Chapter 8

LONG-TERM HEALTH EFFECTS OF NERVE AGENTS AND MUSTARD

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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 long-term 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 long-term 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 Ct (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 • min / m^3 of sarin will produce miosis and rhinorrhea in man.[13] This Ct 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 (Ct of 10–200 mg • 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 $mg \cdot min / m^3$ 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 $mg \cdot min / m^3$.[23] Airway injury occurs at a C*t* of 100 $mg \cdot min / m^3$ 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 a 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 insecti-

cide 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, dis-

turbance 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 be-

fore 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 coworkers 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 Ct of 10 $mg \cdot min / m^3$ 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 disorders), 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. Mus-

cular 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. *Green 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.