin GJ, Young NA. Recovery of

Venezuelan equine encephalomyelitis virus in Panama: A fatal case in man. *Am J Trop Med Hyg*. 1968;17:432–440.

110. Briceño Rossi AL. Rural epidemic encephalitis in Venezuela caused by a group A arbovirus (VEE). In: Melnick JL, ed. *Progress in Medical Virology*. Vol 9. Basel, Switzerland: Karger; 1967: 176–203.

111. Briceño Rossi AL. The frequency of VEE virus in the pharyngeal material of clinical cases of encephalitis. *Gac Med Caracas*. 1964;72:5–22.

112. Calisher CH, El-Kafrawi AO, Al-Deen Mahmud MI, et al. Complex specific immunoglobulin M antibody patterns in humans infected with alphaviruses. *J Clin Microbiol*. 1986;23:155–159.

113. Madalengoitia J, Palacios O, Ubiliuz JC, Alva S. An outbreak of Venezuelan encephalitis virus in man in the Tumbes department of Peru. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 198–200. PAHO Scientific Publication 243.

114. Monath TP. Arthropod-borne encephalitides in the Americas. *Bull WHO*. 1979;57:513–533.

115. Goldfield M, Welsh JN, Taylor BF. The 1959 outbreak of eastern encephalitis in New Jersey, VI: The frequency of prior infection. *Am J Epidemiol*. 1968;87:39–49.

116. Goldfield M, Welsh JN, Taylor BF. The 1959 outbreak of eastern encephalitis in New Jersey, V: The inapparent infection:disease ratio. *Am J Epidemiol*. 1968;87:32–38.

117. Hart KL, Keen D, Belle EA. An outbreak of eastern equine encephalomyelitis in Jamaica, West Indies, Nov–Dec 1962, I: Description of human cases. *Am J Trop Med Hyg*. 1964;13:331–334.

118. Farber S, Hill A, Connerly MI, Dingle JH. Encephalitis in infants and children caused by the virus of the eastern variety of equine encephalitis. *JAMA*. 1940;114:1725–1731.

119. Clarke DH. Two non-fatal human infections with the virus of eastern encephalitis. *Am J Trop Med Hyg*. 1961; 10:67–70.

120. Goldfield M, Taylor BF, Welsh JN. The 1959 outbreak of eastern encephalitis in New Jersey, III: Serologic studies of clinical cases. *Am J Epidemiol*. 1968;87:18–22.

121. Feemster RF. Equine encephalitis in Massachusetts. *N Engl J Med*. 1958;257:107–113.

122. Ayres JC, Feemster RF. The sequelae of eastern equine encephalomyelitis. *N Engl J Med*. 1949;240:960–962.

123. McGowan JE, Bryan JA, Gregg MB. Surveillance of arboviral encephalitis in the United States, 1955–1971. *Am J Epidemiol*. 1973;97:199–207.

124. Hayes RO. Eastern and western encephalitis. In: Beran GW, ed. *Handbook Series in Zoonoses: Viral Zoonoses*. Vol. 1. Boca Raton, Fla: CRC Press; 1981: 29–57.

125. Bianchi TI, Aviles G, Monath TP, Sabatini MS. Western equine encephalomyelitis: Virulence markers and their epidemiologic significance. *Am J Trop Med Hyg*. 1993;49(3):322–328.

126. Hanson RP, Sulkin SE, Buescher EL, Hammon W McD, McKinney RW, Work TH. Arbovirus infections of laboratory workers. *Science*. 1967;158:1283.

127. Sciple GW, Ray CG, Holden P, La Motte LC, Irons JV, Chin TDY. Encephalitis in the high plains of Texas. *Am J Epidemiol*. 1968;87:87–98.

128. Finley KG. Postencephalitis manifestations of viral encephalitides. In: Fields NS, Blattner RF, eds. *Viral Encephalitis*. Springfield, Ill: Charles C Thomas; 1959: 69–91.

129. Earnest MP, Goolishian HA, Calverly JR, Hayes RO, Hill HR. Neurologic, intellectual, and psychologic sequelae following western encephalitis. *Neurology*. 1971;21:969–974.

130. Shinefield HR, Townsend TE. Transplacental transmission of western equine encephalomyelitis. *J Ped*. 1953;43:21–25.

131. Calisher CH, Emerson JK, Muth DJ, Lazuick JS, Monath TP. Serodiagnosis of western equine encephalitis infections: Relationships of antibody titer and test to observed onset of Illness. *J Am Vet Med Assoc*. 1983;183:438.

132. Calisher CH, Karabatsos N. Arbovirus serogroups: Definition and geographic distribution. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. Vol 1. Boca Raton, Fla: CRC Press; 1988: 19–57.

133. Rozdilsky B, Robertson HE, Chorney J. Western encephalitis: Report of eight fatal cases: Saskatchewan epidemic, 1965. *Can Med Assoc J*. 1968;98:79–86.

134. Schmaljohn AL, Johnson ED, Dalrymple JM, Cole GA. Non-neutralizing monoclonal antibodies can prevent lethal alphavirus encephalitis. *Nature*. 1982;297:70–72.

135. Hunt AR, Roerhig, JT. Biochemical and biological characteristics of epitopes on the E1 glycoprotein of western equine encephalitis virus. *Virology*. 1985;142:334–346.

136. Mathews JH, Roehrig JT. Determination of the protective epitopes on the glycoproteins of Venezuelan equine encephalomyelitis virus by passive transfer of monoclonal antibodies. *J Immunol*. 1982;129(6):2763–2767.

137. Jahrling PB, Stephenson EH. Protective efficacies of live attenuated and formaldehyde-inactivated Venezuelan equine encephalitis virus vaccines against aerosol challenge in hamsters. *J Clin Microbiol*. 1984;19(3):429–431.

138. Vilcek J. Production of interferon by newborn and adult mice infected with Sindbis virus. *Virology*. 1964;22:651–652.

139. Finter NB. Interferon as an antiviral agent in vivo: Quantitative and temporal aspects of the protection of mice against Semliki Forest virus. *Br J Exp Pathol*. 1966;47:361–371.

140. Rabinowitz SG, Adler WH. Host defenses during primary Venezuelan equine encephalomyelitis virus infection in mice, I: Passive transfer of protection with immune serum and immune cells. *J Immunol*. 1973;110(5):1345–1353.

141. Tazulakhova EB, Novokhatsky AS, Yershov FI. Interferon induction by, and antiviral effect of, poly(rI)-poly (rC) in experimental viral infection. *Acta Virol*. 1973;17:487–492.

142. Rodda SJ, White DO. Cytotoxic macrophages: A rapid nonspecific response to viral infection. *J Immunol*. 1976;117(6):2067–2072.

143. McFarland HF. In vitro studies of cell-mediated immunity in an acute viral infection. *J Immunol*. 1974;113(1):173–180.

144. Mullbacher A, Blanden RV. Murine cytotoxic T-cell response to alphavirus is associated mainly with H-2Dk. *Immunogenetics*. 1978;7:551–561.

145. Mullbacher A, Blanden RV. H-2-linked control of cytotoxic T-cell responsiveness to alphavirus infection: Presence of H-2Dk during differentiation and stimulation converts stem cells of low responder genotype to T cells of responder phenotype. *J Exp Med*. 1979;149:786–790.

146. Peck R, Brown A, Wust CJ. In vitro heterologous cytotoxicity by T effector cells from mice immunized with Sindbis virus. *J Immunol*. 1979;123(4):1763–1766.

147. Roerhig JT, Mathews JH. The neutralization site on the E2 glycoprotein of Venezuelan equine encephalitis (TC-83) virus is composed of multiple conformationally stable epitopes. *Virology*. 1985;142:346–356.

148. Kinney RM, Esposito JJ, Mathews JH, et al. Recombinant vaccinia virus/Venezuelan equine encephalitis (VEE) virus protects mice from peripheral VEE virus challenge. *J Virol*. 1988;(62):4697–4702.

149. Howitt BF. Equine encephalomyelitis. *J Infect Dis*. 1932;51:493–510.

150. Olitsky PK, Schlesinger RW, Morgan IM. Induced resistance of the central nervous system to experimental infection with equine encephalomyelitis virus, II: Serotherapy in western virus infection. *J Exp Med*. 1943;77:359–375.

151. Wyckoff RWG, Tesar WC. Equine encephalomyelitis in monkeys. *J Immunol*. 1939;37:329–343.

152. Berge TO, Gleiser CA, Gochenour WS, Miesse ML, Tigertt WD. Studies on the virus of Venezuelan equine encephalomyelitis, II: Modification of specific immune serum of response of central nervous system of mice. *J Immunol*. 1961;87:509–517.

153. Griffin DE, Johnson RT. Role of the immune response in recovery from Sindbis virus encephalitis in mice. *J Immunol*. 1978;118(3):1070–1075.

154. Igarashi A, Fukuoka T, Fukai K. Passive immunization of mice with rabbit antisera against Chikungunya virus and its components. *Biken J*. 1971;14:353–355.

155. Seamer JH, Boulter EA, Zlotnik I. Delayed onset of encephalitis in mice passively immunised against Semliki Forest virus. *Br J Exp Path*. 1971;52:408–414.

156. Gold H, Hampil B. Equine encephalomyelitis in a laboratory technician with recovery. *Ann Intern Med*. 1942;16: 556–569.

157. Helwig FC. Western equine encephalomyelitis following accidental inoculation with chick embryo virus. *JAMA*. 1940;115:291–292.

158. Berge TO, Banks IS, Tigertt WD. Attenuation of Venezuelan equine encephalomyelitis virus by in vitro cultivation in guinea-pig heart cells. *Am J Hyg*. 1961;73:209–218.

159. McKinney RW, Berge TO, Sawyer WD, Tigertt WD, Crozier D. Use of an attenuated strain of Venezuelan equine encephalomyelitis virus for immunization in man. *Am J Trop Med Hyg*. 1963;12:597–603.

160. Pittman PR, Makuch RS, Mangiafico JA, Cannon TL, Gibbs PH, Peters CJ. Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. *Vaccine*. 1996;14(4):337–343.

161. McKinney RW. Inactivated and live VEE vaccines—A review. In: Venezuelan Encephalitis: Proceedings of the Workshop-Symposium on Venezuelan Encephalitis Virus; 14–17 September 1971. Washington, DC: Pan American Health Organization; 1972: 369–377. PAHO Scientific Publication 243.

162. Burke DS, Ramsburg HH, Edelman R. Persistence in humans of antibody to subtypes of Venezuelan equine encephalitis (VEE) virus after immunization with attenuated (TC-83) VEE virus vaccine. *J Infect Dis*. 1977;136(3):354–359.

163. Eddy GA, Martin DH, Reeves WC, Johnson KM. Field studies of an attenuated Venezuelan equine encephalomyelitis vaccine (strain TC-83). *Infect Immunol*. 1972;5(2):160–163.

164. Wenger F. Venezuelan equine encephalitis. *Teratology*. 1977;16:359–362.

165. Austin FJ, Scherer WF. Studies of viral virulence, I: Growth and histopathology of virulent and attenuated strains of Venezuelan encephalitis virus in hamsters. *Am J Pathol*. 1971;62:195–209.

166. Spertzel RO, Kahn DE. Safety and efficacy of an attenuated Venezuelan equine encephalomyelitis vaccine for use in equidae. *J Am Vet Med Assoc.* 1971;159(6):731–738.

167. Monlux WS, Luedke AJ, Browne J. Central nervous system response of horses to Venezuelan equine encephalomyelitis vaccine (TC-83). *J Am Vet Med Assoc*. 1972;161(3):265–269.

168. Walton TE, Alvarez O, Buckwalter RM, Johnson KM. Experimental infection of horses with an attenuated Venezuelan equine encephalomyelitis vaccine (strain TC-83). *Infect Immunol*. 1972;5(5):750–756.

169. Jochim MM, Barber TL, Luedke AJ. Venezuelan equine encephalomyelitis: Antibody response in vaccinated horses and resistance to infection with virulent virus. *J Am Vet Med Assoc*. 1973;162(4):280–283.

170. Calisher CH, Sasso DR, Sather GE. Possible evidence for interference with Venezuelan equine encephalitis virus vaccination of equines by pre-existing antibody to eastern or western equine encephalitis virus, or both. *Appl Microbiol*. 1973;26:485–488.

171. Sutton LS, Brooks CC. Venezuelan equine encephalomyelitis due to vaccination in man. *JAMA*. 1954;155(17):1473–1478.

172. Cole FE Jr, May SW, Eddy GA. Inactivated Venezuelan equine encephalomyelitis vaccine prepared from attenuated (TC-83 strain) virus. *Appl Microbiol*. 1974;27(1):150–153.

173. Edelman R, Ascher MS, Oster CN, Ramsburg HH, Cole FE, Eddy GA. Evaluation in humans of a new, inactivated vaccine for Venezuelan equine encephalitis virus (C-84). *J Infect Dis*. 1979;140(5):708–715.

174. Maire LF III, McKinney RW, Cole FE Jr. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture, I: Production and

testing. *Am J Trop Med Hyg*. 1970;19(1):119–122.

175. Bartelloni PJ, McKinney RW, Duffy TP, Cole FE Jr. An inactivated eastern equine encephalomyelitis vaccine propagated in chick-embryo cell culture, II: Clinical and serologic responses in man. *Am J Trop Med Hyg*. 1970;19(1):123–126.

176. Pittman PR. Lieutenant Colonel, Medical Corps, US Army. Chief, Special Immunizations Program, Medical Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, October 1996.

177. Bartelloni PJ, McKinney RW, Calia FM, Ramsburg HH, Cole FE Jr. Inactivated western equine encephalomyelitis vaccine propagated in chick embryo cell culture: Clinical and serological evaluation in man. *Am J Trop Med Hyg*. 1971;20(1):146–149.

Chapter 29

VIRAL HEMORRHAGIC FEVERS

PETER B. JAHRLING, PH.D.*

INTRODUCTION

EPIDEMIOLOGICAL OVERVIEW
- The *Arenaviridae*
- The *Bunyaviridae*
- The *Filoviridae*
- The *Flaviviridae*

CLINICAL FEATURES OF THE VIRAL HEMORRHAGIC FEVER SYNDROME

DIAGNOSIS

MEDICAL MANAGEMENT
- Supportive Care
- Isolation and Containment
- Specific Antiviral Therapy

IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY
- Passive Immunization
- Active Immunization

SUMMARY

* *Senior Research Scientist, Headquarters, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

INTRODUCTION

The concept of a viral hemorrhagic fever (VHF)syndrome is useful in clinical medicine. VHF syndrome can be described as an acute febrile illness characterized by malaise, prostration, generalized signs of increased vascular permeability, and abnormalities of circulatory regulation. Bleeding manifestations often occur, especially in the more severely ill patients, but this does not result in a life-threatening loss of blood volume. Rather, these signs are the result of damage to the vascular endothelium and are an index of how severe the disease is in specific target organs.

The viral agents that cause VHFs are taxonomically diverse; they are all ribonucleic acid (RNA) viruses and are transmitted to humans through contact with infected animal reservoirs or arthropod vectors. They are all natural infectious disease threats although their geographical ranges may be tightly circumscribed. The recent advent of jet travel coupled with human demographics increase the opportunity for humans to contract these infections.

The VHF agents are all highly infectious via the aerosol route, and most are quite stable as respirable aerosols. This means that they satisfy at least one criterion for being weaponized, and some clearly have the potential to be biological warfare threats. Most of these agents replicate in cell culture to concentrations sufficiently high to produce a small terrorist weapon, one suitable for introducing lethal doses of virus into the air intake of an airplane or office building. Some replicate to even higher concentrations, with obvious potential ramifications. Since the VHF agents cause serious diseases with high morbidity and mortality, their existence as endemic disease threats and as potential biological warfare weapons suggests a formidable potential impact on unit readiness. Further, returning troops may well be carrying exotic viral diseases to which the civilian population is not immune, a major public health concern.

EPIDEMIOLOGICAL OVERVIEW

The VHF agents are a taxonomically diverse group of RNA viruses whose major characteristics are summarized in Table 29-1. Four virus families contribute pathogens to the group of VHF agents: the *Arenaviridae, Bunyaviridae, Filoviridae,* and *Flaviviridae.* Despite their diverse taxonomy, all these viruses share some common characteristics. They are all relatively simple RNA viruses, and they all have lipid envelopes. This renders them relatively susceptible to detergents, as well as to low-pH environments and household bleach. Conversely, they are quite stable at neutral pH, especially when protein is present. Thus, these viruses are stable in blood for long periods, and can be isolated from a patient’s blood after weeks of storage at refrigerator or even at ambient temperatures.

These viruses tend to be stable and highly infectious as fine-particle aerosols. These characteristics have great significance in not only the natural transmission cycle for arenaviruses and bunyaviruses (from rodents to man) but also make nosocomial transmission a concern. As a group, the viruses are also linked to the ecology of their vectors or reservoirs, whether rodents or arthropods. In that regard, most of these reservoirs tend to be rural, and a patient’s history of being in a rural locale is an important factor to consider when reaching a diagnosis. Human-to-human spread is possible, but pandemics are unlikely.

TABLE 29-1 RECOGNIZED VIRAL HEMORRHAGIC FEVERS OF HUMANS

			Source of Human Infection		
Virus Family Genus	**Disease (Virus)**	**Natural Distribution**	**Usual**	**Less Likely**	**Incubation(Days)**
Arenaviridae					
Arenavirus	Lassa fever	Africa	Rodent	Nosocomial	5–16
	Argentine HF (Junin)	South America	Rodent	Nosocomial	7–14
	Bolivian HF (Machupo)	South America	Rodent	Nosocomial	9–15
	Brazilian HF (Sabia)	South America	Rodent	Nosocomial	7–14
	Venezuelan HF (Guanarito)	South America	Rodent	Nosocomial	7–14
Bunyaviridae					
Phlebovirus	Rift Valley fever	Africa	Mosquito	Slaughter of domestic animal	2–5
Nairovirus	Crimean-Congo HF	Europe, Asia, Africa	Tick	Slaughter of domestic animal; nosocomial	3–12
Hantavirus	HFRS (Hantaan and related viruses)	Asia, Europe; possibly worldwide	Rodent		9–35
Filoviridae					
Filovirus	Marburg and Ebola HF	Africa	Unknown	Nosocomial	3–16
Flaviviridae					
Flavivirus (Mosquito–borne)	Yellow fever	Tropical Africa, South America	Mosquito		3–6
	Dengue HF	Asia, Americas, Africa	Mosquito		Unknown for dengue HF, but 3–5 for uncomplicated dengue
(Tick–borne)	Kyasanur Forest disease	India	Tick		3–8
	Omsk HF	Soviet Union	Tick	Muskrat–contaminated water	3–8

HF: hemorrhagic fever; HFRS: hemorrhagic fever with renal syndrome

The *Arenaviridae*

The arenaviruses are classified into the Old World and New World groups. All the arenaviruses are maintained in nature by a life-long association with a rodent reservoir. Rodents spread the virus to humans, and outbreaks can usually be related to some perturbation in the ecosystem that brings man into contact with the rodents.

Lassa virus causes Lassa fever, a major febrile disease of West Africa, where it is associated with 10% to 15% of adult febrile admissions to the hospital and perhaps 40% of nonsurgical deaths.[1] In addition, Lassa fever is a pediatric disease and the cause of high mortality in pregnant women. While nosocomial infections do occur, most Lassa virus infections can be traced to contact with the carrier rodent, *Mastomys natilensis.*

The Junin virus that causes Argentine hemorrhagic fever is carried by a field mouse, *Calomys colosus,* and is associated with agricultural activities in the pampas of Argentina, where 300 to 600 cases have occurred every year since 1955.[2] In Bolivia, Machupo virus is the agent associated with Bolivian hemorrhagic fever,[3] a disease that was associated with outbreaks in the 1960s but only with sporadic disease subsequently. Guanarito virus is a newly described arenavirus, first recognized in association with an outbreak of VHF involving several hundred patients in Venezuela beginning in 1989.[4] More recently, yet another VHF arenavirus has been recognized: Sabia virus was associated with a fatal VHF infection in Brazil in 1990, followed by a severe laboratory infection in Brazil in 1992 and another laboratory infection in the United States in 1994.[5]

The *Bunyaviridae*

Among the bunyaviruses, the significant human pathogens include the phlebovirus Rift Valley fever (RVF) virus, which causes Rift Valley fever. This major African disease is frequently associated with unusual increases in mosquito populations.[6] Rift Valley fever is also a disease of domestic livestock, and human infections have resulted from contact with infected blood, especially around slaughter houses.

A nairovirus, Crimean-Congo hemorrhagic fever (C-CHF) virus is carried by ticks, and has been associated with sporadic, yet particularly severe, VHF in Europe, Africa, and Asia.[7] Crimean-Congo hemorrhagic fever has frequently occurred as small, hospital-centered outbreaks, owing to the copious hemorrhage and highly infective nature of this virus via the aerosol route.

Hantaviruses, unlike the other bunyaviruses, are not transmitted via infected arthropods; rather, they infect man via contact with infected rodents and their excreta. Hantavirus disease was described prior to World War II in Manchuria along the Amur River, and later among United Nations troops during the Korean War, where it became known as Korean hemorrhagic fever.[8] The prototype virus from this group, Hantaan, is the cause of Korean hemorrhagic fever as well as the severe form of hemorrhagic fever with renal syndrome (HFRS). Hantaan virus is borne in nature by the striped field mouse, *Apodemus agrarious.*

Hantaan virus is still active in Korea, Japan, and China. Seoul virus causes a milder form of HFRS, and may be distributed worldwide. There are a number of other hantaviruses that are associated with HFRS, including Puumala virus, which is associated with chronically infected bank voles *(Clethrionomys glareolus)*. Recently in the United States, a new hantavirus (Sin nombre virus) has been associated with the hantavirus pulmonary syndrome (HPS).[9]

The *Filoviridae*

The *Filoviridae* includes the causative agents of Ebola and Marburg hemorrhagic fevers. These filoviruses have an exotic, threadlike appearance when observed via electron microscopy. Marburg

virus was first recognized in 1967 when a lethal epidemic of VHF occurred in Marburg, Germany, among laboratory workers exposed to the blood and tissues of African green monkeys that had been imported from Uganda; secondary transmission to medical personnel and family members also occurred.[10] In all, 31 patients became infected, 9 of whom died. Subsequently, Marburg virus has been associated with sporadic, isolated, usually fatal cases among residents and travelers in southeast Africa.[11]

Ebola viruses are taxonomically related to Marburg viruses; they were first recognized in association with explosive outbreaks that occurred almost simultaneously in 1976 in small communities in Zaire[12] and Sudan.[13] Significant secondary transmission occurred through reuse of unsterilized needles and syringes and nosocomial contacts. These independent outbreaks involved serologically distinct viral strains. The Ebola–Zaire outbreak involved 277 cases and 257 deaths (92% mortality), while the Ebola–Sudan outbreak involved 280 cases and 148 deaths (53% mortality). Sporadic cases occurred subsequently. In 1989, a third strain of Ebola virus appeared in Reston, Virginia, in association with an outbreak of VHF among cynomolgus monkeys imported to the United States from the Philippines.[14] Hundreds of monkeys were infected (with high mortality) but no human cases occurred, although four animal caretakers seroconverted without overt disease. Recently, small outbreaks involving new strains of Ebola virus occurred in human populations in Côte d'Ivorie in 1994 and Gabon in 1995; a larger outbreak involving the Ebola-Zaire strain involved more than 300 people, with 75% mortality, in Zaire in 1995.[15]

Very little is known about the natural history of any of the filoviruses. Animal reservoirs and arthropod vectors have been aggressively sought without success.

The *Flaviviridae*

Finally, the flaviviruses include the agents of yellow fever, found throughout tropical Africa and South America; and dengue, found throughout the Americas, Asia, and Africa, both transmitted by mosquitoes.[16] Both yellow fever and dengue have had major impact on military campaigns and military medicine. The tick-borne flaviviruses include the agents of Kyasanur Forest disease, which occurs in India,[17] and Omsk hemorrhagic fever, which occurs in the former Soviet Union.[18] Both diseases have a biphasic course; the initial phase includes a prominent pulmonary component, followed by a neurological phase with central nervous system manifestations.

CLINICAL FEATURES OF THE VIRAL HEMORRHAGIC FEVER SYNDROME

The VHF syndrome develops to varying degrees in patients infected with these viruses. The exact nature of the disease depends on viral virulence and strain characteristics, routes of exposure, dose, and host factors. For example, dengue hemorrhagic fever is typically seen only in patients previously exposed to heterologous dengue serotypes.[19] The target organ in the VHF syndrome is the vascular bed; correspondingly, the dominant clinical features are usually a consequence of microvascular damage and changes in vascular permeability.[20] Common presenting complaints are fever, myalgia, and prostration; clinical examination may reveal only conjunctival injection, mild hypotension, flushing, and petechial hemorrhages. Full-blown VHF typically evolves to shock and generalized bleeding from the mucous membranes, and often is accompanied by evidence of neurological, hematopoietic, or pulmonary involvement. Hepatic involvement is common, but a clinical picture dominated by jaundice and other evidence of hepatic failure is seen in only a small percentage patients with Rift Valley fever, Crimean-Congo hemorrhagic fever, Marburg hemorrhagic fever, Ebola hemorrhagic fever, and yellow fever. Renal failure is proportional to cardiovascular compromise, except in HFRS caused by hantaviruses, where it is an integral part of the disease process; oliguria is a prominent feature of the acutely ill patient.[8] VHF mortality may be substantial, ranging from 5% to 20% or higher in recognized cases. Ebola outbreaks in

Africa have had particularly high case fatality rates, from 50% up to 90%.[12,13]

The clinical characteristics of the various VHFs are somewhat variable. For Lassa fever patients, hemorrhagic manifestations are not pronounced, and neurological complications are infrequent, occurring only late and in only the most severely ill group. Deafness is a frequent sequela of severe Lassa fever. For the South American arenaviruses, (Argentine and Bolivian hemorrhagic fevers), neurological and hemorrhagic manifestations are much more prominent. RVF virus is primarily hepatotropic; hemorrhagic disease is seen in only a small proportion of cases. In recent outbreaks in Egypt, retinitis was a frequently reported component of Rift Valley fever.[21]

Unlike Rift Valley fever, where hemorrhage is not prominent, Crimean-Congo hemorrhagic fever infection is usually associated with profound disseminated intravascular coagulation (DIC) (Figure 29-1). Patients with Crimean-Congo hemorrhagic fever may bleed profusely; and since this occurs during the acute, viremic phase, contact with the blood of an infected patient is a special concern: a number of nosocomial outbreaks have been associated with C-CHV virus.

The picture for diseases caused by hantaviruses is evolving, especially now in the context of HPS syndrome. The pathogenesis of HFRS may be somewhat different; immunopathological events seem to be a major factor. When patients present with HFRS, they are typically oliguric. Surprisingly, the oliguria occurs while the patient's viremia is resolving and they are mounting a demonstrable antibody response. This has practical significance in that renal dialysis can be started with relative safety.

Fig. 29-1. Massive cutaneous ecchymosis associated with late-stage Crimean-Congo hemorrhagic fever virus infection, 7 to 10 days after clinical onset. Ecchymosis is indicative of multiple abnormalities in the coagulation system, coupled with loss of vascular integrity. Epistaxis and profuse bleeding from puncture sites, hematemesis, melena, and hematuria often accompany spreading ecchymosis, which may occur anywhere on the body as a result of needlesticks or other minor trauma. The sharply demarcated proximal border of this patient's lesion is not explained. Photograph: Courtesy of Robert Swanepoel, PhD, DTVM, MRCVS, National Institute of Virology, Sandringham, South Africa.

For the diseases caused by filoviruses, little clinical data from human outbreaks exist. Although mortality is high, outbreaks are rare and sporadic. Marburg and Ebola viruses produce prominent maculopapular rashes, and DIC is a major factor in their pathogenesis. Therefore, treatment of the DIC should be considered, if practicable, for these patients.

Among the flaviviruses, yellow fever virus is, of course, hepatotropic: black vomit caused by hematemesis has been associated with this disease. Patients with yellow fever develop clinical jaundice and die with something comparable to hepatorenal syndrome. Dengue hemorrhagic fever and shock are uncommon, life-threatening complications of dengue, and are thought—especially in children—to result from an immunopathological mechanism triggered by sequential infections with different dengue viral serotypes.[19] Although this is the general epidemiological pattern, dengue virus may also rarely cause hemorrhagic fever in adults and in primary infections.[22]

DIAGNOSIS

The natural distribution and circulation of VHF agents are geographically restricted and mechanistically linked with the ecology of the reservoir species and vectors. Therefore, a high index of suspicion and elicitation of a detailed travel history are critical in making the diagnosis of VHF. Patients with arenaviral or hantaviral infections often recall having seen rodents during the presumed incubation period, but, since the viruses are spread to humans by aerosolized excreta or environmental contamination, actual contact with the reservoir is not necessary. Large mosquito populations are common during the seasons when RVF virus and the flaviviruses are transmitted, but a history of mosquito bite is sufficiently common to be of little assistance in making a diagnosis, whereas tick bites or nosocomial exposure are of some significance when Crimean-Congo hemorrhagic fever is suspected. History of exposure to animals in slaughterhouses should raise suspicions of Rift Valley fever and Crimean-Congo hemorrhagic fever in a patient with VHF. When large numbers of military personnel present with VHF manifestations in the same geographical area over a short period of time, medical personnel should suspect either a natural outbreak (in an endemic setting) or possibly a biowarfare attack (particularly if the virus causing the VHF is not endemic to the area).

VHF should be suspected in any patient presenting with a severe febrile illness and evidence of vascular involvement (subnormal blood pressure, postural hypotension, petechiae, hemorrhagic diathesis, flushing of the face and chest, nondependent edema) who has traveled to an area where the etiologic virus is known to occur, or where intelligence suggests a biological warfare threat. Signs and symptoms suggesting additional organ system involvement are common (headache, photophobia, pharyngitis, cough, nausea or vomiting, diarrhea, constipation, abdominal pain, hyperesthesia, dizziness, confusion, tremor), but they rarely dominate the picture. A macular eruption occurs in most patients who have Marburg and Ebola hemorrhagic fevers; this clinical manifestation is of diagnostic importance.

Laboratory findings can be helpful, although they vary from disease to disease and summarization is difficult. Leukopenia may be suggestive, but in some patients, white blood cell counts may be normal or even elevated. Thrombocytopenia is a component of most VHF diseases, but to a varying extent. In some, platelet counts may be near normal, and platelet function tests are required to explain the bleeding diathesis. A positive tourniquet test has been particularly useful in diagnosing dengue hemorrhagic fever, but this sign may be associated with other hemorrhagic fevers as well. Proteinuria or hematuria or both are common in VHF, and their absence virtually rules out Argentine hemorrhagic fever, Bolivian hemorrhagic fever, and hantaviral infections. Hematocrits are usually normal, and if there is sufficient loss of vascular integrity perhaps mixed with dehydration, hematocrits may be increased. Liver enzymes such as aspartate aminotransferase (AST) are frequently elevated. VHF viruses are not primarily hepatotropic, but livers are involved and an elevated AST may help to distinguish VHF from a simple febrile disease.

For much of the world, the major differential diagnosis is malaria. It must be borne in mind that parasitemia in patients partially immune to malaria does not prove that symptoms are due to malaria. Typhoid fever and rickettsial and leptospiral diseases are major confounding infections; nontyphoidal salmonellosis, shigellosis, relapsing fever, fulminant hepatitis, and meningococcemia are some of the other important diagnoses to exclude. Ascertaining the etiology of DIC is usually surrounded by confusion. Any condition leading to DIC could be mistaken for diseases such as acute leukemia, lupus erythematosus, idiopathic or thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome.

Definitive diagnosis in an individual case rests on specific virological diagnosis. Most patients have readily detectable viremia at presentation (the exception is those with hantaviral infections). Infectious virus and viral antigens can be detected and identified by a

number of assays using fresh or frozen serum or plasma samples. Likewise, early immunoglobulin (Ig) M antibody responses to the VHF-causing agents can be detected by enzyme-linked immunosorbent assays (ELISA), often during the acute illness. Diagnosis by viral cultivation and identification requires 3 to 10 days for most (longer for the hantaviruses); and, with the exception of dengue, specialized microbiologic containment is required for safe handling of these viruses.[23] Appropriate precautions should be observed in collection, handling, shipping, and processing of diagnostic samples.[24] Both the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia.) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID, Fort Detrick, Frederick, Maryland.) have diagnostic laboratories operating at the maximum Biosafety Level (BL-4; see Chapter 19, The U.S. Biological Warfare and Biological Defense Programs, for further discussion of BLs). Viral isolation should not be attempted without BL-4 containment.

In contrast, most antigen-capture and antibody-detection ELISAs for these agents can be performed with samples that have been inactivated by treatment with β-propiolactone (BPL).[25] Likewise, diagnostic tests based on reverse transcriptase polymerase chain reaction (RT-PCR) technology are safely performed on samples following RNA extraction using chloroform and methanol. RT-PCR has been successfully applied to the real-time diagnosis of most of the VHF agents.[26,27] When isolation of the infectious virus is difficult or impractical, RT-PCR has proven to be extremely valuable; for example, with HPS, where the agent was recognized by PCR months before it was finally isolated in culture.[9]

When the identity of a VHF agent is totally unknown, isolation in cell culture and direct visualization by electron microscopy, followed by immunological identification by immunohistochemical techniques is often successful.[14] Immunohistochemical techniques are also useful for retrospective diagnosis using formalin-fixed tissues, where viral antigens can be detected and identified using batteries of specific immune sera and monoclonal antibodies. Although intensive efforts are being directed toward the development of simple, qualitative tests for rapid diagnosis in the field, definitive diagnosis for these diseases today requires, at a minimum, an ELISA capability coupled with specialized immunological reagents, supplemented (ideally) with an RT-PCR capability.

MEDICAL MANAGEMENT

Patients with VHF syndrome require close supervision, and some will require intensive care. Since the pathogenesis of VHF is not entirely understood and availability of specific antiviral drugs is limited, treatment is largely supportive. This care is essentially the same as the conventional care provided to patients with other causes of multisystem failure. The challenge is to provide this support while minimizing the risk of infection to other patients and medical personnel.

Supportive Care

Patients with VHF syndrome generally benefit from rapid, nontraumatic hospitalization to prevent unnecessary damage to the fragile capillary bed. Transportation of these patients, especially by air, is usually contraindicated because of the effects of drastic changes in ambient pressure on lung water balance. Restlessness, confusion, myalgia, and hyperesthesia occur frequently and should be managed by reassurance and other supportive measures, including the judicious use of sedative, pain-relieving, and amnestic medications. Aspirin and other antiplatelet or anticlotting-factor drugs should be avoided.

Secondary infections are common and should be sought and aggressively treated. Concomitant malaria should be treated aggressively with a regimen known to be effective against the geographical strain of the parasite; however, the presence of malaria,

particularly in immune individuals, should not preclude management of the patient for VHF syndrome if such is clinically indicated.

Intravenous lines, catheters, and other invasive techniques should be avoided unless they are clearly indicated for appropriate management of the patient. Attention should be given to pulmonary toilet, the usual measures to prevent superinfection, and the provision of supplemental oxygen. Immunosuppression with steroids or other agents has no empirical and little theoretical basis, and is contraindicated except possibly for HFRS.

The diffuse nature of the vascular pathological process may lead to a requirement for support of several organ systems. Myocardial lesions detected at autopsy reflect cardiac insufficiency antemortem. Pulmonary insufficiency may develop, and, particularly with yellow fever, hepatorenal syndrome is prominent.[16]

Treatment of Bleeding

The management of bleeding is controversial. Uncontrolled clinical observations support vigorous administration of fresh frozen plasma, clotting factor concentrates, and platelets, as well as early use of heparin for prophylaxis of DIC. In the absence of definitive evidence, mild bleeding manifestations should not be treated at all. More-severe hemorrhage indicates that appropriate replacement therapy is needed. When definite laboratory evidence of DIC becomes available, heparin therapy should be employed if appropriate laboratory support is available.

Treatment of Hypotension and Shock

Management of hypotension and shock is difficult. Patients often are modestly dehydrated from heat, fever, anorexia, vomiting, and diarrhea, in any combination. There are covert losses of intravascular volume through hemorrhage and increased vascular permeability.[28] Nevertheless, these patients often respond poorly to fluid infusions and readily develop pulmonary edema, possibly due to myocardial impairment and increased pulmonary vascular permeability. Asanguineous fluids—either colloid or crystalloid solutions—should be given, but cautiously. Although it has never been evaluated critically for VHFs, dopamine would seem to be the agent of choice for patients with shock who are unresponsive to fluid replacement. α-Adrenergic vasoconstricting agents have not been clinically helpful except when emergent intervention to treat profound hypotension is necessary. Vasodilators have never been systematically evaluated. Pharmacological doses of corticosteroids (eg, methylprednisolone 30 mg/kg) provide another possible but untested therapeutic modality in treating shock.

Particular Problems With Dengue and Hantaviral Infections

Two hemorrhagic fevers should be clearly separated from the other VHF diseases. Severe consequences of dengue infection are largely due to systemic capillary leakage syndrome and should be managed initially by brisk infusion of crystalloid, followed by albumin or other colloid if there is no response.[29]

Severe hantaviral infections have many of the management problems of the other hemorrhagic fevers but will culminate in acute renal failure with a subsequent polyuria during the patient’s recovery. Careful fluid and electrolyte management, and often renal dialysis, are necessary for optimal treatment.

Isolation and Containment

Patients with VHF syndrome generally have significant quantities of virus in their blood, and perhaps in other secretions as well (with the exceptions of dengue and classic

hantaviral disease). Well-documented secondary infections among contacts and medical personnel not parenterally exposed have occurred. Thus, caution should be exercised in evaluating and treating patients with suspected VHF syndrome. Over-reaction on the part of medical personnel is inappropriate and detrimental to both patient and staff, but it is prudent to provide isolation measures as rigorous as feasible.[30] At a minimum, these should include the following:

- stringent barrier nursing;
- mask, gown, glove, and needle precautions;
- hazard-labeling of specimens submitted to the clinical laboratory;
- restricted access to the patient; and
- autoclaving or liberal disinfection of contaminated materials, using hypochlorite or phenolic disinfectants.

For more intensive care, however, increased precautions are advisable. Members of the patient care team should be limited to a small number of selected, trained individuals, and special care should be directed toward eliminating all parenteral exposures. Use of endoscopy, respirators, arterial catheters, routine blood sampling, and extensive laboratory analysis increase opportunities for aerosol dissemination of infectious blood and body fluids. For medical personnel, the wearing of flexible plastic hoods equipped with battery-powered blowers provides excellent protection of the mucous membranes and airways.

Specific Antiviral Therapy

Ribavirin is a nonimmunosuppressive nucleoside analogue with broad antiviral properties,[31] and is of proven value for some of the VHF agents. Ribavirin reduces mortality from Lassa fever in high-risk patients,[32] and presumably decreases morbidity in all patients with Lassa fever, for whom current recommendations are to treat initially with ribavirin 30 mg/kg, administered intravenously, followed by 15 mg/kg every 6 hours for 4 days, and then 7.5 mg/kg every 8 hours for an additional 6 days.[30] Treatment is most effective if begun within 7 days of onset; lower intravenous doses or oral administration of 2 g followed by 1 g/d for 10 days also may be useful.

The only significant side effects have been anemia and hyperbilirubinemia related to a mild hemolysis and reversible block of erythropoiesis. The anemia did not require transfusions or cessation of therapy in the published Sierra Leone study[32] or in subsequent unpublished limited trials in West Africa. Ribavirin is contraindicated in pregnant women, but, in the case of definite Lassa fever, the predictability of fetal death and the need to evacuate the uterus justify its use. Safety of ribavirin in infants and children has not been established.

A similar dose of ribavirin begun within 4 days of disease is efficacious in patients with HFRS.[33] In Argentina, ribavirin has been shown to reduce virological parameters of Junin virus infection (ie, Argentine hemorrhagic fever), and is now used routinely as an adjunct to immune plasma. However, ribavirin does not penetrate the brain and is expected to protect only against the visceral, not the neurological phase of Junin infection.

Small studies investigating the use of ribavirin in the treatment of Bolivian hemorrhagic fever and Crimean-Congo hemorrhagic fever have been promising, as have preclinical studies for Rift Valley fever.[33] Conversely, ongoing studies conducted at USAMRMC predict that ribavirin will be ineffective against both the filoviruses and the flaviviruses. No other antiviral compounds are currently available for the VHF agents.

Interferon alpha has no role in therapy, with the possible exception of Rift Valley fever,[34] where fatal hemorrhagic fever has been associated with low interferon responses in

experimental animals. However, as an adjunct to ribavirin, exogenous interferon gamma holds promise in treatment of arenaviral infections.

IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY

Passive immunization has been attempted for treatment of most VHF infections. This approach has often been taken in desperation, owing to the limited availability of effective antiviral drugs. Anecdotal case reports describing miraculous successes are frequently tempered by more systematic studies, where efficacy is less obvious. For all VHF viruses, the benefit of passive immunization seems to be correlated with the concentration of neutralizing antibodies, which are readily induced by some—but not all—of these viruses.

Passive Immunization

Antibody therapy (ie, passive immunization) also has a place in the treatment of some VHFs. Argentine hemorrhagic fever responds to therapy with two or more units of convalescent plasma that contain adequate amounts of neutralizing antibody (or an equivalent quantity of immune globulin), provided that treatment is initiated within 8 days of onset.[35] Antibody therapy is also beneficial in the treatment of Bolivian hemorrhagic fever. Efficacy of immune plasma in treatment of Lassa fever[36] and Crimean-Congo hemorrhagic fever[37] is limited by low neutralizing antibody titers and the consequent need for careful donor selection.

In the future, engineered human monoclonal antibodies may be available for specific, passive immunization against the VHF agents. In HFRS, a passive immunization approach is contraindicated for treatment, since an active immune response is usually already evolving in most patients when they are first recognized, although plasma containing neutralizing antibodies has been used empirically in prophylaxis of high-risk exposures.

Active Immunization

The only established and licensed virus-specific vaccine available against any of the hemorrhagic fever viruses is yellow fever vaccine, which is mandatory for travelers to endemic areas of Africa and South America. For prophylaxis against Argentine hemorrhagic fever (AHF) virus, a live-attenuated Junin vaccine strain (Candid #1) was developed at USAMRMC and is available as an Investigational New Drug (IND). Candid #1 was proven to be effective in Phase III studies in Argentina, and plans are proceeding to obtain a New Drug license. This vaccine also provides some cross-protection against Bolivian hemorrhagic fever in experimentally infected primates. Two IND vaccines were developed at USAMRMC against Rift Valley fever; an inactivated vaccine that requires three boosters, which has been in use for 20 years; and a live-attenuated RVF virus strain (MP-12), which is presently in Phase II clinical trials.

For Hantaan virus, a formalin-inactivated rodent brain vaccine is available in Korea, but is not generally considered acceptable by U.S. standards. Another USAMRMC product, a genetically engineered vaccinia construct, expressing hantaviral structural proteins, is in Phase II safety testing in U.S. volunteers. For dengue, a number of live attenuated strains for all four serotypes are entering Phase II efficacy testing. However, none of these vaccines in Phase I or II IND status will be available as licensed products in the near term. For the remaining VHF agents, availability of effective vaccines is more distant.

SUMMARY

The VHF agents are a taxonomically diverse group of RNA viruses that cause serious diseases with high morbidity and mortality. Their existence as endemic disease threats or their use in biological warfare could have a formidable impact on unit readiness. Significant human pathogens include the arenaviruses (Lassa, Junin, and Machupo viruses, the agents of Lassa fever and Argentinean and Bolivian hemorrhagic fevers, respectively). Bunya virus pathogens include RVF virus, the agent of Rift Valley fever; C-CHF virus, the agent of Crimean-Congo hemorrhagic fever; and the hantaviruses. Filovirus pathogens include Marburg and Ebola viruses. The flaviviruses are arthropod-borne viruses and include the agents of yellow fever, denque, Kyasanur Forest disease, and Omsk hemorrhagic fever.

The dominant clinical features of VHF are a consequence of microvascular damage and changes in vascular permeability. Patients commonly present with fever, myalgia, and prostration. Full-blown VHF syndrome typically evolves to shock and generalized mucous membrane hemorrhage, and often is accompanied by evidence of neurological, hematopoietic, or pulmonary involvement. A viral hemorrhagic fever should be suspected in any patient who presents with a severe febrile illness and evidence of vascular involvement (subnormal blood pressure, postural hypotension, petechiae, easy bleeding, flushing of the face and chest, nondependent edema), and who has traveled to an area where the virus is known to occur, or where intelligence suggests a biological warfare threat.

Definitive diagnosis rests on specific virological diagnosis, including detection of viremia or IgM by ELISA at presentation. Diagnosis by viral cultivation and identification requires 3 to 10 days or longer and specialized microbiologic containment. Appropriate precautions should be observed in collection, handling, shipping, and processing of diagnostic samples. It is prudent to provide isolation measures that are as rigorous as feasible.

Patients with viral hemorrhagic fevers generally benefit from rapid, nontraumatic hospitalization to prevent unnecessary damage to the fragile capillary bed. Aspirin and other antiplatelet or anticlotting-factor drugs should be avoided. Secondary and concomitant infections including malaria should be sought and aggressively treated. The management of bleeding includes administration of fresh frozen plasma, clotting factor concentrates and platelets, and early use of heparin to control DIC. Fluids should be given cautiously, and asanguineous colloid or crystalloid solutions should be used. Multiple organ system support may be required.

Ribavirin is an antiviral drug with efficacy for treatment of the arenaviruses and bunyaviruses. Passively administered antibody is also effective in therapy of some viral hemorrhagic fevers. The only licensed vaccine available for VHF agents is for yellow fever. Experimental vaccines exist for Junin, RVF, hantaan, and dengue viruses, but these will not be licensed in the near future.

REFERENCES

1. McCormick JB, Webb PA, Krebs JW, Johnson KM, Smith E. A prospective study of epidemiology and ecology of Lassa fever. *J Infect Dis.* 1987;155:437–444.

2. Maiztegui J, Feuillade M, Briggiler A. Progressive extension of the endemic area and changing incidence of Argentine hemorrhagic fever. *Med Microbiol Immunol.* 1986;175:149–152.

3. Johnson KM, Wiebenga NH, Mackenzie RB, et al. Virus isolations from human cases of hemorrhagic fever in Bolivia. *Proc Soc Exp Biol Med.* 1965;118:113–118.

4. Salas R, De Manzione N, Tesh RB, et al. Venezuelan haemorrhagic fever. *Lancet.* 1991;338:1033–1036.

5. Coimbra TLM, Nassar ES, Burattini MN, et al. New arenavirus isolated in Brazil. *Lancet*. 1994;343:391–392.

6. Easterday BC. Rift Valley fever. *Adv Vet Sci.* 1965;10:65–127.

7. van Eeden PJ, van Eeden SF, Joubert JR, King JB, van de Wal BW, Michell WL. A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital, II: Management of patients. *S Afr Med J.* 1985;68:718–721.

8. Lee HW. Hemorrhagic fever with renal syndrome in Korea. *Rev Infect Dis.* 1989;11(May–Jun):S864–S876.

9. Butler JC, Peters CJ. Hantaviruses and Hantavirus Pulmonary Syndrome. *Clin Infect Dis.* 1994;19:387–395.

10. Martini GA, Siegert R, eds. *Marburg Virus Disease*. New York, NY: Springer-Verlag; 1971.

11. Gear JHS. Clinical aspects of African viral hemorrhagic fevers. *Rev Infect Dis*.1989;11(May–Jun):S777–S782.

12. World Health Organization International Study Team. Ebola haemorrhagic fever in Zaire, 1976. *Bull WHO*. 1978;56:271–293.

13. World Health Organization International Study Team. Ebola haemorrhagic fever in Sudan, 1976. *Bull WHO.* 1978;56:247–270.

14. Jahrling PB, Geisbert TW, Dalgard DW, et al. Preliminary report: Isolation of Ebola virus from monkeys imported to the USA. *Lancet*. 1990;335:502–505.

15. Sanchez A, Ksiazek TG, Rollin PE, et al. Reemergence of Ebola virus in Africa. *Emerging Infectious Diseases.* 1995;1:96–100.

16. Monath TP. Yellow fever: Victor, Victoria? Conqueror, conquest? Epidemics and research in the last forty years and prospects for the future. *Am J Trop Med Hyg.* 1991;45(1):1–43.

17. Pavri K. Clinical, clinicopathologic, and hematologic features of Kyasanur Forest disease. *Rev Infect Dis.* 1989;11(May–Jun):S854–859.

18. Chumakov MP. Studies of virus hemorrhagic fevers. *J Hyg Epidemiol Microbiol Immunol.* 1959;7:125–135.

19. Halstead SB. Antibody, macrophages, dengue virus infection, shock, and hemorrhage: A pathogenetic cascade. *Rev Infect Dis.* 1989;11(May–Jun):S830–S839.

20. McKay DG, Margaretten W. Disseminated intravascular coagulation in virus diseases. *Arch Intern Med.* 1967;120:129–152.

21. WHO Collaborating Centre for Research and Training in Veterinary Epidemiology and Management. *Report of the WHO/IZSTE Consultation on Recent Developments in Rift Valley Fever (With the Participation of FAO and OIE).* 1993;128:1–23. Civitella del Tronto, Italy; 14–15 September 1993. WHO/CDS/VPH.

22. Rosen L. Disease exacerbation caused by sequential dengue infections: Myth or reality? *Rev Infect Dis.* 1989;11(May–Jun):S840–S842.

23. Centers for Disease Control and Prevention, National Institutes of Health. *Biosafety in Microbiology and Bio-medical Laboratories.* Washington, DC: US Government Printing Office; 1993. HHS Publication (CDC) 93-8395.

24. 49 CFR, Ch 1, § 173.196. Infectious substances (etiologic agents). 1 October 1994.

25. van der Groen G, Elliot LH. Use of betapropiolactone inactivated Ebola, Marburg and Lassa intracellular antigens in immunofluorescent antibody assay. *Ann Soc Belg Med Trop*. 1982;62:49–54.

26. Trappier SG, Conaty AL, Farrar BB, Auperin DD, McCormick JB, Fisher-Hoch SP. Evaluation for the polymerase chain reaction for diagnosis of Lassa virus infection. *Am J Trop Med Hyg.* 1993;49:214–221.

27. Ksiazek TG, Rollin PE, Jahrling PB, Johnson E, Dalgard DW, Peters CJ. Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. *J Clin Microbiol.* 1992;30(4):947–950.

28. Fisher-Hoch SP. Arenavirus pathophysiology. In: Salvato MS, ed. *The Arenaviridae.* New York, NY: Plenum Press; 1993: Chap 17: 299–323.

29. Bhamarapravati N. Hemostatic defects in dengue hemorrhagic fever. *Rev Infect Dis.* 1989;11(4):S826–S829.

30. Centers for Disease Control. Management of patients with suspected viral hemorrhagic fever. *MMWR.* 1988;37(suppl 3):1–16.

31. Canonico PG, Kende M, Luscri BJ, Huggins JW. In-vivo activity of antivirals against exotic RNA viral infections. *J Antimicrob Chemother*. 1984;14(suppl A):27–41.

32. McCormick JB, King IJ, Webb PA, et al. Lassa fever: Effective therapy with ribavirin. *N Engl J Med.* 1986;314:20–26.

33. Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev Infect Dis.* 1989;11(4):S750–S761.

34. Morrill JC, Jennings GB, Cosgriff TM, Gibbs PH, Peters CJ. Prevention of Rift Valley fever in rhesus monkeys with interferon-α. *Rev Infect Dis*. 1989;11(May–Jun):S815–825.

35. Enria DA, Fernandez NJ, Briggiler AM, Lewis SC, Maiztegui JI. Importance of neutralizing antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet*. 1984;4:255–256.

36. Jahrling PB, Frame JD, Rhoderick JB, Monson MH. Endemic Lassa fever in Liberia, IV: Selection of optimally effective plasma for treatment by passive immunization. *Trans R Soc Trop Med Hyg.* 1985;79:380–384.

37. Shepherd AJ, Swanepoel R, Leman PA. Antibody response in Crimean-Congo hemorrhagic fever. *Rev Infect Dis.* 1989;11(May–Jun):S801–S806.

Chapter 30

DEFENSE AGAINST TOXIN WEAPONS

DAVID R. FRANZ, D.V.M., PH.D *

INTRODUCTION

UNDERSTANDING THE THREAT
- Toxins Compared With Chemical Warfare Agents
- Toxicity, Ease of Production, and Stability
- Sources of Toxins and Their Mechanisms of Action
- Mechanisms of Action and the Development of Countermeasures
- Populations at Risk

COUNTERMEASURES
- Physical Protection
- Real-Time Detection of an Attack
- Diagnosis: General Considerations
- Approaches to Prevention and Treatment
- Decontamination and Protection of Medical Personnel
- Sample Collection: General Rules for Toxins
- Toxin Analysis and Identification
- Water Treatment

THE FUTURE
- Toxins as Weapons
- Countermeasures to Toxins

SUMMARY

*Colonel, Veterinary Corps, U.S. Army; Commander, U.S. Army Medical Research

INTRODUCTION

Toxins are biological agents that are produced by living organisms: bacteria, plants, or animals. Toxins differ significantly from replicating agents (viruses and bacteria) and from classic chemical agents. The physical characteristics and mechanisms of action of toxins, as a group, dictate how they must be used as weapons and how they may be defended against. The terminology used in the field of toxin weapons is specific, and the definitions given in Exhibit 30-1 will be used in this textbook.

Some of the toxins described in the chapters that follow have been identified by the intelligence community as biological warfare threats. The likely route of intoxication for soldiers or victims of terrorist attack is through the lung by respirable aerosols; another possibility is through the gastrointestinal tract by contamination of food or water supplies, although the latter would be difficult in chlorinated water; or in rivers, lakes, or reservoirs because of dilution effects. The effects of most toxins are more severe when inhaled than when consumed in food or injected by bites or stings. Other toxins can elicit a significantly different clinical picture when the route of exposure is changed, a phenomenon that may confound diagnosis and delay treatment. For the most part, physical measures, such as the protective mask and decontamination systems developed for the chemical threat, can protect against toxins.

EXHIBIT 30-1
TOXIN WEAPONS TERMINOLOGY

Toxin	Any toxic substance that can be produced by an animal, plant, or microbe. Some toxins can also be produced by molecular biological techniques (protein toxins) or by chemical synthesis (low-molecular-weight toxins). Chemical agents, such as soman, sarin, VX, cyanide, and mustard agents, typically man-made for weaponization, are not included in this discussion except for comparison.

Mass Casualty Biological (Toxin) Weapon (MCBW)	Any toxin weapon capable of causing death or disease on a large scale, such that the military or civilian infrastructure of the state or organization being attacked is overwhelmed. (NOTE: The commonly accepted term for this category of weapons is “weapons of mass destruction,” although the term brings to mind destroyed cities, bomb craters, and great loss of life; MCBWs might cause loss of life only. I do not anticipate that “MCBW” will replace the term “weapons of mass destruction” in common usage, but it is technically more descriptive of toxin, and other biological, weapons.)
Militarily Significant (or Terrorist Weapon)	Any weapon capable of affecting—directly or indirectly, physically, or through psychological impact—the outcome of a military operation.

Source: Franz DR. *Defense Against Toxin Weapons*. Fort Detrick, Frederick, Md: US Army Medical Research Institute of Infectious Diseases; 1996: 4–5.

UNDERSTANDING THE THREAT

Replicating agents (bacteria and viruses) are clearly accepted throughout the medical defense community to be biological agents—and there is no argument that classic chemicals are chemical agents. Toxins, however, have sometimes been claimed to be chemicals (saxitoxin and ricin are included in the chemical weapons convention as placeholders) and at other times to be biological agents. Even Article I of the 1972 Biological Weapons Convention contributes to this ambiguity by describing the agents in question as “Microbial or other biological agents, or toxins.”[1]

The purpose of this chapter is to introduce toxins and describe their physical and biochemical characteristics, and the implications for medical defense, in the context of the clearly defined, and universally accepted, mass-casualty–producing agent classes. The following theoretical discussion is based on an understanding of physical and bio-chemical characteristics of toxins. It is not an intelligence assessment of the threat.

Toxins Compared With Chemical Warfare Agents

Toxins differ from classical chemical agents by source and physical characteristics. When considering them as biological warfare agents, the physical characteristics of the toxins are much more important than their source. Table 30-1 compares both types of agents. These are generalizations, and there are exceptions. The most important differences are in the areas of volatility and dermal activity. Toxins also differ from bacterial agents (eg, those causing anthrax or plague) and viral agents (eg, those causing viral equine encephalitides, or smallpox) in that toxins do not reproduce themselves.

Because toxins are not volatile, as are chemical agents, and with rare exceptions, do not directly affect the skin, an aggressor would have to present toxins to target populations in the form of respirable aerosols, which allow contact with the more vulnerable inner surfaces of the lung. This, fortunately, complicates an aggressor's task by limiting the number of toxins available for an arsenal. Aerosol particles between 0.5 and 5 µm in diameter are typically retained within the lung. Smaller particles can be inhaled, but most are exhaled. Particles larger than 5 to 15 µm lodge in the nasal passages or trachea and do not reach the lung. A large percentage of aerosol particles larger than 15 to 20 µm simply drop harmlessly to the ground. Because they are not volatile they are no longer a threat—even to unprotected troops. Although there are few data on aerosolized toxins, it is unlikely that secondary aerosol formation (ie, formation of 1–5 µm particles from larger, previously deposited droplets) caused by vehicular or troop movement over ground previously exposed to a toxin aerosol would generate a respirable toxin aerosol within the breathing zone of mounted or dismounted troops. (However, this may not be true with very heavy contamination with infectious agents such as anthrax spores, which might occur near the point of agent release from a munition.)

TABLE 30-1
COMPARISON OF TOXINS AND CHEMICAL AGENTS

Characteristics	Toxins	Chemical Agents
Origin	Natural	Man-made
Production	Difficult, small-scale	Large-scale industrial
Volatility	**None volatile**	**Many volatile**
Relative Toxicity	Many are more toxic	Less toxic than many toxins
Dermal Activity	**Not dermally active***	**Dermally active**
Use	Legitimate medical use	No use other than as weapons
Odor and Taste	Odorless and tasteless	Noticeable odor or taste
Toxic Effects	Diverse toxic effects	Fewer types of effects
Immunogenicity	Many are effective immunogens †	Poor immunogens
Delivery	Aerosol delivery	Mist/droplet/aerosol delivery

* Exceptions are trichothecene mycotoxins, lyngbyatoxin, and some of the blue-green algal toxins. The latter two cause dermal injury to swimmers in contaminated waters, but are generally unavailable in large quantities and have low toxicity, respectively.
† The human body recognizes them as foreign material and makes protective antibodies against them.
Adapted from Franz DR. *Defense Against Toxin Weapons*. Fort Detrick, Frederick, Md: US Army Medical Research Institute of Infectious Diseases; 1996: 6.

Toxicity, Ease of Production, and Stability

A toxin's toxicity, ease of production, and stability are inextricably interconnected. Regardless of its toxicity, a toxin that cannot be produced in sufficient quantity or is too unstable to survive as an aerosol after delivery cannot be an effective mass casualty biological weapon (MCBW).

Slightly less toxic toxins that are easy and inexpensive to produce and deliver, and that are stable as aerosols, could be real threats, however.

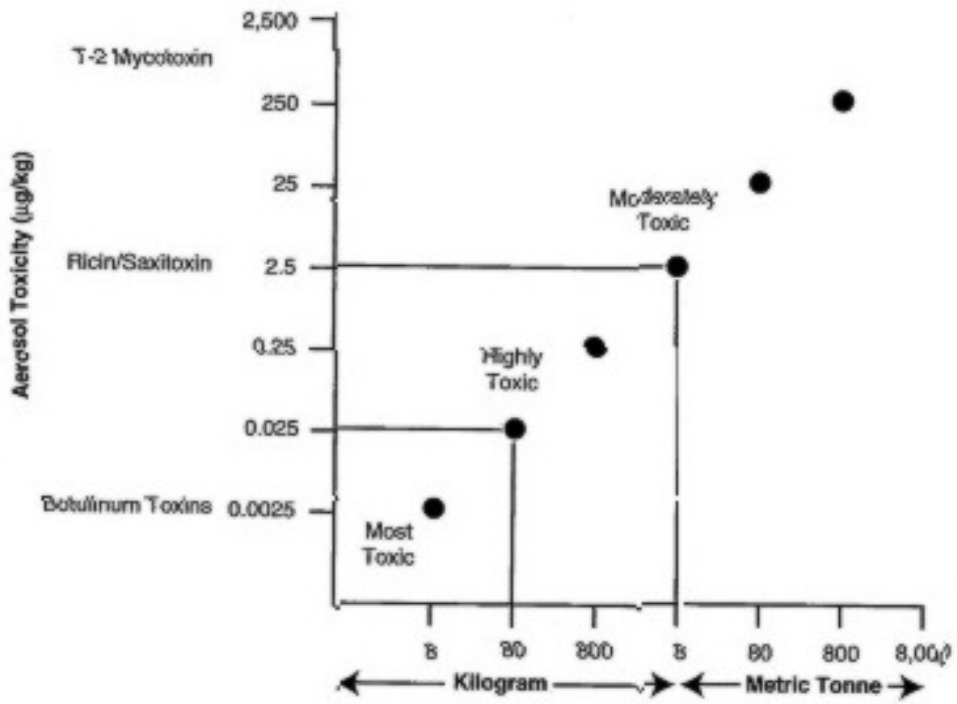


Fig. 30-1. Toxicity, in mouse LD_{50} (see Table 30-2), plotted against the quantity of toxin required to provide a theoretically effective open-air aerosol exposure, under ideal meteorological conditions, to an area of 100 km^2. Although the toxicity is based on direct studies with mice, it is believed to be very similar in humans. The mathematical model corrects for human parameters such as respiration. Ricin, saxitoxin, and botulinum, and trichothecene mycotoxins (T-2) kill at the concentrations depicted. Adapted from Spertzel RO, Wannemacher RW, Patrick WC, Linden CD, Franz DR. *Technical Ramifications of Inclusion of Toxins in the Chemical Weapons Convention (CWC)*. Alexandria, Va: Defense Nuclear Agency; 1992: 18. DNA Technical Report 92-116.

Because it must be delivered as a respirable aerosol, the utility of a toxin as an MCBW is limited by its toxicity and ease of production. The laws of physics dictate how much toxin of a given toxicity is needed to fill a given space with a small-particle aerosol. Figure 30-1 is a schematic representation of a theoretical calculation of the approximate quantities of toxins of varying toxicities required to intoxicate people exposed in large, open areas on the battlefield under optimal meteorological conditions. This figure is based on a mathematical model that was field tested in the 1960s (open-air testing) and found to be valid. It shows that a toxin with an aerosol toxicity of 0.025 mg/kg would require 80 kg of toxin to cover 100 km^2 with an effective cloud that exposes individuals within the cloud to a

dose that would be lethal to approximately 50% of those exposed (LD_{50}). For example, a typical 70-kg soldier would have a 50% chance of surviving after receiving a 70-mg dose of a toxin with an LD_{50} of 1.0 mg/kg. Note that for toxins less toxic than botulinum or the staphylococcal enterotoxins, hundreds of kilograms or even tons would be needed to cover an area of 100 km^2 with an effective aerosol. Table 30-2 shows the mouse LD_{50}s of 25 toxins and chemical warfare agents.

TABLE 30-2
COMPARATIVE LETHALITY OF SELECTED TOXINS AND CHEMICAL AGENTS IN LABORATORY MICE

Agent	LD_{50}(μg/kg)*	Molecular Weight †	Source
Botulinum Toxin	0.001	150,000	Bacterium
Shiga Toxin	0.002	55,000	Bacterium
Tetanus Toxin	0.002	150,000	Bacterium
Abrin	0.04	65,000	Plant (rosary pea)
Diphtheria Toxin	0.10	62,000	Bacterium
Maitotoxin	0.10	3,400	Marine dinoflagellate
Palytoxin	0.15	2,700	Marine soft coral
Ciguatoxin	0.40	1,000	Fish, marine dinoflagellate
Textilotoxin	0.60	80,000	Elapid snake
Clostridium perfringens toxins	0.1–5.0	35,000–40,000	Bacterium
Batrachotoxin	2.0	539	Arrow-poison frog
Ricin	3.0	64,000	Plant (castor bean)
α-Conotoxin	5.0	1,500	Cone snail
Taipoxin	5.0	46,000	Elapid snake
Tetrodotoxin	8.0	319	Puffer fish

α-Tityustoxin	9.0	8,000	Scorpion
Saxitoxin	10.0 (Inhal: 2.0)	299	Marine dinoflagellate
VX	15.0	267	Chemical agent
Staphylococcus Enterotoxin B (Rhesus/Aerosol)	27.0	28,494	Bacterium
Anatoxin-A(s)	50.0	500	Blue-green algae
Microcystin	50.0	994	Blue-green algae
Soman (GD)	64.0	182	Chemical agent
Sarin (GB)	100.0	140	Chemical agent
Aconitine	100.0	647	Plant (monkshood)
T-2 Toxin	1,210.0	466	Fungal mycotoxin

*LD_{50}s are approximate, drawn from numerous published and unpublished sources. Routes of administration are typically intraperitoneal or intravenous.
†Note the general inverse relation between toxicity and molecular weight.
Reprinted from *Medical Management of Biological Casualties Handbook.* Fort Detrick, Frederick, Md: US Army Medical Research Institute of Infectious Diseases; Aug 1993: Appendix.

During the U.S. biological warfare program, which ended in 1969, toxicity calculations were based on LD_{50} values as described above. The mathematical formulae developed by Calder and validated in field trials used the LD_{50} as a measure of toxicity.[2] Calculation of the LD_{50} of an aerosol requires a number of assumptions regarding respiratory minute volume of the experimental animal, and the percentage of the inhaled aerosol retained in the lung and airways during the period of exposure. In an attempt to improve accuracy, reproducibility, and data comparability within and between species, values called LCt_{50} values have been generated in recent years for aerosols. LCt_{50} is the product of the average concentration (C, in mg/m^3) and the exposure time (*t*, in min) that is lethal (L) to 50% of the population exposed (the units are expressed as

mg•min/m^3). LCt_{50} values for selected toxins in mice and rhesus monkeys are shown in Table 30-3.

Ignoring other characteristics, if a toxin is not adequately toxic, sufficient quantities cannot be produced to make even one weapon. Because of their low toxicity, therefore, hundreds of toxins can be eliminated as ineffective as MCBWs. Certain plant toxins with marginal toxicity could be produced in large (ton) quantities. These toxins could possibly be weaponized. At the other extreme, several bacterial toxins are so lethal that MCBW quantities are measured not in tons, but in kilograms—quantities more easily produced. Such toxins are potential threats to our soldiers on the battlefield.

TABLE 30-3
LCt_{50}s FOR SELECTED TOXINS IN MICE AND RHESUS MONKEYS

Toxin	Mouse LCt_{50} (mg•min/m^3)	Rhesus Monkey LCt_{50} (mg•min/m^3)
Botulinum A	0.0225	0.0225
Ricin	3–7	114
Saxitoxin	3	—
T-2 Toxin	200	—
Staphylococcus Enterotoxin B	NA	80–100

Source: Pitt L, PhD. Chief, Department of Aerobiology and Product Evaluation, Toxinology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, July 1996.

Stability of toxins after aerosolization is also an important factor, because it further limits toxin weapon effectiveness. Some toxins are adequately toxic and can be produced in sufficient quantities for weapons, but are too unstable in the atmosphere to be candidates for weaponization. Although stabilization of naturally unstable toxins and enhanced production of those toxins now difficult to produce are possibilities for the future, no evidence exists at this time for successful amplification of toxicity of a naturally occurring toxin.

Incapacitation as well as lethality to humans must be considered. A few toxins cause illness at levels many times less than the concentration needed to kill. For example, toxins that directly affect the membranes, fluid balance, or both within the lung may greatly reduce gas transport without causing death. Less-potent toxins could also be significant threats as

aerosols in a confined space such as a building; delivery could be into the filtration, heating, and air-conditioning systems. Finally, breakthroughs in delivery-vehicle efficiency or toxin “packaging” by an aggressor might alter the relation between toxicity and quantity; but even at best, the quantities needed could probably be reduced by only one half for a given toxicity. For now, however, the relation shown in Figure 30-1 provides a reasonable and valid way to sort potential threat toxins.

Militarily significant weapons need not be MCBWs. Thirty-nine Iraqi-modified Scud missiles reached Israel from 18 January through 28 February 1991. Although many of the Scuds were off target or malfunctioned, some of them landed in and around Tel Aviv. Approximately 1,000 people were treated as a result of the missile attacks, but only 2 died. Anxiety was listed as the reason for admitting 544 patients and atropine overdose for hospitalization of 230 patients.[3] The remainder (226 patients) suffered traumatic injury. Clearly, these Scuds were not effective mass casualty weapons, yet they caused significant disruption to the population of Tel Aviv. Approximately 75% of the 1,000 casualties were injured as a result of their own inappropriate actions or reactions. Had one of the warheads contained a toxin that killed or intoxicated a few people, the “terror effect” would have been even greater. Therefore, many toxins that are not sufficiently toxic for use in an *open-air* MCBW could probably be used to produce a *militarily significant weapon*. However, the likelihood that such a toxin weapon will cause panic among military personnel decreases when the leaders and troops become better educated regarding toxins.

Sources of Toxins and Their Mechanisms of Action

Toxins vary as to their source of production, molecular structure and size, and mechanism of action. Article I of the 1972 Biological Weapons Convention includes the concept of “toxins whatever their origin or method of production”[1] for good reason. Although in nature toxins are produced by microorganisms, plants, and animals, many of them can also be produced synthetically; this is generally not true of replicating agents. Ease of production—whether natural or synthetic—is obviously an important factor in evaluating a threat toxin, but a toxin’s method of production does not change its molecular structure or mechanism of action.

Regarding molecular structure and size, the terms “low-molecular-weight” and “protein” toxins are commonly used. Low-molecular-weight toxins are typically less than 1,000 dalton (d), or approximately 10 amino acids, and may be either organic molecules or peptides. Protein toxins are proteins generally greater than approximately 10 amino acids.

The mechanism of action of a toxin does not necessarily correlate with

either its source or its molecular structure or size. Understanding the mechanism of toxicity by the threat route of challenge is, however, the first step in developing medical countermeasures for a toxin, and is often the most important factor influencing what approach will be taken to protect soldiers. For toxins, there are two general categories of mechanism of action:

1. Neurotoxins exert direct effects on nervous system function, most often the peripheral nervous system. These effects are typically temporary or reversible.
2. Membrane-damaging toxins actually destroy or damage tissues or organs, directly or indirectly through the release of mediators of disease. The effects of membrane-damaging toxins are less commonly reversible.

Although each of these factors will be discussed in detail in individual agent chapters, the concepts and their implications for the protection of soldiers are introduced here. There is little correlation between the artificial groupings (source, molecular structure, and mechanism of action) commonly used to categorize toxins. The natural source and the implications of mechanism of action of toxins on the development of medical countermeasures are discussed below.

Bacterial Toxins

The most toxic biological materials known are protein toxins produced by bacteria. They are generally more difficult to produce on a large scale than are the plant toxins, but they are many, many times more toxic. Botulinum toxins (seven related toxins), the staphylococcal enterotoxins (also seven different toxins), diphtheria, and tetanus toxin are well-known examples of bacterial toxins.

The botulinum toxins are so very toxic that lethal aerosol MCBW weapons could be produced with quantities of toxin that are relatively easily attainable with present technology. They cause death through paralysis of respiratory muscles without producing microscopic change in the tissues.

Staphylococcal enterotoxins, when inhaled, cause fever, headache, diarrhea, nausea, vomiting, muscle aches, shortness of breath, and a nonproductive cough within 2 to 12 hours after exposure. They can also kill, but only at much higher doses. Staphylococcal enterotoxin B (SEB) can incapacitate at levels at least 100-fold lower than the lethal level. These toxins, too, would probably be delivered as respirable aerosols.

Other bacterial toxins, classified generally as membrane-damaging, are derived from *Escherichia coli* (which produces hemolysins), *Aeromonas, Pseudomonas,* and *Staphylococcus,* (which also produce cytolysins and phospholipases), and are moderately easy to produce, but they vary a great deal in stability. Many of these toxins affect bodily functions or even kill by forming pores in cell membranes. In general, their lower toxicities make them less likely battlefield threats.

Marine Toxins

A number of the toxins produced by marine organisms, or by bacteria that live in marine organisms, might be used in terrorist biological weapons (where less agent would be needed to achieve the desired effect), but they are unlikely threats on the open battlefield. For many of these low-molecular-weight marine toxins, either difficulty of production or lack of sufficient toxicity limits the likelihood of their use as MCBWs.

Saxitoxin, the best known example of this group, is a potent neurotoxin found in shellfish such as mussels, clams, and scallops. Saxitoxin is a sodium channel–blocking agent and is more toxic by inhalation than by other routes of exposure. Unlike oral intoxication with saxitoxin (paralytic shellfish poisoning), which has a relatively slow onset, inhalational intoxication with saxitoxin can be lethal in a few minutes. Saxitoxin could be used against our troops as an antipersonnel weapon, but because it cannot currently be chemically synthesized efficiently, or produced easily in large quantities from natural sources, it is unlikely to be seen as an area aerosol weapon on the battlefield.

Tetrodotoxin, from the puffer fish and other members of the order Tetraodontiformes, is a neurotoxin much like saxitoxin in its mechanism of action, toxicity, and physical characteristics. Palytoxin, from the soft coral *Palythoa tuberculosa*, is extremely toxic and quite stable in impure form, but difficulty of production or harvest from nature reduces the likelihood that an aggressor would use it as an MCBW. The brevetoxins, commonly associated with “red tide” dinoflagellate blooms, and the blue-green algal toxins like microcystin, a hepatotoxin, have limited toxicity.

Fungal Toxins

The trichothecene mycotoxins, which are toxins produced by various species of fungi, are also examples of low-molecular-weight toxins (MW < 1,000 d). The yellow rain incidents in Southeast Asia in the early 1980s are believed to have demonstrated the utility of one of the trichothecene mycotoxins, T-2, as a biological warfare agent.

T-2 is one of the more stable toxins, retaining its bioactivity even when heated to high temperatures. High concentrations of sodium hydroxide

and sodium hypochlorite are required to detoxify it. Aerosol toxicities are generally too low to make this class of toxins useful to an aggressor as an MCBW as defined in Figure 30-1; however, unlike most toxins, these are dermally active. Clinical presentation includes nausea, vomiting, weakness, low blood pressure, and burns in exposed areas.

Plant Toxins

Toxins derived from plants are generally very easy to produce in large quantities at minimal cost in a low-technology environment. Ricin, a protein derived from the bean of the castor plant, and abrin, a very similar toxin from *Abrus precatorius* are typical plant toxins.

Worldwide, approximately 1 million tons of castor beans are processed annually in the production of castor oil. The resulting waste mash is approximately 3% to 5% ricin by weight. Because of its marginal toxicity, at least 1 tonne (1,000 kg) of the toxin would be necessary to produce an MCBW (see Figure 30-1). Unfortunately, the precursor raw materials are available in these quantities throughout the world.

Venom Toxins

Animal venoms often contain a number of toxic and nontoxic proteins. Until recently, it would have been practically impossible to collect enough of these materials to develop them as biological weapons. However, many of the venom toxins have now been *sequenced* (ie, their molecular structure has been determined), and some have been cloned and *expressed* (ie, produced by molecular biological techniques). Some of the smaller ones could also be produced by relatively simple chemical synthesis methods. The following are examples of the mechanisms of action and sources of venom toxins:

- ion channel (cationic) toxins, such as those found in the venoms of the rattlesnake, scorpion, and cone snail;
- presynaptic phospholipase A_2 neurotoxins of the banded krait, Mojave rattlesnake, and Australian taipan snake;
- postsynaptic (curare-like alpha toxin) neurotoxins of the coral, mamba, cobra, and sea snakes, and the cone snail;
- membrane-damaging toxins of the Formosan cobra and rattlesnake; and
- coagulation/anticoagulation toxins of the Malayan pit viper and carpet viper.

Some of the toxins in this group must be considered potential future threats to our soldiers as large-scale production of peptides becomes more efficient. However, because many of these toxins are difficult to produce in large quantities, their threat potential may be limited.

Mechanisms of Action and the Development of Countermeasures

Unlike chemical agents, toxins differ widely in their mechanisms of action. The medical protection of soldiers is therefore difficult; seldom will a vaccine or therapy be effective against more than one toxin. (NOTE: We can prepare for a battlefield threat—unlike a terrorist threat—by developing specific medical countermeasures. Vaccines and other prophylactic measures can be given before combat, and therapies can be kept at the ready.) Countermeasures are discussed in general later in this chapter and in detail in specific agent chapters in this textbook.

Neurotoxins

Saxitoxin. Some neurotoxins, such as saxitoxin and tetrodotoxin, can kill an individual very quickly after inhalation of a lethal dose (within minutes). These toxins act by blocking nerve conduction directly and cause death by paralyzing muscles of respiration. Yet, at just less than a lethal dose, the exposed individual may not even feel ill, or may only feel dizzy.

Because of the rapid onset of signs after inhalation, prophylaxis (either immunization or pretreatment) would be required to protect soldiers from these two rapidly acting neurotoxins. Unprotected soldiers who inhale a lethal dose would probably die before they could be helped, unless they could be intubated and artificially ventilated immediately. Although the mechanism of death after inhalation of saxitoxin is believed to be the same as when the toxin is administered intravenously, it is more toxic if inhaled.

Botulinum Toxins. Other neurotoxins, such as the botulinum toxins, must enter nerve terminals before they can block the release of neurotransmitters, which normally cause muscle contraction. These large-protein neurotoxins generally kill by relatively slow onset respiratory failure (within hours to days). The intoxicated individual may not show signs of disease for 24 to 72 hours. The toxin blocks biochemical action in the nerves that activate the muscles necessary for respiration, leading to suffocation.

Intoxications such as this can be treated with antitoxin injected hours after exposure to a lethal dose of toxin (≤ 24 h in monkeys, and probably also in humans), and still prevent illness and death. Although the mechanisms of toxicity of the botulinum toxins appear to be the same after

any route of exposure, the actual toxicity of the botulinum toxins is less by inhalation.

Membrane-Damaging Toxins

While neurotoxins effectively stop nerve and muscle function without causing microscopic damage to the tissues, membrane-damaging toxins destroy or damage tissue directly. For these toxins, prophylaxis is important, because the point at which the pathological change becomes irreversible often occurs within minutes to a few hours after exposure.

Microcystin. An example of this type of toxin is microcystin (produced by blue-green algae), which binds covalently to a phosphatase inside liver cells; this toxin does not damage other cells of the body. Unless uptake of the toxin by the liver is blocked, irreversible damage to the organ occurs within 15 to 60 minutes after exposure to a lethal dose. When this happens, the tissue damage to the liver is so severe that therapy may have little or no value. For microcystin, unlike most toxins, the toxicity is the same, no matter what the route of exposure.

Ricin. When dealing with membrane-damaging toxins, the consequences of intoxication, thus the pathogenesis of disease, may vary widely with route of exposure, even with the same toxin. Ricin, a plant toxin, kills by blocking protein synthesis in many cells of the body, but no lung damage occurs with any exposure route except inhalation. If ricin is inhaled, however, as would be expected during a biological attack, microscopic damage is limited primarily to the lung, and death is caused by a mechanism different from that of injected toxin. Furthermore, when equivalent doses of toxin are used, much more protective antibody must be injected to protect from inhalational exposure than from intravenous injection. Finally, although signs of intoxication may not be noted for 12 to 24 hours, microscopic damage to lung tissue begins within 8 to 12 hours or less. Irreversible biochemical changes may occur within 60 to 90 minutes after exposure, again making therapy difficult.

Trichothecene Mycotoxins. Only one class of easily produced, membrane-damaging toxins, the trichothecene mycotoxins, is dermally active. Therefore, they must be considered by standards different from those for all other toxins. Trichothecenes can cause skin lesions and systemic illness without being inhaled and absorbed through the respiratory system. Skin exposure and ingestion of contaminated food are the two likely routes of exposure of soldiers; oral intoxication is unlikely in modern, well-trained armies. Nanogram quantities per square centimeter of skin cause irritation, and microgram quantities cause necrosis. If the eye is exposed, microgram doses can cause irreversible injury to the cornea.

The aerosol toxicity of even the most toxic trichothecene is low enough that the large-quantity production required (approximately 80 tonnes to expose a 10-km^2 area with respirable aerosol) makes an inhalational threat unlikely on the battlefield. These toxins, therefore, might be dispersed as larger particles, probably visible in the air and on the ground and foliage.

In contrast to treatment for exposure to any of the other toxins, simply washing the skin with soap and water within 1 to 3 hours after exposure to the trichothecene mycotoxins will eliminate or greatly reduce the risk of illness or injury.

Populations at Risk

Because there are hundreds of toxins available in nature, the job of protecting troops against them seems overwhelming. It might seem that an aggressor would need only to discover the toxins against which we can protect our troops, and then pick a different one to weaponize. In reality, however, it is not that simple. The utility of toxins as MCBWs is limited by their toxicity (see Figure 30-1). This criterion alone reduces the list of potential open-air, weaponizable toxins for MCBWs from hundreds to fewer than 20. Issues related to stability and weaponization will not be addressed here, but would further reduce the list and make the aggressor's job more difficult.

An armored or infantry division in the field is not at great risk of exposure to a marine toxin whose toxicity is so low that 80 tonnes is needed to produce an MCBW covering 10 km^2. Most marine toxins are simply too difficult to produce in such quantities. Military leaders on today's battlefield should be concerned first about the most toxic bacterial toxins.

The more confined the military or terrorist target (eg, inside shelters, buildings, ships, or vehicles), the greater the list of potential toxin threats that might be effective. This concern is countered, however, by the fact that toxins are not volatile like the chemical agents and are thus more easily removed from air-handling systems. It is probably most cost effective to protect our personnel from these less-toxic toxins through the use of collective filtration systems.

Nonetheless, we must consider subpopulations of troops and areas within which they operate when we estimate vulnerability to a given toxin threat. Because of differences in operational environments, situations could well occur in which different populations of troops require protection from different toxins. To protect them effectively, military decision makers and leaders must understand the nature of the threat and the physical and medical defense solutions.

Table 30-4 gives the approximate number of known toxins by toxicity

level and source. To simplify our approach to the development of medical countermeasures, we have divided them into “Most Toxic,” “Highly Toxic,” and “Moderately Toxic” categories (also see Figure 30-1). The most toxic toxins could probably be used in an MCBW; it is feasible to develop individual medical countermeasures against them. The highly toxic toxins could probably be used in closed spaces such as the air-handling system of a building or as relatively ineffective terror weapons in the open; collective filtration would be effective against these toxin aerosols targeted to enclosed spaces. The moderately toxic toxins would likely be useful only as assassination weapons, which would require direct attack against an individual; it is not feasible to develop medical countermeasures against all of the toxins in this group. Such reasoning allows us to use limited resources most effectively and to maximize protection, and thus effectiveness, of our fighting force.

TABLE 30-4
ARBITRARY CATEGORIZATIONS* OF TOXIN TOXICITY

Source of Toxin	Most Toxic	Highly Toxic	Moderately Toxic	Total
	(Number of toxins in each category)			
Bacteria	17	12	> 20	> 49
Plants		5	> 31	> 36
Fungi			> 26	> 26
Marine organisms		> 46	> 65	>111
Snakes		8	>116	>124
Algae		2	> 20	> 22
Insects			> 22	> 22
Amphibians			> 5	> 5
Total	**17**	**> 73**	**>305**	**>395**

* Most toxic (LD_{50} < 0.025 µg/kg), highly toxic (LD_{50} 0.025–2.5 µg/kg), moderately toxic (LD_{50}> 2.5 µg/kg)
Adapted from Spertzel RO, Wannemacher RW, Patrick WC, Linden CD, Franz DR. *Technical Ramifications of Inclusion of Toxins in the Chemical Weapons Convention (CWC)*. Alexandria, Va: Defense Nuclear Agency; 1992: 13. DNA Technical Report 92-116.

COUNTERMEASURES

Physical Protection

As stated above, most toxins are neither volatile nor dermally active. Therefore, an aggressor would most likely attempt to present them as respirable aerosols. Toxin aerosols should pose neither a significant residual environmental threat nor remain on the skin or clothing. The typical toxin cloud would, depending on meteorological conditions, either drift with the wind close to the ground or rise above the surface of the Earth and be diluted in the atmosphere. There may, however, be residual contamination near the munition-release point. Humans in the path of a true aerosol cloud would be exposed as the agent drifts through that area. The principal way humans are exposed to such a cloud is through breathing. Aerosol particles must be drawn into the lungs and retained to cause harm.

The protective mask, worn properly, is effective against toxin aerosols. Its efficacy, however, depends on two factors: (1) mask-to-face or hood-to-head fit and (2) use during an attack. Proper fit is vital. Because of the extreme toxicity of some of the bacterial toxins, a relatively small leak could result in a significant exposure. Eyes should be protected when possible. Definitive studies have not been done to assess the effects of aerosolized toxins on the eyes. In general, however, ocular exposure to a toxin aerosol, unless the exposed individual is near the release point, would be expected to cause few systemic effects because of the low doses absorbed. A few toxins have direct effects on the eyes, but these are generally not toxins we would expect to be used as aerosols. Donning the protective mask prior to exposure would, of course, protect the eyes.

Because important threat biological warfare agents are not dermally active and must be presented as respirable aerosols, special protective clothing other than the mask is less important in a toxin attack than in a chemical attack. Presently available protective clothing should be effective against biological threats as we know them. Commanders should carefully consider the relative impact of thermal load and the minimal additional protection provided by protective clothing.

Real-Time Detection of an Attack

Because of the nature of the threat, soldiers may be dependent on a mechanical detection-and-warning system to notify them of impending or ongoing attack. Without timely warning, their most effective generic countermeasure, the protective mask, may be of limited value. Real-time detectors of a chemical agent attack have been successfully developed.

Biological agent detectors will be more difficult to develop, for several reasons. As stated above, these agents must be presented as respirable aerosols, which act as a cloud, not as droplets (as the chemical agents are delivered) that fall to the ground and evaporate with time. The toxin cloud, typically delivered at night with a slight wind, would be expected to move across the battlefield until it either rises into the atmosphere to be diluted or settles, relatively harmlessly, to the ground. Unlike chemical agents, which might be detectable for hours, toxins might be detectable in the air *at one location only* for a few minutes. Definitive, specific toxin detectors would have to sample continuously or be turned on by a continuous sampler of some kind.

Furthermore, toxin detectors (assuming the present state of technology) would probably require the specificity of immunoassays to identify a toxin and differentiate it from other organic material in the air. Continuous monitoring by such equipment would be extremely costly, reagent-intensive, and very difficult to support logistically because of the reagent requirements. Identifying each toxin would require a different set of reagents if an immunoassay system were used.

Analytical assays would necessarily be more complex and less likely to identify distinct toxins, but they might detect that something unusual was present. Imagine the difficulty of developing a detection system based on molecular weight or other physical characteristics to differentiate among the seven botulinum toxins (molecular weight is the same for all, but each requires a different, specific antibody for identification or therapy).

Finally, to be effective, a detector would have to be located where it could “sniff” a toxin cloud in time to warn the appropriate population. This might be possible on a battlefield but would be nearly impossible, except in selected high-risk facilities, in the case of a terrorist attack. However, if all the capabilities described were developed and available at the right place and time, it is possible that an aerosol cloud of almost any of the toxins of concern could be detected and identified. Future advances in technology could well resolve our present technical difficulties.

Diagnosis: General Considerations

Medical personnel often ask whether they will be able to tell the difference among cases of inhalational botulinum, staphylococcal enterotoxin intoxication, and chemical nerve agent poisoning. Table 30-5 describes these differences. In general, nerve agent poisoning has a rapid onset (minutes) and induces increased body secretions (saliva, airways secretions), pin-point pupils, and convulsions or muscle spasms. Botulinum intoxication has a slow onset (12–72 h) and manifests as visual disturbance, skeletal muscle weakness and/or paralysis of oropharyngeal

muscles. Staphylococcal enterotoxin B poisoning has an intermediate (few hours) time of onset and is typically not lethal but is severely incapacitating. Chemical nerve agent poisoning is a violent illness resulting in respiratory failure because of muscle spasm, airway constriction, and excessive fluid in the airways. Botulinum-intoxicated patients simply get very tired and very weak; if they die, it is because the muscles of respiration fail. Staphylococcal enterotoxin B–intoxicated patients become very sick but typically survive with supportive therapy.

Medical personnel should consider toxins in the differential diagnosis, especially when multiple patients present with similar clinical syndromes. Patients should be viewed epidemiologically and asked about where they were, whom they were with, what they observed, how many other soldiers were and are involved, and so forth. Inhaled and retained doses of toxins will differ among soldiers exposed to the same aerosol cloud. Those who received the highest dose typically will show signs and symptoms first. Others will present somewhat later, while still others in the same group may be unaffected. The distribution of severities within the group of soldiers may vary with type of exposure and type of toxin. For example, exposing a group of individuals to the staphylococcal enterotoxins by inhalation would likely make a large percentage (80%) of them sick, but would result in few deaths. Exposing a group of soldiers to a cloud of botulinum toxin might kill 50%, make 20% very sick, and leave 30% unaffected.

Medical personnel must consider the varying latent periods before onset of clinical signs. For patients exposed to toxins by aerosol, the latent period varies from minutes (saxitoxin, microcystin) to hours (the staphylococcal enterotoxins), even to days (ricin, the botulinum toxins).

Medical personnel must also save clinical and environmental samples for diagnosis. Immunoassays and analytical tests are available for many of the toxins. Environmental toxin samples taken directly from a weapon or other hardware are often easier to test than biological samples because they do not contain body proteins and other interfering materials.

The best early diagnostic sample for most toxins is a swab from the nasal mucosa. In general, the more-toxic toxins are more difficult to detect in tissues and body fluids, because so little toxin needs to be present in the body to exert its effect. The capability exists however, to identify most of the important toxins in biological fluids or tissues, and many other toxins in environmental samples. Definitive laboratory diagnosis might take 48 to 72 hours; however, prototype field assays that can identify some toxins within 30 minutes have been developed recently. For individuals who survive an attack with toxins of lower toxicity, immunoassays that detect immunoglobulins M or G offer a means of diagnosis, identification, or confirmation of agent within 2 to 3 weeks after exposure.

TABLE 30-5
DIFFERENTIAL DIAGNOSIS OF CHEMICAL NERVE AGENT, BOTULINUM TOXIN, AND STAPHYLOCOCCAL ENTEROTOXIN B INTOXICATION FOLLOWING INHALATIONAL EXPOSURE

Signs and Symptoms	Chemical Nerve Agent (Organophosphate)	Botulinum Toxin	Staphylococcal Enterotoxin B
Time to Onset	Minutes	Hours (12–72)	Hours (2–12)
Nervous	Convulsions, fasciculations	Progressive paralysis	Headache, muscle aches
Cardiovascular	Bradycardia	Normal rate	Normal rate or tachycardia
Respiratory	Difficult breathing, constricted airways	Normal, then progressive paralysis	Nonproductive cough In severe cases: chest pain, difficult breathing
Gastrointestinal	Increased motility, pain, diarrhea	Decreased motility	Nausea, vomiting and/or diarrhea
Ocular	Small pupils	Droopy eyelids	Conjunctival injection possible
Salivary	Profuse, watery saliva	Normal, but swallowing difficult	Slightly increased quantities of saliva possible
Death	Minutes	2–10 d	Unlikely
Response to Atropine/ 2-PAM Cl	Yes	No	Atropine may reduce gastrointestinal symptoms

2-PAM Cl: 2-pyridine aldoxime methyl chloride

Approaches to Prevention and Treatment

In developing medical countermeasures, each toxin must be considered individually. Some incapacitate so quickly that there would be little time for therapy after an attack. Others cause few or no clinical signs for many hours, but they set off irreversible biochemical processes in minutes or a few hours that lead to severe debilitation or death several days later. Fortunately, some of the most potent bacterial protein toxins act slowly enough that, if they are identified, therapy initiated 12 to 24 hours after exposure is usually successful. Active and passive immunoprophylaxes are currently available but are not licensed for all high-threat toxins. Immunization, pretreatment, and specific drug therapies are the subjects of considerable research interest.

Active Immunization

It is always better to prevent casualties than to treat injured soldiers. For most of the significant threat toxins in military situations, vaccination is the most effective means of preventing casualties. Unlike the chemical warfare agents, many of the important threat toxins are highly immunogenic. Immunized laboratory animals are totally protected from high-dose aerosols of these toxins. Immunization requires a knowledge of the threat, an available vaccine, and time. The minimum time needed to allow the body to make its own protective antibodies to a toxin may range from 4 to 6 weeks, to 12 to 15 weeks, or longer. Some vaccines currently in use require multiple injections, often administered weeks apart. The logistical burden of assuring that troops are given booster immunizations at the correct time could be overwhelming in a fast-moving buildup to hostilities.

The time and effort required for immunization can possibly be reduced. For example, antigens are being microencapsulated to form timed-release vaccines that might provide the primary immunization, a booster dose 2 weeks later, and another booster dose 10 weeks after that—all with one injection. Another approach is being evaluated with current Medical Biological Defense Research Program vaccines. Soldiers could be given a priming dose and the first booster dose 2 weeks apart, while in basic training. The response generated by the immune system’s memory cells (ie, the B lymphocytes) might last for many months or even years, although not all soldiers would develop fully protective immunity after only two immunizations. Shortly before the onset of hostilities, or when the soldier is assigned to a rapidly deployable unit, one booster dose could provide protective immunity quickly, and preclude the need for additional booster doses after deployment. Preliminary data suggest that a booster dose administered up to 24 months (the greatest interval thus far tested)

after two initial priming doses will be effective, even with moderately immunogenic vaccines such as the current botulinum toxoid.

Passive Antibody Prophylaxis

Passive antibody prophylaxis is generally quite effective in protecting laboratory animals from toxin exposure. However, this option is of little real utility for large groups of people for several reasons. The protection provided by human antibody may last for only 1 to 2 months, and protection afforded by despeciated horse antibody may last for only a few weeks. Therefore, antibody prophylaxis would be practical only when the threat is both clearly understood and imminent. Furthermore, it is unlikely that animal antibody would be used in an individual before intoxication because of the risk, albeit small, of an adverse reaction to foreign protein. The latter problem may be overcome within the next few years, as the production of human monoclonal antibodies or the “humanization” of mouse monoclonal antibodies becomes practical. Unfortunately, single monoclonal antibodies are seldom as effective against toxins as polyclonal antibodies, such as those produced naturally in other humans or horses. However, combined antibody therapy, or “cocktails” of more than one monoclonal antibody, may overcome this problem in the future.

Postexposure prophylaxis (ie, treatment after exposure but before signs and symptoms develop) with antibodies from human or animal sources is feasible for some of the threat toxins. Passive immunotherapy is very effective after exposure to botulinum toxin if treatment is begun soon enough, up to 24 hours after high-dose aerosol exposure to the toxin. The utility of antibody therapy drops sharply at or shortly after the onset of the first signs of disease. It appears that a significant amount of the toxin has, at that time, been taken up by areas of the body that cannot be reached by circulating antibodies. Antibody therapy given after the onset of signs may shorten the time that a patient must be given ventilatory support. The available antibody to botulinum toxin is produced in horses, and then despeciated to make a product with a reduced risk of adverse reaction that can be given to humans. Human monoclonal antibodies, or cocktails of two or more monoclonal antibodies, may be the next generation of antibody therapy. Passive antibody therapy such as that described here is more likely to be effective against neurotoxins like the botulinum toxins, which do not cause tissue damage, than against membrane-damaging toxins that induce mediator release (eg, staphylococcal enterotoxins), directly damage tissues (eg, ricin), or both.

Specific Therapy

Specific therapy with drugs presently has little value: most of the toxins

either physically damage cells and tissues very quickly (eg, ricin), or affect such basic mechanisms within the cell (eg, the neurotoxins) that drugs designed to reverse their effects are toxic themselves. Nevertheless, at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland, we have shown that rifampin stops the lethal intoxication by microcystin if it is given therapeutically to laboratory animals soon (15–30 min) after the toxin is administered.

Development of therapeutic drugs for toxins is presently aimed at several more general approaches. When the toxin's mechanism of action is understood and covalent (permanent) binding of the toxin to cellular protein does not occur (eg, ion-channel toxins), attempts are being made to discover drugs that compete with or block the toxin from binding to its site of action. For toxins with enzymatic activities, such as ricin and the botulinum toxins, drugs that serve as surrogate targets of such enzymatic action may be developed. For toxins such as botulinum, which block the release of a neural transmitter, there have been attempts to enhance the release of the needed transmitter by other means; the diamino pyridines are temporarily effective in reversing botulinum intoxication by this mechanism. Finally, for toxins like the staphylococcal enterotoxins and ricin, which induce the release of secondary mediators, specific mediator-blocking agents are being studied. In the future, drugs may well find a place in the therapy of some intoxications as adjuncts to vaccination or passive antibody therapy, or they may be used to delay onset of toxic effects.

Symptomatic Therapy

General supportive measures are likely to be effective in therapy of intoxication. Artificial ventilation could be lifesaving in the case of neurotoxins such as the botulinum toxins and saxitoxin. Oxygen therapy, with or without artificial ventilation, may be beneficial for intoxication with toxins such as ricin that directly damage the alveolar-capillary membrane of the lung. Vasoactive drugs and volume expanders could be used to treat the shocklike state that accompanies some intoxications (eg, with staphylococcal enterotoxin B). These measures could be used in conjunction with more specific therapies.

Decontamination and Protection of Medical Personnel

Recall that a true respirable aerosol will leave less residue on clothing and environmental objects than would the larger particles produced by a chemical munition. This suggests that decontamination would be relatively unimportant after a toxin aerosol attack. Because we lack field experience,

however, prudence dictates that soldiers decontaminate themselves after an attack.

As a general rule, the decontamination procedure recommended for chemical warfare agents[4] effectively destroys toxins. Exposure to 0.1% sodium hypochlorite solution (household bleach) for 10 minutes destroys most protein toxins. The trichothecene mycotoxins require more stringent measures to inactivate them, but even they can be removed from the skin (although not inactivated) simply by washing with soap and water. Soap and water, or even just water, can be very effective in removing most toxins from skin, clothing, and equipment.

For the same reason that decontamination is only moderately important after individuals are exposed to a respirable toxin aerosol, medical personnel are probably at only limited risk from secondary aerosols. Because toxins are not volatile, casualties of a toxin attack can, for the most part, be handled safely and moved into closed spaces or buildings, unless they were very heavily exposed. Prudence dictates, however, that patients be handled as if they were chemical casualties or, at a minimum, that they be washed with soap and water. The risk to medical personnel is of greater concern with some agents. Secondary exposure might be a hazard with very potent bacterial protein toxins, such as botulinum toxin or the staphylococcal enterotoxins. (NOTE : Decontamination and isolation of patients or remains could be much more important and difficult after an attack with a bacteria or virus that replicates within the body.)

Remains of persons possibly contaminated with toxins should be handled the same as chemically contaminated remains. For the most part, toxins are more easily destroyed than chemical agents, and they are much more easily destroyed than anthrax spores. Chemical disinfection of remains in 0.2% sodium hypochlorite solution for 10 minutes would destroy all surface toxin (and even anthrax spores), greatly reducing the risk of secondary exposure.

Sample Collection: General Rules for Toxins

Identifying toxins or their metabolites (break-down products) in biological samples (blood, urine, feces, saliva, or body tissues) is difficult for several reasons. First, for most toxic toxins, relatively few molecules of toxin need be present in the body to cause an effect; therefore, finding them requires extremely sensitive assays. Second, the most toxic toxins, and those most likely to be seen on the battlefield, are proteins. Therefore, after they break down, these toxins and toxin fragments can be unidentifiable in the human body.

Third, we must generally look for the toxin itself or its metabolites, not an antibody response, as can be done with infectious agents. Anyone

receiving a lethal dose of any of the toxins would be unlikely to live long enough to be able to mount an antibody response. However, with certain protein toxins (ricin and the staphylococcal enterotoxins) that are highly immunogenic and less lethal, we might see antibodies produced in soldiers who received a single exposure and survived. These might be seen as early as 2 weeks after exposure.

Certain toxins can be identified in the sera of animals, and therefore probably of humans, exposed by inhalation. Blood samples should be collected in sterile tubes and kept frozen, or at least cold, preferably after clotting and removal of cells. If collected within the first day, swab samples taken from the nasal mucosa are the best early diagnostic samples in which to identify several of the toxins. These too, should be kept cold. As a general rule, all samples that are allowed to remain at room temperature (approximately 75°F–80°F) or higher for any length of time will have little value.

Biological samples from patients are generally not as useful for diagnosis of intoxications as they are for diagnosis of infectious diseases or chemical intoxications. The same is true of postmortem samples. Ricin can be identified with immunoassays in extracts of lung, liver, stomach, and intestines up to 24 hours after aerosol exposure. High doses of ricin can be identified in fixed lung tissue of aerosol-exposed laboratory animals by immunohistochemical methods. The staphylococcal enterotoxins can be detected by immunoassay in bronchial washes. Like blood and swab samples, postmortem tissue or fluid samples should be kept cold, preferably frozen, until they can be assayed.

Environmental samples from munitions or swabs from environmental materials should be placed in sealed glass or Teflon (polytetrafluoroethylene, manufactured by Du Pont Polymers, Wilmington, Delaware) containers, and kept dry and as cold as possible. CAUTION: Handling a dry or powdered toxin can be very dangerous because the toxin may adhere to skin and clothing and could be inhaled.

Toxin Analysis and Identification

Immunological or analytical assays or both are available for most of the toxins discussed in this chapter. Immunological methods, typically enzyme-linked immunosorbent assays (ELISAs) or receptor-binding assays, are sensitive to 1 to 10 ng/mL and require approximately 4 hours to complete; these are being developed as the definitive diagnostic tests for deployed units. Analytical (chemical) methods are sensitive at low-microgram to high-nanogram amounts, and take approximately 2 hours to run plus time for instrument setup and isolation or matrix removal (ie, removal of normal body proteins and other contaminating material) when necessary; the

latter can add days to the process. A small, sensitive, far-forward, fieldable assay for several toxins has been developed, and similar kit assays are being developed for many of the other toxins described in this chapter. The polymerase chain reaction (PCR) technique, which provides very sensitive means of detecting and identifying the genetic material of any living organism, can be used to detect remnants of the bacterial, plant, or animal cells that might remain in the crude, impure toxin that we would expect to find in a weapon. Finally, a new method of combining immunoassays with PCR may allow us to detect extremely small quantities of the toxins themselves. In their present state, PCR assays are best suited for use in the reference laboratory.

Water Treatment

Questions often arise regarding the protection of water supplies from toxins. It is unlikely that a small-particle aerosol attack with toxins of military concern would significantly contaminate water supplies. Furthermore, as a general rule, direct contamination of water supplies by pouring toxins into the water would need to be done downstream of the processing plant and near the end user, even for the most toxic bacterial toxins—and ordinary chlorination methods are effective against some of the most potent toxins. Because of dilution, adding toxins to a lake or reservoir would be unlikely to cause human illness. Natural production of algal toxins (eg, microcystin) in stagnant bodies of water could produce enough toxin to cause illness if that water were used for drinking. Three methods of water purification have been tested for the threat toxins (Table 30-6).[5]

TABLE 30-6
WATER PURIFICATION METHODS EFFECTIVE AGAINST TOXINS

Method	Toxin (MW in d)	Effectiveness

Reverse Osmosis	Ricin (64,000) Microcystin (1,000) T-2 mycotoxin (466) Saxitoxin (294) Botulinum toxins (150,000) Staphylococcal Enterotoxin B (28,494)	Effective Effective Effective Effective — * — *
Coagulation/Flocculation	Ricin Microcystin T-2 mycotoxin Saxitoxin Botulinum toxins Staphylococcal Enterotoxin B	Not effective Not effective Not effective Not effective — † — †
Free Chlorine (household bleach) 5 mg/L (5 ppm) for 30 min	Ricin Microcystin T-2 mycotoxin Saxitoxin Botulinum toxins Staphylococcal Enterotoxin B	Not effective Not effective Not effective Not effective Destroys the toxins — †

* Not tested but expected to be effective
† Not tested but not expected to be effective
Data source: Wannemacher RW Jr, Dinterman RE, Thompson WL, Schimdt MO, Burrows WD. *Treatment for Removal of Biotoxins From Drinking Water.* Fort Detrick, Frederick, Md: US Army Biomedical Research and Development Laboratory; Sept 1993. Technical Report 9120.

THE FUTURE

Toxins as Weapons

Research literature suggests that the majority of the “most toxic” (LD_{50} < 0.025 µg/kg) naturally occurring toxins have already been discovered. New toxins of lesser toxicity, especially the venom toxins, are being discovered at the rate of perhaps 10 to 20 per year. There is little precedence in the literature for artificially increasing the toxicities of naturally occurring toxins; however, it might be possible to increase the physical stability of toxins that are toxic enough but too unstable to weaponize. This could increase the effectiveness of a toxin that is currently considered to be a low threat.

It is unlikely that chemical synthesis of complex nonprotein toxins will become significantly easier in the near future. It is likely, however, that large-scale biosynthesis of peptide toxins of 10 to 15 amino acids (some of the venom toxins) will become possible in the next few years.

Countermeasures to Toxins

Although the threat of toxin weapons of the future is formidable, the prospect of new and better medical countermeasures is brighter than ever before. Biotechnology may have more value to those of us who are developing countermeasures than to those who would use toxins maliciously. Molecular biological techniques that have been developed in the last few years now allow us to produce more-effective and less-expensive vaccines against the protein and peptide toxins. Such vaccines will likely be available for the most important toxins within the next few years.

We are making good progress on developing recombinant vaccines for certain high-threat toxins. In the future, protection of our soldiers from toxin threats will be limited only by our willingness to use the vaccines. Similar technology allows us to produce human antibodies, which will eventually replace those now produced in animals. Human antibodies will be a significant advance over despeciated horse antibodies, possibly allowing us to protect unvaccinated soldiers by simply giving them an injection before they go into battle, thereby providing immediate protection. Human antibodies could also be used therapeutically in treating victims of a terrorist attack.

SUMMARY

Protecting soldiers on the battlefield from toxins—and replicating agents—is possible if we use our combined resources effectively. Physical

countermeasures such as the protective mask, protective clothing, and decontamination capabilities exist and are effective. As we improve our battlefield detection systems, early warning of our soldiers may become a reality, at least in subpopulations within our forces. These assets, unlike most medical countermeasures, are generally generic and protect against most or all of the agents.

Among the medical countermeasures, vaccines are available and effective for some of the most important agents, and therapies exist for others. Because of limited resources available to develop vaccines, diagnostic methodologies, and therapies, we can field specific medical countermeasures only to a relatively small group of threat agents. Our efforts in this area must be carefully focused.

A third and complementary element of our defensive program must be good intelligence. Only through knowledge of specific threat agents, delivery systems, and national capabilities can we assure the effective development and use of our physical and medical countermeasures.

Finally, our renewed understanding of the real strengths and weaknesses of toxins as weapons allows us to put them in perspective in educating our soldiers, removing much of the mystique—and associated fear—surrounding toxins. Knowledge of the threat thus reduces the threat to our soldiers.

REFERENCES

1. *The 1972 Biological Weapons Convention.* Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. Signed at London, Moscow, and Washington on 10 April 1972. Entered into force on 26 March 1975. Depositaries: UK, US, and Soviet governments.

2. Calder KL. Mathematical models for dosage and casualty coverage resulting from single point and line source release of aerosol near ground level. BWL Tech Study 3. Defense Technical Information Center AD310-361; Dec 1957. Cited in: Spertzel RO, Wannemacher RW, Patrick WC, Linden CD, Franz DR. *Technical Ramifications of Inclusion of Toxins in the Chemical Weapons Convention (CWC)*. Alexandria, Va: Defense Nuclear Agency; 1992: 18. DNA Technical Report 92-116.

3. Karsenty E, Shemer J, Alshech I, et al. Medical aspects of the Iraqi missile attacks on Israel. *Isr J Med Sci*. 1991; 27(11–12):603–607.

4. Department of the Army. *Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries.* Washington, DC: DA; Feb 1990. Army Training Manual 8-285.

5. Wannemacher R. Assistant Chief, Toxinology Division, US Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, July 1994.

Chapter 31

STAPHYLOCOCCAL ENTEROTOXIN B AND RELATED PYROGENIC TOXINS

ROBERT G. ULRICH, PH.D.*; SHELDON SIDELL, M.D.†; THOMAS J. TAYLOR, M.D.‡; CATHERINE L. WILHELMSEN, D.V.M., PH.D.§; AND DAVID R. FRANZ, D.V.M, PH.D.¥

INTRODUCTION

DESCRIPTION OF THE AGENT

PATHOGENESIS

CLINICAL DISEASE
- Fever and Myalgia
- Respiratory Signs and Symptoms
- Headache
- Gastrointestinal Symptoms
- Other Signs and Symptoms

DETECTION AND DIAGNOSIS

MEDICAL MANAGEMENT

PROPHYLAXIS
- Immunotherapy
- Vaccines

SUMMARY

*Microbiologist, Department of Immunology and Molecular Biology, Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011

†Diablo Internal Medical Group, Inc., 2121 Ygnacio Valley Road, Suite 206-E, Walnut Creek, California 94598

‡Colonel, Medical Corps, U.S. Army; Chief, Endocrinology, Department of Medicine, Tripler Army Medical Center, Honolulu, Hawaii 96859-5000

§Lieutenant Colonel, Veterinary Corps, U.S. Army; formerly, Department of Comparative Pathology, Pathology Division; currently, Chief, Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011

¥Colonel, Veterinary Corps, U.S. Army; Commander, U.S. Army Medical Research Institute of

INTRODUCTION

Staphylococcal enterotoxin B (SEB) is one of seven enterotoxins produced by strains of *Staphylococcus aureus*. SEB, the best understood of the staphylococcal enterotoxins, can be regarded as the "type" enterotoxin. Staphylococcal enterotoxins, toxic shock syndrome toxin–1 (TSST-1), and certain other bacterial products (such as streptococcal pyrogenic exotoxins [SPE]) and viral products (which are not discussed in this chapter) are commonly referred to as *superantigens* because of their profound effects on the immune system. Minute concentrations of superantigens can activate the immune system receptors because they bind with strong avidity to T-cell antigen receptors and class II molecules of the major histocompatibility complex (MHC).

The staphylococcal enterotoxins are the most frequent cause of food poisoning. However, more severe physiological consequences, such as a life-threatening toxic shock–like syndrome, may result from exposure to these toxins through a nonenteric route. Although high-dose exposures may well cause fatalities, it is the *incapacitating* consequences of inhalational exposure to agents such as SEB on the battlefield that are of most concern in the context of biological defense.

During the 1960s, when the United States had an offensive biological warfare program, SEB (then code-named PG) was studied extensively as a biological incapacitant. This toxin was especially attractive as a biological agent because much lower quantities were needed to produce the desired effect than were required with synthetic chemicals. The dose that is incapacitating for 50% of the human population exposed (also called the effective dose [ED_{50}]) was found to be 0.0004 µg/kg, and the dose that is lethal for 50% of the human population exposed (LD_{50}) was estimated to be approximately 0.02 µg/kg, both by the inhalational route.[1]

DESCRIPTION OF THE AGENT

The staphylococcal and streptococcal toxins with superantigen-like properties are 23- to 29-kilodalton (kd) proteins (referred to here as pyrogenic toxins) that can be categorized into three distinct amino acid–sequence homology groups[2]:

1. The staphylococcal enterotoxin serotypes SEA, SED, and SEE are closely related; they form the first homology group.
2. Staphylococcal enterotoxin serotypes SEB, SEC1, SEC2, SEC3, and the streptococcal pyrogenic exotoxins A and C (SPE-A and SPE-C) form the second homology group.
3. The third homology group comprises toxic shock syndrome toxin (TSST-1) and SPE-B, which share key amino acid residues with the other toxins but exhibit only weak sequence homology overall. However, data from X-ray crystallography of SEB[2] and TSST-1[3,4] indicate that these representatives of the two groups have considerable similarities in their three-dimensional structures.

The pyrogenic toxins bind to MHC class II molecules and this complex, in turn, stimulates T cells. [5–8] In contrast, MHC-independent binding induces T-cell anergy. It is

likely that all pyrogenic toxins share a common mode for binding MHC class II molecules, with additional stabilizing interactions that are unique to each toxin.[9] A second, zinc-dependent molecular binding mode for SEA and SEE increases T-cell signaling and may account for the greater toxicities of these toxins. In conventional antigen-specific responses, the cluster of differentiation 4 (CD4) molecule stabilizes interactions between T-cell antigen receptors and MHC class II molecules on antigen presenting cells. The pyrogenic toxins may mimic CD4 binding[10] and, by so doing, stimulate large numbers of T cells in a manner independent of antigen recognition.

In addition, each superantigen stimulates T cells bearing characteristically distinct variable domain-b (Vb) subsets of antigen receptors (Table 31-1). It is thought that a massive release of cytokines (such as interferon gamma, interleukin-6, and tumor necrosis factor–alpha) is responsible for the systemic effects of the toxins.[11] In contrast, the gastrointestinal illness especially prominent after ingestion of staphylococcal enterotoxins is associated with histamine and leukotriene release from mast cells.[12] Endotoxin from Gram-negative bacteria may act in concert with pyrogenic toxins to greatly amplify lethality. For example, mice are ordinarily unaffected by SEB and related toxins, but they become susceptible to doses in nanogram amounts when coadministered with nonlethal amounts of endotoxin.[11]

Primates, including humans, are most sensitive to pyrogenic toxins because the binding affinity of their MHC class II molecules is greater. Lymphocyte responses of chimpanzees and humans are very similar, while rhesus monkeys are more sensitive to toxin stimulation. These differences in primate lymphocyte responses appear to be more dependent on the T cell than the cells presenting the MHC class II receptor.[13] However, pyrogenic toxins do vary in their affinity toward different MHC class II isotypes and alleles. Therefore, it is possible that MHC polymorphisms within the human population may also contribute to individual differences in susceptibility to the toxic effects of the pyrogenic toxins. Inbred mice differing in MHC alleles, for example, have widely varying sensitivities to the staphylococcal enterotoxins.

TABLE 31-1
HUMAN T-LYMPHOCYTE RESPONSE TO BACTERIAL SUPERANTIGENS

Superantigen (Pyrogenic Toxin)	T-Cell Receptor Vβ Used by Responding Lymphocytes *
SEA	1.1, 5.3, 6.3, 6.4, 7.3, 7.4, 9.1
SEB	3, 12, 14, 15, 17, 20
SEC1	12
SEC2	12, 13.1, 13.2, 14, 15, 17, 20
SEC3	5, 12
SED	5, 12
SEE	5.1, 6.1, 6.2, 6.3, 8.1, 18
TSST-1	2

Although little information is available concerning environmental reservoirs, domestic infections of rodents and other animals with strains that produce TSST-1 and the staphylococcal enterotoxins have been demonstrated.[14,15] Both ovine- and bovine-specific staphylococcal toxins, which are associated with mastitis, are almost identical to TSST-1 in their amino acid sequence.[16] Pyrogenic toxin–producing strains of *Staphylococcus aureus* and group A streptococcus are appearing with increasing frequency in clinical isolates. Approximately 50% of nonmenstrual toxic shock syndrome cases are linked to TSST-1, while the remaining cases are attributable to enterotoxins, with SEB predominating.[17] Kawasaki disease and some forms of arthritis have been causally linked to organisms producing streptococcal pyrogenic exotoxins, SEA, and TSST-1, although this is still controversial.[18] In addition, a severe form of streptococcal pneumonia, with accompanying toxic shock syndrome–like symptoms is caused by SPE-producing bacterial strains.[19] It is of interest also to note that the majority of group A streptococcal cultures produce pyrogenic exotoxins.

* These T cells are the predominant phenotypes stimulated by each toxin, independent of the donor.
Vβ: variable domain-β
SE: staphylococcal enterotoxin
TSST-1: toxic shock syndrome toxin 1
Data sources: (1) Kappler J, Kotzin B, Herron L, et al. Vβ-specific stimulation of human T cells by staphylococcal toxins. *Science*. 1989;244:811–813. (2) Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science.* 1990;248:705–711. (3) Champagne E, Huchenq A, Sevin J, Casternan N, Rubin B. An alternative method for T-cell receptor repertoire analysis: Clustering of human V-beta subfamilies selected in responses to staphylococcal enterotoxins B and E. *Mol Immunol*. 1993;30(10):877–886.

Most of the pyrogenic toxins are encoded by mobile genetic elements. SPE-A, SPE-C, SEA, and SEE are all phage-borne, while SED is plasmid-encoded. Insufficient data are available to clearly define the nature of SEB and TSST-1 genetic elements.[20] Because there is little evidence of genetic drift, it has been suggested that the majority of staphylococcal and streptococcal toxic shock–like syndrome bacterial isolates have each descended from single clones.[21] Production of the various staphylococcal pyrogenic toxins is dependent on the phase of cell growth cycle, environmental pH, and glucose concentration. Transcriptional control of TSST-1, SEB, SEC, and SED is mediated through the accessory gene regulator *(agr)* locus,[20] while SEA expression appears to be independent of *agr.* Strains that are *agr* negative are generally low toxin producers. However, there are also considerable differences in production levels between *agr* positive isolates.

PATHOGENESIS

Rhesus macaques *(Macaca mulatta)* have been used extensively as a model for lethal inhaled intoxication of SEB. Efforts to develop lethal aerosolized SEB animal models in rabbits and endotoxin-primed mice are ongoing. The following previously unpublished information is derived from a study of dose ranging during the development of a sublethal model conducted by one of the authors of this chapter (C.L.W.) in 1994 at U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland. Similar information, from another study using different monkeys, was published in 1995 from the same laboratory.[22]

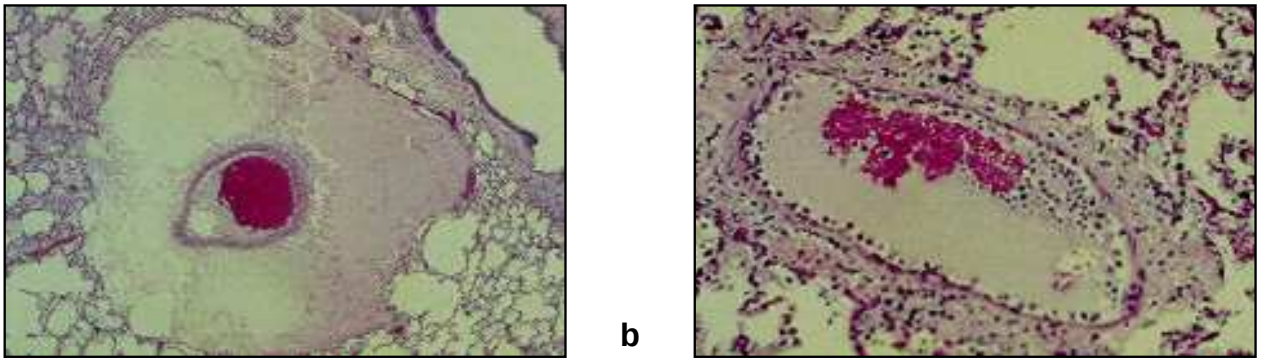


Fig. 31-1. Lung of a rhesus monkey that died of inhaled staphylococcal enterotoxin B (SEB) intoxication. **(a)** Marked perivascular interstitial edema and focal loss of bronchial epithelium can be seen (hematoxylin-eosin stain, original magnification × 10). **(b)** The intravascular mononuclear cells include lymphocytes, lymphoblasts, monocytes, and mononuclear phagocytes (hematoxylin-eosin stain, original magnification × 50).

In recent studies, young and mature adult male and female rhesus monkeys developed signs of SEB intoxication after being exposed to a lethal dose of aerosolized SEB for 10 minutes in a modified Henderson head-only aerosol exposure chamber.[23] These animals demonstrated no detectable anti-SEB antibody prior to exposure.

Generally, SEB-intoxicated monkeys developed gastrointestinal signs within 24 hours after the exposure. Clinical signs were mastication, anorexia, emesis, and diarrhea. Following mild, brief, self-limiting gastrointestinal signs, the monkeys had a variable period of up to 40 hours of clinical improvement. At approximately 48 hours after the exposure, intoxicated monkeys generally had an abrupt onset of rapidly progressive lethargy, dyspnea, and facial pallor, culminating in death or euthanasia within 4 hours of onset.

At postmortem examination, most monkeys had similar gross pulmonary lesions. Lungs were diffusely heavy and wet, with multifocal petechial hemorrhages and areas of atelectasis. Clear, serous-to-white, frothy fluid often drained freely from the laryngeal orifice. The small and large intestines frequently had petechial hemorrhages and mucosal erosions. Typically, monkeys had mildly swollen lymph nodes, with moist and bulging cut surfaces.

Most monkeys had similar microscopic pulmonary lesions (Figure 31-1). The most obvious lesion was marked multifocal-to-coalescing interstitial pulmonary edema involving multiple lung lobes. Peribronchovascular connective tissue spaces were distended by pale, eosinophilic, homogeneous, proteinaceous material (edema); variably accompanied by entrapped, beaded fibrillar (fibrin) strands; and extravasated erythrocytes, neutrophils, macrophages, and small and large lymphocytes. Perivascular lymphatics were generally distended by similar eosinophilic material and inflammatory cells.

Most monkeys had intravascular circulating and marginated neutrophils, monocytes, mononuclear phagocytes, and lymphocytes, including large lymphocytes with prominent nucleoli (lymphoblasts), some in mitosis. Extravascular extension of these cell types was interpreted as exocytosis/chemotaxis.

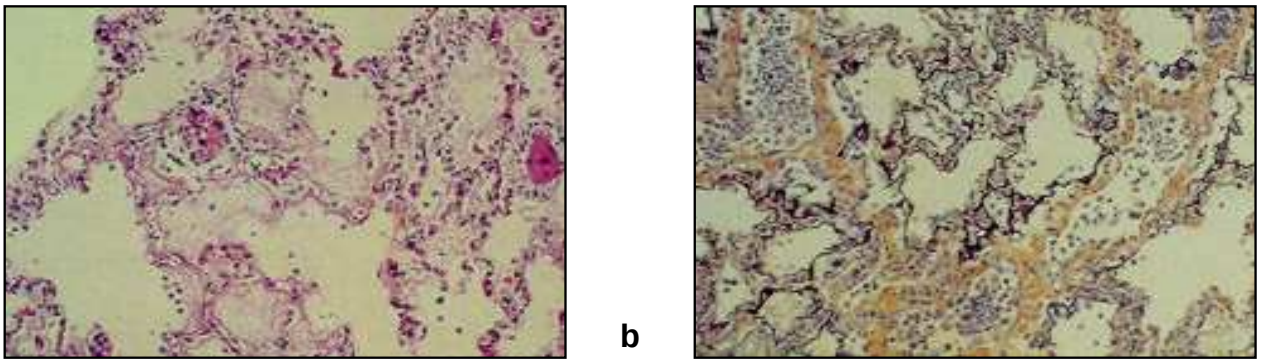
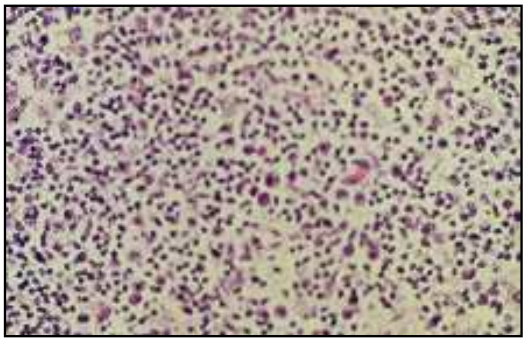


Fig. 31-2. Lung of a rhesus monkey that died of inhaled staphylococcal enterotoxin B (SEB) intoxication. **(a)** Intraalveolar fibrin deposition and hyaline membrane formation can be seen (hematoxylin-eosin stain, original magnification × 50). **(b)** Intraalveolar deposition of polymerized fibrin can be seen (phosphotungstic acid hematoxylin stain, original magnification × 50).

Loss of airway epithelium was inconsistent. Some monkeys had multifocal, asymmetric denudation of bronchial epithelium, with near total loss of the bronchiolar epithelium. Former bronchioles were recognized only by their smooth-muscle walls. Scant bronchial intraluminal exudate consisted of mucoid material, neutrophils, macrophages, and sloughed, necrotic cells.

A common finding was the combination of multifocal alveolar flooding and acute purulent alveolitis. Alveolar septa were distended by congested alveolar capillaries. Alveolar spaces were filled with eosinophilic, pale, homogeneous material (edema); with embedded, more deeply eosinophilic, beaded fibrillar strands (fibrin); or with condensed, eosinophilic, curvilinear deposits hugging the alveolar septal contours (hyaline membranes) (Figure 31-2). A variably severe, intraalveolar, cellular infiltrate of neutrophils, eosinophils, small lymphocytes, large lymphocytes (lymphoblasts), erythrocytes, and alveolar macrophages filled the alveolar spaces. Replicate pulmonary microsections stained with phosphotungstic acid–hematoxylin demonstrated alveolar fibrin deposition. Replicate microsections stained with Giemsa revealed scarce, sparsely granulated connective tissue mast cells.

In the upper respiratory tract, the tracheal and bronchial lamina propria was thickened by clear space or pale, eosinophilic, homogeneous material (edema); and neutrophils, small and large lymphocytes, and (possibly preexisting) plasma cells. The edema and cellular infiltrate extended transtracheally into the mediastinum, with moderate-to-marked mediastinal lymphangiectasia.



Lymphoid tissues of the respiratory tract had depletion of B-cell dependent areas and hyperplasia of T-cell dependent areas. The bronchus-associated lymphoid tissue (BALT) in some of the monkeys had follicular lymphocytic depletion. Most monkeys' mediastinal lymph nodes had subcapsular and medullary sinus edema and histiocytosis and paracortical lymphoid hyperplasia, characterized by numerous, closely packed, small lymphocytes, with interspersed macrophages bearing tingible bodies and large lymphocytes having prominent nucleoli (lymphoblasts) (Figure 31-

Fig. 31-3. Mediastinal lymph node of a rhesus monkey that died of inhaled staphylococcal enterotoxin B (SEB) intoxication. Paracortical lymphoproliferation with lymphoblasts can be seen (hematoxylin-eosin stain, original magnification × 100).

3). There were scattered mitoses, including atypical mitoses. Cortical follicles had small, solid centers, or had hypocellular, hyalinized (depleted) centers.

Microscopic changes of lymphoid tissues elsewhere in the body mirrored changes in the respiratory mucosal lymphoid tissue. Mesenteric, axillary, inguinal, and retropharyngeal lymph nodes had sinus edema and histiocytosis, paracortical lymphocytic and lymphoblastic hyperplasia, and unstimulated or depleted follicular centers. Also depleted were follicular germinal centers of gut-associated lymphoid tissue (GALT). Splenic T-cell dependent periarteriolar sheath zones were hypercellular, populated by a mix of small and large lymphocytes and macrophages, whereas B-cell dependent follicular areas were not recognized. Several monkeys had marked diffuse depletion of cortical thymocytes, with a “starry sky” appearance attributed to the presence of numerous thymic macrophages bearing tingible bodies.

Many monkeys had a mild, erosive enterocolitis, with slight multifocal, superficial mucosal loss, and with numerous lamina proprial macrophages bearing engulfed cellular debris. Crypt enterocytes had a high nuclear-to-cytoplasmic ratio and numerous mitoses. The crypt epithelium had a conspicuous population of large, mononuclear, intraepithelial leukocytes interpreted as lymphoblasts (Figure 31-4). Some monkeys’ colons contained many small crypt abscesses.

Generalized vascular changes in most monkeys were congestion; swollen endothelial cells, with many intravascular large lymphocytes or lymphoblasts; and inconsistent widening of perivascular connective tissue spaces (by edema). Hepatic lesions were portal infiltrates of lymphocytes, lymphoblasts, macrophages, and occasional neutrophils. The choroid plexus was slightly thickened (by edema).

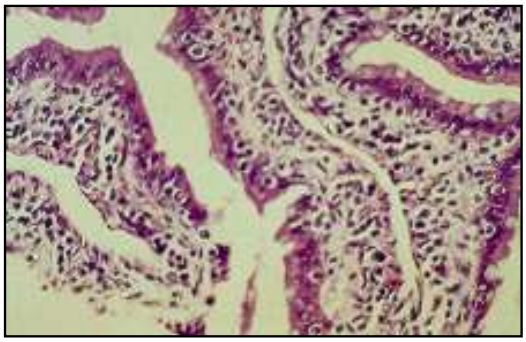


Fig. 31-4. Small intestine of a rhesus monkey that died of inhaled staphylococcal enterotoxin B (SEB) intoxication. Intraepithelial lymphoblastic leukocytes can be seen (hematoxylin-eosin stain, original magnification × 100).

True inhaled SEB pathogenesis studies, in which monkeys are killed and necropsied at sequential times after the exposure, have not been conducted in recent years. To the best of our knowledge, there are no recently published reports of SEB pathogenesis studies in monkeys in the open literature.

Inhaled SEB appears to be a potent stimulant of the immune system of rhesus monkeys. Following inhalational exposure, there was microscopic lymphoproliferation of T-cell dependent areas of the lymphoid system. Recent immunohistochemical analysis of the large lymphocytes present in the pulmonary vasculature of rhesus monkeys lethally intoxicated with aerosolized SEB identified them as T cells, using anti-CD3 antibody.[22]

CLINICAL DISEASE

The clinical documentation of toxic shock syndrome provides perhaps the most comprehensive source of information on the pathology of pyrogenic toxin exposure. To meet the strict Centers for Disease Control criteria for toxic shock syndrome,[24] negative (except for *S aureus*) cultures of the blood, throat, or cerebrospinal fluid should be obtained; negative serologic tests for Rocky Mountain spotted fever, leptospirosis, and measles should also be obtained. Most of the pyrogenic toxins produce the same signs and symptoms as toxic shock syndrome: a rapid drop in blood pressure, elevated temperature, and multiple organ failure. However, the respiratory route of exposure to toxins may involve some unique mechanisms. The profound hypotension characteristic of toxic shock syndrome is not observed, and respiratory involvement is rapid. Fever, prominent after aerosol exposure, is generally not observed in cases of SEB ingestion.

A previously unpublished report from the laboratory of one of the authors of this chapter (S.S.) documents an accidental laboratory inhalational exposure of nine laboratory workers to SEB, and best exemplifies the clinical disease. At least one and perhaps two other workers were in the building at the time of the accident. They were examined but remained completely asymptomatic; they might not have been exposed. These additional workers are not included in this report; the nine mentioned are those who are known to have been exposed (because they became ill).

This report describes a severely incapacitating illness of rapid onset (3–4 h) but modest duration (3–4 d). The degree of illness can be categorized as follows: three of the nine patients were seriously ill, and one of these three was profoundly ill; two of nine were moderately ill; and four of nine were mildly ill.

Fever and Myalgia

Fever was prominent in all nine of those exposed. Eight of the individuals experienced at least one shaking chill that heralded the onset of illness. Using the morning peak level of SEB aerosol generation in the laboratory as the most likely time of exposure, onset of fever occurred from 8 to 20 hours after the initial exposure, with a mean time of onset of 12.4 ± 3.9 (standard deviation) hours. Duration of fever was from 12 to 76 hours after onset, with a mean duration of 50 ± 22.3 hours. Fever reached as high as 106°F acutely.

Myalgias were often associated with the initial fever. Onset of myalgia was from 8 to 20 hours, with a mean onset of 13 ± 5 hours. Duration of myalgia was from 4 to 44 hours, and the mean duration was 16 ± 15 hours.

Respiratory Signs and Symptoms

All nine patients were admitted to the hospital with a generally nonproductive cough. Onset of respiratory symptoms was 10.4 ± 5.4 hours, and duration was 92 ± 41 hours. Five of the nine patients had inspiratory rales with dyspnea. The three most seriously compromised patients had dyspnea, moist inspiratory and expiratory rales, and orthopnea; these signs gradually cleared. One of these three patients had profound dyspnea for the first 12 hours, which moderated to exertional dyspnea and rales that persisted for 10 days.

Chest radiographs taken on admission showed, in the three patients with inspiratory rales, densities compatible with “patches of pulmonary edema” and Kerley lines suggesting interstitial edema. During recovery, discoid atelectasis was noted. Moderate

compromise of the respiratory system was often accompanied by radiographic evidence of peribronchial accentuation or “cuffing.” The three mildly ill patients had normal radiographs.

The one profoundly ill patient had severe pulmonary compromise and profound dyspnea, and received only slight relief when treated with an aminophylline suppository. Moderately intense chest pain, of a substernal pleuritic type, occurred in seven of the nine individuals. Onset of chest pain was 12 ± 6.5 hours; duration ranged from 4 to 84 hours, with a mean duration of 23 ± 27 hours.

Headache

Eight of the nine patients experienced headache. Onset ranged from 4 to 36 hours, and the mean time of onset was 13.3 ± 10 hours. Duration ranged from 8 to 60 hours, with a mean duration of 30.6 ± 19 hours. The headaches ranged from severe to mild, but usually were mild by the second hospital day. Five individuals’ headache responded to Darvon (propoxyphene hydrochloride, manufactured by Eli Lilly and Company, Indianapolis, Indiana) or codeine.

Gastrointestinal Symptoms

Gastrointestinal symptoms occurred in most of the individuals: nausea and anorexia in six and vomiting in four. The onset of nausea ranged from 8 to 24 hours, with a mean onset of 17 ± 6.3 hours. Duration ranged from 4 to 20 hours, with a mean of 9 ± 5.5 hours. Onset of anorexia ranged from 8 to 24 hours, with a mean onset of 18.5 ± 5.6 hours. Duration of anorexia ranged from 4 to 136 hours, and the mean duration was 44.5 ± 45 hours. Vomiting occurred in four patients and sometimes occurred after prolonged paroxysms of coughing. The range of onset of vomiting was 8 to 20 hours, with a mean time to onset of 14 ± 5.1 hours. Duration was not prolonged and usually consisted of one episode. Compazine (prochlorperazine, manufactured by SmithKline Beecham Pharmaceuticals, Philadelphia, Pennsylvania) and Benadryl were employed successfully.

Only one individual demonstrated hepatomegaly and bile in the urine, although another patient also demonstrated mildly elevated liver function tests. (The liver function tests on this last patient remained elevated for years, but it is unclear whether this is actually a sequela of this illness. In addition, this individual had previous exposures to staphylococcal enterotoxins while working at a different laboratory.) No diarrhea was reported in any of the exposed individuals.

Other Signs and Symptoms

All patients who experienced chest pain had normal electrocardiograms. Throughout the illness, all were normotensive. Vomiting was of relatively brief duration and no patients, including those vomiting, required intravenous fluid administration. Pulse rate, when elevated, paralleled temperature elevation.

Leukocytosis was observed in most patients 12 to 24 hours after exposure to the toxin.

None of these individuals experienced conjunctivitis, although one individual later remembered that his eyes had “burned” during the believed time of exposure.

DETECTION AND DIAGNOSIS

The staphylococcal enterotoxins are moderately stable proteins; therefore, immunological evaluation should be possible on samples collected in either deployed or fixed medical treatment facilities. Immunoassays can detect picogram quantities of toxins in environmental samples. For comparison, 440 pg/mL was reported as the mean concentration of TSST-1 in human sera from patients with toxic shock syndrome.[25] Anti-TSST-1 antibody titers are either suppressed or depleted in patients with toxic shock syndrome[26,27] and the levels only recover during convalescence. In addition, most normal human serum samples contain detectable levels of antibody reacting with several different bacterial pyrogenic toxins, including SEB. Therefore, serum antibody titers are of little diagnostic value. If actual bacterial involvement is suspected, and if cultures can be obtained, the detection of extremely minute quantities of potentially toxigenic strains is possible by using (1) polymerase chain reaction (PCR) amplification and (2) toxin gene–specific oligonucleotide primers. The results from both methods are rapid, allowing quantitative or qualitative measurements in less than 24 hours.

Finally, for at least 12 to 24 hours after the exposure, toxins should be identifiable in nasal swabs from individuals exposed to a respirable aerosol. This may be the best approach to early diagnosis on the battlefield.

MEDICAL MANAGEMENT

Supportive therapy seems to have been adequate care in the nine patients described above with mild, accidental, respiratory exposure to aerosolized SEB. Symptoms of fever, muscle aches, and arthralgias may respond to cool compresses, fluids, rest, and judicious use of acetaminophen or aspirin. For nausea, vomiting, and anorexia, symptomatic therapy should be considered. Antihistamines (eg, diphenhydramine) and phenothiazine derivatives (eg, prochlorperazine) were used parenterally or as suppositories. The success of these drugs in controlling nausea may have been augmented by the relatively short duration of nausea and vomiting induced by aerosolized SEB. Because of the brevity of vomiting episodes, fluid replacement was not considered or required in the series discussed. However, in the event of prolonged vomiting resulting in fluid and electrolyte depletion, replacement may be necessary. Diarrhea was not observed in the nine human accidental exposure cases, but deposition of toxin on foodstuff could produce the syndrome. Diarrhea should be treated symptomatically.

Cough suppressants containing dextromethorphan or codeine should be employed initially for symptomatic therapy of respiratory symptoms. Prolonged coughing unrelieved by codeine might benefit from a semisynthetic, centrally acting narcotic antitussive containing hydrocodone (dihydro-codeinone). Monitoring of pulmonary status by pulse oximetry with prompt evacuation to a site with the capacity for intensive respiratory care by mechanical ventilation should be considered when respiratory status is compromised. No specific therapy has been identified or described for managing the respiratory effects of intoxication with aerosolized SEB. Experimental intervention in cytokine cascades has been a proposed therapy. Animal models have not yet indicated any therapeutic value for steroids.

PROPHYLAXIS

Immunotherapy

Infusion of intravenous gamma globulins has been successfully used[28,29] to treat episodes of Kawasaki disease (which is linked to the staphylococcal enterotoxins and TSST-1). Unpublished studies have documented prophylaxis and the therapeutic value of affinity-purified chicken anti-SEB antibody administered intravenously, in rhesus monkey inhalation of SEB. Monkeys given antibody just prior to, or 4 hours after, lethal challenge (5 LD_{50}, or 135 µg/kg) were protected from death. Although the animals survived, they did develop signs of intoxication. Efforts to devise both passive and active immunoprotection are being pursued. Because of the rapidity of MHC class II receptor binding by these toxins (apparent saturation < 5 min, in vitro), active immunity should be considered the best defense.[30]

Vaccines

A formalin-treated SEB toxoid has demonstrated some degree of efficacy in animal trials,[31] but has not yet been approved for human use. In an attempt to stimulate the requisite secretory antibody titers, a variety of different immunization routes and adjuvants have been explored, although the intramuscular immunization route has proven effective. Vaccines produced by genetic inactivation of the toxins have shown promising results in animal trials.[32] This strategy is based on substitution of active receptor-binding amino acid side chains to reduce affinities and consequential T-cell activation,[9] without altering the three-dimensional structure of the antigen. It is anticipated that this second-generation vaccine will be more antigenic and more likely to induce neutralizing antibodies than the toxoid.

SUMMARY

The staphylococcal enterotoxins are a family of superantigen protein toxins produced by strains of *Staphylococcus aureus*. Staphylococcal enterotoxin B (SEB), a toxin often associated with food poisoning, was weaponized as an incapacitating agent by the United States during in the 1960s. When inhaled as a respirable aerosol, SEB causes fever, severe respiratory distress, headache, and sometimes nausea and vomiting. The mechanism of intoxication is thought to be from a massive release of cytokines such as interferon-gamma, interleukin-6 and tumor necrosis factor–alpha. Diagnosis can be confirmed through the use of enzyme-linked immunosorbent assays of tissues or body fluids. Prophylactic administration of an investigational vaccine protects laboratory animals from inhalational challenge. Supportive care is useful in reducing toxicity in unprotected individuals.

REFERENCES

1. Hursh S, McNally R, Fanzone J Jr, Mershon M. *Staphylococcal Enterotoxin B Battlefield Challenge Modeling with Medical and Non-Medical Countermeasures.* Joppa, Md: Science Applications International Corp; 1995. Technical Report MBDRP-95-2.

2. Ulrich RG, Bavari S, Olson M. Bacterial superantigens in human diseases: Structure, function and diversity. *Trends Microbiol.* 1995;3:463–468.

3. Prasad GS, Earhart CA, Murray DL, Novick RP, Schlievert PM, Ohlendorf DH. Structure of toxic shock toxin. *Biochemistry.* 1993;32:13761–13766.

4. Acharya RK, Passalacqua EF, Jones EY, et al. Structural basis of superantigen action inferred from crystal structure of toxic-shock syndrome toxin-1. *Nature.* 1994;367:94–97. Letter.

5. Fraser JD. High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR. *Nature.* 1989;339:221–223.

6. Mollick JA, Cook RG, Rich RR. Class II MHC molecules are specific receptors for staphylococcal enterotoxin A. *Science.* 1989;244:817–820.

7. Uchiyama T, Tadakuma T, Imanishi K, et al. Activation of murine T cells by toxic shock syndrome toxin–1: The toxin-binding structures expressed on murine accessory cells are MHC class II molecules. *J Immunol.* 1989;43:3175–3183.

8. Kappler J, Kotzin B, Herron L, et al. V β-specific stimulation of human T cells by staphylococcal toxins. *Science.* 1989;244:811–813.

9. Ulrich RG, Bavari S, Olson M. Staphylococcal enterotoxins A and B share a common structural motif for binding class II major histocompatibility complex molecules. *Nat Struct Biol.* 1995;2:554–560.

10. Bavari S, Ulrich RG. Staphylococcal enterotoxin A and toxic shock syndrome toxin compete with CD4 for human major histocompatibility complex class II binding. *Infect Immun.* 1995;63(2):423–429.

11. Stiles BG, Bavari S, Krakauer T, Ulrich RG. Toxicity of staphylococcal enterotoxins potentiated by lipopolysaccharide: Major histocompatibility complex class II molecule dependency and cytokine release. *Infect Immun.* 1993;61:5333–5338.

12. Scheuber PH, Denzlinger C, Wilker D, Beck G, Keppler D, Hammer DK. Cysteinyl leukotrienes as mediators of staphylococcal enterotoxin B in the monkey. *Eur J Clin Invest.* 1987;117:455–459.

13. Bavari S, PhD. Research Chemist, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, 1994.

14. Kenny K, Reiser RF, Bastida-Corcuera FD, Norcross NL. Production of enterotoxins and toxic shock syndrome toxin by bovine mammary isolates of *Staphylococcus aureus. J Clin Microbiol.* 1993;31:706–707.

15. Ho G, Campbell WH, Bergdoll MS, Carlson E. Production of a toxic shock syndrome toxin variant by *Staphylococcus aureus* strains associated with sheep, goats, and cows. *J Clin Microbiol.* 1989;27(9):1946–1948.

16. Lee PK, Kreiswirth BN, Deringer JR, et al. Nucleotide sequences and biologic properties of toxic shock syndrome toxin 1 from ovine- and bovine-associated *Staphylococcus aureus. J Infect Dis.* 1992;165:1056–1063.

17. Crass BA, Bergdoll MS. Involvement of staphylococcal enterotoxins in non-menstrual toxin shock syndrome. *J Clin Microbiol.* 1986;23:1138–1139.

18. Freedman JD, Beer DJ. Expanding perspectives on the toxic shock syndrome. *Adv Intern Med.* 1991;36:363–397.

19. Reichardt W, Muller-Alouf H, Alouf JE, Kohler W. Erythrogenic toxins A, B and C: Occurrence of the genes and exotoxin formation from clinical *Streptococcus pyogenes* strains associated with streptococcal toxic shock–like syndrome. *FEMS Microbiol Lett.* 1992;79:313–322.

20. Betley MJ, Borst DW, Regassa LB. Staphylococcal enterotoxins, toxic shock syndrome toxin and streptococcal pyrogenic exotoxins: A comparative study of their molecular biology. *Chem Immunol.* 1992;55:1–35.

21. Lee PK, Schlievert PM. Molecular genetics of pyrogenic exotoxin superantigens of group A streptococci and *Staphylococcus aureus. Curr Top Microbiol Immunol.* 1991;174:1–19.

22. Mattix ME, Hunt RE, Wilhelmsen CL, Johnson AJ, Baze WB. Aerosolized staphylococcal enterotoxin B–induced pulmonary lesions in rhesus monkeys *(Macaca mulatta). Toxicol Pathol.* 1995;23:262–268.

23. Henderson DW. An apparatus of the study of airborne infection. *J Hyg.* 1952;50:53–68.

24. Centers for Disease Control. Modifications: Toxic shock syndrome, United States, 1970–1982. *MMWR.* 1982;31:201–204.

25. Miwa K, Fukuyama M, Kunitomo T, Igarashi H. Rapid assay for detection of toxic shock syndrome toxin 1 from human sera. *J Clin Microbiol.* 1994;32:539–542.

26. Crass BA, Bergdoll MS. Toxin involvement in toxic shock syndrome. *J Infect Dis.* 1986;153:918–926.

27. Chesney PJ, Bergdoll MS, Davis JP, Vergeront JM. The disease spectrum, epidemiology, and etiology of toxic-shock syndrome. *Annu Rev Microbiol.* 1984;38:315–338.

28. Takei S, Arora YK, Walker SM. Intravenous immunoglobulin contains specific antibodies inhibitory to activation of T cells by staphylococcal toxin superantigens. *J Clin Invest.* 1993;91:602–607.

29. Leung DY, Meissner HC, Fulton DR, Murray DL, Kotzin BL, Schlievert PM. Toxic shock syndrome toxin–secreting S*taphylococcus aureus* in Kawasaki syndrome. *Lancet.* 1993;342:1385–1388.

30. Lemley P, PhD, Lieutenant Colonel, US Army. Principal Investigator, Immunology and Molecular Biology Department, Toxinology Division, US Army Medical Research Institute of Infectious Disease, Fort Detrick, Frederick, Md. Personal communication, June 1995.

31. Tseng J, Komisar JL, Trout RN, et al. Humoral immunity to aerosolized staphylococcal enterotoxin B (SEB), a superantigen, in monkeys vaccinated with SEB toxoid–containing microspheres. *Infect Immun.* 1995;63(8):2880–2885.

32. Bavari S, Olson M, Dyas B, Ulrich RG. Bacterial superantigen vaccines. In: Brown F, Burton D, Collier J, McKalanos J, Norrby E, eds. *Vaccines.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1996: 135.

Chapter 32

RICIN TOXIN

DAVID R. FRANZ, D.V.M., PH.D.*; AND NANCY K. JAAX, D.V.M.†

INTRODUCTION

HISTORY AND MILITARY SIGNIFICANCE

DESCRIPTION OF THE AGENT
- Toxicity
- Pathogenesis

CLINICAL SYMPTOMS, SIGNS, AND PATHOLOGY
- Oral Intoxication
- Injection
- Inhalation
- Cause of Death

DIAGNOSIS

MEDICAL MANAGEMENT
- Immunization and Passive Protection
- Supportive and Specific Chemotherapy

SUMMARY

*Colonel, Veterinary Corps, U.S. Army; Commander, U.S. Army Medical Research Institute of Infectious Diseases
†Colonel, Veterinary Corps, U.S. Army; Chief, Pathology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011

INTRODUCTION

Ricin toxin, found in the bean of the castor plant, *Ricinis communis*, is one of the most toxic and easily produced plant toxins. It is a lectin consisting of two polypeptide chains, the A-chain and the B-chain, linked by a disulfide bond. It is one of a group of dichain ribosome-inactivating proteins, which are specific for the depurination of a single adenosine in ribosomal ribonucleic acid (RNA).[1] The active chain (ie, the A-chain) has the ability to modify catalytically the 28S subunit of eucaryotic ribosomes to block protein synthesis. The toxicity of castor beans has been known since ancient times, and more than 750 cases of intoxication in humans have been described.[2] Although ricin's lethal toxicity is approximately 1,000-fold less than that of botulinum toxin, ricin may have significance as a biological weapon because of its heat stability and worldwide availability, in massive quantities, as a by-product of castor oil production.

HISTORY AND MILITARY SIGNIFICANCE

Ricinis communis was cultivated in ancient Egypt for its oil's lubricating and laxative effects; both the oil and the whole seeds have been used in various parts of the world in the treatment of other diseases as well. During World War I and World War II, the lubricating oil was used in aircraft. Because of shortages of castor oil during World War II, the United States government subsidized the cultivation of castor beans in the San Joaquin Valley of California until the 1960s, when artificial oils replaced castor oil in the aircraft industry.

Although the industry is no longer active in this country, castor oil is still produced in large quantities throughout the world. The toxin, which remains in the castor meal after the oil has been extracted with hexane or carbon tetrachloride, is easily extracted through a simple salting-out procedure.[3]

In the late 1800s, Stillmark[4] discovered that the beans of the castor plant contained a toxic protein, which he named ricin. He discovered that ricin caused agglutination of erythrocytes and precipitation of serum proteins. (The lectin properties of ricin and abrin [a closely related toxin from the bean of *Abrus precatorius*] and their use as tools for research were described in 1972 by Sharon and Lis.[5])

Paul Ehrlich studied ricin[6] and abrin[7] during the 1890s; Ehrlich's work with these lectins became the very foundation of the discipline of immunology. Since the toxins are much less toxic when given by mouth than by injection, Ehrlich was able to induce immunity by feeding mice or rabbits small amounts of the seeds. This seminal work provided evidence

that specific serum proteins are induced, capable of precipitating and neutralizing the toxin antigens. Using the same model, he also showed that, during pregnancy, specific antitoxin activity is transferred from mother to offspring through the blood and, during lactation, the same protective components are transferred through the milk.

Native ricin was first shown to inhibit tumor growth in 1951. The toxin was tested by various routes—local application, intratumor, and intra-arterial—in patients with tumors, with varying results.[8] In recent years, with the advent of new immunotherapeutic techniques, ricin has once again found a niche in the armamentarium of the medical profession. It has been studied as a component of antitumor agents called immunotoxins or, more specifically, chimeric toxins.[9]

The native ricin, or just the ricin A-chain, is conjugated to tumor cell–specific monoclonal antibodies (technically, to other ligands, which target the active component of the toxin to tumor cells for selective killing).[10] A number of these compounds have undergone Phase I or Phase II clinical trails as anticancer agents.[11,12] Although results have been promising, two factors appear to limit ricin immunotoxin efficacy: (1) lack of specificity of the antibody and (2) significant immunogenicity of the toxin moiety, which results in relatively rapid onset of refractory immunity to the therapeutic agent.[13,14]

Because of its relatively high toxicity and its extreme ease of production, ricin, code-named Compound W, was considered for weaponization by the United States during its offensive Biological Warfare Program. The U.S. Chemical Warfare Service began studying ricin as a weapon of war near the end of World War I. Work done in collaboration with the British resulted in the development of a W bomb in World War II. The weapon was tested but apparently never used in battle.[15] Ricin was used in the highly publicized assassination of Bulgarian defector Georgi Markov[16]; this incident is discussed in greater detail later in this chapter. However, because ricin intoxication is a relatively uncommon occurrence in human medicine, no concerted effort was made to produce specific therapies or prophylactic measures until the early 1990s, when it was perceived to be a significant biological warfare threat.

In recent years, ricin has become a favorite tool of extremist individuals or groups who seek to harm others, as the following examples demonstrate:

- Two tax protesters were convicted in February 1995 of possessing ricin as a biological weapon. This was the first case of prosecution under the 1989 Biological Weapons Anti-terrorism Act.[17]

- A retired electrician who had worked on the trans-Alaska pipeline recently committed suicide in an Arkansas jail after being arrested under the antiterrorism act for possessing castor beans. Two years before, a large quantity of ricin toxin and weapons, ammunition, and gold were found in his car by Canadian customs officials as he crossed the border from Alaska to Canada.[18,19]

Ricin's appeal to individuals such as these is likely related to its ready availability, relative ease of extraction, and its popularization by the press.[20]

DESCRIPTION OF THE AGENT

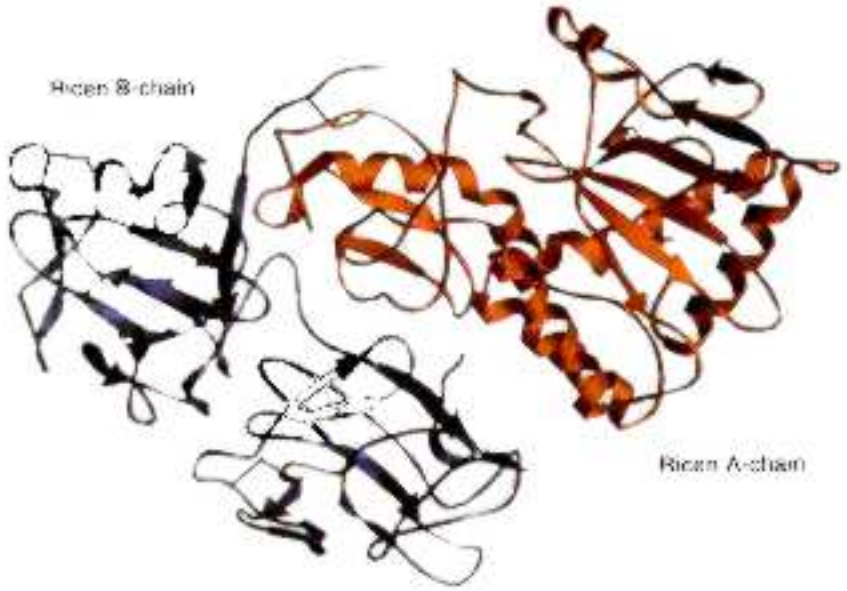


Fig. 32-1. The crystallographically determined structure of ricin. The molecule is depicted using a ribbon representation, with the A-chain colored red; the B-chain, blue; and the disulfide bonds, yellow. Illustration: Courtesy of Mark A. Olson, PhD, Toxinology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md.

Ricin is a 66-kilodalton (kd) globular protein that makes up 1% to 5% by weight of the bean of the castor plant, *Ricinis communis*. The toxic heterodimer consists of a 32-kd A-chain that is disulfide-bonded to a 32-kd B-chain.[21] The toxin is stored in the matrix of the castor bean, together with a 120,000-d ricinus lectin.[22] Both chains are glycoproteins containing mannose carbohydrate groups; the two 32-kd chains must be associated for toxicity.

Several investigators have purified and characterized ricin[22]and have succeeded in crystallizing it. The crystal structure has been determined to 2.5 Å.[23] The A- and B-chains are globular proteins, with the A-chain tucked into a gap between two roughly spherical domains of the B-chain. A lactose disaccharide moiety is bound to each of the spherical domains of the B-chain. The disulfide bond links amino acid 259 of the A-chain and

amino acid 4 of the B-chain. The crystal structure demonstrates a putative active cleft in the A-chain, which is believed to be the site of the enzymatic action of the toxin. Recombinant A- and B-chains, as well as mutants of the chains, have been expressed in *Escherichia coli* and other expression systems. The chains have been crystallized and their structures derived (Figure 32-1).[24-27]

Toxicity

There is a 100-fold variation in the lethal toxicity of ricin for various domestic and laboratory animals, per kilogram of body weight. Of animals tested, the chicken and frog are least sensitive, while the horse is the most.[28]

Toxicity of ricin also varies with route of challenge. In laboratory mice, the approximate dose that is lethal to 50% of the exposed population (LD_{50}) and time to death are, respectively, 3 to 5 µg/kg and 60 hours by inhalation, 5 µg/kg and 90 hours by intravenous injection, 22 µg/kg and 100 hours by intraperitoneal injection, 24 µg/kg and 100 hours by subcutaneous injection, and 20 mg/kg and 85 hours by intragastric administration. Low oral toxicity reflects poor absorption of the toxin from the gastrointestinal tract. Higher toxicities by other routes may be directly related to accessibility of target-cell populations and the ubiquity of “toxin receptors” throughout the cells of the body. When skin tests were performed on mice, no dermal toxicity was observed at the 50-µg spot.[29]

Pathogenesis

The B-chain has lectin properties that allow it to bind to complex galactosides of cell-surface carbohydrates, while the A-chain has enzymatic activity. Binding of the B-chain to glycoside residues on glycoproteins

and glycolipids appears to trigger endocytotic uptake of the protein. Internalization of the toxin occurs, primarily via uncoated pits,[30,31] within a few hours; the dissociation rate of ricin for its binding sites is increased in the presence of lactose.[32] Almost all of the toxin entering the cytosol does so via the Golgi apparatus.[33] As with other protein toxins such as diphtheria toxin, *Pseudomonas* exotoxin A, and modeccin, transport to the cytosol is the rate-limiting step during the decline of protein synthesis.[34] Presence of the B-chain facilitates transport of the A-chain into the cytosol.[35] The latent periods of 1 to 3 hours in vitro and 8 to 24 hours in vivo, before cell death or clinical signs, respectively, are probably related to the necessary transport of the toxin into cells, the site of activity. Once in the cytoplasm of a eukaryotic cell, the A-chain enzymatically attacks the 28S ribosomal subunit. Ricin has a Michaelis constant (K_M) of 0.1 µmol/L for ribosomes and an enzymatic constant (K_{cat}) of 1,500/min. Ricin cleaves one adenine residue (A4324) near the 3 ' end of 28S RNA. This deletion causes elongation factor-2 to fail to bind and thereby blocks protein synthesis.[36,37]

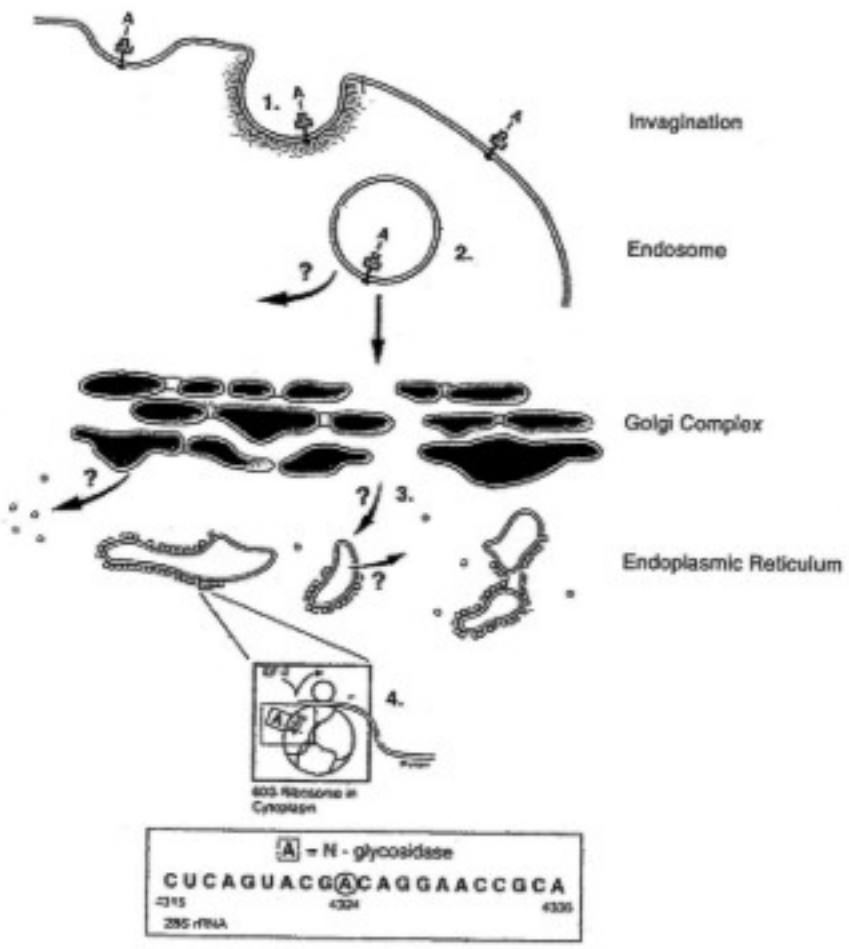


Fig. 32-2. Cartoon depicting ricin (A—B) binding to coated pits on the surface of the cell (1), internalization via endocytosis (2), and transport through the Golgi complex (3); and enzymatic inactivation of protein synthesis via cleavage of adenine residue (A4324), and blockage of elongation factor-2 binding (4). Arrows with "?" indicate other possible routes of entry to the cytosol. Illustration: Courtesy of Bob Wellner, PhD, Toxinology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md.

At the cellular level, ricin kills through inhibition of protein synthesis (Figure 32-2).

CLINICAL SYMPTOMS, SIGNS, AND PATHOLOGY

The clinical signs, symptoms, and pathological manifestations of ricin toxicity vary with the dose and the route of exposure.[38] Experimental animal studies indicate that clinical signs and pathological changes are largely route specific; for example, inhalation results in respiratory distress and airway and pulmonary lesions; ingestion causes gastrointestinal signs and gastrointestinal hemorrhage with necrosis of liver, spleen, and kidneys; and intramuscular intoxication causes severe localized pain, muscle and regional lymph node necrosis, and moderate involvement of visceral organs. The route-specific pathology is probably due to the lectin properties of ricin, which cause it to bind rapidly to complex galactosides of cell-surface carbohydrates. Transient leukocytosis appears to be a constant feature in humans, whether intoxication is via injection or oral ingestion. Leukocyte counts 2- to 5-fold higher than the normal value are characteristic findings in cancer patients, and also occurred in the Markov case.[16]

Oral Intoxication

Ricin is less toxic by oral ingestion than by other routes, probably because of poor absorption and some enzymatic digestion in the digestive tract. In oral (and parenteral) intoxication, cells in the reticuloendothelial system, such as Kupffer cells and macrophages, are particularly susceptible, due to the mannose receptor present exclusively in macrophages.[39] In 1985, A. Rauber and J. Heard[2] summarized the findings from their study of 751 cases of castor bean ingestion. There were 14 fatalities in this study, constituting a death rate of 1.9%—much lower than traditionally believed. Twelve of the 14 cases resulting in death occurred before 1930. Even with little or no effective supportive care, the death rate in symptomatic patients has been low—in the range of 6%. The reported number of beans taken by patients who died varied greatly. Of the two cases of lethal oral intoxication documented since 1930, one was of a 24-year-old man who ate 15 to 20 beans, and the other was a 15-year-old boy who ate 10 to 12 beans. All of the reported serious or fatal cases of castor bean ingestion have the same general clinical history: rapid (less than a few hours) onset of nausea, vomiting, and abdominal pain; followed by diarrhea; hemorrhage from the anus; anuria; cramps; dilation of the pupils; fever; thirst; sore throat; headache; vascular collapse; and shock. Death occurred on the third day or later. The most common autopsy findings in oral intoxication are multifocal ulcerations and hemorrhages of gastric and small-intestinal mucosa, which may be quite severe; lymphoid necrosis in the mesenteric lymph nodes, gut-associated lymphoid tissue (GALT), and spleen; Kupffer cell and liver necrosis; diffuse nephritis; and diffuse splenitis.

Injection

In one large clinical trial,[40] low doses (18–20 µg/m^2) of intravenous ricin administered to cancer patients were well tolerated. Flulike symptoms with fatigue—in some cases very pronounced fatigue—and muscular pain were common, and sometimes nausea and vomiting occurred. The symptoms began 4 to 6 hours after administration and lasted for 1 to 2 days. Two toxic deaths were reported in Phase I clinical trails of the closely related protein toxin, abrin; these patients had general seizures and other signs of central nervous system toxicity.

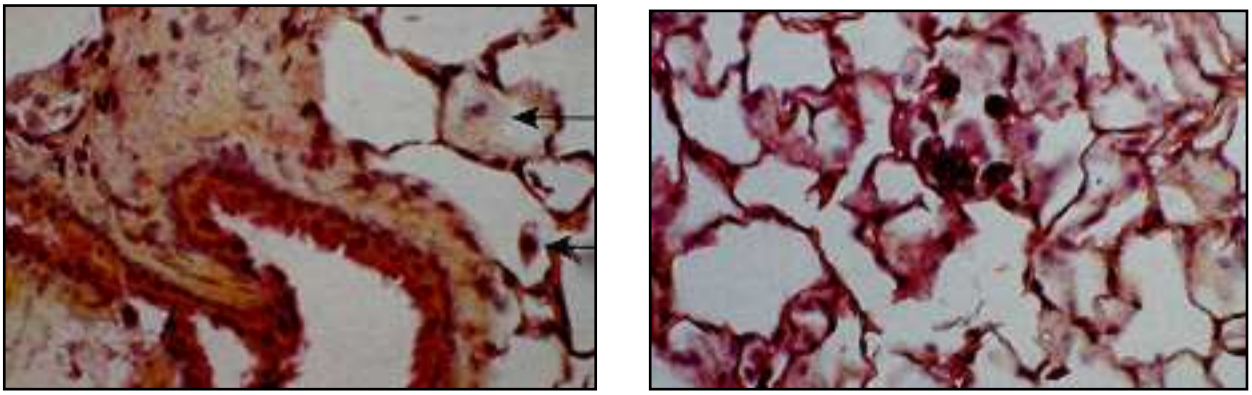
In the case of Mr. Markov, whose assassination was mentioned earlier,[16] injection of a lethal dose of ricin, estimated to be as much as 500 µg, resulted in almost immediate local pain, then a feeling of weakness within about 5 hours. Fifteen to 24 hours later, he had a high temperature, nausea, and vomiting. Thirty-six hours after the incident, he was admitted to the hospital feeling very ill. He had fever, tachycardia, and normal blood pressure; lymph nodes in the affected groin were swollen and sore; and a 6-cm diameter area of induration and inflammation was observed at the injection site on his thigh. Just over 2 days after the attack, he became suddenly hypotensive and tachycardic; the pulse rate was 160 beats per minute, and vascular collapse and shock had set in. His white blood count was 26,300/mm^3. Early on the third day after the attack, he became anuric and began vomiting blood. An electrocardiogram demonstrated complete atrioventricular conduction block. Mr. Markov died shortly thereafter; at the time of death, his white blood count was 33,200/mm^3.

Intramuscular or subcutaneous injection of high doses of the toxin in humans, as occurred in the assassination, results in severe local lymphoid necrosis, gastrointestinal hemorrhage, liver necrosis, diffuse nephritis, and diffuse splenitis. In the case of Mr. Markov, a mild pulmonary edema was thought to have been secondary to cardiac failure. Similar results have been reported in experimental animal studies.

Inhalation

Although data on aerosol toxicity exposure are not available for humans, lesions induced by oral and parenteral exposure are consistent with those seen in experimental animal studies, suggesting that the same would hold true for aerosol exposures. The only information on inhalation of ricin in humans is an allergic syndrome reported in workers exposed to castor bean dust in or around castor oil processing plants.[41] The clinical picture is characterized by sudden onset of congestion of the nose and throat, itchiness of the eyes, urticaria, and tightness of the chest. In more severe cases, wheezing, leading to bronchial asthma, may also occur, and may last for several hours. Affected individuals respond to symptomatic therapy and removal from the source of exposure.

Inhalational exposure of rats to lethal ricin challenge results in a diffuse necrotizing pneumonia of the airways, with interstitial and alveolar inflammation and edema.[42] No notable changes in lung injury parameters occur before 8 hours after the challenge. By 12 hours, inflammatory cell counts and total protein (both from fluid obtained via bronchoalveolar lavage) increase, suggesting both increased permeability of the air–blood barrier and cytotoxicity; these findings are associated with a blood-cell analysis indicative of inflammation. By 18 hours after the challenge, alveolar flooding is present, and extravascular lung water is increased. Both continue to increase up to 30 hours after the challenge. At 30 hours after the challenge, arterial hypoxemia and acidosis are present and histopathological evidence of alveolar flooding becomes significant. Recently completed immunohistochemical studies[43] in rats exposed to ricin via aerosol indicate that aerosolized ricin binds to ciliated bronchiolar lining cells, alveolar macrophages, and alveolar lining cells (Figure 32-3).



a **b**

Fig. 32-3. Lung from a rat exposed to ricin by aerosol. Immunocytochemical stain for ricin demonstrates strong reactivity for **(a)** airway epithelial cells and alveolar macrophages (arrows) and **(b)** alveolar lining cells. Original magnification × 50, immunocytochemical stain. Photographs: Courtesy of CL Wilhelmsen, DVM, PhD, Lieutenant Colonel, Veterinary Corps, US Army; Division of Pathology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md.

In a recent study[44] of nonhuman primates, inhalational toxicity was characterized by a dose-dependent preclinical period of 8 to 24 hours, followed by anorexia and progressive decrease in physical activity. Death occurred 36 to 48 hours after the challenge, time to death also being dose dependent. Relevant gross and histopathological changes were confined to the thoracic cavity (Figure 32-4). All monkeys had acute marked-to-severe fibrinopurulent pneumonia, with variable degrees of diffuse necrosis and acute inflammation of airways. There were also diffuse, severe alveolar flooding and peribronchovascular edema (Figure 32-5), acute tracheitis, and marked-to-severe purulent mediastinal lymphadenitis. Two monkeys had acute adrenalitis.

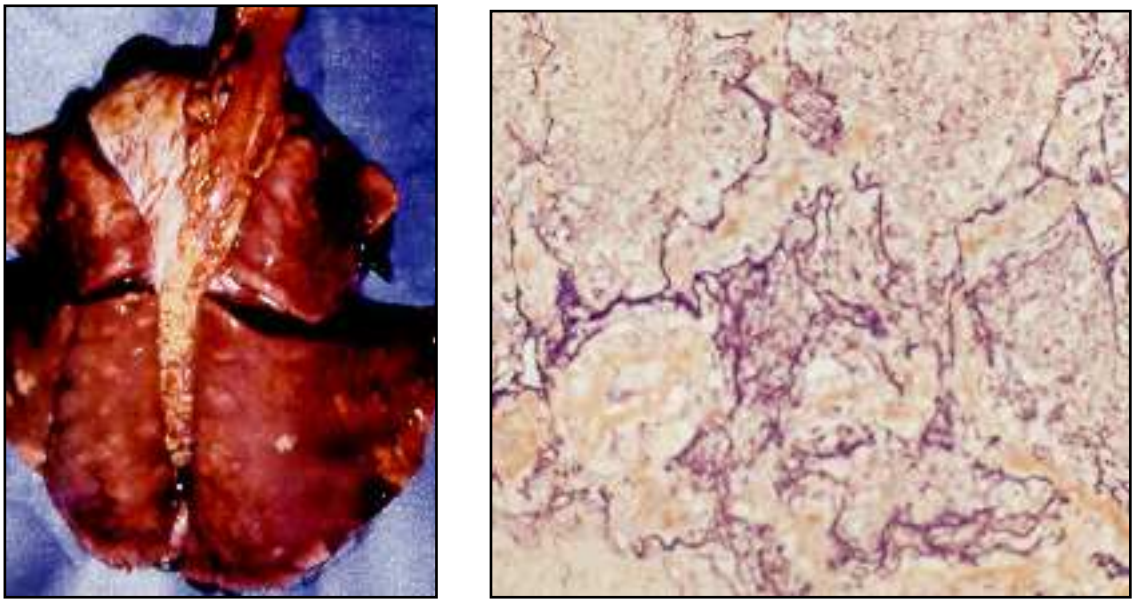


Fig. 32-4. Lungs from a monkey exposed to ricin by aerosol. **(a)** The lungs are edematous, with accompanying hemorrhage and necrosis. **(b)** Histologically, the microscopical changes are characterized by fibrinopurulent pneumonia. The fibrin has been specifically stained by phosphotungstic acid hematoxylin to appear purple (original magnification x 25). Photographs: Courtesy of CL Wilhelmsen, DVM, PhD, Lieutenant Colonel, Veterinary Corps, US Army; Division of Pathology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md.

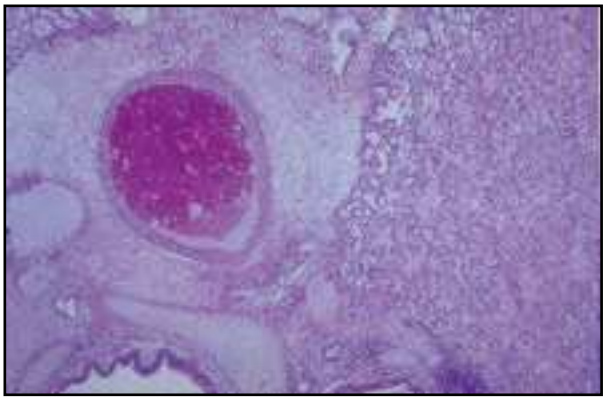


Fig. 32-5. Widespread perivascular and peribronchiolar edema in a monkey, a characteristic finding in aerosol ricin intoxication (hematoxylineosin stain; original magnification × 10). Photograph: Courtesy of CL Wilhelmsen, DVM, PhD, Lieutenant Colonel, Veterinary Corps, US Army; Division of Pathology, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md.

Cause of Death

The exact cause of death is unknown and probably varies with route of intoxication. Results of ricin A-chain immunotoxin clinical trial studies demonstrated that ricin A-chain caused a vascular leak syndrome characterized by hypoalbuminemia and edema; subsequent in vitro studies with human umbilical vein endothelial cells showed that ricin damaged endothelial cells.[45] In mice, rats, and primates, high doses via inhalation appear to produce severe enough

pulmonary damage to cause death, probably due to hypoxemia resulting from massive pulmonary edema and alveolar flooding. High doses administered intravenously in experimental animals are associated with disseminated intravascular coagulation[8]; it has been suggested that hepatocellular and renal lesions are the result of vascular disturbances induced by the toxin rather than a direct effect of the toxin itself.[46] Studies published in 1987[47] clearly establish that in intravenous administration of ricin toxin to rats, diffuse Kupffer cell damage occurred within 4 hours, followed by endothelial cell damage, formation of thrombi in the liver vasculature, and finally, hepatocellular necrosis, confirming studies that had been published in 1976.[48]

DIAGNOSIS

Like other potential intoxications on the unconventional battlefield, epidemiological findings will likely play a central role in diagnosis. The observation of multiple cases of very severe pulmonary distress in a population of previously healthy young soldiers, linked with a history of their having been at the same place and time during climatic conditions suitable for biological warfare attack, would be suggestive.

The differential diagnoses of aerosol exposure to ricin would include staphylococcal enterotoxin B, exposure to pyrolysis by-products of organofluorine polymers (eg, Teflon [polytetrafluoroethylene, manufactured by Du Pont Polymers, Wilmington, Delaware], Kevlar [polyparaphenyleneterephthalamide, manufactured by Du Pont Advanced Fiber Systems, Wilmington, Delaware]) or other organohalides, oxides of nitrogen, and phosgene. Insecticides such as paraquat and anaphthylthiourea (ANTU), although not expected in a battlefield scenario, can be spread aerially over large geographical areas and are also potent edemagenic agents.

Confirmation of ricin inhalational intoxication would most likely be through enzyme-linked immunosorbent assay analysis of a swab sample from the nasal mucosa; ricin can be identified by this method for at least 24 hours after the challenge.[49] Because ricin is extremely immunogenic, individuals surviving a ricin attack would likely have circulating antibody within 2 weeks after the exposure; serum samples should be obtained from survivors. Following inhalational intoxication in laboratory animals, laboratory findings are generally nonspecific.

Enzyme-linked immunosorbent assays (for blood or other body fluids)[50] or immunohistochemical techniques (for direct analysis of tissues) may be useful in confirming ricin intoxication. However, because ricin is bound very quickly regardless of route of challenge, and metabolized before excretion, identification in body fluids or tissues is difficult. In rats exposed

to ricin labeled with iodine 125 by intravenous injection, the radioactive label was found in liver (46%), muscle (13%), and spleen (9%) 30 minutes after intravenous injection.[51] Ricin was quickly cleared from the animals, with only 11% remaining after 24 hours; 70% was excreted in the urine as low-molecular-weight metabolites. Attempts at identification of the toxin may also include introduction of biological autopsy materials into mice or cultured cells and neutralization through the use of specific antibodies.

MEDICAL MANAGEMENT

The most likely scenarios in which ricin intoxication might be seen by military medical personnel are (1) small-scale battlefield or terrorist delivery of an aerosol and (2) parenteral administration of the toxin to an individual as an assassin's tool. Because ricin acts rapidly and irreversibly (directly on lung parenchyma after inhalation, or is distributed quickly to vital organs after parenteral exposure), postexposure therapy is more difficult than with slowly processed, peripherally acting agents (such as the botulinum toxins or bacterial agents) that can be treated with antibiotics. Therefore, immunization of personnel at risk for ricin exposure is even more important than it is for some of the other potential biological warfare agents.

Immunization and Passive Protection

Animal studies have shown that either active immunization or passive prophylaxis or therapy (if the therapy is given within a few hours) is extremely effective against intravenous or intraperitoneal intoxication with ricin. On the other hand, inhalational exposure is best countered with active immunization or prophylactic administration of aerosolized specific antiricin antibody. Active prophylaxis through immunization is the only effective medical countermeasure within our grasp at this time. Prophylactic immunization of mice, rats, and sub-human primates with two to three doses of the toxoid (3–5 µg per dose), with or without adjuvant (aluminum hydroxide), protects against death and incapacitation following inhalational exposure to multiple lethal doses of ricin toxin.[52] Either a toxoid of the native toxin or a preparation of the purified A-chain produces a measurable antibody response that correlates with protection from lethal aerosol exposure. The toxoid has been microencapsulated in glactide-glycolyde microparticles and, in mice, provides effective immune protection after one immunization.[53] As this is written, the only potential approved medical countermeasures for human use are the formalin-treated toxoid, which has gone through preclinical testing and has been submitted to the Food and Drug Administration as an Investigational New

Drug, and the deglycosylated A-chain, which has also shown promise as an antigen in preclinical trials. The toxoid has proven safe and effective in rodents and nonhuman primates exposed to ricin aerosol by inhalation, the route of challenge believed to be the most likely threat on the battlefield.

In seeking an approach to passive protection of soldiers without immunization, animal studies have been conducted in the laboratory to evaluate the short-term efficacy of prophylactically inhaled specific antibody. Preliminary data[54] suggest that *(a)* when mice are exposed for 20 minutes to aerosolized specific immunoglobulin G (about 24 µg antibody per mouse, the lowest dose tested), they are completely protected from lethal aerosol challenge 1 hour later; and *(b)* more than 95% of them are protected from pulmonary lesions. These findings suggest that inhalation of protective antibody from a portable nebulizer just before an attack might provide some protection in unimmunized individuals. These studies also suggest that intravenous administration or inhalation of specific antibody after exposure to aerosolized ricin will be of little value in blocking or reversing the toxin's pathological effects.

Supportive and Specific Chemotherapy

As is the case in toxicity and pathogenesis of intoxication, the route of exposure is important in relation to possible modes and their likelihood of success of prophylaxis and therapy. For oral intoxication, supportive therapy includes activated charcoal administration and intravenous fluid and electrolyte replacement. For inhalational intoxication, supportive therapy to counteract acute pulmonary edema and respiratory distress is indicated. Symptomatic care is the only intervention presently available to clinicians for the treatment of incapacitating or lethal doses of inhaled ricin. Positive end-expiratory ventilatory therapy, fluid and electrolyte replacement, antiinflammatory agents, and analgesics would likely be of benefit in treating aerosol-intoxicated humans. As we learn more about the pathogenesis of intoxication by this route, specific mediator blocking agents may prove valuable, as well.

In recent years, a wide variety of chemotheraputic agents has systematically been screened in an in vitro Vero cell inhibition of protein synthesis assay for efficacy against ricin toxicity.[55] More than 150 agents, including cellular membrane effectors, calcium channel–blocking agents, sodium–calcium exchangers, reducing agents, antioxidants, effectors of endocytosis, nucleoside derivatives, antibacterials, ricin analogs, effectors of cellular metabolism, and competitors for binding have been tested. Of these, only five agents showed promise in vitro and were screened in mouse-protection assays:

- D-galactose and its derivatives, which are competitors for binding,
- azidothymidine (AZT) and a purine derivative, BM33203, both of which are nucleoside derivatives, and
- brefeldin-A, which is a Golgi transport inhibitor.

None of the compounds have proven useful for protecting laboratory animals, even from intravenous exposure to the toxin. Efforts are also underway to synthesize very specific compounds, transition-state inhibitors, which block the enzymatic effects of the A-chain.

SUMMARY

Ricin is a large, moderately toxic, protein dichain toxin from the bean of the castor plant, *Ricinis communis*. It can be produced easily in relatively large quantities. Ricin was developed as a biological weapon by the United States and its allies during World War II. Although ricin is toxic by several routes, when inhaled as a respirable aerosol, it causes severe necrosis of the airways and increased permeability of the alveolar–capillary membrane. The inhalational route is presumed to be the likeliest threat on the battlefield.

Death after inhalation of a lethal dose appears to be caused by hypoxemia resulting from massive pulmonary edema and alveolar flooding. Diagnosis can be confirmed through the use of enzyme-linked immunosorbent assays of tissues or body fluids. Prophylactic administration of an investigational vaccine protects laboratory animals from inhalational and other routes of challenge.

REFERENCES

1. Barbieri L, Baltelli M, Stirpe F. Ribosomes-inactivating proteins from plants. *Biochemica Biophysica Acta*. 1993;1154:237–282.

2. Rauber A, Heard J. Castor bean toxicity re-examined: A new perspective. *Vet Hum Toxicol*. 1985;27:498–502.

3. Wannemacher R, Hewetson J, Lemley P, et al. Comparison of detection of ricin in castor bean extracts by bioassays, immunoassays, and chemistry procedures. In: Gopalakrishnakone P, Tan C, eds. *Recent Advances in Toxinology Research*. Singapore: National University of Singapore; 1992: 108–119.

4. Stillmark. Ueber Ricen. Arbeiten des Pharmacologischen Institutes zu Dorpat, iii, 1889. Cited in: Flexner J. The histological changes produced by ricin and abrin intoxications. *J Exp Med*. 1897;2:197–216.

5. Sharon N, Lis H. Cell-agglutinating and sugar-specific proteins. *Science*. 1972;177:949–959.

6. Ehrlich P. Experimentelle Untersuchungen über Immunität, I: Euber Ricin. *Deutsch Med Wochenschr*. 1891;17:976–979.

7. Ehrlich P. Experimentelle Untersuchungen über Immunität, II: Euber Abrin. *Deutsch Med Wochenschr*. 1891;17:1218–1219.

8. Olsnes S, Pihl A. Abrin, ricin, and their associated agglutinins. In: Cuatrecasas P, ed. *Receptors and Recognition: The Specificity and Action of Animal, Bacterial, and Plant Toxins*. London, England: Chapman and Hall; 1976: 129–173.

9. Olsnes S, Pihl A. Construction and properties of chimeric toxins target specific cytotoxic agents. In: Dorner F, Drews J, eds. *Pharmacology of Bacterial Toxins.* New York, NY: Pergamon Press; 1986: 709-739.

10. Magerstadt M. Therapeutic aminoconjugates. In: *Antibody Conjugates and Malignant Disease.* Boca Raton, Fla: CRC Press; 1991: Chap 3.

11. Ucken F, Frankel A. The current status of immunotoxins: An overview of experimental and clinical studies as presented at the 3rd International Symposium on Immunotoxins. *Leukemia*. 1993;7:341–348.

12. Vitetta E, Thorpe P, Uhr J. Immunotoxins: Magic bullets or misguided missiles? *Trends Pharmacol Sci*. 1993;14(5):148–154.

13. Vitetta E, Krolick K, Muneo M, Cushley W, Uhr J. Immunotoxins: A new approach to cancer therapy. *Science*. 1983;219:644–649.

14. Thorpe PE, Mason DW, Brown AN, et al. Selective killing of malignant cells in leukemic rat bone marrow using an antibody–ricin conjugate. *Nature*. 1982;297:594–596.

15. Cookson J, Nottingham J. *A Survey of Chemical and Biological Warfare.* New York, NY: Monthly Review Press. 1969: 6.

16. Crompton R, Gall D. Georgi Markov: Death in a pellet. *Med Leg J*. 1980;48:51–62.

17. Sharn L. Probe aims at sale of deadly bacteria. *USA Today*. 11 Jul 1995;2-A.

18. Kifner J. Man is arrested in a case involving deadly poison. *New York Times*. 23 Dec 1995;A-7.

19. Goodman PS. Seized poison set off few alarms. *Anchorage Daily News*. 4 Jan 1996;B-1.

20. Nelan BW. The price of fanaticism. *Time*. 3 Apr 1995;38–41.

21. Robertus J. Toxin structure. In: Frankel A, ed. *Immunotoxins*. Boston, Mass:

Kluwer Academic Publishers; 1988: 11–24.

22. Youle R, Huang A. Protein bodies from the endosperm of castor beans, subfractionation, protein components, lectins, and changes during germination. *Plant Physiol*. 1976;58:703.

23. Rutenber E, Katzin B, Collins E, et al. The crystallographic refinement of ricin at 2.5 Å resolution. *Proteins*. 1991;10:240–250.

24. Wales R, Richardson PT, Roberts LM, Woodland HR, Lord JM. Mutational analysis of the galactose binding ability of recombinant ricin B chain. *J Biol Chem*. 1991;266(29):19172–19179.

25. Afrin LB, Gulick H, Vesely J, Willingham M, Frankel AE. Expression of oligohistidine-tagged ricin B chain in *Spodoptera frugiperda. Bioconjug Chem*. 1994;5(6):539–546.

26. Frankel A, Roberts H, Afrin L, Vesely J, Willingham M. Expression of ricin B chain in *Spodoptera frugiperda*.*Biochem J*. 1994;303(pt 3):787–794.

27. Robertus J, Piatak M, Ferris R, Houston L. Crystallization of ricin A chain obtained from a cloned gene expressed in *Escherichia coli*. *J Biol Chem*. 1987;262:19–20.

28. Balint GA. Ricin: The toxic protein of castor oil seeds. *Toxicology*. 1974;2(1):77–102.

29. Wannemaker R. Assistant Division Chief, Toxinology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, September 1994.

30. Ghosh P, Wellner R, Cragoe E, Wu H. Enhacement of ricin cytotoxicity in Chinese hamster ovary cells by depletion of intracellular K^+: Evidence for Na^+/H^+ exchange system in Chinese hamster ovary cells. *J Cell Biol*. 1985;101:350–357.

31. Moya M, Dautry-Varsat A, Goud B, Louvard D, Boquet P. Inhibition of coated pit formation in Hep_2 cells blocks the cytotoxicity of diptheria toxin but not that of ricin toxin. *J Cell Biol*. 1985;101(2):548–559.

32. Sandvig K, Olsnes S, Pihl A. Kinetics of binding of the toxic lectins abrin and ricin to surface receptors of human cells. *J Biol Chem*. 1976;251(13):3977–3984.

33. Lord JM, Roberts LM, Robertus JD. Ricin: Structure, mode of action, and some current applications. *FASEB J*. 1994;8:201–208.

34. Hudson T, Neville D. Temporal separation of protein toxin translocation from processing events. *J Biol Chem*. 1987;262:16484–16494.

35. Youle R, Neville D. Kinetics of protein synthesis inactivation by ricin-anti-thy.1.1 monoclonal antibody hybrids: Role of the ricin B subunit demonstrated by

reconstitution. *J Biol Chem*. 1982;267:1598–1601.

36. Endo Y, Mitsui K, Motizuki M, Tsurugi K. The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes: The site and the characteristics of the modification in 28S ribosomal RNA caused by the toxins. *J Biol Chem*. 1987;262(12):5908–5912.

37. Olsnes S. Closing in on ricin action. *Nature.* 1987;328:474–475.

38. Olsnes S, Pihl A. Toxic lectins and related proteins. In: Cohen P, van Heyningen S, eds. *Molecular Action of Toxins and Viruses*. Amsterdam, Netherlands: Elsevier Biomedical Press; 1982: 51–105.

39. Zenilman ME, Fiani M, Stahl P, Brunt E, Flye MW. Use of ricin A-chain to selectively deplete Kupffer cells. *J Surg Res*. 1988;45(1):82–89.

40. Fodstad O, Kvalheim G, Godal A, et al. Phase I study of the plant protein ricin. *Cancer Res*. 1984;44:862–865.

41. Brugsch HG. Toxic hazards: The castor bean. *Mass Med Soc*. 1960;262:1039–1040.

42. Assaad A. Principal Investigator, Aerobiology and Product Evaluation Department, Toxinology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, December 1994.

43. Davis K. Lieutenant Colonel, Veterinary Corps, US Army. Chief, Experimental Pathology Department, Pathology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, July 1994.

44. Wilhelmsen C, Pitt L. Lesions of acute inhaled lethal ricin intoxication in rhesus monkeys. *Vet Pathol*. 1993;30:482.

45. Soler-Rodriguez A, Ghetie M, Oppenheimer-Marks N, Uhr J, Vitetta E. Ricin A-chain and ricin A-chain immunotoxins rapidly damage human endothelial cells: Implications for vascular leak syndrome. *Experimental Cell Research*. 1993;206:227–234.

46. Howat AJ. The toxic plant proteins ricin and abrin induce apoptotic changes in mammalian lymphoid tissues and intestine. *J Pathol*. 1988;154:29–33.

47. Bingen A, Creppy EE, Gut JP, Dirheimer G, Kirn A. The Kupffer cell is the first target in ricin-induced hepatitis. *J Submicrosc Cytol*. 1987;19(2):247-256.

48. Derenzini M, Bonetti E, Marionozzi V, Stirpe F. Toxic effects of ricin: Studies on the pathogenesis of liver lesions. *Virchows Arch B Cell Pathol*. 1976;20:15–28.

49. Hewetson J. Principal Investigator, Immunology and Molecular Biology Department, Toxinology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, June 1994.

50. Poli MA, Rivera VR, Hewetson JF, Merrill GA. Detection of ricin by colorimetric and chemiluminescence ELISA. *Toxicon*. 1994;32(11):1371–1377.

51. Ramsden C, Drayson M, Bell E. The toxicity, distribution, and excretion of ricin holotoxin in rats. *Toxicology*. 1989;55:161–171.

52. Hewetson J, Rivera V, Lemley P, Pitt M, Creasia D, Thompson W. A formalinized toxoid for protection of mice from inhaled ricin. *Vaccine Research*. 1996;4:179–187.

53. Yan C, Resau JH, Hewetson J, West M, Rill W, Kende M. Characterization and morphological analysis of protein-loaded poly(lactide-co-glycolide) microparticles prepared by water-in-oil-in-water emulsion technique. *Journal of Controlled Release*. 1994;32:231–241.

54. Poli M, Virera V, Pitt L, Vogel P. Aerosolized specific antibody protects mice from lung injury associated with aerosolized ricin exposure. In: 11th World Congress on Animal, Plant, and Microbial Toxins; 1994; Tel Aviv, Israel. Abstract.

55. Thompson W, Scovill J, Pace J. Drugs that show protective effects from ricin toxicity in in-vitro protein synthesis assays. *Natural Toxins*. 1995;3:369–377.

Chapter 33

BOTULINUM TOXINS

JOHN L. MIDDLEBROOK, PH.D.*; AND DAVID R. FRANZ, D.V.M., PH.D.†

INTRODUCTION

HISTORY AND MILITARY SIGNIFICANCE

DESCRIPTION OF THE AGENT
- Serology
- Genetics

PATHOGENESIS
- Relation to Other Bacterial Toxins
- Stages of Toxicity

CLINICAL DISEASE

DIAGNOSIS

MEDICAL MANAGEMENT

SUMMARY

* *Chief, Life Sciences Division, West Desert Test Center, Dugway Proving Ground, Dugway, Utah 84022; formerly, Scientific Advisor, Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

† *Colonel, Veterinary Corps, U.S. Army; Commander, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

INTRODUCTION

The clostridial neurotoxins are the most toxic substances known to science. The neurotoxin produced from *Clostridium tetani* (tetanus toxin) is encountered by humans as a result of wounds and remains a serious public health problem in developing countries around the world. However, nearly everyone reared in the western world is protected from tetanus toxin as a result of the ordinary course of childhood immunizations. Humans are usually exposed to the neurotoxins produced by *Clostridium botulinum* (ie, the botulinum toxins, of which there are seven in all) by means of food poisoning, although there are rare incidents of wound botulism and a colonizing infection of neonates known as infant botulism.[1] Since the incidence of botulinum poisoning by all routes is very rare, immunization of the general population is not warranted on the basis of cost and the expected rates of adverse reactions to even the best vaccines.

Thus, humans are not protected from botulinum toxins and, because of their relative ease of production and other characteristics, these toxins are likely biological warfare agents. Indeed, the United States itself explored the possibility of weaponizing botulinum toxin after World War II, as is discussed elsewhere in this textbook. Although the United States disavowed any further research on developing the botulinum toxins as biological warfare agents, great concern remains that other nations might employ them, and ongoing research seeks ways to protect our armed forces from their use.

HISTORY AND MILITARY SIGNIFICANCE

Because of the extreme toxicity of botulinum toxin, it was one of the first agents to be considered as a biological weapons agent. Before offensive research on biological warfare was renounced, researchers in the United States worked on the weaponization of botulinum toxin for over two decades. Efforts began early during World War II (Exhibit 33-1). Intelligence information indicated that Germany was attempting to develop botulinum toxin as a cross-channel weapon to be used against invasion forces.[2] At the time the Allied work began, the composition of the toxic agent produced by *C botulinum* was not clear, nor was the mechanism of lethality in animals and man. Therefore, the earliest goals of research on botulinum toxin were to isolate and purify the toxin and to determine its pathogenesis. Botulinum toxin was referred to as agent X.

Strains that produced each of the five serotypes known at the time were obtained and those expressing the most toxin were selected for use in further study, although most of the research involved serotype A.[2]

Culture conditions required to produce maximal levels of toxin were established, and techniques appropriate for purification and concentration were perfected. An important advance was “crystallization” of the toxin. The preferred method of toxin purification involved an initial acid precipitation from the culture supernatants, followed by redissolving the toxin in an aqueous buffer. At that point, the addition of ammonium sulfate produced toxin in a form called “crystalline.” To a protein chemist of today, this term means a highly purified protein that may be suitable for three-dimensional structure determination. The scientists of that time thought that their crystalline preparations were pure, but we now know that these preparations were far from pure—although the procedure did put the toxin in a physical state of high stability. The crystalline toxin they produced was authentic neurotoxin with an accompanying protein or proteins (hemagglutinin, in most cases) that stabilizes the toxin from thermal and proteolytic degradation. Further technical advances in analytical protein chemistry were required before the true physical state of the toxin became evident. However, a good deal of work was carried out with this form of the toxin, and nearly all the insights and conclusions derived from the work remain valid.

One of the more lasting legacies of the early botulinum toxin biowarfare research was the development of the botulinum vaccine that is used even today. It was clear that the scientists working with large quantities of the toxin needed to be protected from possible laboratory exposures and that a vaccine would serve them, as well as the armed forces at risk of biological warfare attack. A formalin-inactivated *toxoid* (ie, a toxin that has been treated so as to destroy its toxicity but retain its antigenicity) proved effective in animal studies, and large quantities were prepared for human use.[3] A large store of vaccine was shipped to England for possible use by the expeditionary forces, but for reasons that are not elaborated in the official history,[2] the decision was made not to vaccinate the troops. Many humans have since been vaccinated with this and similarly prepared botulinum toxin vaccines, and clinical experience has indicated that they are safe and effective.

EXHIBIT 33-1
A FOOTNOTE TO HISTORY: WAS BOTULINUM TOXIN USED IN THE ASSASSINATION OF REINHARD HEYDRICH?

Reinhard Heydrich, head of the Gestapo and Security Service in Germany during World War II, was arguably second only to Hitler as the chief perpetrator of the Holocaust. He was assassinated in Prague in the spring of 1942 by Czech patriots who were trained and equipped by the British. The fatal injury resulted from the detonation of a bomb, which drove fragments through a seat in Heydrich's car and into his left flank, injuring the lung, diaphragm, and spleen.

The surgical care he received was surprisingly good, even when judged by today's standards. Heydrich's initial postoperative course was satisfactory, although he was modestly febrile and there was drainage from the wound of entrance. His condition worsened suddenly on the seventh postoperative day and he died early the next day.

An autopsy showed no apparent gross or microscopic cause of death; specifically, there was no missed injury, evidence of peritonitis, abscess, wound tract infection, or retained foreign bodies; and the heart and lungs appeared normal.[1] The senior German pathologists in attendance wrote that "…death occurred as consequence of ... bacteria and possibly by poisons carried ... by the bomb splinters.…"[2(p17)] Although when we use modern terminology their assessment can be interpreted to mean that death was due to septic shock or multiple organ failure, looking at the incident from the vantage point of 50 years also allows for a more diabolical interpretation.

The extraordinary efforts made by the British and Americans to develop biological weapons in World War II are not generally known. For instance, by 1944, it would have been possible for the Allies to drop tens of thousands of bombs containing *Bacillus anthracis* (ie, anthrax) spores on major German cities.[3] Other potential biological warfare agents were also being investigated, among them the neurotoxins of *Clostridium botulinum.* It is here that Heydrich's death becomes relevant. Although we have no official written documentation, the chief scientist in charge of the British biological warfare program, Paul Fildes, is recorded as having made remarks to colleagues that can only be interpreted to mean that he and, by implication, a biological warfare agent, played a role in Heydrich's death: "[I] had a hand [in Heydrich's death]"[3(p94)] and "[Heydrich] was the first notch on my pistol."[3(p94)]

There is reason to believe that Fildes's research group was actively developing botulinum toxin as a weapon. That the British were very knowledgeable about the potential use of botulinum toxin in war is apparent from their request to the Canadian government for several hundred thousand doses of toxoid as a defense against possible German use. Although not carrying the weight of written documentation, Fildes's recorded statements, together with the known British interest in botulinum toxin, have led two British historians to propose that the bomb used to assassinate Heydrich contained botulinum toxin in addition to the usual explosive charge.[3]

How likely is it that botulinum toxin played a role in Heydrich's death? Certain observations are possible:

- The bomb used in the assassination was not of standard issue but instead was of distinctly unusual design: the upper third of a British hand grenade had been cut off and the open end and sides wrapped with tape.[2] This strange modification becomes understandable if a way was needed to contaminate its contents with a foreign substance.
- Heydrich's clinical course does not explain his death. Although infection was likely to accompany his injury, his sudden deterioration and death do not conform to the usual expression of fatal sepsis. It is noteworthy that infection was not a prominent finding at autopsy. Heydrich's death is actually much more suggestive of a massive pulmonary embolism, yet his heart and lungs were said to be normal.[1]
- Heydrich's death is not especially suggestive of botulism. The clinical course of wound botulism (albeit with a more-rapid onset) probably comes close to what should have happened if Heydrich's wound was actually contaminated with botulinum toxin. However, the apparent absence of such expected signs and symptoms as ptosis, diplopia, dysphonia, dysarthria, dysphagia, facial paralysis, and generalized muscular weakness culminating in respiratory insufficiency developing over several days speak against botulism.

The answer will probably never be known, although the British archives for this period, which are scheduled to be opened early in the 21st century, may contain relevant information.

(1) Davis RA. The assassination of Reinhard Heydrich. *Surg Gynecol Obstet*. 1971;August:304–318. (2) Ramsey WG, ed. The assassination of Reinhard Heydrich. *After the Battle*. 1979;24:cover 2–37. (3) Harris R, Paxman J. *A Higher Form of Killing*. New York, NY: Hill and Wang; 1982.

DESCRIPTION OF THE AGENT

C botulinum and *C tetani* are spore-forming, anaerobic bacteria found worldwide in soil. As mentioned previously, however, the organisms produce their toxicoses in very different manners. Victims of tetanus present clinical symptoms of a rigid tetanic paralysis, whereas the victims of botulinum poisoning present with a radically different symptom: peripheral, flaccid paralysis. Poisoning by tetanus toxin is a result of wound contamination and is an infectious disease like cholera or diphtheria, in which invading organisms multiply within the body and produce their toxin. The disease is very old, with descriptions and drawings of victims going back to the Middle Ages.

In contrast, poisoning by botulinum toxin (ie, the disease we call *botulism*) seems to have been more rare, especially during ancient times. Although there may have been ancient cases of wound botulism, there is little or no evidence for such infections until much more recent times. Food poisoning due to botulinum toxin emerged as a problem when food preservation became a widespread practice. Since the outbreaks were so dramatic, they soon received the attention of scientists and the etiology of the poisoning was elucidated. It is now clear that *C botulinum* grows and produces neurotoxin in the anaerobic conditions frequently encountered in the canning or preservation of foods. The spores are very hardy, and special efforts in sterilization are required to ensure that the organisms are inactivated and unable to grow and synthesize their toxin. Modern commercial procedures have virtually eliminated the problem of food poisoning by botulinum toxin, and most of the cases now seen are associated with home-canned foods or meals produced by restaurants.

One other mode of botulinum toxin poisoning has a significant number of cases in the United States: infant botulism.[1] These cases involve an ongoing colonization of the intestines of infants, usually in the first year of life, by the usually benign *C botulinum* organism. Apparently, the flora of newborns, their intestinal environment, or both is such that the organism can grow and produce toxin; there are no well-documented cases of intestinal infections in adult humans.

Serology

The initial identification of botulinum toxin as the etiologic agent in poisoning came after isolation of organisms from the victims, followed by growth in the laboratory and demonstration of toxigenicity by injection of animals with culture filtrates. After inactivation, the culture filtrates were used to raise (ie, produce) toxin-neutralizing antiserum. This antiserum was used to confirm poisoning by *C botulinum* until victims with similar symptoms appeared, but the antiserum did not neutralize the toxin in animal experiments. However, when the entire process of antiserum production was repeated with the new isolates, neutralization was observed. It soon became clear that medicine was dealing with a family of toxins that produced related poisoning sequelae, but that differed immunologically. Thus, seven distinct serotypes of botulinum toxin have now been isolated, designated A through G. Interestingly, not all serotypes have been associated with poisoning of humans. Serotypes A, B, E, and F have been clearly identified in numerous human poisoning episodes. Serotype G is the most recently isolated toxin and has only been identified in a few outbreaks. For serotypes C and D, respectively, only a single anecdotal human case of intoxication has been reported. These serotypes have been found in outbreaks involving various animals including chickens and mink in domestic settings and ducks in wild environments. Why it is that humans are typically not poisoned by serotypes C and D is not clear.

Because the clostridial toxins are so potent, they have been the subjects of many studies by laboratories throughout the world. In nearly every case, multiple strains have been isolated that produce the same serotype of botulinum toxin. (Strangely, however, *C tetanus* strains all produced the same serotype of tetanus toxin.) Many of the strains are available from microbiological repositories such as the American Type Culture Collection. However, due to the ubiquitous nature of the organisms, we could simply isolate anaerobic organisms from the soil in nearly any country and expect to obtain one or more serotypes of toxin-producing *C botulinum*. In fact, there is recent evidence that other clostridia can carry and express the genes for the botulinum neurotoxins.[4] In addition, with the emergence of molecular genetics as a readily available technology, nearly any laboratory with such expertise could move the gene for botulinum toxin into other organisms. Although such research is forbidden by law in most western nations, including the United States, there is no international legal prohibition of such work.

Genetics

Our understanding of many important details of the action of botulinum and tetanus toxins has been slow in progressing. However, enormous strides have been made during the last 5 years, and science is now closing in on a detailed description of how these toxins work. The most important breakthrough involved cloning and sequencing of the genes for tetanus and all seven serotypes of botulinum neurotoxins.[5–14] With that information, the amino acid sequences could be deduced, and this led to other important insights into the molecular mechanisms of action.

The family of structural genes for the clostridial neurotoxins is unrestricted in its location, being both chromosomal and extrachromosomal. The structural gene for tetanus toxin is on a plasmid, as is probably the structural gene for botulinum toxin serotype G.[15] The structural genes for botulinum toxin serotypes C and D are found on bacteriophages.[16,17] The remainder are believed to be chromosomal in location, but this is not definitely proven.

The isolation of clostridia not classified as *C botulinum*, yet expressing botulinum toxin and involved in human disease,[18,19] raises several interesting questions. Does the gene for botulinum neurotoxin move from species to species in the clostridia family? Have the toxin genes always been resident in clostridia other than *C botulinum* and either not expressed or expressed at low levels, therefore remaining unrecognized? Why is the gene for tetanus toxin not found (thus far) in other clostridial species? Why are there multiple serotypes of botulinum, but not of tetanus toxin? Have we found all existing serotypes of botulinum toxin, or are there additional serotypes lurking in the environment waiting to be discovered? And probably the most intriguing question of all: What is the real function of these neurotoxins? Surely not to poison humans. Humans are not predators of clostridia and can hardly be viewed as prey, either. The neurotoxins probably serve some important function in the natural environment or life cycle of clostridia, and humans just happen to get in the way.

PATHOGENESIS

As mentioned earlier, botulinum toxins are the most poisonous substances known. The dose that is lethal to 50% of the population exposed (ie, the LD_{50}) has been estimated[20] to be approximately 1 ng/kg. This is similar to LD_{50}s reported for most laboratory animal species when the toxin is administered intravenously, subcutaneously, or intraperitoneally. All of the botulinum toxins are slightly less toxic when

exposure is by the pulmonary route: a recent estimate for the human LD_{50} by inhalation is 3 ng/kg.[21]

The extreme toxicity of the botulinum toxins would lead us to believe that they must have some highly potent and efficient mechanism of action. This probability made botulinum toxin the subject of work by many laboratories, especially after we learned that it is a neurotoxin. Experiments with in vitro neuromuscular models established that the toxin acts presynaptically to prevent the release of acetylcholine. In many of those same models, very high doses of botulinum toxin will block the release of neurotransmitters other than acetylcholine, but there seems to be a marked toxin specificity involving acetylcholine.[22]

Relation to Other Bacterial Toxins

With the development of such techniques as sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, it became evident that the crystalline toxin was really an aggregate of proteins, and that the molecular species responsible for poisoning was a single protein of about MW 150,000. However, because the molecular events underlying neurotransmitter release were poorly understood, little progress was made in understanding the details involved in botulinum toxin's pathogenesis during the 1970s and 1980s. As is true for science in general, it was research in related areas that provided the framework for the next round of advances with botulinum toxin. Studies with another microbial toxin, that produced by *Corynebacterium diphtheriae*, provided several important insights that some scientists believed could be applied to botulinum toxin. Most importantly, the realization that diphtheria toxin is an enzyme permitted researchers to understand how certain toxins could be so much more potent than others. Curare, for example, binds to the acetylcholine receptor and acts in a stoichiometric relationship to exert toxicity. Diphtheria toxin, being an enzyme, can act many times over on (or, more properly, *in*) a cell and therefore exert a much more powerful effect (for a given number of molecules) than a stoichiometrically acting toxin. Because botulinum toxin is much more potent than even diphtheria toxin, many laboratories embarked on a search for the (putative) enzymatic activity expressed by botulinum toxin.

Stages of Toxicity

The second insight provided by the work on diphtheria toxin was the recognition that microbial toxins have structural domains (or subunits) that serve common general functions related to a three-stage mechanism of action: binding, internalization, and enzymatic activity.[23] Figure 33-1 depicts these stages.

All three functions are normally required for expression of toxicity in cells or animals, but under certain experimental circumstances these functions can be blocked or overcome. Thus, if the enzyme domain of a toxin is removed or specifically inactivated, the toxin is rendered inactive and could be used as a vaccine. It could bind to a target cell and enter the interior, but without its functional component, no toxicity would result. A derivative of diphtheria toxin called CRM-197 is a perfect example of this type of alteration. On the other hand, the active enzyme domain by itself is virtually nontoxic if it is added to cells or given to animals. The enzyme domain alone is unable to recognize and bind specifically to target cells. If, however, the enzyme domain is linked to other cell-binding ligands, the toxicity can be redirected to new target cells. This type of construction is the basis for many attempts to treat cancer or other diseases (eg, the fusion of diphtheria toxin fragment A, its enzymatic component, with tumor-directed antibodies).

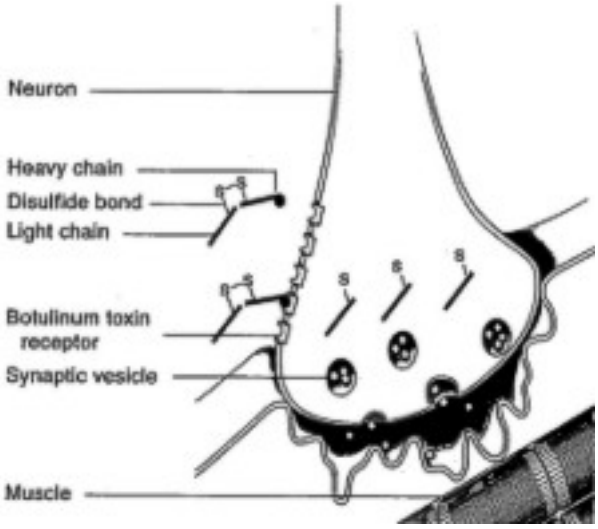


Fig. 33-1. The three stages in the mechanism of action of dichain microbial toxins (ie, two protein chains connected by a disulfide bond). In the first stage, a domain (the sphere at the end of the heavy chain) at the carboxyl end of the holotoxin recognizes and binds to a receptor or acceptor on the surface of the target neuron. The internalization stage follows, which results in delivery of the light chain of the toxin into the cytoplasm. The internalization stage is directed by a domain adjacent to the receptor-recognition domain on the heavy chain. Whether all or part of the heavy chain also enters the cytoplasm remains unclear, but the light chain certainly gains

entry into that compartment. Finally, once in the cytoplasm, the light chain acts like an enzyme and catalyzes specific reactions described in the text, preventing both *(a)* the vesicle from fusing to the membrane and *(b)* neurotransmitter release.

Binding

What about *(a)* the receptor for botulinum toxin, *(b)* the means by which the toxin enters neurons, and *(c)* the toxin's enzymatic activity? First, the receptor. For many years, the observation that complex sphingolipids prevented toxicity was taken to indicate that gangliosides were receptors for the toxins. However, there is as much evidence suggesting that gangliosides *are not* the receptor (or receptors) as there is that they *are*.[24] An important study[25] published in 1986 presented good evidence that the different serotypes of botulinum toxin do not share the same receptor. It is not yet clear if there are distinct receptors for each serotype.

As this is written (1996), it is fair to say that science does not know the structure of the receptors for either botulinum or tetanus toxins. A report published in 1994[26] suggesting that a synaptic vesicle–associated protein called synaptotagmin may be the receptor for botulinum toxin serotype B. This intriguing work has yet to be confirmed, and as it stands, it lacks one key piece of information. The researchers elegantly demonstrate that serotype B binds to synaptotagmin, but the crucial evidence showing that synaptotagmin binding results in internalization and toxicity is not yet available.

Internalization

A substantial body of evidence indicates that botulinum toxin enters neurons by the general pathway used by several other bacterial toxins, a number of polypeptide hormones or growth factors, and even some viruses.[27] This pathway has come to be known as receptor-mediated endocytosis (RME).

Briefly, ligands are concentrated on the cell surface by virtue of binding to receptors that are localized in specialized regions called coated pits. The pits invaginate, becoming vesicles, and are transported to one or more sites in the cell interior, carrying along the contents. At various stages of vesicle trafficking, some of the contents escape or are released into the cytoplasm, where, in the case of bacterial toxins, they act on the intracellular substrates. This action leads to toxicity.

One general feature of the process is a gradual decrease in the

intravesicular pH as a result of a protein pump in the membrane; this may drop as low as pH 4.5. Certain drugs or compounds called lysosomotropic amines prevent this pH drop and, in nearly all cases, inhibit the release of ligands. Because of this known effect, inhibition of a specific biological process by lysosomotropic amines is widely accepted as a hallmark of the presence of an RME process. Indeed, this is the case for botulinum toxin. The presence of lysosomotropic drugs will inhibit, or at least delay, the onset of botulinum toxin paralysis[23]; this fact has led most scientists in the field to believe that the toxin enters the neurons by RME.

Enzymatic Activity

Finally, the enzymatic activity of botulinum toxin. A critical key to the identification of this enzymatic activity was provided by the cloning and sequencing of tetanus toxin and all seven serotypes of botulinum toxin—work that was performed by several laboratories. Initially, a scientist not in the neurotoxin field noted[28] that tetanus toxin has an amino acid sequence in its light chain that is similar to that seen in zinc-dependent proteases. When the amino acid sequences of the botulinum toxins became available, they, too, were seen to have this sequence in the light chain. This remarkable similarity led several laboratories to seek to determine if botulinum and tetanus toxins might exhibit a zinc-dependent proteolytic activity.[29]

Demonstration of this activity was initially difficult because the toxins are very specific for their substrates. However, it is now clear that *(a)* the clostridial neurotoxins express proteolytic activity and *(b)* this activity is absolutely required for toxicity.[30] The substrate proteins for this action appear to be part of a hetero-oligomeric assembly associated with the synaptic vesicles. Interestingly, the specific target site for cleavage seems to be different for each serotype of the botulinum toxins: in some cases, different locations on the same protein; in others, different proteins of the assembly.[30] The basis of this marked specificity is not yet clear and remains fascinating to scientists interested in neurosecretion.

CLINICAL DISEASE

Botulism is a feared and dramatic disease and is frequently fatal for animals and humans alike. In food poisoning, the symptoms appear several hours to 1 or 2 days after contaminated food is consumed. The earliest symptoms are difficult to associate with poisoning and, depending on their severity, might result in a number of clinical effects: blurred vision, ptosis, dysphagia, dysarthria, and apparent muscle weakness. As the neuromuscular symptoms progress and respiratory distress begins,

healthcare providers usually consider botulism. A confirmatory diagnosis comes from mouse bioassays demonstrating toxin in blood or stool, neutralized by the appropriate antisera. Many times, the organism can be isolated from the offending food, and toxin and neutralizing tests can then be run again using food samples. Established effective treatments are few or none, save artificial ventilation and other forms of life support.

Inhalational botulism, the syndrome most likely to be seen on the battlefield, is rare. One incident involving accidental exposure of humans to botulinum toxin occurred in a laboratory in Germany and was reported in 1962.[31] After conducting a postmortem examination of laboratory animals that had been exposed, whole-body, to botulinum toxin type A, three laboratory workers experienced symptoms of botulinum intoxication. Three days after exposure, they described having *(a)* a “mucous plug in the throat,” *(b)* difficulty in swallowing solid food, and *(c)* “the beginning of a cold without fever” and were hospitalized. On the fourth day, their signs were more severe. The patients complained of “mental numbness” and retarded ocular motions; their pupils were moderately dilated with slight rotary nystagmus. Speech became indistinct and gait uncertain as patients complained of extreme weakness. The patients were given antibotulinum serum on the fourth and fifth days. Between the sixth and tenth days after exposure, the patients experienced steady reductions in their visual disturbances, numbness, and difficulties in swallowing. They were discharged from the hospital less than 2 weeks after the exposure, with only a mild general weakness remaining. The signs and symptoms of inhalational botulinum intoxication, listed in order of onset, are found in Exhibit 33-2.

EXHIBIT 33-2
SIGNS AND SYMPTOMS OF INHALATIONAL BOTULISM, IN ORDER OF ONSET

Humans[1] (sublethal dose)	**Monkeys[2] (lethal dose)**
Third day postexposure:	Mild muscular weakness
Mucous in the throat	Intermittent ptosis
Difficulty swallowing solid food	Severe weakness of postural muscles of the neck
Feeling of catching a cold but without fever	Occasional mouth breathing
	Serous nasal discharge
	Salivation, dysphagia
	Mouth breathing
	Rales
	Anorexia

Fourth day postexposure: Mental numbness Retarded ocular motions Pupils moderately dilated with slight nystagmus Indistinct speech Uncertain gait Extreme weakness	Severe generalized weakness Lateral recumbency

Data sources: (1) Holzer E. Botulism caused by inhalation. *Med Klin.* 1962;41:1735–1740. (2) Franz DR, Pitt LM, Clayton MA, Hanes MA, Rose KJ. Efficacy of prophylactic and therapeutic administration of antitoxin for inhalation botulism. In: Das Gupta B, ed. *Botulinum and Tetanus Neurotoxins and Biomedical Aspects*. New York, NY: Plenum Press; 1993: 473–476.

More data are available on exposure of animals to toxin aerosols. Rhesus monkeys were exposed by inhalation to botulinum toxin, type A, in conjunction with toxoid and hyperimmune globulin efficacy trials.[32] Exposure to 5 to 10 monkey LD_{50} (ie, 5 to 10 times the LD_{50} for monkeys) resulted in death in 2 to 4 days. Clinical signs of intoxication were noted 12 to 18 hours before death; they are also listed in order of onset in Exhibit 33-2. Preliminary studies with small numbers of animals (N = 3 per serotype) have recently demonstrated that serotypes C, D, and G are also toxic to rhesus monkeys.[33]

DIAGNOSIS

Making a diagnosis of botulism under battlefield conditions might be very difficult during the early stages of a biological warfare attack. A history of simultaneous onset of bulbar and neuromuscular disease in a group of soldiers would alert medical personnel to botulism. The symptoms listed in Exhibit 33-2 are nearly pathognomonic of botulism prior to development of respiratory failure. The absence of convulsions would differentiate botulinum intoxication from chemical nerve agent poisoning. However, a wide variety of natural neurotoxins not related to botulinum toxin could produce roughly the same sequelae. For example, the bite of a snake that produces venom neurotoxins would lead to many, if not all, of the symptoms of botulinum toxin poisoning. However, the patient or soldier or his buddies would probably mention the bite if it had occurred, the telltale fang puncta would be seen on physical examination, and numerous patients simultaneously exhibiting these signs and symptoms can hardly all have been bitten by snakes.

Medical personnel must remember, however, that with the advances of

molecular genetics, it is possible to clone and produce many natural neurotoxins in relatively large quantities. For the present (1996), botulinum poisoning is a much more likely biological warfare agent than snake venom toxins. In future years, other (cloned) neurotoxins should be considered in the diagnosis.

Because of the small quantity of toxin protein needed to kill, botulinum toxin exposure does not typically induce an antibody response after exposure. The most likely means of laboratory diagnosis is through enzyme-linked immunosorbent assay identification of botulinum toxin from swabs taken from the nasal mucosa within 24 hours of inhalational exposure.

MEDICAL MANAGEMENT

Because the incidence of botulinum poisoning is so low in the United States, vaccination of the general public is unwarranted. It is only because of the possible use of the toxin as a biological warfare agent that vaccine and antiserum development have taken place, and this work has been done almost entirely by the U.S. Army. For this reason, nearly all stocks of these products are presently held by the army. There are two basic alternatives for prophylaxis from botulinum poisoning: active immunization using a vaccine, or passive immunotherapy using immunoglobulin.

The vaccine currently available is a toxoid that protects from serotypes A through E. This material is used under Investigational New Drug (IND) status, with a license held by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. The toxoid was developed by scientists at Fort Detrick, Frederick, Maryland, during the 1950s.[3] It is a formalin-fixed crude culture supernatant from strains of *C botulinum* that produce the appropriate serotypes. Vaccinations are administered at 0, 2, and 12 weeks, followed by annual booster doses.

Nearly 80% of recipients exhibit protective titers (according to the CDC standard of > 0.25 international units per milliliter) at 14 weeks.[3] However, hardly anyone has a measurable titer just prior to receiving the first annual booster dose. The kinetics of this loss are imperfectly understood at the present. A booster dose administered at 1 year leads to a robust response from approximately 90% of the recipients. Although currently an IND vaccine, the botulinum toxoid has been administered to hundreds of humans over many years and is widely regarded as safe. (Approximately 8,000 service members received the toxoid between 23 January and 28 February 1991, as part of the U.S. force deployed to the Persian Gulf War. A significant fraction of recipients experience stinging immediately after injection and a sore arm for 2 to 4 days. However, in the experience of most recipients, including the authors, the short-lived symptoms are not

significantly different from those produced by tetanus vaccination. Work is currently underway in U.S. Army laboratories to develop a new generation recombinant botulinum vaccine that would protect from all known serotypes.

Passive protection can be afforded by administration of immunoglobulin products of various types. The earliest developed was a horse antibotulinum serum (ie, globulin). Because of the relatively high risk of serum sickness, a despeciated globulin (ie, the species-specific antigenic properties were removed from the equine immunoglobulin) was produced. Thus, the product currently held in quantity is horse antibotulinum toxin immunoglobulin that has been treated with pepsin to produce the fragment $F(ab')_2$ (ie, the basic immunoglobulin molecule has been altered by removal of the complement fixing [Fc] region to concentrate the antigen binding sites). This material has been tested for efficacy in studies with monkeys. Animals given one human dose, or one tenth of one human dose of the $F(ab')_2$ antitoxin, and challenged with approximately 10 LD_{50} of serotype A by inhalation, survived without signs of intoxication.[32] Antibody titers ranging from 0.6 to 0.38 international units per milliliter, in those given one human dose, and from 0.02 to not measurable after one tenth of one human dose of $F(ab')_2$, were fully protective.

When given as therapy after exposure, one tenth of one human dose of the $F(ab')_2$ product was protective if given before onset of clinical signs of disease. If given after onset of signs, however, even a dose 3-fold greater than the recommended human dose was not protective. We believe that toxin is already inside neurons and producing the symptoms of poisoning, and cannot be reached by circulating antibody. These data demonstrate that, at least in monkeys, titers much lower (< 0.02) than 0.25 international units (as recommended for the vaccine) are protective and suggest that humans may be more easily protected by vaccination than previously believed. In addition to the recombinant vaccine presently in development, research on cocktails of monoclonal antibodies is being conducted at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Frederick, Maryland, to replace the despeciated horse serum. The cocktail approach will enhance the safety of the immunotherapy, and recombinant techniques will probably also reduce the cost of therapeutic antibody.

Although some investigators[34] have reported limited success in treating human serotype A poisoning with aminopyridines, controlled animal experiments[35] performed at USAMRIID showed no effect on non–type A poisoning and only delays in time to death with type A. Obviously, no controlled human trials can be done, so whether the drugs helped at all remains unclear. Since the drugs have significant toxicity themselves, they

have largely been abandoned as a treatment for botulism.

Research is also ongoing (by J.L.M.) to target therapy directly to intoxicated nerves by making a chimer of the receptor binding portion of the botulinum molecule and either monoclonal antibodies or drugs that neutralize intracellularly. Just as the available toxoid and prototype second-generation vaccines provide solid protection against this most toxic of agents, we hope that second-generation therapeutics will allow us to reverse the lethal effects of this toxin after it has been internalized in the cell.

SUMMARY

The seven serotypes of botulinum toxin produced by *Clostridium botulinum* are the most toxic substances known. They are associated with lethal food poisoning after the consumption of canned foods. This family of toxins was evaluated by the United States as a potential biological weapon in the 1960s and is believed to be an agent that could be used against our troops. Unlike other threat toxins, botulinum neurotoxin appears to cause the same disease after inhalation, oral ingestion, or injection. Death results from skeletal muscle paralysis and resultant ventilatory failure. Because of its extreme toxicity, the toxin typically cannot be identified in body fluids, other than nasal secretions, after inhalation of a lethal dose. The best diagnostic sample for immunologic identification of the toxin is from swabs taken from the nasal mucosa within 24 hours after inhalational exposure. Because of the small quantity of toxin protein needed to kill, botulinum toxin exposure does not typically induce an antibody response after exposure.

Prophylactic administration of a licensed pentavalent vaccine fully protects laboratory animals from all routes of challenge. Passive immunotherapy with investigational hyperimmune plasma also prevents illness if it is administered before the onset of clinical intoxication.

REFERENCES

1. Tacket CO, Rogawski MA. Botulinism. In: Simpson LL, ed. *Botulinum Neurotoxin and Tetanus Toxin*. New York, NY: Academic Press; 1989: 351–378.

2. Cochrane RC. *Biological Warfare Research in the United States.* Vol 2. In: *History of the Chemical Warfare Service in World War II.* Historical Section, Plans, Training and Intelligence Division, Office of Chief, Chemical Corps, US Department of the Army; 1947. Unclassified.

3. Middlebrook JL. Contributions of the U.S. Army to botulinum toxin research. In:

Das Gupta B, ed. *Botulinum and Tetanus Neurotoxins and Biomedical Aspects.* New York, NY: Plenum Press; 1993: 515–519.

4. McCroskey LM, Hathaway CL, Fenicia L, Pasolini B, Aureli P. Characterization of an organism that produces type E botulinal neurotoxin but which resembles *Clostridium butyricum* from the feces of an infant with type E botulism. *J Clin Microbiol*. 1986;23:201–202.

5. Eisel U, Jarausch W, Goretzki K, et al. Tetanus toxin: Primary structure, expression in *E coli*, and homology with botulinum toxins. *EMBO J*. 1986;10:2495–2502.

6. Fairweather NF, Lyness VA. The complete nucleotide sequence of tetanus toxin. *Nucleic Acids Res*. 1986;14:7809–7812.

7. Binz T, Kurazono H, Wille M, Frevert J, Wernars K, Nieman H. The complete sequence of botulinum neurotoxin type A and comparison with other clostridial neurotoxins. *J Biol Chem*. 1990;265:9153–9158.

8. Thompson DE, Brehm JK, Oultram JD, et al. The complete amino acid sequence of the *Clostridium botulinum* type A neurotoxin, deduced by nucleotide sequence analysis of the encoding gene. *Eur J Biochem*. 1990;189:73–81.

9. Whelan SM, Elmore MJ, Bodsworth NJ, Brehm JK, Atkinson T, Minton NP. Molecular cloning of the *Clostridium botulinum* structural gene encoding the type B neurotoxin and determination of its entire nucleotide sequence. *Appl Environ Microbiol*. 1992;58:2345–2354.

10. Hausor D, Popoff MR, Kurazono H, et al. Complete sequence of botulinal C1 neurotoxin. *Nucleic Acids Res*. 1990;18:4924–4928.

11. Binz T, Kurazono H, Popoff MR, et al. Nucleotide sequence of the gene encoding *Clostridium botulinum* neurotoxin type D. *Nucleic Acids Res*. 1990;18:5556–5557.

12. Whelan SM, Elmore MJ, Bodsworth NJ, Atkinson T, Minton NP. The complete amino acid sequence of the *Clostridium botulinum* type-E neurotoxin, derived by nucleotide-sequence analysis of the encoding gene. *Eur J Biochem*. 1992;204:657–667.

13. Alison K, Richardson PT, Allaway D, Collins MD, Roberts TA, Thompson DE. Sequence of the gene encoding type F neurotoxin of *Clostridium botulinum*. *FEMS Microbiol Lett*. 1992;96:225–230.

14. Campbell KD, Collins MD, East AK. Nucleotide sequence of the gene coding for *Clostridium botulinum (Clostridium argentinese)* type G neurotoxin: Genealogical comparison with other clostridial neurotoxins. *Biochim Biophys Acta*. 1993;1216:487–491.

15. Eklund MW, Poysky FT, Mseitif LM, Strom MS. Evidence for plasmid-mediated toxin and bacteriocin production in *Clostridium botulinum* type G. *Appl Environ*

Microbiol. 1988;54:1405–1408.

16. Eklund MW, Poysky FT, Reed SM, Smith CA. Bacteriophages and toxigenicity of *Clostridium botulinum C. Science.* 1971;172:480–482.

17. Eklund MW, Poysky FT, Reed SM. Bacteriophages and toxigenicity of *Clostridium botulinum* type D. *Nature*. 1976;235:16–18.

18. Aureli P, Fenicia L, Pasolini B, Gionfranceschi M, McCroskey LM, Hathaway CL. Two cases of type E infant botulism in Italy caused by neurotoxigenic *Clostridium butyricum. J Infect Dis*. 1986;154:201–211.

19. Hall JD, McCroskey LM, Pincomb BJ, Hathaway CL. Isolation of an organism which produces type E botulinal toxin from an infant with botulism. *J Clin Microbiol*. 1985;21:654–655.

20. Gill DM. Bacterial toxins: A table of lethal amounts. *Microbiol Rev*. 1982;46(1):86–94.

21. McNally RE, Morrison MB, Berndt JE, et al. *Effectiveness of Medical Defense Interventions Against Predicted Battlefield Levels of Botulinum Toxin A*. Vol 1. Joppa, Md: Science Applications International Corporation; 1994: 3.

22. Habermann E. Clostridial neurotoxins and the central nervous system: Functional studies on isolated preparations. In: Simpson LL, ed. *Botulinum Neurotoxin and Tetanus Toxin*. New York, NY: Academic Press, Inc; 1989: 53–67.

23. Simpson LL. Peripheral actions of the botulinum toxins. In: Simpson LL, ed. *Botulinum Neurotoxin and Tetanus Toxin*. New York, NY: Academic Press, Inc; 1989: 153–178.

24. Middlebrook JL. Cell surface receptors for protein toxins. In: Simpson LL, ed. *Botulinum Neurotoxin and Tetanus Toxin*. New York, NY: Academic Press, Inc; 1989: 95–119.

25. Black JD, Dolly O. Interaction of ^{125}I-botulinum neurotoxins with nerve terminals: Ultrastructural autoradio-graphic localization and quantitation of distinct membrane acceptors for types A and B on motor nerves. *J Cell Biol*. 1986;103:521–534.

26. Nishiki T-I, Kamata Y, Nemoto Y, et al. Identification of protein receptor for *Clostridium botulinum* type B neurotoxin in rat brain synaptosomes. *J Biol Chem*. 1994;269(14):10498–10503.

27. Goldstein JL, Anderson RGW, Brown MS. Coated pits, coated vesicles and receptor-mediated endocytosis. *Nature*. 1979;279:679–689.

28. Jongeneel CV, Bouvier J, Bairoch A. A unique signature identifies a family of zinc-dependent metallopeptidases. *FEBS Lett*. 1989;242:211–214.

29. Schiavo G, Benfenati F, Poulain B, et al. Tetanus and botulinum-B neurotoxins

block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature.* 1992;359:832–835.

30. Nieman H, Blasi J, Jahn R. Clostridial neurotoxins: New tools for dissecting exocytosis. *Trends in Cell Biology*. 1994;4:179–185.

31. Holzer E. Botulism caused by inhalation. *Med Klin*. 1962;41:1735–1740.

32. Franz DR, Pitt LM, Clayton MA, Hanes MA, Rose KJ. Efficacy of prophylactic and therapeutic administration of antitoxin for inhalation botulism. In: Das Gupta B, ed. *Botulinum and Tetanus Neurotoxins and Biomedical Aspects*. New York, NY: Plenum Press; 1993: 473–476.

33. Hunt R. Lieutenant Colonel, Veterinary Corps, US Army. Principal Investigator, Toxinology Division, Department of Aerobiology and Product Development, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Personal communication, February 1996.

34. Ball AP, Hopkinson RB, Farrell ID, et al. Human botulism caused by *Clostridium botulinum* type E: the Birmingham outbreak. *Q J Med*. 1979;48(191):473–491.

35. Siegel LS, Johnson-Winegar AD, Sellin LC. Effect of 3,4-diaminopyridine on the survival of mice injected with botulinum neurotoxin type A, B, E or F. *Toxicol Appl Pharmacol*. 1986;84:255–263.

Chapter 34

TRICHOTHECENE MYCOTOXINS

ROBERT W. WANNEMACHER, JR., PH.D.*; AND STANLEY L. WIENER, M.D.†

INTRODUCTION

HISTORY AND MILITARY SIGNIFICANCE
- Use in Biological Warfare
- The Yellow Rain Controversy
- Weaponization

DESCRIPTION OF THE AGENT
- Occurrence in Nature
- Chemical and Physical Properties

TOXICOLOGY AND TOXICOKINETICS
- Mechanism of Action
- Metabolism

CLINICAL DISEASE
- Acute Effects
- Chronic Toxicity

DIAGNOSIS
- Battlefield Diagnosis
- Confirmatory Procedures

MEDICAL MANAGEMENT
- Individual and Unit
- Specific or Supportive Therapy
- Prophylaxis

SUMMARY

* *Assistant Chief, Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011*

† Colonel, Medical Corps, U.S. Army Reserve; Professor of Medicine and Chief, Section of General Internal Medicine, Department of Medicine, University of Illinois College of Medicine, 840 Wood Street, Chicago, Illinois 60612

INTRODUCTION

Mycotoxins, by-products of fungal metabolism, have been implicated as causative agents of adverse health effects in humans and animals that have consumed fungus-infected agricultural products.[1,2] Consequently, fungi that produce mycotoxins, as well as the mycotoxins themselves, are potential problems from both public health and economic perspectives. The fungi are a vast assemblage of living organisms, but mycotoxin production is most commonly associated with the terrestrial filamentous fungi called the molds.[3] Various genera of toxigenic fungi are capable of producing such diverse mycotoxins as the aflatoxins, rubratoxins, ochratoxins, fumonisins, and trichothecenes.[1,2]

The trichothecenes are a very large family of chemically related toxins produced by various species of *Fusarium, Myrotecium, Trichoderma, Cephalosporium, Verticimonosporium,* and *Stachybotrys*.[4] They are markedly stable under different environ mental conditions. The distinguishing chemical feature of trichothecenes is the presence of a trichothecene ring, which contains an olefinic bond at C-9, 10; and an epoxide group at C-12, 12.[5] All trichothecenes are mycotoxins, but not all mycotoxins are trichothecenes. This family of mycotoxins causes multiorgan effects including emesis and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, decreased reproductive capacity, and bone marrow damage.[4,6]

In this chapter, we will concentrate on T-2 mycotoxin, a highly toxic trichothecene that, together with some closely related compounds, has been the causative agent of a number of illnesses in humans and domestic animals.[1,2,4] During the 1970s and 1980s, the trichothecene mycotoxins gained some notoriety as putative biological warfare agents when they were implicated in “yellow rain” attacks in Southeast Asia.[7–11]

HISTORY AND MILITARY SIGNIFICANCE

Fungi that produce trichothecenes are plant pathogens and invade various agricultural products and plants. Since *Fusarium* and other related fungi infect important foodstuff, they have been associated worldwide with intoxication of humans and animals. Thus, these fungi have potential as biological weapons.

Use in Biological Warfare

From 1974 to 1981, toxic agents were used by the Soviet Union and its client states in such Cold War sites as Afghanistan, Laos, and Kampuchea (Cambodia). Aerosol-and-droplet clouds were produced by delivery systems in the Soviet arsenal such as aircraft spray tanks, aircraft-launched rockets, bombs (exploding cylinders), canisters, a Soviet hand-held weapon (DH-10), and booby traps. Aircraft used for delivery included L-19s, AN-2s, T-28s, T-41s, MiG-21s (in Laos) and Soviet MI-24 helicopters (in Afghanistan and Laos).

Attacks in Laos (1975–1981) were directed against Hmong villagers and resistance forces who opposed the Lao People's Liberation Army and the North Vietnamese. In Kampuchea, North Vietnamese troops used 60-mm mortar shells; 120-mm shells; 107-mm rockets; M-79 grenade launchers containing chemicals; and chemical rockets, bombs, and sprays delivered by T-28 aircraft (1979–1981) against Khmer Rouge troops. The chemical munitions were supplied by the Soviets and delivered by North Vietnamese or Laotian pilots. In Afghanistan, the chemical weapons were delivered by Soviet or Afghan pilots against Mujahidin guerrillas (1979–1981). Lethality of the attacks is documented by a minimum of 6,310 deaths in Laos (from 226 attacks); 981 deaths in Kampuchea (from 124 attacks); and 3,042 deaths in Afghanistan (from 47 attacks).[7] Trichothecenes appear to have been used in some of these attacks.

The air attacks in Laos have been described as "yellow rain" and consisted of a shower of sticky, yellow liquid that sounded like rain as it fell from the sky. Other accounts described a yellow cloud of dust or powder, a mist, smoke, or insect spray–like material. Liquid agent rapidly dried to a powder. In Laos, 50% to 81%[7] of attacks involved material associated with a yellow pigment. Other attacks were associated with red, green, white, or brown smoke or vapor. More than 80%[7] of attacks were delivered by air-to-surface rockets; the remainder, from aircraft spray tanks or bombs. Intelligence information and some of the victims' descriptions of symptoms raised the possibility that chemical warfare agents such as phosgene, sarin, soman, mustards, CS, phosgene oxime, or BZ may also have been used. These agents may have been used in mixtures or alone, and with or without the trichothecenes.

Unconfirmed reports have implicated the use of trichothecenes in the 1964 Egyptian (or Russian) attacks on Yemeni Royalists in Yemen[12] and in combination with mustards during chemical warfare attacks in the Iran–Iraq War (1983–1984).[13] According to European sources, Soviet–Cuban forces in Cuba are said to have been equipped with mycotoxins, and a Cuban agent is said to have died of a hemorrhagic syndrome induced by a mycotoxin agent.[14]

The Yellow Rain Controversy

Actual biological warfare use of trichothecenes in Southeast Asia and Afghanistan is strongly supported by the epidemiological and intelligence assessments and trichothecene assays, although reports in the open literature have discounted this contention. An article written by L. R. Ember,[15] published in 1984 in Chemical Engineering News, is the most exhaustive and authoritative account of the controversy surrounding the use of trichothecene mycotoxins in Southeast Asia during the 1970s.

The United States government, its allies, and journalists exhaustively studied the possibility that yellow rain attacks had occurred, based on evidence[7,14,15] such as the following:

- interviews of Hmong survivors of and eyewitnesses to lethal yellow rain attacks in Laos, who provided consistent descriptions of the episodes;
- interrogations of a defecting Laotian Air Force officer and North Vietnamese ground troops, who corroborated the descriptions of attacks and admitted using the chemicals;
- interrogations of prisoners of war, who admitted being involved in attacks where unconventional weapons were used (ie, in Afghanistan);
- laboratory confirmations of Soviet use of chemical agents, and
- the presence of Soviet-manufactured chemical agents and Soviet technicians in Laos.

The evidence supports the contention that trichothecene mycotoxins were used as biological warfare agents in Southeast Asia and Afghanistan by the former Soviet Union and its surrogates. The Russians have not recently denied such use but have declined to discuss the subject.

In addition to the evidence stated above, elevated levels and naturally rare mixtures of trichothecene toxins were recovered from the surfaces of plants, fragments of plastic, and rocks in areas attacked[9,11,15,16] ; and were detected in the blood of attack survivors and the tissues of a dead casualty.[10,15] Control samples that were taken *(a)* from an environment that had not been attacked, and during another season of the year,[15] and *(b)* from Hmong who had never been exposed to an attack were consistently negative.

The evidence that trichothecenes were used in Southeast Asia has been challenged: questions have been raised about the interview methodology used by U.S. Army physicians and U.S. State Department personnel in Hmong refugee camps in Thailand to obtain descriptions of the attacks. Some inconsistencies of specific individuals' stories were demonstrated, but the frequency of unreliable information has not been reported and is unlikely to be large enough to discredit all witnesses.[15] Symptom descriptions are generally consistent with known trichothecene effects.

The paucity of positive evidence of the presence of trichothecenes (5 positive environmental and 20 positive biomedical samples) has been used to

challenge the belief that biological warfare attacks occurred, since only 10% of samples were positive. However, 32% of samples from victims were positive, a value too high for natural causes (eg, food contamination) to be used as an explanation, since 98% of controls in nonattack areas of Thailand were negative.[17] The 2% of samples that were positive could represent either a nonspecific result or low-prevalence food contamination. The paucity and type of control samples have also been questioned.

Some experts[18–21] have claimed that yellow rain was not a biological warfare attack at all, but that the yellow residue was caused by showers and deposits of bee feces—the result of massive bee swarming and cleansing–defecation flights over some areas of Southeast Asia. The presence of pollen in bee feces and some samples has not only added confusion[18] but is also the supporting evidence used by the skeptics. It is important to remember that persons caught in a shower of bee feces do not get sick and die. Although bee flights have occurred before and since 1982, reports of attacks of yellow rain and death in Asia have not.

Then what explains the symptoms consistent with trichothecene effects in the casualties, and the pollen and bee feces in some of the yellow spots on vegetation in the area? Bee feces do not contain trichothecenes, yet pollen and trichothecenes without mold are found together in some samples from attack areas. The most likely explanation is that during biological warfare attacks, dispersed trichothecenes landed in pollen-containing areas.

French scientists have reported the simultaneous synthesis of three trichothecene toxins by *Fusarium* growing on corn, but actual production of these toxins by *Fusarium* species in Southeast Asia has not been demonstrated, presumably because of high environmental temperature (ie, toxin production usually increases at low temperatures). Whether or not *Fusarium* toxin is produced in the high-mountain temperate regions of Laos inhabited by the Hmong remains unanswered. The presence of toxin on leaves without accompanying mold also is unexplained by critics of the trichothecene weapon hypothesis. In vivo studies have demonstrated that *F semitectum* var *semitectum* will grow on leaves in Southeast Asia, but have not shown that it will produce toxin in vivo.[15]

In support of the weapon hypothesis are the positive trichothecene analyses performed by two leading researchers[9,10] in the detection of trichothecenes; the Defense Research Establishment, Ottawa, Canada[11,22]; and the U.S. Army Chemical Research and Development Center, Edgewood, Maryland.[23] Negative results of analyses of biomedical and environmental samples from Southeast Asia have come from Porton Down Laboratory in England,[17,24] but according to the British, such results do not exclude sampling problems, including delay in sample collection after an attack, as a cause of the negative results.[15]

Proponents have been accused of analyzing samples that were purposely contaminated with toxin, either after collection or during the analysis. Other methodological criticisms include poor recovery (< 10% of one sample spiked

with T-2 toxin); low precision of quantitative data when analyzing two portions of the same leaf; and lack of well-documented, confirming, replicate analyses in Mirocha's or a similarly equipped second laboratory.[15] The presence of polyethylene glycol in the sample analyzed by Rosen[9] also indicates that the trichothecene mixture detected was manufactured, not natural.

Many experts in the intelligence community,[16] academia,[8,9] the U.S. Department of State,[7] and the authors of this chapter believe that trichothecenes were used as biological weapons in Southeast Asia and Afghanistan. However, a weapon containing trichothecenes was not found in Southeast Asia, and the Soviets have not declared any stockpiles of trichothecenes among their chemical or biological weapons. Thus, it has not been possible for the United States to prove unequivocally that trichothecene mycotoxins were used as biological weapons.

Weaponization

Trichothecene mycotoxins can be delivered as dusts, droplets, aerosols, or smoke from aircraft, rockets, missiles, artillery, mines, or portable sprayers. Because of their antipersonnel properties, ease of large-scale production, and apparent proven delivery by various aerial dispersal systems, the trichothecene mycotoxins (especially T-2 toxin) have an excellent potential for weaponization.

When delivered at low doses, trichothecene mycotoxins cause skin, eye, and gastrointestinal problems. In nanogram amounts,[4,25] they (T-2 toxin, in particular) cause severe skin irritation (erythema, edema, and necrosis).[4,6] Skin vesication has been observed in a number of humans exposed to yellow rain attacks.[4,14,15] T-2 toxin is about 400-fold more potent (50 ng vs 20 µg) than mustard in producing skin injury.[26] Lower-microgram quantities of trichothecene mycotoxins cause severe eye irritation, corneal damage, and impaired vision.[4,16,26,27] Emesis and diarrhea have been observed at amounts that are one fifth to one tenth the lethal doses of trichothecene mycotoxins.[26]

Depending on the species of experimental animal tested and the exposure procedure,[28,29] the lethality of T-2 toxin by aerosol exposure can be 10- to 50-fold greater than when injected parenterally.[30] With larger doses in humans, aerosolized trichothecenes may produce death within minutes to hours.[7,14,15] The term LCt_{50} (the concentration • time that is lethal to 50% of the exposed population) is used to describe exposure to vapors and aerosols; milligrams • minutes per cubic meter is the conventional unit of measurement. LCt_{50} and its relation to LD_{50} (the dose that is lethal to 50% of the exposed population) are discussed in detail in Chapter 5, Nerve Agents, and will not be further explicated here.

The toxicity of T-2 toxin by the inhalational route of exposure (LCt_{50} range: 200–5,800 mg•min/m^3) [28–30] is similar to that observed for mustards or Lewisite (LCt_{50} range: 1,500–1,800 mg•min/m^3). [31] However, the lethality of T-

2 toxin by the dermal route (LD_{50} range: 2–12 mg/kg[6]) is higher than that for liquid Lewisite (LD_{50}: approximately 30 mg/kg[31(p39)]) or liquid mustards (LD_{50}: approximately 100 mg/kg[31(p32)]). Therefore, the trichothecene mycotoxins are considered to be primarily blister agents that, at lower exposure concentrations, can cause severe skin and eye irritation, and at larger doses can produce considerable incapacitation and death within minutes to hours.

By solid substrate fermentation, T-2 toxin can be produced at approximately 9 g/kg of substrate, with a yield of 2 to 3 g of crystalline product.[32] Several of the trichothecene mycotoxins have been produced in liquid culture at medium yields and large volumes of culture for extraction.[33] Thus, using existing state-of-the-art fermentation processes that were developed for brewing and antibiotics, it would be fairly simple to produce ton quantities of a number of the trichothecene mycotoxins.

In Southeast Asia, most of the yellow rain attacks were delivered by aircraft or helicopter spray, bombs, and air-to-surface rockets. The attacks were described as a shower of sticky liquid, a yellow cloud of dust or powder, or a mist (like an insect spray).[7,15] The delivery of the trichothecene mycotoxins was similar in many aspects to the spraying of pesticides on agricultural crops. This would result in a very low-efficiency respiratory aerosol (1–5 µm particles)[34] but a highly effective droplet aerosol that could cause severe skin and eye irritation.

DESCRIPTION OF THE AGENT

Occurrence in Nature

Potentially hazardous concentrations of the trichothecene mycotoxins can occur naturally in moldy grains, cereals, and agricultural products.[4,35] Toxigenic species of *Fusarium* occur worldwide in habitats as diverse as deserts, tidal salt flats, and alpine mountain regions.[35] For example, a food-related disease has been recorded in Russia from time to time, probably since the 19th century.[36] Over the period 1942 through 1947, more than 10% of the population in Orenburg, near Siberia, were fatally affected by overwintered millet, wheat, and barley.[4,36] The syndrome was officially named alimentary toxic aleukia (ATA). Extensive investigations in Russia indicated that a toxin from *Fusarium* species of fungi was the causative agent of ATA.[36,37] Subsequently, it was demonstrated that T-2 toxin, a potent trichothecene mycotoxin, was the likely agent.[37]

Stachybotryotoxicosis has been reported among farm workers in Russia, Yugoslavia, and Hungary.[38,39] This disease is caused by the presence of a mold, *Stachybotrys atra (S alternans)*, on the hay fed to domestic animals. A macrocyclic trichothecene (satratoxin) produced by the *Stachybotrys* species of the mold may be in part responsible for this toxicosis.[40] The only literature citation on apparent human cases of stachybotryotoxicosis in the United States occurred in people living in a water-damaged house with a heavy infestation of

S atra.[41]

Russian scientists have reported a case of “cotton lung disease,” which was brought about by the inhalation of cotton dust that was contaminated with *Dendrochium toxicum*. This fungus is considered to be synonymous with *Myrothecium verrucaria* (a natural producer of the verrucarin class of trichothecenes).[42]

The “red mold disease” of wheat and barley in Japan is prevalent in the region that faces the Pacific Ocean.[4] Toxic trichothecenes, including nivalenol, deoxynivalenol, and monoacetylnivalenol (fusarenon-X) from *Fusarium nivale*, can be isolated from moldy grains. In the suburbs of Tokyo, an illness similar to “red mold disease” was described in an outbreak of a food-borne disease, as a result of the consumption of *Fusarium*-infected rice.[35] Ingestion of moldy grains that are contaminated with trichothecenes has been associated with mycotoxicosis in domestic farm animals.[4]

Chemical and Physical Properties

The trichothecenes make up a family of closely related chemical compounds called *sesquiterpenoids* (Figure 34-1). The structures of close to 150 derivatives of the trichothecenes are described in the scientific literature.[35,43] The specific side structures of the most abundant of the naturally occurring trichothecenes are shown in Table 34-1. Because of its availability and relatively high toxicity, T-2 toxin has been the most extensively studied trichothecene mycotoxin.

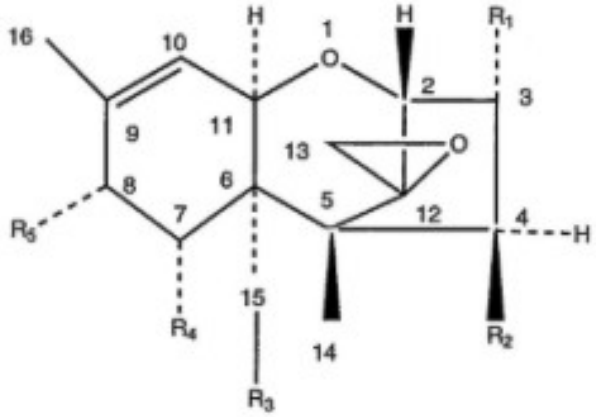


Fig. 34-1. The general structure, numbering system, and variable side groups of the tetracyclic trichothecene nucleus.

The trichothecene mycotoxins are nonvolatile, low-molecular-weight (MW 250–550) compounds.[43] This group of mycotoxins is relatively insoluble in water but highly soluble in acetone, ethyl acetate, chloroform, dimethyl sulfoxide (DMSO), ethanol, methanol, and propylene glycol.[43] Purified trichothecenes generally have a low vapor pressure, but they do vaporize when heated in organic solvents. Extraction of trichothecene mycotoxins from fungal cultures with organic solvents yields a yellow-brown liquid, which, if allowed to evaporate, forms a greasy, yellow crystalline product. Some experts[10,16] believe this extract to be the yellow contaminate of yellow rain. In contrast, highly purified toxins form white, crystalline products that have characteristic melting points.[35]

When maintained as either crystalline powders or liquid solutions,

trichothecene mycotoxin compounds are stable when exposed to air, light, or both.[35,44] Moreover, these mycotoxins are not inactivated by autoclaving but require heating at 900°F for 10 minutes or 500°F for 30 minutes for complete inactivation. A 3% to 5% solution of sodium hypochlorite is an effective inactivation agent for them.[44] The efficacy of this solution can be increased by the addition of small amounts of alkali.

TABLE 34-1
SPECIFIC SIDE GROUPS OF THE MOST ABUNDANT TRICHOTHECENE MYCOTOXINS

Trichothecene	R_1	R_2	R_3	R_4	R_5
T-2 Toxin	–OH	$–OCOCH_3$	$–OCOCH_3$	–H	$–OCOCH_2CH(CH_3)_2$
HT-2 Toxin	–OH	–OH	$–OCOCH_3$	–H	$–OCOCH_2CH(CH_3)_2$
4,15-Diacetoxyscripenol (DAS, also called anguidine)	–OH	$–OCOCH_3$	$–OCOCH_3$	–H	–H
Nivalenol	–OH	–OH	–OH	–OH	=O
Deoxynivalenol (DON)	–OH	–H	–OH	–OH	=O
Macrocyclics	–H	–O–R '	–O–	–H	–H

R ': Macrocyclic ester or ester–ether bridge between carbons 4 and 15. The most abundant macrocyclic trichothecenes are verrucarins, roridins, and satratoxin H. Source for this statement: Jarvis BB. Macrocyclic trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 361–421.

TOXICOLOGY AND TOXICOKINETICS

The trichothecene mycotoxins are toxic to humans, other mammals, birds, fish, a variety of invertebrates, plants, and eukaryotic cells in general. The acute toxicity of the trichothecene mycotoxins varies somewhat with the particular toxin and animal species studied (Table 34-2). Differences are noted among the various species in their susceptibility to trichothecene mycotoxins, but they are small compared with the divergence obtained by diverse routes of administration of the toxins (Table 34-3). Once the trichothecene mycotoxins enter the systemic circulation, regardless of the route of exposure, they affect rapidly proliferating tissues.[1,2,4,6,35,42,45]

TABLE 34-2
RELATIVE ACUTE PARENTERAL TOXICITY OF THE MOST ABUNDANT TRICHOTHECENE MYCOTOXINS

	Mammals Tested							
	Mouse	Rat	Guinea Pig	Rabbit	Cat	Dog	Pig	Monkey
Trichothecenes Tested	LD_{50} (mg/kg)							
T-2 Toxin	5.2 (IV)	0.9 (IV)	1.0 (IV)	1.0 (IM)	< 0.5 (SC)	—	1.2 (IV)	0.8 (IM)
HT-2 Toxin	9.0 (IP)	—	—	—	—	—	—	—
4,15-Diacetoxy-scripenol (DAS)	12.0 (IV)	1.3 (IV)	—	1.0 (IV)	—	1.1 (IV)	0.38 (IV)	—
Nivalenol	6.3 (IV)	—	—	—	—	—	—	—
Deoxynivalenol (DON)	43 (SC)	—	—	—	—	—	—	—
Verrucarin A	1.5 (IV)	0.8 (IV)	—	0.54 (IV)	—	—	—	—
Roridin A	1.0 (IV)	—	—	—	—	—	—	—
Satratoxin H	1.0 (IP)	—	—	—	—	—	—	—

Routes of administration of the mycotoxin: IV: intravenous; IM: intramuscular; SC: subcutaneous; IP: intraperitoneal
—: Not determined
Data sources: (1) Ueno Y. Trichothecene mycotoxins: Mycology, chemistry, and toxicology. *Adv Nut Res.* 1989;3:301–353. (2) Wannemacher RW Jr, Bunner DL, Neufeld HA. Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: Smith JE, Henderson RS, eds. *Mycotoxins and Animal Foods.* Boca Raton, Fla: CRC Press; 1991: 499–552. (3) Sharma RP, Kim Y-W. Trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins.* Boca Raton, Fla: CRC Press; 1991: 339–359. (4) Jarvis BB. Macrocyclic trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins.* Boca Raton, Fla: CRC Press; 1991: 361–421.

TABLE 34-3
COMPARATIVE TOXICITY OF T-2 TOXIN BY VARIOUS ROUTES OF ADMINISTRATION

Route of Administration	Mammals Tested						
	Mouse	Rat	Guinea Pig	Rabbit	Cat	Pig	Monkey
	T-2 Toxin LD_{50} (mg/kg)						
Intravenous	4.2–7.3	0.7–1.2	1.0–2.0	—	—	1.2	—
Intraperitoneal	5.2–9.1	1.3–2.6	—	—	—	—	—
Subcutaneous	2.1–3.3	0.6–2.0	1.0–2.0	—	<0.5	—	—
Intramuscular	—	0.5–0.9	1.0	1.1	—	—	0.8
Intragastric	9.6–10.5	2.3–5.2	3.1–5.3	—	—	—	—
Intranasal	—	0.6	—	—	—	—	—
Intratracheal	0.16	0.1	—	—	—	—	—
Inhalational	0.24	0.05	0.6–2.0	—	—	—	—
Intracerebral	—	0.01	—	—	—	—	—
Dermal in DMSO	6.6	4.3	2.2	10	—	—	>8.0
Dermal in Methanol	—	>380	>80	—	—	—	—

DMSO: dimethyl sulfoxide
—: Not determined
Data sources: (1) Ueno Y. Trichothecene mycotoxins: Mycology, chemistry, and toxicology. *Adv Nut Res.* 1989;3:301–353. (2) Wannemacher RW Jr, Bunner DL, Neufeld HA. Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: Smith JE, Henderson RS, eds. *Mycotoxins and Animal Foods.* Boca Raton, Fla: CRC Press; 1991: 499–552. (3) Sharma RP, Kim Y-W. Trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins.* Boca Raton, Fla: CRC Press; 1991: 339–359.

Mechanism of Action

The trichothecene mycotoxins are cytotoxic to most eukaryotic cells.[46] A number of cytotoxicity assays have been developed and include survival and cloning assays, measuring protein and deoxyribonucleic acid (DNA) synthesis by radiolabeling procedures, and a neutral red cell–viability assay. A minimum of 24 to 48 hours is required to measure the effects of the trichothecene mycotoxins on cell viability.

These mycotoxins inhibit protein synthesis in a variety of eukaryotic cells.[46–48] Similar sensitivity to T-2 toxin was observed in established cell lines and primary cell cultures.[46,48] Inhibition of protein synthesis is observed 5 minutes after exposure of Vero cells to T-2 toxin, with a maximal response noted by 60 minutes after the exposure.[46] Researchers[47] have concluded that the trichothecene mycotoxins act by inhibiting either the initiation or the elongation process of translation, by interfering with peptidyl transferase activity.

Substantial inhibition of ribonucleic acid (RNA) synthesis (86% inhibition) by trichothecene mycotoxin was observed in human (HeLa) cells,[47] but T-2 toxin had minor effects (15% inhibition) on RNA synthesis in Vero cells.[46] The trichothecene mycotoxin– related inhibition of RNA synthesis is probably a secondary effect of the inhibition of protein synthesis. Scheduled DNA synthesis is strongly inhibited in various types of cells that are exposed to trichothecene mycotoxins. In mice or rats treated with trichothecene mycotoxins, DNA synthesis in all tissues studied was suppressed, although to a lesser degree than protein synthesis.[49] The pattern by which DNA synthesis is inhibited by the trichothecene mycotoxins is consistent with the primary effect of these toxins on protein synthesis. In appropriate cell models, for the most part, trichothecene mycotoxins demonstrate neither mutagenic activity nor the capacity to damage DNA.[50]

Studies with radiolabeled trichothecene mycotoxins suggest that the toxin interaction with cells is best viewed as (1) a free, bidirectional movement of these low-molecular-weight chemicals across the plasma membrane; and (2) specific, high-affinity binding to ribosomes.[51] Thus, further evidence indicates that the primary toxic effects of the trichothecene mycotoxins is caused by their properties as potent inhibitors of protein synthesis.

Since the trichothecene mycotoxins are amphophilic molecules, an investigation[52] that focused on various kinds of interaction with cellular membranes concluded that T-2 exerts multiple effects on the cell membrane. Lipid peroxidation is increased in liver, spleen, kidney, and thymus; and bone marrow when rats are treated with a single, oral dose of T-2 toxin.[53] These observations led to the suggestion that the trichothecene mycotoxins might induce some alterations in membrane structure, which consequently stimulates lipid peroxidation. Once trichothecene mycotoxins cross the plasma membrane barrier, they enter the cell, where they can interact with a number of targets, including ribosomes [47] and mitochondria.[54]

These toxins inhibit electron transport activity, as the inhibition of succinic dehydrogenase activity and mitochondrial protein synthesis implies. Toxin-stimulated alteration in mitochondrial membranes contributes to the effects on cellular energetics and cellular cytotoxicity. Although initial investigations on the mechanism of action of the trichothecene mycotoxins suggested that the inhibition of protein synthesis as the principal mechanism of action, the above observations indicate that the effects of these toxins are much more diverse.

Metabolism

Compared with some of the other mycotoxins such as aflatoxin, the trichothecenes do not appear to require metabolic activation to exert their biological activity.[50] After direct dermal application or oral ingestion, the trichothecene mycotoxins can cause rapid irritation to the skin or intestinal mucosa. In cell-free systems or single cells in culture, these mycotoxins cause a rapid inhibition of protein synthesis and polyribosomal disaggregation.[35,47,50] Thus, we can postulate that the trichothecene mycotoxins have molecular capability of direct reaction with cellular components. Despite this direct effect, it is possible to measure the toxicokinetics and the metabolism of the trichothecene mycotoxins.

The lipophilic nature of these toxins suggests that they are easily absorbed through skin, gut, and pulmonary mucosa. Absorption of a single, oral dose of T-2 toxin is rapid, with concentration of labeled toxin peaking in the blood within 1 hour.[55] This indicates that the trichothecene mycotoxins rapidly pass through the intestinal mucosa. The inhaled median lethal dose of T-2 toxin is equal to [29] or less than[28,30] the systemic dose. Mice, rats, and guinea pigs die rapidly (within 1–12 h) after exposure to high concentrations of aerosolized mycotoxin, with no apparent lung lesions or pulmonary edema.[28–30] This finding is in contrast to the effect of an oral dose of T-2 toxin, which causes direct damage to the intestinal mucosa.[55]

From these data, we can conclude that the trichothecene mycotoxins very rapidly cross the pulmonary and intestinal mucosa and enter the systemic circulation to induce the toxin-related toxicoses. In contrast, trichothecene mycotoxins are only slowly absorbed through skin, especially when applied as a dust or powder.[56] Systemic toxicity and lethality can be produced by dermal exposure to higher concentrations of T-2 toxin, however, especially if the mycotoxin is dissolved in a penetrant such as DMSO.[6]

Various cell culture lines and ruminal bacteria metabolize T-2 toxin by deacylation of specific deepoxidylation (ie, removal of the oxygen from the epoxide ring at the C-12, 13 position to yield a carbon–carbon double bond) and oxidization of the C-3 ' and C-4 ' positions on the isovaleryl side chains of T-2 toxin and HT-2 toxin, a metabolite (Figure 34-2).[57–59] A number of different cell types contain the metabolic processes necessary to metabolize trichothecene mycotoxins.

Pharmacokinetic studies[60,61] have demonstrated T-2 toxin in the plasma of animals that were administered this mycotoxin both intravascularly and by aerosol. As plasma concentrations of the parent trichothecene mycotoxin decrease, the deacylated and hydroxylated metabolites and their glucuronide conjugates rapidly appear and disappear from circulation. From these various observations, we can conclude that the pharmacokinetics of the trichothecene mycotoxins are functions of the rate of absorption into the general circulation, metabolism, tissue distribution, and excretion.

Tissue-distribution studies[55] suggest that the liver is the major organ for metabolism of the trichothecene mycotoxins. The bile and the gastrointestinal tract contained large amounts of radioactivity after intravascular, intramuscular, oral, or dermal administration of radiolabeled T-2 toxin. Although the liver is the major organ for the metabolism of the trichothecene mycotoxins, other tissues such as the intestine are capable of metabolic

alteration of these toxins. After an intravenous dose of T-2 toxin, 95% of the total radioactivity was excreted in the urine and feces, in a ratio of 3 to 1.[61] The majority of the excreted products were either metabolites or glucuronide conjugates of the metabolites.

Regardless of the route of administration or the species of animal tested, the trichothecene mycotoxins were rapidly metabolized and excreted in urine and feces. The route of exposure to the toxins and the species can, however, influence the pattern of metabolites that are excreted in the urine. The deacetylated and hydroxylated metabolites appear to be present in most of the species that have been evaluated to date.

A microsomal, nonspecific carboxylesterase [EC 3.1.1.1] from liver selectively hydrolyses the C-4 acetyl group of T-2 toxin to yield HT-2 toxin.[62] In addition to hepatic microsomes, the trichothecene-specific carboxylesterase activity has been detected in brain, kidney, spleen, intestine, white blood cells, and erythrocytes. These findings emphasize the importance of carboxylesterase in detoxifying the trichothecene mycotoxins. A hepatic cytochrome, P-450, is responsible for catalyzing the hydroxylation of the C-3 ' and C-4 ' positions on the isovaleryl side chain of the T-2 and HT-2 toxins.[59] When oxygen is removed from the epoxide group of a trichothecene mycotoxin to yield the carbon–carbon bond, deepoxy metabolites are formed. The deepoxy metabolites are essentially nontoxic.[58] This latter observation indicates that epoxide reduction is a single-step detoxification reaction for trichothecene mycotoxins.

Four hours after swine received intravenous tritium-labeled T-2 toxin, glucuronide conjugates represented 63% of the metabolic residues in urine, and 77% in bile.[63] The formation of glucuronide conjugates generally results in the elimination of toxicological activity of xenobiotics, which in certain species could represent a major route of detoxification of trichothecene mycotoxins. In summary, then, very little of the parent trichothecene mycotoxin is excreted intact. Rather, elimination by detoxification of the toxin is the result of extensive and rapid biotransformation.

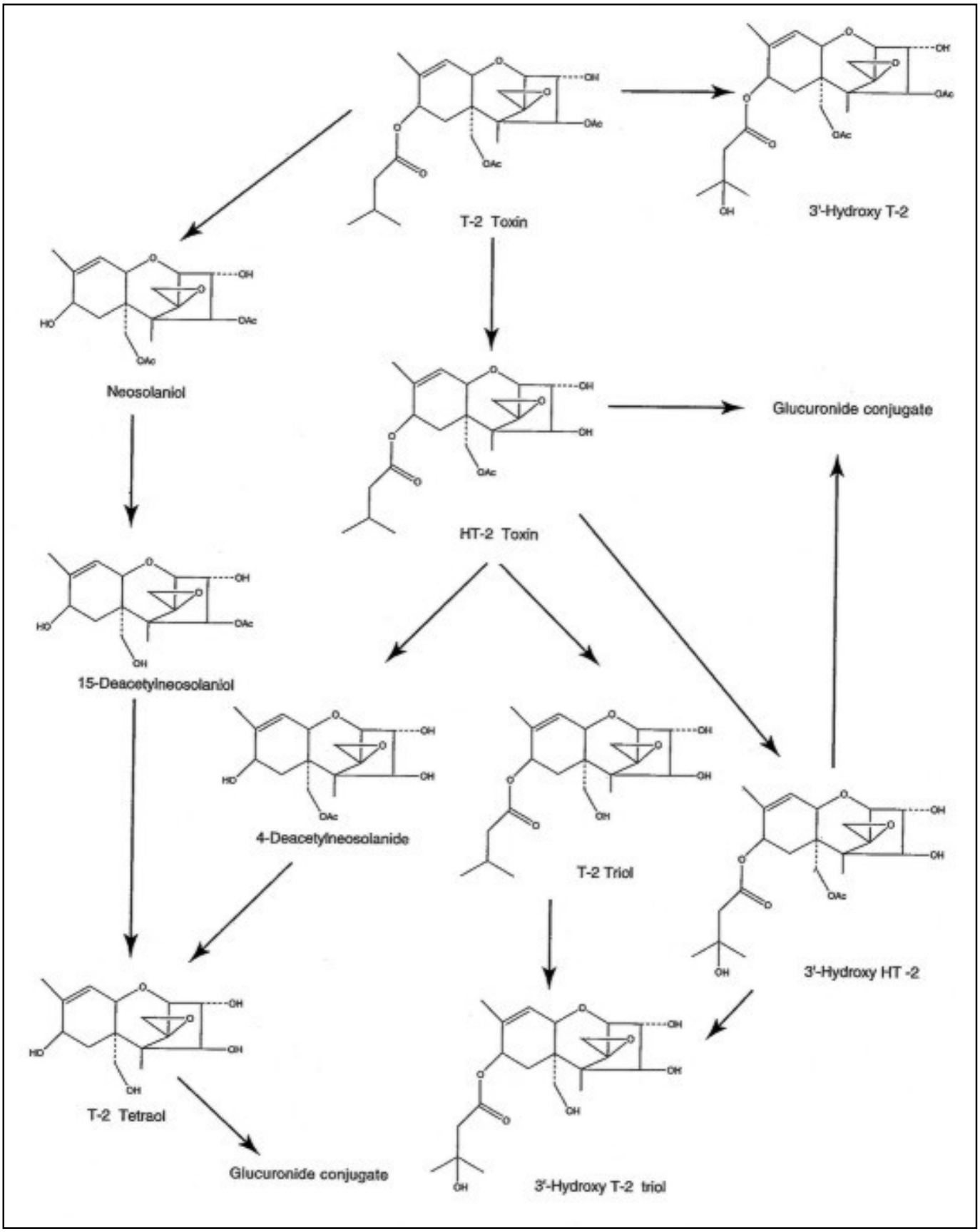


Fig. 34-2. Metabolic pathway of T-2 toxin both in vitro and in vivo.

CLINICAL DISEASE

The degree of illness in an individual exposed to trichothecene mycotoxins could be affected by a number of factors, including the nutritional status of the host, liver damage, intestinal infections, route of toxin administration, and stress.

The pathological effects and clinical signs for many toxic materials can vary with the route and type (acute, single dose vs chronic, subacute doses) of exposure. For the trichothecene mycotoxins, however, a number of the toxic responses are similar, regardless of the route of exposure. As we discussed earlier in this chapter, once they enter the systemic circulation, trichothecene mycotoxins affect rapidly proliferating tissue regardless of the route of exposure.

In contrast, the symptoms and clinical signs of trichothecene intoxication can vary depending on whether the exposure is acute or chronic. Acute exposure to trichothecene mycotoxins used as biological warfare agents is the major concern for military medicine, but for continuity and historical implications, chronic intoxication will also be addressed in this chapter.

Acute Effects

Acute oral, parenteral, dermal, or aerosol exposures to trichothecene mycotoxins produce gastric and intestinal lesions. Hematopoietic and immunosuppressive effects are radiomimetic. Central nervous system toxicity causes anorexia, lassitude, and nausea; suppression of reproductive organ function; and acute vascular effects leading to hypotension and shock. While a number of toxic effects are common to different routes of exposure, route-specific effects have been observed in animal models. Examples of local, route-specific effects include the following:

- dermal exposure: local cutaneous necrosis and inflammation[6];
- oral exposure: lesions to the upper gastrointestinal tract[64]; and
- ocular exposure: corneal injury.[6]

In Southeast Asia during the 1970s, symptoms began within minutes after an exploding munition (air-to-surface rocket, aerial bomb, cylinder) caused a yellow, oily, droplet mist to fall on individuals within 100 m of the explosion site. The falling droplet rain was inhaled, swallowed, and collected on skin and clothing; contaminated the terrain and food and water supply; and caused humans and animals to become acutely ill and to die after a variable period.[7] Massive cutaneous contact was prevalent when the sources of exposure were sprays or coarse mists that were used deliberately to contaminate humans and the environment. Although the suspected trichothecene mycotoxin attacks in Southeast Asia would have involved multiple routes of exposure, we can

postulate that the skin would have been the major site for deposition of a aerosol spray or coarse mist.

Early symptoms and signs included severe nausea, vomiting, burning superficial skin discomfort, lethargy, weakness, dizziness, and loss of coordination. Within minutes to hours, diarrhea—at first watery brown and later grossly bloody—began. During the first 3 to 12 hours, dyspnea, coughing, sore mouth, bleeding gums, epistaxis, hematemesis, abdominal pain, and central chest pain could occur. The exposed cutaneous areas could become red, tender, swollen, painful, or pruritic, in any combination. Small or large vesicles and bullae might form; and petechiae, ecchymoses, and black, leathery areas of necrosis might appear. After death, the necrotic areas might slough easily when the corpse was moved.

Marked anorexia and dehydration were frequent. Dying patients became hypothermic and hypotensive, and developed tachycardia. A bloody ooze from the nares and mouth and an associated hematochezia occurred in severely poisoned individuals. Death could occur within minutes, hours, or days, and was often preceded by tremors, seizures, and coma, in any combination.

The most common symptoms in both Southeast Asia and Afghanistan included vomiting (71%); diarrhea (53%); skin irritation, burning, and itching (44%); rash or blisters (33%); bleeding (52%); and dyspnea (48%).[7,15,27] All of the symptoms listed could be attributed to trichothecene mycotoxin toxicity.

Dermal Exposure

Similar cutaneous irritations have been observed in numerous accidental and experimental settings:

- Individuals who were exposed to hay or hay dust contaminated with trichothecene-producing molds developed severe cutaneous irritations.[38]
- In working up large batches of fungal cultures from trichothecene-producing organisms, laboratory personnel suffered facial inflammation followed by desquamation of the skin and considerable local irritation.[65]
- When trichothecene mycotoxins of relatively low toxicity (crotocin and trichotecin) were applied to the volar surface of human forearm or to the human head, reddening and irritation occurred within a few hours of exposure, and was followed by inflammation or scrabbling that healed in 1 to 2 weeks.[66]
- The hands of two laboratory workers were exposed to crude ethyl acetate extracts containing T-2 toxin (approximately 200 µg/ mL) when the extract accidently got inside their plastic gloves.[66] Even though the workers thoroughly washed their hands with a mild detergent within 2 minutes after contact, they experienced severe cutaneous irritations.

These observations provide evidence that when human skin is exposed in vivo

to small amounts of trichothecene mycotoxins, severe cutaneous irritations develop and can last 1 to 2 weeks after acute exposure.

A number of animal models have been used to assess local and systemic toxicity and lethality from skin exposure to trichothecenes.[6] In a dermal study that used a mouse model, necrosis in the skin was present by 6 hours after dermal application of T-2 toxin, with inflammation observed by 12 hours. The hairless guinea pig is an excellent model to illustrate the local skin lesions produced by a dermal application of T-2 toxin (Figure 34-3). The lesions are easily identified by 24 hours after the exposure, with maximal response at 48 hours. Some small lesions are still present 14 days after exposure to the toxin. From this experimental evidence, we can postulate that dermal exposure to trichothecene mycotoxins played a major role in the clinical illnesses that were seen following the yellow rain attacks.

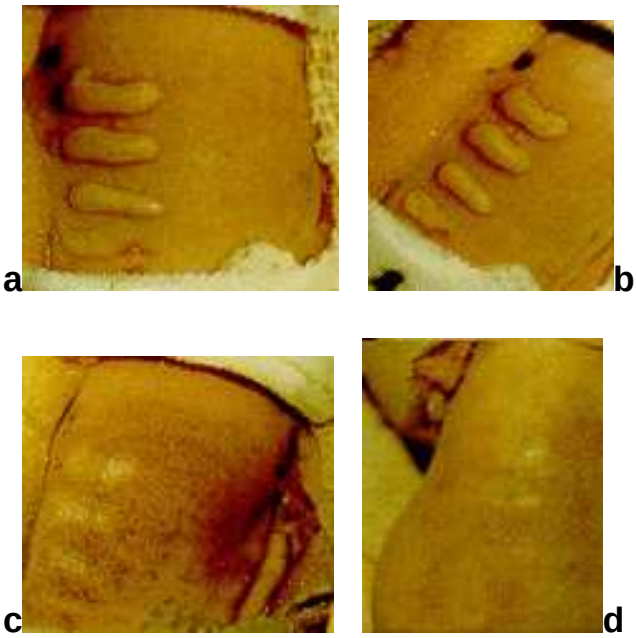


Fig. 34-3. Skin lesions on the back of a hairless guinea pig at **(a)** 1, **(b)** 2, **(c)** 7, and **(d)** 14 days after application of (bottom to top) 25, 50, 100, or 200 ng of T-2 toxin in 2 µL of methanol.

Ocular Exposure

Victims of yellow rain attacks frequently reported tearing, eye pain, conjunctivitis, burning sensations about the eyes, and blurred vision for up to 1 week.[7,16] A Canadian Forces medical team interviewed Khmer Rouge causalities after a chemical/toxin attack at Tuol Chrey, Kampuchea.[27] Soldiers located 100 to 300 m from the artillery impact had onset of symptoms 2 to 5 minutes after exposure; these, likewise, included tearing, burning sensations, and blurred vision that lasted from 8 to 14 days. Analysis of autopsy samples from one of the casualties identified T-2, HT-2, and diacetoxy-scripenol (DAS, also called anguidine) in his tissues. When the culture filtrates containing trichothecenes were instilled into the conjunctival sacs of rabbits, reddening and edema of the conjunctive membrane were observed within 1 or 2 days. Later, the cornea became opaque and developed scars that persisted for as long as 5 months.[67]

From these reports, we conclude that trichothecene mycotoxins can cause severe eye injury that can lead to a marked impairment of vision. This could be a severe incapacitating problem for unprotected military personnel. No systemic toxicity has been documented from the instillation of trichothecene mycotoxins

into the eye of experimental animals, however.

Respiratory Exposure

Victims of yellow rain reported a variety of upper respiratory signs and symptoms.[7,27] The major subdivisions of the respiratory tract that were affected include the nose (itching, pain, rhinorrhea, and epistaxis); the throat (sore/pain, aphonia, and voice change); and the tracheobronchial tree (cough, hemoptysis, dyspnea, and deep chest pain or pressure or both). Agricultural workers who were exposed to hay or hay dust contaminated with trichothecene mycotoxins developed similar signs and symptoms of upper respiratory injury. The descriptions of the yellow rain attacks in Southeast Asia (ie, the droplets, heavy mist, vapor), suggest that the aerosols were larger than 1 to 4 µm—the particle size required for deposition in the alveoli. Thus, respiratory tract exposure from the larger-particle aerosols would involve mycotoxin deposition in the upper respiratory and tracheobronchial region, followed by secondary gastrointestinal tract exposure after clearance from the lungs.

We can postulate that multiple routes of exposure (topical, upper respiratory, and secondary enteral) to trichothecene mycotoxins occurred in victims of the yellow rain attacks. The symptoms of vomiting, diarrhea, melena, abdominal pain, and acute gastroenteritis with hematemesis[7] could be related to ingestion of toxin that was deposited in the upper respiratory tract and tracheobronchial region. Autopsies in the field of victims who died 24 to 48 hours after a yellow rain attack disclosed severe gastroenteritis with bleeding in the lower esophagus, stomach, and duodenum.[27] In humans, many of the acute enteral effects (from either yellow rain or contaminated hay and dust particles) of the trichothecene mycotoxins are probably the result of secondary ingestion of toxins that originally were deposited in the respiratory tract by large-particle aerosol.

Chronic Toxicity

Chronic exposure to subacute doses of trichothecene mycotoxins is not thought to be an effect of biological warfare. This type of exposure, however, was responsible for ATA toxicosis in humans and mycotoxicosis in domestic animals. In addition, chronic toxicity has been iatrogenically induced when repeated subacute doses of a trichothecene mycotoxin were administrated intravenously to cancer patients as a chemotherapy for colon adenocarcinoma.

Alimentary Toxic Aleukia Toxicosis

The clinical course of ATA is divided into four stages. The *first stage* develops immediately or several days after consumption of grain products that are contaminated with trichothecene mycotoxins. Inflammation of the gastric and intestinal mucosa causes vomiting, diarrhea, and abdominal pain. In most cases, excessive salivation, headache, dizziness, weakness, fatigue, and

tachycardia accompany this stage, and fever and sweating may also be present.[36]

The disease progress to the *second stage*—the leukopenic or latent stage—which is characterized by leukopenia, granulopenia, and progressive lymphocytosis. When the ingestion of the toxin-contaminated food is not interrupted or if large doses are consumed, the next stage develops.[36]

The *third stage* is characterized by the appearance of a bright red, or dark cherry-red, petechial rash on the skin of the chest and other areas of the body. At first, the petechiae are localized in small areas, but they then spread and become more numerous.

In the most severe cases, intensive ulceration and gangrenous processes develop in the larynx, leading to aphonia and death by strangulation. At the same time, affected individuals have severe hemorrhagic diathesis of the nasal, oral, gastric, and intestinal mucosa.[36]

As the necrotic lesions heal and the body temperature falls, the *fourth stage*—the recovery stage—begins. During this period, exposed patients are susceptible to various secondary infections, including pneumonia. Convalescence is prolonged and can last for several weeks. Usually, 2 months or more are required for the blood-forming capacity of the bone marrow to return to normal.[36]

Cancer Chemotherapy

The inhibitory effect of trichothecene mycotoxins on rapidly dividing cells was the basis for their evaluation as antitumor chemotherapy drugs during the late 1970s and early 1980s.[68] Phase I and phase II clinical evaluations of DAS (anguidine) in patients with cancer disclosed significant toxicity with intravenous doses 3.0 mg/m ^{2} (0.077 mg/kg) daily for 5 days, particularly in patients with hepatic metastases. The signs and symptoms included nausea, vomiting, diarrhea, burning erythema, confusion, ataxia, chills, fever, hypotension, and hair loss.[69,70] Antitumor activity of the trichothecenes was minimal or absent in the patients treated with DAS. Because of the marked toxicity of the drug, the life-threatening hypotensive effects, and the poor tolerance by patients, the evaluation of trichothecenes as chemotherapeutic drugs was discontinued.

DIAGNOSIS

Battlefield Diagnosis

In the absence of a biological detector or a particular characteristic of the aerosol (such as color or odor), diagnosis of an attack with trichothecene would depend on clinical observations of casualties and identification of the toxins in biological or environmental samples. This would involve a combined effort between the medical and chemical units in the field. The early signs and symptoms of an aerosol exposure to trichothecene mycotoxins would depend on particle size and toxin concentration. For a large-particle aerosol (particles > 10 µm, found in mist, fog, and dust; similar to that used in Southeast Asia), the signs and symptoms would include rhinorrhea, sore throat, blurred vision, vomiting, diarrhea, skin irritation (burning and itching), and dyspnea. Early (0–8 h) signs and symptoms from a deep-respiratory aerosol exposure (from aerosol particles in the 1- to 4-µm range) have not been fully evaluated but could include vomiting, diarrhea, skin irritation, and blurred vision.

Later signs and symptoms (8–24 h) would probably be similar (except for the degree of skin rash and blisters) for both large-particle and deep-respiratory aerosol exposure to trichothecene mycotoxins. They could include continued nausea and vomiting, diarrhea, burning erythema, skin rash and blisters, confusion, ataxia, chills, fever, hypotension, and bleeding.

Nonspecific changes in serum chemistry and hematology occurred in monkeys exposed to an acute dose of T-2 toxin. Alterations in serum chemistry included elevations in serum creatinine, serum enzymes (especially creatine kinase), potassium, phosphorous, and serum amino acids; and, due to decreased coagulation factors, elevations in prothrombin time and partial thromboplastin time. An initial rise in the absolute number of neutrophils and lymphocytes may occur within hours, followed by a decrease in lymphocyte counts by 48 hours. Survival beyond several days may be associated with a fall in all blood cellular elements.[6] Although it is likely that these acute changes will also be seen in humans, careful clinical observations of human victims of acute trichothecene mycotoxicosis have not been reported to date. In patients with chronic toxicity (ie, ALA) resulting from repeated ingestion of contaminated bread, pancytopenia is an important part of the clinical picture.[36]

In the yellow rain attacks in Southeast Asia, diagnosis of the causative agent was difficult and involved ruling out the presence of conventional chemical warfare agents. Contamination of the environment and clothing by nerve and blistering agents would be absent, and these were, in fact, not detectable in such samples from Southeast Asia. Sarin, soman, or other nerve agents could be missed unless thickened soman or VX was used.

The following events should suggest to medical officers that a biological warfare attack with trichothecene mycotoxins has occurred:

- clinical findings that match the symptoms listed above;

- high attack and fatality rates;
- all types of dead animals; and
- onset of symptoms after a yellow rain or red, green, or white smoke or vapor attack.

At present, we do not have a fieldable identification kit for any of the trichothecene mycotoxins. Several commercial immunoassay kits are marketed for the detection of trichothecene mycotoxins (T-2 toxin, deoxynivalenol, and their metabolites) in grain extracts or culture filtrates of Fusarium species.[71,72] These kits have not been evaluated against biomedical samples that contain typical concentrations of the mycotoxins, however. Screening tests for presumptive identification of trichothecene mycotoxins in the biomedical samples would probably involve bioassays, thin-layer chromatography, or immunological assays, in any combination. At a national laboratory, confirmatory methodology would involve the use of various combinations of gas chromatography, high-performance liquid chromatography, mass spectrometry, and nuclear magnetic resonance spectrometry.

In areas that have experienced a yellow rain attack, environmental assays have been in the range of 1 to 150 parts per million (ppm) and blood samples in the range of 1 to 296 parts per billion (ppb).[8–10,16,22] In the laboratory, at 10 and 50 minutes after an intramuscular exposure to 0.4 mg/kg of T-2 toxin in the dog, plasma concentrations of T-2 toxin were 150 and 25 ppb, and for HT-2 toxin were 50 and 75 ppb, respectively.[60] Thus, any screening procedure for trichothecene mycotoxins in biomedical samples must have detection limits of 1 to 100 ppb. Most of the analytical procedures require extraction and cleanup treatment to remove interfering substances.[73]

Screening tests for the trichothecene mycotoxins are generally simple and rapid but, with the exception of the immunochemical methods, are nonspecific. A number of bioassay systems have been used for the identification of trichothecene mycotoxins.[73] Although most of these systems are very simple, they are not specific, their sensitivity is generally relatively low compared to other methods, and they require that the laboratory maintain vertebrates, invertebrates, plants, or cell cultures. Thin-layer chromatography (TLC) is one of the simplest and earliest analytical methods developed for mycotoxin analysis. Detection limits for trichothecene mycotoxins by TLC is 0.2 to 5 ppm (0.2 to 5 µg/ mL). Therefore, extracts from biomedical samples would have to be concentrated 10- to 1,000-fold to screen for trichothecene mycotoxins.

To overcome the difficulties encountered with the bioassays and TLC methods, immunoassays using specific polyclonal and monoclonal antibodies have been developed for most of the major trichothecene mycotoxins and their metabolites.[73] These antibodies have been used to produce simple, sensitive, and specific radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs) for the mycotoxins. In the presence of the sample matrix, the lower detection limits for identification of trichothecene mycotoxins by RIA is about 2 to 5 ppb[73] and by ELISA, 1 ppb.[74] We conclude that immunoassays

are useful tools for screening biomedical samples for evidence of a biological warfare attack with trichothecene mycotoxins.

Confirmatory Procedures

Gas-liquid chromatography (GLC) is one of the most commonly used methods for the identification of the trichothecene mycotoxins in both agricultural products and biomedical samples.[75] Before GLC analysis, the polar groups in mycotoxin molecules must first be converted to their esters or ethers. Extensive treatment to clean up the sample is required before derivatization and subsequent analysis can be performed. By the most sensitive procedures, the detection limit for trichothecene mycotoxins is 10 ppb. If the analysis is on a sample that contains an unknown toxic material, such as those from the yellow rain attacks, then the GLC method can only provide presumptive evidence of a trichothecene mycotoxin exposure. Confirmation will require the identification with more definitive physicochemical procedures.

Mass spectrometry (MS) is the physicochemical method of choice for characterizing, identifying, and confirming the presence of trichothecene mycotoxins.[76,77] Picogram quantities of trichothecene mycotoxins are readily detectable by MS methods. In some cases, extensive cleanup steps are unnecessary.

The combination of GLC and MS techniques (GLC–MS) has proven to be a more-specific method for identifying mycotoxins than is GLC alone.[76,77] As a result, the GLC–MS method has become the standard for identifying trichothecene mycotoxins in agricultural products as well as in biomedical samples. As little as 1 ppb of T-2 toxin can be identified without extensive cleanup.[76] One major drawback of this methodology is the time-consuming derivatization step that trichothecene mycotoxin identification by GLC–MS requires. A high-performance liquid chromatography–mass spectrometry (HPLC–MS) procedure was described in 1991 and provides a specific and reliable method for the identification of trichothecene mycotoxins without derivatization.[78] The HPLC–MS procedure achieves sensitivity at the 0.1-ppb level. This technology will require further evaluation and development, but it appears to be a promising approach for the rapid confirmation of trichothecene mycotoxins in a biomedical sample.

MEDICAL MANAGEMENT

Individual and Unit

The immediate use of protective clothing and mask at the first sign of a yellow rain–like attack should protect an individual from the lethal effects of this mycotoxin. The mask can be applied in less than 9 seconds and can be worn at first sighting of an incoming rocket or enemy aircraft. Contaminated battle dress uniforms (BDUs) should be removed before protective clothing is donned. Since the area covered with agent is likely to be small, another helpful tactic is to leave the area after taking samples to document the attack. Vulnerability is increased by lack of protective clothing, mask, or training (as was demonstrated in Laos) or by a surprise biological warfare attack (such as a night or an undetected attack). A lightweight face mask, outfitted with filters that block the penetration of aerosol particles 3 to 4 µm or larger, should provide respiratory protection against yellow rain. Only 1% or 2% of aerosolized T-2 toxin penetrated nuclear, biological, chemical protective covers (NBC–PC).[79] Regular BDUs would offer some protection, but the degree would be functions of the age and condition of the fabric, and the type of environmental conditions.

Two topical skin protectants (TPS1 and TSP2) are in advanced development for protection against chemical warfare agents. When applied to the skin of rabbits 60 minutes before exposure to 50 µg of T-2 toxin, both topical skin protectants completely protected the rabbits from the dermal irritating effects of this mycotoxin for at least 6 hours.[80]

As soon as individuals or units suspect that they have been exposed to a mycotoxin attack, they should remove their BDUs, wash their contaminated skin with soap and water, and then rinse with water. Washing the contaminated area of the skin within 4 to 6 hours after exposure to T-2 toxin removed 80% to 98% of the toxin and prevented der-mal lesions and death in experimental animals.[25] Contaminated BDUs as well as wash waste from personnel decontamination should be exposed to household bleach (5% sodium hypochlorite) for 6 hours or more to inactivate any residue mycotoxin.

Two skin decontamination kits, the M258A1 and the M238A1, have been designed for the removal and detoxification of chemical warfare agents. The M258A1 kit is the currently fielded standard. When evaluated against trichothecene mycotoxins, however, the M238A1 kit effectively removed T-2 toxin from the skin of rats but did not detoxify this biological warfare agent.[81] Several of the components of the M258A1 kit are themselves highly toxic, caustic compounds that caused dermal irritation and lethality in rats and rabbits.[82]

A second-generation skin decontamination kit, the XM291, has been developed, and contains an XE-555 resin material as the active component. This skin decontamination kit is efficacious against most chemical warfare agents and presents no serious human factor or human safety problems. The XE-556 resin, a similar but different formulation, was effective in the physical

removal of T-2 toxin from the skin of rabbits and guinea pigs.[83] The foregoing observations suggest that the skin decontamination kits that were designed specifically for removal and detoxification of chemical warfare agents could also afford a significant degree of protection through the physical removal of mycotoxins from the skin of exposed individuals.

Specific or Supportive Therapy

No specific therapy for trichothecene-induced mycotoxicosis is known or is presently under experimental evaluation. Several therapeutic approaches have been evaluated in animal models. It is perhaps significant, however, that although experimental procedures for treatment of systemic exposure have been successful in reducing mortality in animal models, they have not been tested in primates. Thus, these treatments are not available for field use for humans exposed to trichothecene mycotoxins.

Individuals exposed to a yellow rain–like attack should be treated with standardized clinical toxicology and emergency medicine practices for ingestion of toxic compounds. After an aerosol exposure to a yellow rain–like attack, mycotoxins will be trapped in the nose, throat, and upper respiratory tract. The particles will be returned by ciliary action to be swallowed, resulting in a significant oral exposure. Superactive charcoal has a very high maximal binding capacity (0.48 mg of T-2 toxin per 1 mg of charcoal), and treatment either immediately or 1 hour after oral or parenteral exposure to T-2 toxin significantly improves the survival of mice.[84] Superactivated charcoal with magnesium sulfate is stocked in the chemical and biological warfare kits of U.S. Army field hospitals.

Symptomatic measures for the treatment of exposure to trichothecene mycotoxins are modeled after the care of casualties of mustard poisoning.[85] Irrigation of the eyes with large volumes of isotonic saline may assist in the mechanical removal of trichothecene mycotoxins, but would have limited useful therapeutic effects. After the skin has been decontaminated, some erythema may appear, accompanied by burning and itching. Most casualties whose skin has been treated with soap and water within 12 hours of exposure will have mild dermal effects; these should be relieved by calamine and other lotion or cream, such as 0.25% camphor and methanol.

Limited data are available on the respiratory effects of inhaled trichothecene mycotoxins, although acute pulmonary edema is one of the serious, often lethal consequences of a yellow rain attack.[16,27] One of the major symptoms following the yellow rain attacks was an upper respiratory irritation (sore throat, hoarseness, nonproductive cough),[7,16,27] which can be relieved by steam inhalation, codeine, or another substance to suppress the cough, and other simple measures.[85] A casualty who develops severe respiratory symptoms should be under the care of a physician skilled in respiratory care.

The early use of high doses of systemic glucocorticosteriods increases survival time by decreasing the primary injury and the shocklike state that

follows exposure to trichothecene mycotoxins.[86] A selective platelet activating factor antagonist, BN 52021, can prolong the survival of rats exposed to a lethal intravenous dose of T-2 toxin.[87] This finding suggests that platelet activating factor is an important mediator of T-2 toxicosis. Dosing before and after the exposure with diphenhydramine (an antihistaminic agent) or naloxone (an opioid antagonist) prolonged the survival times of mice exposed subcutaneously or topically with lethal doses of T-2 toxin.[88]

We can postulate that a number of bioregulators are the mediators of the shocklike state of trichothecene mycotoxicosis. Methylthiazolidine-4-carboxylate increased hepatic glutathione content and enhanced the survival of mice after an acute intraperitoneal exposure to T-2 toxin.[89] The protective effects of this drug may be the result of increased detoxification and excretion of the glucuronide conjugate of T-2 toxin. A general therapeutic protocol that included combinations of metoclopramide, activated charcoal, magnesium sulfate, dexamethasone, sodium phosphate (which had very little effect), sodium bicarbonate, and normal saline as the therapeutic agents was evaluated in swine given an intravenous LD_{50} dose of T-2 toxin.[90] All treatment groups showed improved survival times when compared with the nontreated T-2 controls.

Prophylaxis

The mycotoxins are low-molecular-weight compounds that must be conjugated to a carrier protein to produce an effective antigen.[73] When T-2 toxin is conjugated to a protein, it develops relatively low antibody titers and is still a marked skin irritant.[91] This would preclude mycotoxins' use as immunogens in the production of protective immunity. To circumvent such problems, a deoxyverrucarol (DOVE)–protein conjugate was used to immunize rabbits.[92] Antibody titers to DOVE developed rapidly after immunization, but they were highly specific for DOVE rather than a common trichothecene backbone.[92]

Another approach was to develop antibody-based vaccines (anti-idiotype) against T-2 toxin. Protective monoclonal antitoxin antibodies were first generated and then used to induce specific monoclonal anti-idiotype antibodies. When mice were immunized with specific monoclonal anti-idiotype antibodies, they developed neutralizing antibodies and were protected against challenge with a lethal dose of T-2 toxin.[93]

Thus, it would be feasible to develop a despeciated monoclonal anti-idiotype antibody that could be a vaccine candidate against T-2 toxin. Several monoclonal antibodies against T-2 toxin will protect against the T-2–induced cytotoxicity in various cell lines.[94,95] When a monoclonal antibody against T-2 toxin (15H6) was given to rats (250 mg/kg) 30 minutes before or 15 minutes after a lethal dose of mycotoxin, it conferred 100% survival.[94] Thus, monoclonal antibodies do have some prophylactic and therapeutic value against T-2 toxicosis, but very large quantities are required for protection.

Prophylactic induction of enzymes involved in the conjugation of xenobiotics reduced or prevented the acute toxic effects of T-2 toxin in the rat, while inhibition of these enzymes resulted in a higher toxicity for this trichothecene.[96] Pretreatment with flavonoids,[97] ascorbic acid,[98] vitamin E,[99] selenium,[100] or chemoprotective compounds such as emetine[101] that block trichothecene–cell association all reduce acute toxicity of these mycotoxins. However, none of these chemoprotective treatments have undergone extensive efficacy studies to evaluate their ability to protect against an aerosol or dermal exposure to trichothecene mycotoxins.

SUMMARY

Trichothecene mycotoxins are noted for their marked stability under different environmental conditions. On a weight-for-weight basis, they are less toxic than other toxins such as ricin, botulinum, and staphylococcal enterotoxin B, but trichothecene mycotoxins are proven lethal agents in warfare. Symptoms include vomiting, pain, weakness, dizziness, ataxia, anorexia, diarrhea, bleeding, skin redness, blistering, and gangrene, as well as shock and rapid death. Sensitive immunoassays and chemical procedures are available for the identification of trichothecene mycotoxins in biological samples, but no detection kits have been fielded.

Prevention of exposure is the only current defense, with a protective mask and clothing worn when under attack. Previous successful lethal attacks have always occurred against unprotected civilians and soldiers. Skin decontamination with water and soap can be used effectively up to 6 hours after exposure. Experimental treatments for systemic toxicity are being investigated, but no therapy is available for humans.

REFERENCES

1. Ciegler A. Mycotoxins: Occurrence, chemistry, biological activity. *Lloydia*. 1975;38(1):21–35.
2. Ciegler A, Bennett JW. Mycotoxins and mycotoxicoses. *Bioscience*. 1980;30(8):512–515.
3. Moss MO. Mycotoxins of *Aspergillus* and other filamentous fungi. *J Appl Bacteriol.* 1989;67(symposium suppl):69S–81S.
4. Ueno Y. Trichothecene mycotoxins: Mycology, chemistry, and toxicology. *Adv Nutr Res*. 1989;3:301–353.
5. Godtfredsen WO, Grove JF, Tamm Ch. Trichothecenes. *Hev Chim Acta*. 1967;50:1666–1668.
6. Wannemacher RW Jr, Bunner DL, Neufeld HA. Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: Smith JE, Henderson RS, eds. *Mycotoxins and Animal Foods*. Boca Raton, Fla: CRC Press; 1991: 499–552.

7. Haig AM Jr. *Chemical Warfare in Southeast Asia and Afghanistan*. Washington, DC: US Government Printing Office; March 22, 1982. Report to the Congress.

8. Mirocha CJ. Hazards of scientific investigation: Analysis of samples implicated in biological warfare. *Journal of Toxicology-Toxin Reviews*. 1982;1(1):199–203.

9. Rosen RT, Rosen JD. Presence of four *Fusarium* mycotoxins and synthetic material in “yellow rain”: Evidence for the use of chemical weapons in Laos. *Biomed Mass Spectrom*. 1982;9(10):443–450.

10. Mirocha CJ, Pawlosky RA, Chatterjee K, Watson S, Hayes W. Analysis for *Fusarium* toxins in various samples implicated in biological warfare in Southeast Asia. *J Assoc Off Anal Chem*. 1983;66(6):1485–1499.

11. Greenhalgh R, Miller JD, Neish GA, Schiefer HB. Toxigenic potential of some *Fusarium* isolates from Southeast Asia. *Appl Environ Microbiol*. 1985;50(2):550–552.

12. Ricaud D. Les Recherche de Défense Contre les Armés Biologique et Chimiques. Paris, France: École Polytechique; 1983. ISBN 2–7170–0738–5.

13. Ember LR, Sorenson WG, Lewis DM. Charges of toxic arms use by Iraq escalate. *Chemical and Engineering News*. 1984;62(12):16–18.

14. Seagrave S. *Yellow Rain: A Journey Through the Terror of Chemical Warfare.* New York, NY: M Evans; 1981.

15. Ember LR. Yellow rain. *Chemical and Engineering News*. 1984;62(2):8–34.

16. Watson SA, Mirocha CJ, Hayes AW. Analysis for trichothecenes in samples from Southeast Asia associated with “Yellow Rain.” *Fundam Appl Toxicol*. 1984;4(5):700–717.

17. Marshall E. Yellow rain evidence slowly whittled away. *Science*. 1986;233(4759):18–19.

18. Marshall E. Bugs in the yellow rain theory. *Science*. 1983;220(4604):1356–1358.

19. Nowicke JW, Meselson M. Yellow rain—A palynological analysis. *Nature*. 1984;309(5965):205–207.

20. Seeley TD, Nowicke JW, Meselson M, Guillemin J, Akratanakul P. Yellow rain. *Sci Am*. 1985;253(3):128–137.

21. Dashek WV, Mayfield JE, Llewellyn GC, O’Rear CE, Bata A. Trichothecenes and yellow rain: Possible biological warfare agents. *Bioessays*. 1986;4(1):27–30.

22. Yellow rain report. *NBC Defense Technology International*. 1986;1(2):11–12.

23. Marshall E. The apology of yellow rain. *Science*. 1983;221(4608):242.

24. Yellow rain: British analyses find no toxin. *Nature*. 1986;321(6069):459. News.

25. Wannemacher RW, Bunner DL, Pace JG, Neufeld HA, Brennecke LH, Dinterman RE. Dermal toxicity of T-2 toxin in guinea pigs, rats, and cynomolgus monkeys. In: Lacey J, ed. *Trichothecenes and Other Mycotoxins*. Chichester, England: John Wiley

& Sons Ltd; 1985: 423–432.

26. Bunner DL, Upshall DG, Bhatti AR. Toxicology data on T-2 toxin. In: Report of Focus Officers Meeting on Mycotoxin Toxicity, September 23–24, 1985. Suffield, Alta, Canada: Defense Research Establishment at Suffield; 1985.

27. Stahl CJ, Green CC, Farnum JB. The incident at Tuol Chrey: Pathological and toxicological examination of a casualty after chemical attack. *J Forensic Sci*. 1985;30(2):317–337.

28. Creasia DA, Thurman JD, Wannemacher RW Jr, Bunner DL. Acute inhalation toxicity of T-2 mycotoxin in the rat and guinea pig. *Fundam Appl Toxicol*. 1990;14(1):54–59.

29. Marrs TC, Edginton JA, Price PN, Upshall DG. Acute toxicity of T2 mycotoxin to the guinea-pig by inhalation and subcutaneous routes. *Br J Exp Path*. 1986;67(2):259–268.

30. Creasia DA, Thurman JD, Jones LJ, et al. Acute inhalation toxicity of T-2 mycotoxin in mice. *Fundam Appl Toxicol*. 1987;8(2):230–235.

31. US Department of Defense. *Potential Military Chemical/Biological Agents and Compounds*. Washington, DC: Headquarters, Departments of the Army, Navy, and Air Force; 1990. Field Manual 3-9, Air Force Regulation 355-7, NAVFAC P-467.

32. Burmeister HR. T-2 toxin production by *Fusarium tricinctum* on solid substrate. *Appl Microbiol*. 1971;21(4):739–742.

33. Miller JD, Taylor A, Greenhalgh R. Production of deoxynivalenol and related compounds in liquid culture by *Fusarium graminearum. Can J Microbiol*. 1983;29(9):1171–1178.

34. Spertzel RO, Wannemacher RW Jr, Patrick WC, Linden CD, Franz DR. *Technical Ramifications of Inclusion of Toxins in the Chemical Weapons Convention (CWC)*. Alexandria, Va: Defense Nuclear Agency; 1993. DNA Technical Report 92–116.

35. Committee on Protection Against Mycotoxins, Board on Toxicology and Environmental Health Hazards, Commission on Life Sciences, National Research Council. *Protection Against Trichothecene Mycotoxins*. Washington, DC: National Academy Press; 1983.

36. Joffe AZ. Alimentary toxic aleukia. In: Kadis S, Ciegler A, Ajl SJ, eds. *Microbiol Toxins*. Vol 7. In: *Algal and Fungal Toxins*. New York, NY: Academic Press; 1971: 139–189.

37. Yagen B, Joffe AZ, Horn P, Mor N, Lutsky II. Toxins from a strain involved in ATA. In: Rodericks JV, Hesseltine CW, Mehlman MA, eds. *Mycotoxins in Human and Animal Health*. Park Forest South, Ill: Pathotox Publishers; 1977: 329–336.

38. Forgacs J. Stachybotryotoxicosis. In: Kadis S, Ciegler A, Ajl SJ, eds. *Microbial Toxins*. Vol 8. New York, NY: Academic Press; 1972: 95–128.

39. Hintikka E-L. Stachybotryotoxicosis as a veterinary problem. In: Rodricks JV, Hesseltine CW, Mehlman MA, eds. *Mycotoxins in Human Health*. Park Forest South, Ill: Pathotox Publishers; 1977: 277–284.

40. Eppley RM. Chemistry of stachybotryotoxicosis. In: Rodericks JV, Hesseltine CW,

Mehlman MA, eds. *Mycotoxins in Human and Animal Health*. Park Forest South, Ill: Pathotox Publishers; 1977: 285–293.

41. Croft WA, Jarvis BB, Yatawara CS. Airborne outbreak of trichothecene toxicosis. *Atmos Environ*. 1986;20(3):549–552.

42. Jarvis BB. Macrocyclic trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 361–421.

43. Cole RJ, Cox RH. The trichothecenes. In: Cole RJ, Cox RH. *Handbook of Toxic Fungal Metabolites*. New York, NY: Academic Press; 1981: 152–263.

44. Wannemacher RW Jr, Bunner DL, Dinterman RE. Inactivation of low molecular weight agents of biological origin. In: Proceedings for the Symposium on Agents of Biological Origins. Aberdeen Proving Ground, Md: US Army Chemical Research Development and Engineering Center; 1989.

45. Sharma RP, Kim Y-W. Trichothecenes. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 339–359.

46. Thompson WL, Wannemacher RW Jr. Detection and quantitation of T-2 mycotoxin with a simplified protein synthesis inhibition assay. *Appl Environ Microbiol*. 1984;48(6):1176–1180.

47. McLaughlin CS, Vaughan MH, Campbell IM, Wei CM, Stafford ME, Hansen BS. Inhibition of protein synthesis by trichothecenes. In: Rodericks JV, Hesseltine CW, Mehlman MA, eds. *Mycotoxins in Human and Animal Health*. Park Forest South, Ill: Pathotox Publishers; 1977: 263–275.

48. Yoshizawa T, Morooka N. Trichothecenes from mold infested cereals in Japan. In: Rodericks JV, Hesseltine CW, Mehlman MA, eds. *Mycotoxins in Human and Animal Health*. Park Forest South, Ill: Pathotox Publishers; 1977: 309–321.

49. Thompson WL, Wannemacher RW Jr. In vivo effects of T-2 mycotoxin on synthesis of proteins and DNA in rat tissues. *Toxicol Appl Pharmacol*. 1990;105(3):482–491.

50. Busby WF Jr, Wogan GN. Trichothecenes. In: Shank RC, ed. *Mycotoxins and N-Nitroso Compounds: Environmental Risks*. Vol 2. Boca Raton, Fla: CRC Press; 1981: 29–41.

51. Middlebrook JL, Leatherman DL. Specific association of T-2 toxin with mammalian cells. *Biochem Pharmacol*. 1989;38(18):3093–3102.

52. Bunner DL, Morris ER. Alteration of multiple cell membrane functions in L–6 myoblasts by T-2 toxin: An important mechanism of action. *Toxicol Appl Pharmacol*. 1988;92(1):113–121.

53. Suneja SK, Wagle DS, Ram GC. Effect of oral administration of T-2 toxin on glutathione shuttle enzymes, microsomal reductase and lipid peroxidation in rat liver. *Toxicon*. 1989;27(9):995–1001.

54. Pace JG, Watts MR, Canterbury WJ. T-2 mycotoxin inhibits mitochondrial protein synthesis. *Toxicon*. 1988; 26(1):77–85.

55. Matsumoto H, Ito T, Ueno Y. Toxicological approaches to the metabolites of fusaria, XII: Fate and distribution of T-2 toxin in mice. *Japan Journal of Experimental*

Medicine. 1978;48(5):393–399.

56. Kemppainen BW, Riley RT. Penetration of [^{3}H]T-2 toxin through excised human and guinea-pig skin during exposure to [^{3}H]T-2 toxin adsorbed to corn dust. *Food Chem Toxicol*. 1984;22(11):893–896.

57. Westlake K, Mackie RI, Dutton MF. T-2 toxin metabolism by ruminal bacteria and its effect on their growth. *Appl Environ Microbiol*. 1987;53(3):587–592.

58. Swanson SP, Helaszek C, Buck WB, Rood HDJ, Haschek WM. The role of intestinal microflora in the metabolism of trichothecene mycotoxins. *Food Chem Toxicol*. 1988;26(10):823–830.

59. Yoshizawa T, Sakamoto T, Okamkoto K. In vitro formation of 3 ' -hydroxy T-2 and 3 ' -hydroxy HT-2 toxins from T-2 toxin by liver homogenates from mice and monkeys. *Appl Environ Microbiol*. 1984;47(1):130–134.

60. Yagen B, Bialer M. Metabolism and pharmacokinetics of T-2 toxin and related trichothecenes. *Drug Metab Rev*. 1993;25(3):281–323.

61. Wannemacher RW Jr, Pace JG. Medical defense against biological warfare: Exploratory immunotherapy studies on toxins of potential BW threat. In: *US Army Medical Research Institute of Infectious Diseases Annual Report 1987*. Fort Detrick, Frederick, Md: USAMRIID; 1987: 129–135.

62. Johnsen H, Odden E, Lie O, Johnsen BA, Fonnum F. Metabolism of T-2 toxin by rat liver carboxylesterase. *Biochem Pharmacol*. 1986;35(9):1469–1473.

63. Corley RA, Swanson SP, Buck WB. Glucuronide conjugates of T-2 toxin and metabolites in swine bile and urine. *J Agric Food Chem*. 1985;33(6):1085–1089.

64. Hoerr FJ, Carlton WW, Tuite J, Vesonder RF, Rohwedder WK, Szigett G. Experimental trichothecene mycotoxicosis produced in broiler chickens by *Fusarium sporotrichiella var sporotrichioides. Avian Pathology*. 1982;11(3):385–405.

65. Bamburg JR, Marasas WFO, Riggs NV, Smalley EB, Strong FM. Toxic spiroepoxy compounds from fusaria and other hyphomycetes. *Biotechnol Bioeng*. 1968;10(4):445–455.

66. Bamburg JR, Strong FM. 12,13-Epoxytrichothecenes. In: Kadis S, Ciegler A, Ajl SJ, eds. *Microbial Toxins*. Vol 7. In: *Algal and Fungal Toxins*. New York, NY: Academic Press; 1971: 207–292.

67. Mortimer PH, Campbell J, Di Menna ME, White EP. Experimental myrotheciotoxicosis and poisoning in ruminants by verrucarin A and roridin A. *Res Vet Sci*. 1971;12(6):508–515.

68. Claridge CA, Schmitz H, Bradner WT. Antitumor activity of some microbial and chemical transformation products of anguidine (4,15-diacetoxyscirpene–3-ol). *Cancer Chemother Pharmacol*. 1979;2(3):181–182.

69. Goodwin W, Hass CD, Fabian C, Heller-Bettinger I, Hoogstraten B. Phase I evaluation of anguidine (diacetoxyscirpenol, NSC–141537). *Cancer*. 1978;4(1):23–26.

70. Murphy WK, Burgress MA, Valdivieso M, Livingston RB, Bodey GP, Freireich EJ.

Phase I clinical evaluation of anguidine. *Cancer Treat Rep*. 1978;62(10):1497–1502.

71. Chiba J, Kawamura O, Kajii H, Ohtani K, Nagayama S, Ueno Y. A sensitive enzyme-linked immunosorbent assay for detection of T-2 toxin with monoclonal antibodies. *Food Addit Contam*. 1988;5(4):629–639.

72. Hart LP, Pestka JJ, Gendloff EH. Method and test kit for detecting a trichothecene using novel monoclonal antibodies. US patent 4772551, September 20 1988. *Off Gaz US Pat Trademark Off Pat*. 1988;1094:1518.

73. Chu FS. Detection and determination of mycotoxins. In: Sharma RP, Salunkhe DK, eds. *Mycotoxins and Phytoalexins*. Boca Raton, Fla: CRC Press; 1991: 33–79.

74. Fan TSL, Zhang GS, Chu FS. An indirect enzyme-linked immunosorbent assay for T-2 toxin in biological fluids. *Journal of Food Protection*. 1984;47(12):964–967.

75. Romer TR. Chromatographic techniques for mycotoxins. In: Lawrence JF, ed. *Food Constituents and Food Residues: Their Chromatographic Determination*. New York, NY: Marcel Dekker; 1984: 393–415.

76. Mirocha CJ, Panthre SV, Pawlosky RJ, Hewetson DW. Mass spectra of selected trichothecenes. In: Cole RJ, ed. *Modern Methods in the Analysis and Structure Elucidation of Mycotoxins*. New York, NY: Academic Press; 1986: 353–392.

77. Vesonder RF, Rohwedder WK. Gas chromatographic-mass spectrometric analysis of mycotoxins. In: Cole RJ, ed. *Modern Methods in the Analysis and Structure Elucidation of Mycotoxins*. New York, NY: Academic Press; 1986: 335–352.

78. Kostiainen R, Matsuura K, Nojima K. Identification of trichothecenes by frit-fast atom bombardment liquid chromatography–high-resolution mass spectrometry. *J Chromatogr*. 1991;538(12):323–330.

79. Lowe RC, Roberts CE, Martin DD. *International Material Evaluation (IME) of Nuclear, Biological, Chemical Protective Covers (NBC-PC), Ultra-Ply (Japan)*. Final Report, Phase II. Dugway, Utah: US Army Dugway Proving Ground. Memorandum to US Army Material Command, Chemical Research and Development Center, 13 April 1989. US Army Test and Evaluation Command Project 8-ES-825-PCS-004.

80. Wannemacher RW Jr. *Evaluation of Two Topical Skin Protectants (TSP1 & TSP2) in the Rabbit Model*. Fort Detrick, Frederick, Md: US Army Medical Research Institute of Infectious Disease. Memorandum to US Army Medical Material Development Activity, 19 July 1994.

81. Wannemacher RW Jr, Bunner DL. *Evaluation of the Ability of Various Agents to Decontaminate Skin of Rats Exposed to T-2 Toxin*. Fort Detrick, Frederick, Md: US Army Medical Research Institute of Infectious Disease. Memorandum to US Army Armament, Munitions, and Chemical Command, Chemical Research and Development Command, 10 May 1983.

82. Jederberg WW, Fruin JT. *Primary Dermal Irritation Potential of Components of the M256A–1 Decontamination Kit (Study 8)*. San Francisco, Calif: Letterman Army Institute of Research; 1981. LAIR Technical Report 82–27TN.

83. Wannemacher RW Jr, Bunner DL. *Screening AMBERGARD XE–556 Resin Blend as a Candidate Decontaminating Material for Removing T-2 Mycotoxin From Exposed*

Skin of Guinea Pigs and Rabbits. Fort Detrick, Frederick, Md: US Army Medical Research Institute of Infectious Diseases. Memorandum to US Army Medical Material Development Activity, 10 January 1987.

84. Fricke RF, Jorge J. Assessment of efficacy of activated charcoal for treatment of acute T-2 toxin poisoning. *J Toxicol Clin Toxicol*. 1990;28(4):421–431.

85. Sidell FR, Hurst CG. Clinical considerations in mustard poisoning. In: Somani SM, ed. *Chemical Warfare Agents*. New York, NY: Academic Press; 1992: 51–66.

86. Shohami E, Wisotsky B, Kempski O, Feuerstein G. Therapeutic effect of dexamethasone in T-2 toxicosis. *Pharmacol Res*. 1987;4(6):527–530.

87. Feuerstein G, Leader P, Siren AL, Braquet P. Protective effect of a PAF-acether antagonist BN–52021 in trichothecene toxicosis. *Toxicol Lett*. 1987;38(3):271–274.

88. Ryu J, Shiraki N, Ueno Y. Effects of drugs and metabolic inhibitors on the acute toxicity of T-2 toxin in mice. *Toxicon*. 1987;25(7):743–750.

89. Fricke RF, Jorge J. Methylthiazolidine-4-carboxylate for treatment of acute T-2 toxin exposure. *J Appl Toxicol*. 1991;11(2):135–140.

90. Poppenga RH, Lundeen GR, Beasley VR, Buck WB. The assessment of a general therapeutic protocol for the treatment of acute T-2 toxicosis in swine. *Vet Hum Toxicol*. 1987;29(3):237–239.

91. Chu FS. Immunoassays for mycotoxins. In: Cole RJ, ed. *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*. New York, NY: Academic Press; 1986: 207–237.

92. Chu FS, Zhang GS, Williams MD, Jarvis BB. Production and characterization of antibody against deoxy-verrucarol. *Appl Environ Microbiol*. 1984;48(4):781–784.

93. Chanh TC, Siwak EB, Hewetson JF. Anti-idiotype–based vaccines against biological toxins. *Toxicol Appl Pharmacol*. 1991;108(2):183–193.

94. Feuerstein G, Powell JA, Knower AT, Hunter KW. Monoclonal antibodies to T-2 toxin: In vitro neutralization of protein synthesis inhibition and protection of rats against lethal toxemia. *J Clin Invest*. 1985;76(6):2134–2138.

95. Chanh TC, Hewetson JF. Structure/function studies of T-2 mycotoxin with a monoclonal antibody. *Immunopharmacology*. 1991;21(2):83–90.

96. Kravchenko LV, Avreneva LI, Tutelian VA. Lowering the content of SH-glutathione and glutathione transferase activity in the liver as a factor in increasing the toxicity of T-2 toxin. *Vopr Med Khim*. 1983;29(5):135–137. Translated from Russian.

97. Markham RJ, Erhardt NP, Di Ninno VL, Penman D, Bhatti AR. Flavonoids protect against T-2 mycotoxins both in vitro and in vivo. *J Gen Microbiol*. 1987;133(6):1589–1592.

98. Masood A, Ranjan KS. Cumulative effect of vitamin C and T-2 toxin on clinical abnormalities in guinea pigs *(Cavea cavea). Biomed Lett*. 1994;49(195):213–217.

99. Kravchenko LV, Kranauskas AE, Dzhaparidze LM, Avreneva LI, Spirichev VB. Effect of different supplies of vitamin E on biochemical changes in T-2 mycotoxicosis in

rats. *Vopr Med Khim*. 1986;32(6):99–103. Translated from Russian.

100. Tutelyan VA, Kravchenko LV, Kuzmina EE, Avrenieva LI, Kumpulainen JT. Dietary selenium protects against acute toxicity of T-2 toxin in rats. *Food Addit Contam*. 1990;7(6):821–827.

101. Leatherman DL, Middlebrook JL. Effect of emetine on T-2 toxin-induced inhibition of protein synthesis in mammalian cells. *J Pharmacol Exp Ther*. 1993;266(2):741–748.

Chapter 35

MEDICAL CHALLENGES IN CHEMICAL AND BIOLOGICAL DEFENSE FOR THE 21ST CENTURY

ERNEST T. TAKAFUJI, M.D., M.P.H.*; ANNA JOHNSON-WINEGAR, PH.D.†; AND RUSS ZAJTCHUK, M.D., FACS‡

INTRODUCTION

PROLIFERATION OF BIOLOGICAL WEAPONS

BIOTECHNOLOGY
- Enhanced Pathogenicity
- Antibiotic Resistance
- Bioengineered Toxin Production
- Genetic Weaponry
- The Human Genome Project
- New Medical Countermeasures

MILITARY SCENARIOS

SUMMARY

*Colonel, Medical Corps, U.S. Army; Commander, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C. 20307-5100
†Director, Medical Chemical–Biological Defense Research Program, U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, Maryland 21702-5012
‡Brigadier General, Medical Corps, U.S. Army; Commanding General, U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, Maryland 21702-5000

INTRODUCTION

Biological and chemical warfare has long concerned military planners, strategists, and tacticians. Experiences in the Persian Gulf War (1990–1991), the rising concern over terrorist groups and their interest in weapons of mass destruction, and the continuing difficulties with curbing the proliferation of traditional chemical and biological weapons have resulted in continuing modifications in policies that would be employed in future scenarios. The use of such agents against the United States or its allies or both, including military and civilian populations, remains a distinct and perhaps increasing possibility.

Recent events in the world, including the terrorist attack with the nerve agent sarin in a subway in Tokyo, Japan, in March 1995, have demonstrated both the willingness of extremist organizations to use these agents and the ready availability of deadly agents. Biological organisms continue to be readily available throughout the world, obtainable in nature or through biological supply houses or medical laboratories. Dangerous chemicals already exist in local communities and in hardware and gardening shops.

The current threat posed by weapons of biological and chemical origin has been discussed extensively in previous chapters of this textbook. This chapter discusses three issues that will likely have a significant effect on chemical and biological defense in the next century:

- the global proliferation of biological weapons;
- the advances in technology, particularly biotechnology, that will affect the development of weapons and their countermeasures; and
- possible changes in the future use of chemical and biological weapons by the enemy that will make delivery of medical care even more challenging.

Efforts at counterproliferation will not be discussed in this chapter. This should not be construed as a reflection of its reduced importance, for limiting the proliferation of weapons of mass destruction continues to be a large effort of the United States government. Instead, emphasis is placed here on the issue of biotechnology, for this is the area where medical personnel will find the greatest controversy and conflict. For the interested reader who desires a more in-depth review of the subject of future prospects of biological weapons and proliferation, we refer you to two excellent sources: *Biological Weapons: Weapons of the Future?* and

Director's Series on Proliferation (see Recommended Reading at the end of this chapter).

The potential exists for both (*a*) misuse of the biotechnology for refinement of current biological weapons and (*b*) development of new agents with added potency. The profound impact that biotechnology will continue to have on biological weapons and their countermeasures is of particular concern when put in the context of the worldwide deployability of military forces, the potential use of genetic engineering for both peaceful and sinister purposes, and continued attractiveness of biological warfare as an option by adversaries in future conflicts.

PROLIFERATION OF BIOLOGICAL WEAPONS

The limitation and eventual elimination of both chemical and biological weapons are two of the greatest challenges facing the international community. Unfortunately, proliferation of such weapons is continuing despite the best efforts of many nations, including the United States, to prevent proliferation.[1] Biological weaponry is the most worrisome issue because of the relative ease in developing and mass-producing potent agents, the continuing difficulties in identifying enemy capabilities and limiting their development, and the potential ability for adversaries to bioengineer and deliver new organisms using the latest advances in technology.

On 23 February 1993, following the Persian Gulf War and the breakup of the Soviet Union, a panel of the U.S. House of Representatives Committee on Armed Services submitted a special report,[2] *Special Inquiry of the House Armed Services Committee Into the Chemical and Biological Weapons Threat.* This report concluded that despite the decrease in absolute quantities of chemical weapons, the potential diversity and the frequency with which such weapons could be encountered were increasing. The threat had shifted to Third World scenarios, with deployed U.S. military forces facing new threats from chemical and biological weapons. Technological advances have increased the diversity of potential weapons of each variety. The report stated the following concern, which goes to the heart of the problems that the U.S. military medical departments will face in the 21st century:

> Genetic tailoring and the speed of technological innovation create opportunities for the creation of exotic new agents which may be difficult to detect or defend against.[2(p7)]

In addition, the report of the House of Representatives Special Committee alluded to 31 nations that either possess or have the ability to develop an offensive chemical weapons capability, and 11 nations that either possess or have the ability to develop an offensive biological weapons capability. The Special Committee realized that while it would be more difficult for a country to mass-produce classic chemical warfare agents in large quantities without detection, it would be very easy for a country or organized group to develop the technological capabilities to produce other agents.[2]

The former Soviet Union, long suspected of having an aggressive research and development program despite its participation in international agreements to curtail such development, has continued to be a major factor in the global threat. Covert programs continued, at least through 1992, despite open declarations to the contrary. With the dissolution of the Soviet Union, concern has been generated about the export of the scientific technology and weaponry beyond its borders. Cooperation between the Russian Federation and the United States is making progress in reducing this potentially dangerous situation.

North Korea, Iran, and Iraq are three examples of countries with biological warfare potential. North Korea has continued to have a program of cooperation with several countries in the Middle East, and the prospect of biological weapons being used on the Korean peninsula is a genuine concern. Iraq was known to have an active research, development, and weaponization program at the time of the Gulf War, although whether Saddam Hussein intended to use such weapons is controversial. If chemical weapons agreements are any indication of national compliance and intent, it should be noted that Iraq secretly constructed chemical warfare production plants and imported chemical warfare technology from the West in violation of the Geneva Protocol.[3] The use of chemical weapons on Kurdish forces has now been well documented.

The Persian Gulf War and continuing problems with Iraq resulted in the creation of a unique United Nations organization known as The United Nations Special Commission on Iraq (UNSCOM).[3] UNSCOM was established specifically by a United Nations Security Council resolution that spelled out conditions for cease-fire and the destruction, removal, or rendering harmless of chemical, biological, and nuclear weapons and ballistic missiles with a range greater than 150 km. UNSCOM unilaterally

defined the terms for its inspections of Iraqi facilities and has continued to direct and support compliance inspections.

The United States has been active in its participation in international efforts to specifically control the spread of biological and chemical weapons. The Convention on the Prohibition of the Development, Production, and Stockpiling of Bacteriological and Toxin Weapons and their Destruction (also known as the Biological Weapons Convention) and the Chemical Weapons Convention have been discussed in detail in other publications,[4,5] as have various confidence-building measures,[5] which have met with limited success.

It should be noted that UNSCOM activities are not directly associated with the Biological Weapons and Chemical Weapons Convention agreements, although elimination of weapons of mass destruction continues to be a goal of most nations. It is plausible to assume that should international situations arise in the future regarding proliferents with biological warfare capabilities, the United Nations may again take actions similar to those it took with Iraq.

The “dual-use” issue, in which the technologies used to develop and produce biological or chemical weapons are very similar to those that would be needed for human and veterinary healthcare research and production and the agricultural industry, has created many challenges. The technologies involved in pesticide dispersal, for example, could easily be adapted for the delivery of aerosols containing biological agents. Verification inspections and confidence-building measures have had limited success, but efforts continue to strengthen the conventions.

BIOTECHNOLOGY

Since 1953, when James D. Watson and Francis Crick identified the genetic code contained in deoxyribonucleic acid (DNA), discoveries in the field of molecular genetics have skyrocketed. Breakthroughs in genetic engineering have allowed genes to be substantially altered and combined with other genes in ways that have benefited mankind tremendously. Over the last 30 years, for example, the number of recognized human genetic disorders has risen from fewer than 500 to more than 4,200, primarily as a result of the ability to sequence genes quickly and precisely.[6–8] Since 1990, gene therapy experiments have been approved involving numerous human studies aimed at 12 genetic disorders, including cystic fibrosis, severe combined immune deficiency, familial hypercholes-terolemia, acquired immunodeficiency syndrome, and several cancers.

However, the progress in genetic engineering and molecular biology has raised serious ethical issues, such as man’s apparent ability to freely manipulate nature without clear societal controls; the potentially

dangerous effects of bioengineered organisms of plant and animal origin on the environment; the arbitrary use of human embryonic tissue in research; and the control, storage, and access of genetic information.[7,9] These controversies have arisen as scientific accomplishments have proceeded at a more accelerated pace than has society's ability to resolve such complex issues.

For the military, knowledge of man's specific genetic defects or vulnerabilities (or ways to create such defects) and the ability to modify microorganisms or toxins that would increase pathogenicity take on added concern. Biotechnology theoretically provides opportunities for adversaries to modify existing organisms with specific characteristics, such as increased virulence, infectivity, or stability. Modern advances also allow for the inexpensive production of large quantities of replicating microorganisms for weaponization through recombinant methodologies, and the possibility to create "new" agents for future warfare that bypass current preventive or therapeutic interventions. These could be accomplished through secretive research programs that are superimposed on open biomedical research efforts in pharmaceutical firms or government laboratories. Ironically, while such possibilities continue to generate fear, the same technological advances can and do contribute to the development of new medical countermeasures, such as new vaccines, drugs, and diagnostic tests.

Enhanced Pathogenicity

Splicing genes for virulence, infectivity, stability, or other factors into the genome of an existing organism is one possibility for manipulating potential biological warfare agents. Microorganisms are able to cause disease through a variety of mechanisms that may involve interactions at the cellular level or at target organs. An understanding of the basic mechanisms of action that determine or influence cellular attachment, penetration, and genetic alteration is critical in the redesign of microorganisms.

Infectivity

Although influenza A virus has not been known to be bioengineered as an offensive biological weapon, it serves as an example of opportunities for viral modification. Naturally occurring mutations that result in antigenic drifts and shifts suggest that with today's technology, man could achieve deliberately what nature is already accomplishing naturally. If we are to develop effective countermeasures, we must understand how a virus penetrates the host's natural defenses and modifies itself genetically to

promote its spread and ultimate survivability.

Influenza A possesses a neuraminidase that cleaves the terminal neuraminic acid residue at the cell surface carbohydrates. This simple enzymatic action allows the virus to attach to the cell surface and penetrate the membrane. Once infection has occurred, the body gradually develops specific antibodies to the infecting viral strain. Humoral immunity could also be elicited through immunization with an attenuated strain or a nonreplicating portion of the influenza virus itself.

The infectivity of “new” influenza virus strains is based on the ability of such strains to evade the body’s preexisting immune defenses. The virus’s genes and their products—hemagglutinin and neuraminidase—are altered enough that the body perceives it to be a new virus. This antigenic change could theoretically occur through man-made genetic alterations. The concept for developing new biological weapons is a basic one: develop a virus that evades the protective immunological system, reaches a target organ or tissue quickly, and causes significant disease, disability, or death. Viruses that debilitate or cause chronic illnesses may be just as militarily significant as those that cause acute disease, depending on specific scenarios.

Paradoxically, it may be advantageous for a biologically weaponized virus to cause a severe protracted illness with high communicability rather than quick death with little opportunity for continued spread. The only factor that prevents this from becoming an easy option for adversaries is the aggressor’s need to develop a protective vaccine against the modified virus for use in their troops. The fact that new influenza vaccines are needed every year to provide protection against new or modified circulating strains of virus illustrates the difficulties in developing a long-lasting, immunologically based defense against such viruses.

Virulence

Several bacterial agents have virulence factors that would enhance their pathogenicity in man. Such additions to a highly infectious but less pathogenic strain could make the modified organisms more attractive candidates for offensive weapons. Countermeasures, including vaccines and antimicrobial drugs, must be developed with this possibility in mind to provide the broadest measure of protection.

Moreover, when used in combination, microorganisms have the potential to create a more severe disease state. Similarly, infection with one agent with a shorter incubation period that may weaken overall resistance may provide easier opportunities for infection with a second organism with greater morbidity and mortality. The ability of multiple organisms with different levels of virulence to confuse medical officers

looking for a common etiology accentuates the need for sensitive and specific diagnostic tests to be available in the field setting. These diagnostic tests must be able to decipher genetic differences and differentiate endemic from nonendemic forms of microorganisms.

Genetic Recombination

The ability for some genes to transpose themselves on chromosomes, rearrange and combine with other genes in a manner that may result in radical phenotypic and genotypic changes in the original organism, or to form plasmids that may sit quiescently for the right moment to exert their effect, has been demonstrated in the laboratory. The ability for cancer-producing genes (oncogenes) to be produced through genetic insertion or to be “turned on” by enzymes produced by other genes has given rise to the now-proven theory that some cancers are caused by infectious agents.[7]

In addition, retroviruses that attach to and invade specific cells of the body, inserting themselves into host genes and disrupting the normal DNA, can create long-lasting changes in the host that may eventually weaken overall immunity to diseases. While such discoveries lend themselves well to a better understanding of the pathological processes, they also provide opportunities that may not be as readily apparent for the development of sinister weapons.

Immunity

It is now recognized that protection against respiratory challenge by pathogens may require that a certain level of mucosal immunity be maintained. Cytokines released by leukocytes and other cells are extremely important in the development of the immune response; they modulate the differentiation and division of hematopoietic stem cells, activate lymphocytes and phagocytes, and are very much involved in the development of humoral and cellmediated immunity. An understanding of the importance of cytokines in the immunological process, as well as factors leading to immunopotentiation and immunosuppression, could be applied practically to enhancement of vaccine efficacy or the prevention of release of potentially dangerous substances in the body.

These are just a few of the current immunological challenges in research. Advances in our understanding of immunomodulation allow for breakthroughs in cancer therapy or immunodeficient states to be applied to the development of new protective strategies against a broad spectrum of biowarfare agents. We can only speculate that future medical interventions will incorporate new knowledge on such processes.

Antibiotic Resistance

It has been recognized for many years that the uncontrolled use of antibiotics will promote the selective development of certain resistant strains of many microorganisms. Bacterial agents may rely on a variety of mechanisms to increase their virulence or resistance to antibiotics: through more permanent chromosomal mediation; or through plasmids, independently replicating extrachromosomal DNA segments floating freely in the cytoplasm that are capable of being ejected when antibiotic pressures are absent. Modified biological agents with resistance factors may be unaffected by therapeutic and chemoprophylactic regimens directed against sensitive organisms.

Biotechnology allows for the introduction of factors into many replicating organisms that would promote resistance to antibiotics. With human pathogens, concerns center on bacterial, viral, rickettsial, and fungal agents capable of causing acute and chronic infections. An armamentarium of structurally different, broad-spectrum drugs, which have been developed to initiate their specific therapeutic effects via different mechanisms, will allow some circumvention of the resistance threat.

Bioengineered Toxin Production

Taking bacterial organisms one step further, the combination of a known pathogenic biological agent with genes for producing toxin from another organism spliced into it generates a series of potential issues, including the ability of the original organism to retain enough of its previous pathogenicity; and its ability to replicate, spread, and produce enough toxin to cause symptoms. Although this concept may sound intriguing, naturally occurring organisms that already produce toxins may achieve the same desired effect. Recent experiences with *Escherichia coli* 0157:H7 causing hemorrhagic colitis and a hemolytic uremic syndrome as a result of consumption of contaminated meat demonstrate the devastating effect that the presence of a shigalike toxin may have. Identification of specific pathogens and their toxins require current technologies, including polymerase chain reaction and gene probe technologies, to be readily available even in a field setting.

A more worrisome concept involves the hybridization of two or more genes, such as combining a toxin with a monoclonal or polyclonal antibody directed against specific target cells in the body. Oncological immunotherapy is based on this concept and is effective in treating several types of cancers. While cancer therapy may depend on strong affinity of molecules for specific receptors, biological defense may favor deactivation

of receptors or blocking of attachment. Molecular modeling would be helpful in understanding this phenomenon.

Genetic Weaponry

"Genetic warfare" has been raised as an issue, whereby targeting of specific populations or individuals with specific genotypic characteristics could theoretically be accomplished. Fortunately, although several replicating agents and toxins can now be mass produced with relative ease, entirely different types of biological agents are still difficult to create. This may not be the case in the next century, where emerging pathogens will include biological agents.[7,10]

It has been postulated that "genetic weapons" might very well be developed in the wake of increased knowledge about the human genome and genetic diversity. We would hope that the development of genocidal agents is so repulsive a concept that it would never be accepted by the international community. Racial differences do exist with blood-group proteins and histocompatibility proteins, and genetic susceptibilities to specific diseases have been demonstrated. However, it has been estimated that only 0.1% to 1% of the human genome can clearly be associated with pure ethnic differences[10]; whether this diversity is enough for the development of tailored agents is an open question. And whether a nation would find it necessary to specifically pursue a course to develop such targeted offensive weaponry remains to be seen.

It is improbable that such weapons would pose a serious threat to the forces of this nation, since the population of the United States is more heterogeneous than more segregated homogeneous societies. It is unrealistic to consider this a real threat anytime soon.

The Human Genome Project

The Human Genome Project, begun in the mid 1980s, has as its goal to have genetically sequenced over 100,000 genes in the human genome by year 2005. The information will be placed in a gene bank for international access.[6,7,9] The Human Genome Organization (HUGO) was established to coordinate the human genomic analyses being performed internationally and to maintain the database repository of all sequence information. The purpose of the project is to provide information on the chemical structure of humans. This will allow for a better understanding of hereditary diseases, the immune response, and certain chronic diseases. An understanding of what constitutes a healthy state may tempt proponents of offensive warfare to develop agents that create a state of poor health in their enemies. This could be accomplished through minor

alterations in genes that control enzymatic actions in the body or changes in genes that control other genes. Although legal patent issues appear to be the principal difficulty currently, nations that have provided data in the project will claim a right to have access to all information.

New Medical Countermeasures

Advances in technology now allow for more directed and coordinated approaches in vaccine development against biological warfare agents and endemic diseases. The development of combination vaccines that eliminate the need for multiple vaccinations is of practical importance to the military. But efforts to increase the immunogenicity of vaccines—in particular, recombinant subunit vaccines that have traditionally been associated with lower antibody titer responses than live attenuated vaccines—may result in the discovery of better adjuvants or slow-release formulations (eg, microencapsulation) that will result in highly satisfactory and long-lasting immunological responses. Reversion of attenuated strains is always a concern with live vaccines, although this has not been shown to be a significant problem up to now. Recombinant vector vaccines, including those against vaccinia, adenovirus, and *Salmonella*, also offer some safer prospects,[11,12] but when vaccines against specific biological warfare agents are developed, the soldier's immunological responses to other vaccines administered need to be considered.

It is impossible to provide protection against every conceivable agent, but it seems likely that future medical protective measures will need to be more broadly based if they are to provide the best protection against biowarfare agents in the future. Current and future research must evaluate how to best stimulate the immunological response that will protect against categories of agents, while at the same time ensuring that those agents highest on the threat list are adequately covered.

As mentioned earlier, an understanding of the role of the mucosal response is critical, especially for protection via the respiratory or gastrointestinal tracts, as well as the importance of the humoral versus the cell-mediated response. Antimicrobial drug supplementation may also provide added benefit for immediate or short-term protection.

The abilities to develop monoclonal antibodies against specific antigens and to be able to develop transgenic animal models to form chimeras have opened large windows of research opportunity.[7] For example, the ability to microinject human genes into the pronuclei of fertilized mouse eggs and replace original genetic segments permits sophisticated animal models to be developed that can be used in challenge studies with specific infectious agents. The ethical animal-rights issues associated with creating and using such chimeras in research are obvious, but the opportunities for

medical advancement are equally certain. Although transgenic mice currently provide little to new weapons development, they may be extremely helpful in the development of effective countermeasures. Transgenic plant research, leading to the development of resistance to insects and plant diseases, is another extension of genetic research.

MILITARY SCENARIOS

The threat that chemical agents will be used by hostile forces continues to be a military concern. However, effective personal protective measures and environmental detection systems will likely provide satisfactory protection for the forces in nearly all perceived scenarios involving agents described in earlier chapters of this textbook. Carefully followed decontamination procedures will reduce the possibility of further injury and allow medical personnel to render appropriate care to casualties.

Scenarios of the future may be complicated by the possible use of multiple agents, or the delivery of chemical and replicating agents and/or their toxins that have been carefully matched, based on their stability and ability to generate specific symptoms. Health effects could be potentiated. Therefore, from a medical perspective, detection requires the availability of rapid diagnostic methods and procedures to assess illnesses that will be the result of multiple agents.

Stated another way, detecting the presence of one single agent may not be adequate, since detectors can detect only what they were designed to detect. The classic chemical agents described earlier in this textbook will be only part of the concern, for readily available, highly toxic, industrial compounds that are not under the same degree of international monitoring as well-recognized chemical warfare agents could be used as weapons.

The deployment of troops to foreign lands provides opportunities for biological and chemical incidents to occur. In 1993, the United States, along with 71 other countries belonging to the United Nations, contributed troops to over a dozen peace-keeping missions throughout the world. These deployments involved military medical elements from different countries, but many of the smaller nations, in particular, possess limited abilities to deal with a nuclear, biological, or chemical warfare situation. As of this writing (1996), no chemical or biological warfare incidents have occurred during such peace-keeping missions, although sabotage and terrorism have occurred.

Since the military has the greatest capability in this country to address chemical and biological warfare, the missions for the military have now included the domestic front. Military medical practitioners will very likely find it necessary to be familiar with the diagnosis, treatment, and prevention of injuries and illness caused by chemical and biological

agents, for we will frequently be consulted for our expertise.

Biological warfare, in particular, is of great concern for the military for several reasons:

- Many potent agents are readily available. Theoretically, any microorganism or toxin capable of inflicting death or disease has the potential of being adapted for use as a biological weapon.
- Naturally occurring infectious agents could be used to generate epidemics among susceptible troops, creating confusing disease situations on the battlefield. Naturally occurring or deliberately disseminated spore-forming microbes could continue to persist in the environment, and some aerosolization might occur during military maneuvers; environmental detectors may not necessarily be able to differentiate between natural and man-generated contamination.
- Many classic agents can be mass-produced in a short time using very basic laboratory techniques. Large fermenters may not be necessary if a small amount of agent is all that is required.
- Theoretically, biological agents can be genetically altered to escape detection.
- Biological agents require no precursors for development, unlike chemical and nuclear agents, and a covert program is much more difficult to detect.

SUMMARY

The future requires that we carefully and continually assess the evolving threat from chemical and biological weapons. This can be predicted with certainty: the threat will change with time. As stronger countermeasures are developed by the United States and its allies, the employment of certain agents may become less appealing to adversaries on the battlefield. From that standpoint, medical countermeasures may be an effective deterrent. Biotechnology itself may be the threat of the future, and not specific agents, as adversaries may attempt to evade effective preventive measures with bioengineering. The employment of multiple chemical and biological agents is a very likely scenario of the future, thereby challenging the medical community to be much more proactive in its development of appropriate countermeasures.

The missions of the United States military are changing, and deployments will require a capability to address potential chemical and biological incidents on the domestic and international fronts. Military medical personnel must, therefore, be continually prepared to deal with

such contingencies as we become an even more important asset to this nation's defense and healthcare structures.

REFERENCES

1. ter Haar B. *The Future of Biological Weapons*. New York, NY: Praeger; 1991.
2. House of Representatives, 102nd Cong, 2nd Sess. *Report of the Special Inquiry Into the Chemical and Biological Threat of the Committee on Armed Services.* Washington, DC: US Government Printing Office; 23 Feb 1993.
3. Spertzel RO, Wannemacher RW, Linden CD. *Global Proliferation—Dynamics, Acquisition, Strategies, and Responses*. Vol 4. In: *Biological Weapons Proliferation*. Alexandria, Va: Defense Nuclear Agency; September 1994. DNA Technical Report 93-129-V4.
4. Geissler E, ed. *Biological and Toxin Weapons Today*. Oxford, England: Stockholm International Peace Research Institute, Oxford University Press; 1986: 135–137.
5. Roberts B, ed. *Ratifying the Chemical Weapons Convention*. Washington, DC: Center for Strategic and International Studies; 1994.
6. Friedman T. Molecular medicine. In: Davis BD. *The Genetic Revolution*. Baltimore, Md: The Johns Hopkins University Press; 1991.
7. Lee TF. *Gene Future: The Promise and Perils of the New Biology*. New York, NY: Plenum Press; 1993.
8. Weiss R. Gene therapy at a crossroads. *Washington Post*. 18 Oct 1994; Health:12.
9. Drlica KA. *Double-Edged Sword: The Promises and Risks of the Genetic Revolution*. Reeding, Mass: Addison-Wesley; 1994.
10. Dubuis B. *Recombinant DNA and Biological Warfare*. Zurich, Switzerland: Institut für Militarishe Sicherheitstechnik; October 1994.
11. Rabinovich NR, McInnes P, Klein DL, Hall BF. Vaccine technologies: View to the future. *Science*. 1994;265:1401–1404.
12. Ellis RW, Douglas RG Jr. New vaccine technologies. *JAMA*. 1994;271:929–931.

RECOMMENDED READING

Roberts B, ed. *Biological Weapons: Weapons of the Future?* Washington, DC: The Center for Strategic and International Studies; 1993. This 101-page book is a collection

of essays on various aspects of biological warfare and covers in depth some of the concepts presented briefly in this chapter.

Bailey KC, ed. *Director's Series on Proliferation*. University of California: Lawrence Livermore National Library; 1994. This 112-page book is a collection of essays compiled under a US Department of Energy contract (UCRL-LR-11470-4) on various aspects of the biological warfare threat and proliferation. The document is available from the National Technical Information Service, US Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161.