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SUBJECT:USAMRICD Special Publication 98-01, Pyridostigmine

This special publication is intended to provide technical information to health care professionals of the Army, Navy, Air Force and allied nations on the topic of chemical casualty care, in accordance with the postgraduate medical education mission of the Institute. The information is doctrinally consistent and assumes a working knowledge of the tri-service field manual "Treatment of Chemical Agent Casualties and Conventional Military Chemical Injuries," Army FM 8-285, Navy NAVMED P-5041, Air Force AFM 16011, dated February 1990

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INTRODUCTION

☞ 1. What is the purpose of this special publication?

The currently fielded pretreatment for nerve-agent exposure is pyridostigmine bromide (PB). Military physicians and other health-care providers need to be familiar with this medication, with the rationale for its use, and with its effects on the body. They also need to be able to respond intelligently to questions from users of PB, patients, and field commanders. This publication is intended to address the major issues that military health-care providers need to understand concerning nerve-agent pretreatment with PB. It is organized around a series of questions about PB in general and its pharmacokinetics (absorption, distribution, biotransformation, and elimination), pharmacodynamics (mechanism of action), field use, and reported and expected effects.

DESCRIPTION

☞ 2. What is pyridostigmine bromide [PB] and what is pretreatment?

PB is a medication with a long history of civilian use for a) treatment of patients with myasthenia gravis, b) reversal of the effects of nondepolarizing neuromuscular blocking agents administered during general anesthesia, and c) treatment (as a second-line agent if edrophonium supplies are depleted) of envenomation by Asian snakes or of tetrodotoxin poisoning (Leikin and Paloucek 1995). In military settings, PB is used as a *pretreatment* for nerve-agent intoxication. That is, it is administered prophylactically to military personnel judged to be at high risk for exposure to certain kinds of nerve agents (particularly soman [GD] and tabun [GA]) or to nerve agents in general (when the specific kind of nerve agent is not known with an acceptable degree of certainty). Its use in these circumstances has been shown in animal studies to markedly increase the effectiveness of post-exposure treatment with the currently fielded antidotes atropine and 2-pralidoxime chloride. It is important to realize that PB is not an antidote: it has no value when administered after nerve-agent exposure, and it is not a substitute for atropine or 2-pralidoxime chloride (Shiloff and Clement 1986). Strictly speaking, because the action of PB administered prior to nerve-agent exposure is to increase the efficacy of the antidotes atropine and 2-pralidoxime chloride, PB is an *antidote enhancer* rather than a pretreatment capable of acting by itself in the absence of antidotes (Ellenhorn et al. 1997). However, the use of the term *pretreatment* to describe enhancement of nerve-agent antidotes by pre-exposure use of PB has become so entrenched in medical and military settings that this term is unlikely to be supplanted by *antidotal enhancement* in the foreseeable future.

PB, like the structurally similar compound physostigmine, belongs to a class of chemical compounds called carbamates. Nerve agents, by contrast, are organophosphorus esters containing phosphonate groups. Both carbamates and nerve agents may bind to and thus inactivate (for the duration of their binding) a crucial enzyme called acetylcholinesterase (AChE), which is responsible for the normal breakdown of the neurotransmitter acetylcholine (ACh) following its release by cholinergic neurons in the central and peripheral nervous systems. Thus, both carbamates and organophosphorus compounds may have

anticholinesterase activity. An important difference between carbamates and nerve agents is the nature of their binding to AChE. Both kinds of anticholinesterases form covalent bonds with AChE, but AChE slowly breaks down (or *decarbamylates*) the carbamate PB (much more slowly than the almost instantaneous hydrolysis of ACh by the enzyme but far more rapidly than AChE can hydrolyze nerve agent) and is then available to hydrolyze ACh again. On the other hand, the bond between organophosphorus nerve agents and AChE is so stable that no significant restoration of active AChE occurs (Taylor 1996); that is, the binding is for clinical purposes irreversible, although the difference between the "reversible" binding of PB and the "irreversible" binding of nerve agents is a quantitative difference (albeit a very large one) in reaction rates. Even though the resulting nerve-agent/AChE compound does not break down on its own to any appreciable extent, it can react with substances called oximes, which through a series of reactions separate the nerve agent from the enzyme in a process called *reactivation* or *regeneration*.. However, the strength of the bond between the nerve agent and the AChE may eventually change (after a characteristic time that is different for each nerve agent) in a process called *aging*. Aging prevents subsequent reactivation of the AChE by oximes. The carbamate anticholinesterases physostigmine and PB are both effective as pretreatment against nerve-agent exposure, but differences in their chemical structures affect their distribution in the body: Physostigmine, a nonpolar compound, freely crosses the blood-brain barrier and thus has significant side effects involving the central nervous system, whereas the positively charged quaternary ammonium ion in the pyridostigmine portion of PB prevents PB from passing through an intact blood-brain barrier.

HOW SUPPLIED

☞ 3. How is PB supplied for military use?

PB is currently fielded as twenty-one 30-mg PB tablets in a blister pack within a sealed pouch labeled "Nerve Agent Pre-Treatment Tablets" and often referred to by the acronym NAPPS (for *n*erve-*a*gent *p*yridostigmine *p*retreatment *s*et) (Dunn and Sidell 1989). Since the recommended dose of PB for nerve-agent pretreatment is one 30-mg tablet every eight hours, each packet provides a one-week supply of PB for one person. Military personnel are issued two blister packs each. Long-term storage is at 2-8 C, and blister packs removed from refrigeration are to be used within six months (NAPP fact sheet, http://www~napp.html).

PHARMACOKINETICS

☞ 4. How does the human body absorb PB?

Tablets of PB dissolve rapidly in the body, and the released PB is highly soluble in aqueous solution. Estimates of the bioavailability of PB after oral administration have ranged from a low of 3-4% to a high of nearly 30%, with a large variability between subjects: in one study, the mean bioavailability was 29.1% with a range of 14.7 to 51.1% (Aquilonius and Hartvig 1986). A generally accepted estimate is 10% to 20% (Aquilonius and Hartvig 1986; Leikin and Paloucek 1995), and although bioavailability was not measured directly in a 1998 study of ninety subjects taking 30 mg of PB every eight hours in accordance with the military pretreatment regimen, an estimate of the bioavailability from this study was 12% (Marino et al. 1998). It would be logical to assume that the relatively low bioavailability of PB reflects impaired absorption of the hydrophilic and charged pyridostigmine portion of PB across cell membranes, but intravascular hydrolysis and limited metabolism by the liver may also play a role (Aquilonius and Hartvig 1986). In fact, first-pass metabolism by the liver is consistent with the results of studies using 14C-labeled PB (Kornfield et al. 1970). Several studies (Aquilonius and Hartvig 1986) have shown minor secondary peaks of PB in the plasma after oral administration; these peaks may reflect the formation of more easily absorbed ion pairs of PB with intestinal mucin or bile salts (Chan et al. 1981), or, less plausibly, they may indicate a degree of enterohepatic circulation (White et al. 1981). Peak plasma concentrations, estimated at 20 to 30 ng/mL after ingestion of 30 mg of PB, occur in one to two hours after ingestion (Cohan et al. 1977; Aquilonius et al. 1980). Taking PB with food delays time to peak concentration by up to 90 minutes but does not affect the area under the plasma concentration-time curve (AUC) (Aquilonius et al. 1980). Plasma concentrations of PB after the same oral dose may vary 4- to 7-fold between subjects (Aquilonius et al. 1983) with no obvious relationship between oral dose and steady-state plasma concentrations (Aquilonius et al. 1980; Chan et al. 1981; White et al. 1981). However, plasma concentrations of PB during a dosing interval do not vary greatly in the same individual (Aquilonius et al. 1980, 1983; Sörensen et al. 1984).

☞ 5. How is PB distributed in the body?

Once it enters the bloodstream, PB is widely distributed throughout the body, with reported volumes of distribution of 1.0 (Breyer-Pfaff et al. 1985) or 1.1 (Cronnelly et al. 1980) to 1.76 L/kg (Aquilonius and Hartvig 1986). The most recent study to address this issue postulated a two-compartment model with a volume of distribution of 2.09 L/kg for the central compartment and 4240 L for the peripheral compartment (Marino et al. 1998). Estimates of the distribution half-life of PB have included 0.12 (Cronnelly et al. 1980) and 0.14 hours (Breyer-Pfaff et al. 1985). Some tissue sequestration of PB presumably occurs (Calvey et al. 1981), but neither PB nor its main metabolite is bound to plasma proteins or within red blood cells (Kornfeld et al. 1970). Under ordinary conditions, the positively charged nitrogen of the pyridostigmine moiety of PB prevents it from crossing an intact

blood-brain barrier, although this barrier may normally be at least partially permeable to polar compounds such as pyridostigmine, specifically in the fourth ventricle and in the brainstem (Dunn et al. 1997). PB is able to cross the placenta (Leikin and Paloucek 1995) and can be found in amniotic fluid (Lefvert and Osterman 1983). The finding that PB concentrations in breast milk of nursing mothers range from 36% to 113% of the concentration in maternal plasma implies a very low dose (approximately 0.1% of the dose per kilogram taken by the mother) to the nursing infant (Skoglund et al. 1978; Hardell et al. 1982). In elderly patients, volumes of distribution and elimination half-lives for PB are unchanged from their values in younger groups, but plasma clearance of PB is decreased (6.7 mL/kg-min) in 71- to 85-year-olds compared to 21- to 51-year-olds (9.5 mL/kg-min) (Stone et al. 1995).

☞ 6. How does the human body biotransform (metabolize) PB?

PB is biotransformed in the blood and in the liver. Its main metabolite, 3-hydroxy-N-methylpyridinium, is formed from the reaction of PB with plasma cholinesterases in the blood (Kornfeld et al. 1970); this metabolite is then rapidly glucuronidated in the liver (Kornfeld et al. 1970; Somani et al. 1972). However, the enzyme or enzymes responsible for hepatic metabolism of PB have not yet been identified (Marino et al. 1998).

☞ 7. How is PB eliminated from the body?

Analysis of urine from subjects receiving PB shows unchanged PB and 3-hydroxy-N-methylpyridinium in a 4:1 ratio (Nowell et al. 1962; Somani et al. 1972). The kidneys represent the major route of PB excretion, with urinary excretion of unchanged PB reported at 5 to 15% of an orally administered dose (reflecting the degree of oral absorption) (Nowell et al. 1962). From 75% to 90% of the absorbed dose is excreted unchanged in the urine (Kornfeld et al. 1970). Renal clearance occurs both by glomerular filtration and tubular secretion (Chan and Calvey 1977). The elimination half-life of PB has been reported to be 1.0 to 1.9 hours (Aquilonius and Hartvig 1986) after intravenous injection but to be 3.7 hours after oral administration (Breyer-Pfaff et al. 1985; Benet et al. 1996). Elimination mechanisms may be saturable but not at an oral dose of 30 mg. Nevertheless, doubling the oral dose from 30 mg to 60 mg in one patient resulted in a six-fold increase in plasma PB (Calvey and Chan 1977). The data from the recent large-scale study of PB given as a 30-mg oral dose every eight hours were most consistent with a first-order input and a lag time into a two-compartment model with first-order elimination (that is, an elimination phase characterized by a constant elimination half-life or t½) from the central compartment (Marino et al. 1998).

ACTIONS / PHARMACOLOGY / PHARMACODYNAMICS (MECHANISM OF ACTION)

☞ 8. What are nerve agents and what is their mechanism of action?

Nerve agents include the volatile compounds tabun (GA), sarin (GB), soman (GD), and cyclohexylmethylphosphonofluoridate (GF) in addition to a persistent compound, *o*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate (VX). As previously mentioned, nerve agents are organophosphorus esters that inhibit the enzyme AChE. During the initial reaction of a nerve-agent molecule with a molecule of AChE, a portion (the leaving group) of the agent breaks off. The resulting nerve-agent/AChE complex will not readily dissociate spontaneously (that is, the nerve-agent/AChE bond is essentially irreversible), but it can break apart in the presence of compounds called oximes to yield *reactivated* (*regenerated*) AChE. Eventually, however, one of the alkyl groups of the nerve agent leaves. When this dealkylation or aging process is complete, not even oximes can separate the nerve agent from the enzyme; that is, the enzyme can no longer be reactivated (Dunn et al. 1997). When nerve agent binds to AChE, the enzyme is unavailable for catalyzing the hydrolytic breakdown of the acetylcholine (ACh) normally released by the axons (terminal ends) of cholinergic (ACh-releasing) neurons. ACh thus remains in synapses (a synapse is the space between the axon or terminal end of one neuron and the dendrite [acceptor end] or the body of another neuron), neuromuscular junctions (the spaces between neurons and muscle end-plates), and neuroglandular junctions (the spaces between neurons and exocrine or ducted glands). Excess ACh at these sites leads to cholinergic crisis, in which end organs (nerves, skeletal muscle, smooth muscle, cardiac muscle, and exocrine glands) are overstimulated. End-organ overstimulation results in the classic signs and symptoms of fasciculations, twitching, gastrointestinal hypermotility, glandular hypersecretion, and convulsions. Muscle fatigue from overstimulation leads to flaccid paralysis and (in the case of the diaphragm and the accessory muscles of breathing) terminal apnea.

Atropine, one of the currently fielded antidotes for nerve-agent poisoning, acts as a competitive inhibitor of ACh at muscarinic receptors on smooth muscle and glands. Atropine has little to no cholinergic agonist activity and therefore does not stimulate the end organs, but while an atropine molecule is occupying the postjunctional muscarinic receptor, that receptor cannot accept an ACh molecule. The end organ is therefore partially protected from the excess ACh present in the neuromuscular or neuroglandular junction. Of note is the fact that atropine does not bind to the nicotinic receptors on skeletal muscle and therefore cannot alleviate the skeletal-muscle overstimulation and eventual paralysis that nerve agents cause.

2-Pralidoxime chloride (2-PAM Cl), the other nerve-agent antidote, is an oxime that reacts with the nerve-agent/AChE complex, removing the nerve agent and allowing AChE to regain its ability to hydrolyze ACh. Clinical response to this antidote is particularly satisfying for nicotinic effects, which are not relieved by atropine administration. However, the process of AChE

reactivation or regeneration by 2-pralidoxime chloride is possible only during the initial stage of nerve-agent/AChE binding. Once aging (dealklylation) of the nerve-agent/cholinesterase complex has occurred, oximes are no longer effective.

The time required for aging of the nerve-agent/AChE complex varies with the specific nerve agent. Because the aging half-time for VX is 48 hours, only 50% of absorbed VX will have become irreversibly bound to AChE by this time, and 2-pralidoxime chloride administered anytime in the first few days after a VX exposure will be able to remove bound nerve agent from AChE. The half-times for aging of tabun (GA), sarin (GB), and GF are roughly 13 to 14 hours (Doctor et al. 1993; Mager 1984), 5 hours (Sidell and Groff 1974), and 7 to 40 hours (Hill and Thomas 1969; Mager 1984), respectively; but the aging half-time of soman (GD) may be as short as 2 to 6 minutes (Mager 1984). The extremely short aging time for soman means that 2-pralidoxime chloride administered even a few minutes after exposure to this nerve agent may not be effective. PB pretreatment was developed primarily as a response to the ineffectiveness of oxime antidotal therapy against soman (GD).

☞ 9. How does PB work as a pretreatment for nerve-agent intoxication?

Nerve agents and PB both bind to AChE to act as anticholinesterases, so the effects of PB given in excess are qualitatively similar to the signs and symptoms of nerve-agent poisoning. Nevertheless, when PB is administered in such low doses that only about 30% of erythrocyte AChE is inhibited, there are usually no clinical effects. Another crucial difference between nerve agents and PB is that AChE is for all practical purposes unable to hydrolyze nerve agents and that the nerve-agent/AChE bond can be broken only by oximes-and then only before aging has occurred. PB, on the other hand, is a reversible inhibitor of AChE. As a carbamate anticholinesterase given prior to exposure to nerve agent, PB temporarily inactivates in a dose-dependent manner some of the body's AChE by binding to it (in a process called *carbamylation*) at the same site at which ACh normally binds. As long as a molecule of PB occupies the ACh-binding site on an enzyme molecule, that molecule of AChE will be unable to break down ACh. However, AChE slowly decarbamylates bound PB with a half-time of approximately 30 minutes. The restored fully functional AChE is once again free to bind to ACh and hydrolyze it.

Because PB bound to AChE partially blocks the site at which nerve agents attach to the enzyme, those molecules of AChE that are already bound to PB cannot bind to subsequently introduced nerve agent. These AChE molecules therefore act as a reserve force of enzyme, temporarily unable to break down ACh but also protected from the effects of nerve agent and capable of dissociating later to native AChE able to break down ACh again and aid in relieving the cholinergic crisis (ACh excess) induced by the nerve agent.

The aim of PB pretreatment is to achieve a continuous inhibition of enough AChE in the body's synapses and neuromuscular and neuroglandular junctions so that this reversibly bound fraction of AChE will be protected from subsequent nerve-agent challenge and will become available later to enhance the effectiveness of atropine and 2-pralidoxime chloride by helping to break down the excess ACh built up by exposure to the nerve agent. It has been shown that 20% to 40% inhibition of erythrocyte AChE corresponds to this antidote-enhancing effect (Kluwe 1987; Dunn and Sidell 1989; Kerenyi et al. 1990; Dunn et al. 1997; Ellenhorn 1997; Marino et al. 1998), and evidence exists that as little as 10% inhibition may be efficacious (Lennox et al. 1985; Marino et al. 1998).

☞ 10. How effective is PB likely to be as a pretreatment for nerve-agent intoxication?

With the exception of a single laboratory exposure (Sidell 1974), there have been no known human exposures to soman (GD) in Western countries (Dunn et al. 1997). Therefore, most of the work performed to demonstrate the efficacy of PB pretreatment has been in animals (Dirnhuber et al. 1979; Gordon et al. 1978; Kluwe 1987), although an early study in humans (Leopold and McDonald 1948) demonstrated the usefulness of the related carbamate physostigmine in reducing the time course of miosis induced by the nerve-agent-like organophosphate compound diisopropyl fluorophosphate (DFP). Antidotal treatment in PB-pretreated subjects appears to be most successful in primates such as rhesus monkeys; guinea pigs are somewhat less responsive, followed by rabbits and rats (Dirnhuber and Green 1978). Consistent with its chemical structure, PB was found in one study to inhibit brain cholinesterase by only 10% at the same dose that caused 30% inhibition of AChE in whole blood. In this study, the activity of blood AChE reached a nadir after two hours and slowly rose beginning four hours after administration, but 30% of the AChE in the blood was still inhibited after eight hours (Deyi et al. 1981).

A useful concept in comparing efficacies of various combinations of pretreatment and antidotal regimens against a particular agent is to refer to the protective ratio or PR, which is the ratio of the agent LD50 (the dose of agent likely to cause death in 50% of an exposed group) in the experimental (test) group to the LD50 of the agent in a control group receiving neither pretreatment nor postexposure antidotal treatment. A PR of 1 denotes no protective effect, and the higher the PR, the greater the protection afforded by the prophylactic and therapeutic strategy. Another way of expressing efficacy is to report the number of LD50s that a group receiving the pretreatment and treatment medications can survive. Note, however, that the PR value is technically not the same as the number of LD50s that an entire group would survive: A PR of 3 for a particular management regimen means that the LD50 of agent for a group receiving the regimen is three times as high as the LD50 of the agent without treatment; that is, only half (not all) of a group managed with the regimen would be expected to survive three LD50s of agent. It is possible that even with the best training and with good mask discipline, some soldiers on a chemical battlefield could be exposed to five LD50s of an agent (Dunn and Sidell 1989), and research has therefore focused on achieving PRs well over 5 (or

on achieving survivability after exposure to LD50s of 5 or greater).

In one study with male rhesus monkeys exposed to soman (GD), postexposure treatment with atropine and 2-pralidoxime chloride yielded a PR of only 1.64, consistent with the known failure of oxime therapy in soman poisoning. Administration of the same antidotes to soman-challenged monkeys who had been pretreated with enough PB to inhibit their red-blood-cell AChE by 20 to 45% resulted in a PR of well over 40 compared with an untreated group (Kluwe 1987). In a subsequent study, four out of five rhesus monkeys pretreated with PB, exposed to five LD50s of soman (GD), and then treated with atropine and 2-pralidoxime chloride survived for the 48 hours of the experiment (Koplovitz et al. 1992b). In a similar study, postexposure treatment with atropine, benactyzine hydrochloride (a related anticholinergic compound), and an oxime in PB-pretreated rhesus monkeys protected against more than 10 LD50s of soman (GD), although recovery in these cases was prolonged (von Bredow et al. 1991). Significantly, continuation of PB administration even after exposure to the nerve agent did not result in the return of clinical signs of cholinergic excess, although the loss of one monkey during a second exposure to soman (GD) suggested to the authors of the study that a minimum one-week recovery period may be required before a PB-treated subject would be ready to withstand another multilethal dose of soman (von Bredow et al. 1991).

The most dramatic increases in PR with PB pretreatment and postexposure antidotal administration are seen with exposure to soman (GD). PB also significantly enhanced the efficacy of atropine and 2-pralidoxime chloride against tabun (GA) both in mice and in guinea pigs (Koplovitz et al. 1992a) and achieved PRs of 4 to 12 in exposed animals (Joiner et al. 1988; Koplovitz and Stewart 1994), although a subsequent study (Worek et al. 1995b) warned that PB pretreatment in guinea pigs could enhance circulatory depression induced by postexposure treatment with atropine and oximes. The PR of postexposure antidotal treatment with atropine and an oxime in poisoning by GF was found to be 1.4 in the mouse and 2.7 in the guinea pig (Stewart and Koplovitz 1993); the addition of PB pretreatment in these species did not change the PR in the mouse and raised the PR in the guinea pig from 2.7 only minimally (and not significantly), to 3.4 (Stewart and Koplovitz 1993). However, in a separate study, PB pretreatment followed by standard postexposure antidotal therapy protected all ten rhesus monkeys (arguably a better model for human exposure) against five LD50s of GF in a study in which the mortality rate of monkeys not given PB but treated with atropine and 2-pralidoxime chloride was 40% (Koplovitz et al. 1992b). In the guinea pig, atropine and oxime administration after exposure either to sarin (GB) or to VX is very effective in the absence of pretreatment, with PRs of 36 and 59, respectively (Koplovitz et al. 1992a). PB pretreatment adds no benefit in subjects exposed to these agents. The slight decreases in PRs (to 35 and 47, respectively, with greater decreases in PR seen with higher doses of PB) with these agents are not statistically significant and would be unlikely to be clinically significant on the battlefield (Koplovitz et al. 1992a). Similar small but statistically insignificant decreases in PRs with VX were seen in rats (Anderson et al. 1992), and a more recent study (Worek and Szinicz 1995a) showed that PB pretreatment had a slight protective effect in guinea pigs exposed to five LD50s of sarin (GB), reduced the PR in animals exposed to ten LD50s of sarin, and had no effect in VX poisoning.

Pretreatment with PB therefore clearly improves the efficacy of postexposure treatment in subjects exposed to large doses of soman (GD) and tabun (GA), but it probably does not affect survival significantly one way or the other in subjects poisoned by massive doses of sarin (GB) or VX, both of which respond well to standard treatment with atropine and an oxime.

It would be logical to assume that PB pretreatment followed by exposure to sublethal doses of nerve agent might have a cumulative effect on AChE inhibition and might lead to clinical toxicity at lower-than-usual doses of nerve agent. For reasons that are poorly understood, this postulated effect has not been observed (Harris et al. 1984; Blick et al. 1991), and pretreatment with PB does not by itself alter the LD50 of nerve agents (Dunn et al. 1997). Nevertheless, PB pretreatment does not prevent subtle performance decrements (demonstrated in monkeys by the use of the primate equilibrium platform or PEP) associated with low doses of nerve agents (Blick et al. 1994a).

Several studies (Wills 1963; Deyi et al. 1981; Solana et al. 1990; Philippens et al. 1996) have shown that physostigmine is also an effective pretreatment agent, but because it easily crosses the blood-brain barrier it is associated with militarily unacceptable side effects involving the central nervous system (CNS). The advantage of PB over physostigmine on the battlefield is that penetration of the blood-brain barrier by PB is normally minimal. On the other hand, this advantage is also a drawback in terms of protection from the CNS effects of the cholinergic excess induced by nerve agents. PB-pretreated animals exposed to multiple LD50s of soman (GD) and treated with atropine and an oxime may survive only to develop prolonged seizures, long-lasting performance impairments, and histological evidence of brain damage (McLeod 1985). This is true despite the observation (Petrali et al. 1985, 1991) that exposure to soman (GD) rapidly renders the blood-brain barrier more permeable to a variety of substances, including, presumably, PB. The current doctrinal approach to this problem mandates the addition of postexposure anticonvulsant therapy with diazepam for severely intoxicated nerve-agent casualties (Dunn et al. 1997).

USE

☞ 11. How is PB to be used in a military setting?

Legal authorization for use of PB by military units comes from the Food and Drug Administration (FDA), which approved PB use as a wartime contingency measure under an interim rule that waived informed consent as unfeasible for PB administration during Operation Desert Storm (Keeler et al. 1991). The basis for the FDA ruling was twofold: a) the collected

body of experimental evidence from animal studies that indicated that PB pretreatment would save lives in humans by increasing the efficacy of postexposure treatment for soman (GD) and some of the other nerve agents; and b) the impressive safety record of PB in its long history of civilian use in dosages several times higher than those planned for battlefield use (Dunn et al. 1997). Although during the Persian Gulf War pretreatment against nerve agents was not a specifically approved indication for PB and although the status of PB was that of an Investigational New Drug (IND), the FDA waived informed consent in order to allow the best possible life-saving treatment to be given on the battlefield (Nightingale 1992). However, the FDA subsequently approved a modification to the PB product label (Pierson 1998), and additional documentation is currently in preparation to give military personnel the most recent and most accurate information available concerning PB and its use as a nerve-agent pretreatment.

In wartime, division- or equivalent-level commanders make the actual decisions of whether and when troops are to start PB pretreatment; these decisions follow assessments and recommendations from chemical, intelligence, and medical staff officers (Dunn and Sidell 1989). Although only two 21-tablet blister packs (enough for 14 days of pretreatment) are in the current initial issue of PB, current doctrine allows for continuous treatment of up to 21 days as long as periodic reassessments of need are conducted during that time. Because of the paucity of data concerning long-term administration of PB in healthy individuals, extension of the 21-day limit is permitted only after a senior commander has determined that the ongoing threat of nerve-agent exposure is an overriding consideration (Dunn et al. 1997).

In contrast to PB use in patients with myasthenia gravis, where the goal of therapy is to achieve a serum drug concentration of 50 to 100 ng/mL while avoiding the toxic effects seen at serum levels greater than 100 ng/mL, the endpoint of pretreatment with PB is not a specific serum concentration but rather a certain degree of reduction of AChE enzyme activity. The stated aim of PB pretreatment has been to achieve a continuous inhibition of 20% to 40% of erythrocyte AChE (Dunn and Sidell 1989; Kerenyi et al. 1990; Dunn et al. 1997; Ellenhorn 1997); this level of inhibition protects a sufficient amount of tissue AChE against subsequent nerve-agent challenge but does not usually produce significant side effects. However, PB may be just as efficacious at dosages that effect only a 10% inhibition of AChE (Lennox et al. 1985). In two separate studies, inhibition of erythrocyte AChE measured 150 minutes after a single 30-mg oral dose of PB in healthy men was 39% (±7%) and 40% (±7%) (Kolka and Stephenson 1990; Stephenson and Kolka 1990); and in a group of subjects taking PB according to the standard pretreatment regimen, whole-blood AChE inhibition averaged 33% four hours after ingestion of the fourth PB tablet (Epstein et al. 1990b). Troops receiving PB as pretreatment can therefore expect full benefit of the pretreatment within two or three hours after the initial dose.

Given a time to peak concentration of one to two hours after ingestion on an empty stomach and 2.5 to 3.5 hours after ingestion with food, and given an elimination half-life of 3.7 hours after oral administration, it would be logical to expect the PB serum concentration (which peaks at approximately 20 to 40 ng/mL after a single 30-mg PB tablet and probably at not over 60 ng/mL after several doses) to have declined to at least 25% of the peak concentration by the time of the next dose or by eight hours after the last dose (Aquilonius and Hartvig 1986; Benet et al. 1996). Serum concentrations of PB are relevant to a discussion of side effects from the drug; most if not all side effects from pretreatment doses should have disappeared within 8 to 10 hours after the last dose of PB. However, the duration of efficacy of pretreatment with PB is more accurately predicted by AChE inhibition than by plasma concentrations of PB. Following discontinuation of PB, AChE activity has been reported to return to normal within 12 hours (Moylan-Jones et al. 1979), so enhancement of antidotal efficacy by PB pretreatment will also be expected to have diminished significantly by 10 to 12 hours after the last dose of PB. The pharmacokinetic profile developed by Marino et al. (1998) includes an Emax model with an "effect compartment" related to PB-induced inhibition of AChE. Because the half-life ($t_{1/2}$) for the effect compartment is relatively "fast" at about twenty minutes, the delay between peak plasma levels and peak effect is brief, as is the delay between trough plasma levels and effect. The mean AChE inhibition during any given eight-hour dosing interval during this study was greater than 10%, but 30% of subjects exhibited less than 10% inhibition of AChE near the time of trough plasma levels. Despite this somewhat surprising finding, which has yet to be replicated, the study investigators concluded that the current dosage regimen of 30 mg of PB every eight hours still has utility in protecting soldiers against nerve agents.

Administration of PB at eight-hour intervals rather than simply as a thrice-daily (TID) dosage regimen (for example, at breakfast, lunch, and dinner) would be expected to avoid slightly greater daytime inhibition of AChE and slightly lower nighttime AChE inhibition (because of fluctuations in atmospheric conditions, chemical- and biological-warfare agents are probably more likely to be disseminated between dusk and dawn) and is still the recommended method of prescribing PB. However, because of the relatively low frequency of significant side effects from PB dosages slightly in excess of 30 mg and because of the experimental evidence suggesting that PB may still be effective at doses that inhibit less than 20% of AChE (Lennox et al. 1985), the consequences of substituting a TID regimen for a q 8h regimen may not be so serious as once believed.

Military personnel in the field will on occasion encounter situations that force them to delay or even miss a scheduled dose of PB. For example, chemical contamination may prevent soldiers from removing their masks to take a PB tablet. The most prudent course of action in such situations is probably to take a scheduled dose up to two hours late, according to the recommendation for patients with myasthenia gravis who forget a dose (Griffith 1996; Arky 1998), and then to take the next dose at its regularly scheduled time. Beyond two hours, either the dosing schedule should be reset for the individual or (the more practical course of action in a military setting and the recommendation of this publication) the affected individual should simply wait for the next scheduled dose. Doubling the next regularly scheduled dose may lead to an up-to-six-fold increase in plasma

PB, perhaps from saturation of PB metabolism (Calvey and Chan 1977), with a correspondingly steep drop in AChE activity. In a neurologically normal subject without myasthenia gravis, these changes could theoretically lead to the appearance of the nerve-agent-like signs and symptoms of a PB overdose, and while these effects are distressing enough by themselves they may also lead to a false diagnosis of nerve-agent exposure when the threat level is high.

ADVERSE EFFECTS

IN HEALTHY SUBJECTS

☞ 12. What are the effects of PB in healthy subjects?

PB can create cholinergic effects (that is, effects to be expected from ACh accumulation) by two separate but complementary mechanisms: a) overinhibition of AChE by PB may cause ACh to accumulate and to stimulate other nerves, smooth muscle, cardiac muscle, and exocrine glands; and b) PB, as a cholinergic agonist (that is, a compound that acts in the same way that ACh does), may directly stimulate the same end organs that ACh does (Gallagher et al. 1987; Shinnick-Gallagher 1987). Effects of overstimulation of ACh receptors either by excess ACh or directly by high concentrations of PB are the well-known signs and symptoms of nerve-agent poisoning. Increased stimulation of nicotinic receptors at the neuromuscular junctions of skeletal muscle may induce muscle cramps, fasciculations, and weakness (Anderson and Chamberlain 1988); classic muscarinic effects include hypersecretion (lacrimation, salivation, increased nasal and tracheobronchial secretions, and sweating), gastrointestinal hypermotility (leading to nausea, vomiting, abdominal cramping, flatus, and diarrhea), miosis, and bronchoconstriction (Keeler 1990). Headache, blurred vision, and urinary incontinence are also commonly seen (Almog et al. 1991; Leikin and Paloucek 1995). Very high doses may cause agitation, restlessness, confusion, visual hallucinations, and paranoid delusions; death may follow respiratory paralysis, pulmonary edema, and cardiac dysrhythmias (Ellenhorn 1997), although intestinal intussusception has been reported as the cause of death in beagles succumbing to overdoses of orally administered PB (Kluwe et al. 1990). Clinically evident toxicity is rare with serum levels of PB less than 100 ng/mL (Benet et al. 1996) and when erythrocyte AChE activity is reduced by less than 30 to 50% (Ellenhorn 1997).

In an Israeli study of nine cases of self-administered PB overdose during the Persian Gulf War, overdoses involving 390 to 900 mg of PB produced mild to moderate cholinergic effects that included abdominal cramps, diarrhea, emesis, nausea, hypersalivation, urinary incontinence, fasciculations, muscle weakness, and blurred vision. However, no central-nervous-system (CNS) effects were evident (the blurred vision was considered to represent a local effect on the eyes). Symptoms developed within several minutes of ingestion and lasted up to 24 hours. All nine soldiers underwent gastric emptying followed by administration of activated charcoal, but atropine (at doses of 1 to 8 mg) was required in only three cases. As with nerve-agent intoxication, inhibition of serum cholinesterase was a sensitive indicator of poisoning but was not clearly correlated with the incidence or severity of cholinergic signs. Treatment of PB overdose included general supportive care, gastric decontamination, and administration of 2 mg of atropine every five to 30 minutes as needed. Recovery of cholinesterase activity lagged behind clinical recovery. The authors of the study concluded that PB overdose is self-limiting and is well tolerated by young healthy adults (Almog et al. 1991).

Although nerve-agent poisoning has an unpredictable effect upon heart rate, PB overdose more commonly causes bradycardia than a normal or increased heart rate (Leikin and Paloucek 1995); and slowing of atrioventricular conduction may also occur. PB-induced bradycardia is not clearly related to the degree of AChE inhibition (Stein et al. 1997). In a study (Caldwell et al. 1989) that investigated the cardiopulmonary responses to PB, intravenous doses of 0.5 mg/kg (roughly equivalent to five to ten 30-mg NAPPS tablets in a 70-kg man given a bioavailability of 10% to 20%), 2 mg/kg, and 5 mg/kg were given to dogs. An increase in airway resistance was the first observable sign but occurred only with the two larger doses, which also decreased tidal volume but increased respiratory rate for a net increase in minute volume. These doses also decreased the heart rate, but stroke volume rose sufficiently to keep cardiac output constant. Because the lowest dose (0.5 mg/kg) produced minimal effects on either the respiratory system or the cardiovascular system, the investigators concluded that PB would not cause important effects in healthy humans when used for pretreatment. Hypertension following oral administration of PB is rare (Keeler et al. 1991).

When PB was administered at doses sufficient to inhibit 60% to 70% of whole-blood AChE in rats, electron micrography of neuromuscular junctions revealed changes in nerve-terminal branches, presynaptic and postsynaptic mitochondria, and sarcomeres (Hudson et al. 1985). The investigators were careful to point out that the PB-induced reduction in the area of presynaptic and postsynaptic surfaces did not necessarily imply a concomitant reduction in neuromuscular function. Two later studies in rats disclosed a PB-associated decrement in contractile force generated during tetanic stimulation of skeletal muscle and found evidence for a presynaptic action of PB on neurotransmitter release. This action opposed PB-induced inhibition of AChE, and in fact exposure of PB-treated rats to soman (GD) showed a skeletal-muscle response that was largely unchanged from the effects of PB alone (Anderson et al. 1986; Anderson and Chamberlain 1988). In contrast, Bowman et al. (1989) found that PB given to rats in doses sufficient to inhibit AChE by 74% to 91% had little effect on the ultrastructure of presynaptic areas of neuromuscular junctions but caused pronounced changes postsynaptically. However, these effects were not cumulative and had greatly diminished by 15 days even with constant PB administration and inhibition of AChE. Neither of two studies designed to investigate the effects of PB pretreatment upon skeletal muscle found any significant neuromuscular changes

at a dosage of 30 mg every eight hours for eight days in human subjects (Glikson et al. 1991) or for fourteen days in rats (Adler et al. 1992). In a more recent experiment (Lintern et al. 1997), subcutaneous injection of PB to mice caused variable changes in the activities of the predominant (G1, G4, and G12) molecular forms of AChE in the diaphragm and two other skeletal muscles. In experiments designed to study the effect in rat skeletal muscle of PB given at doses that would inhibit whole-blood AChE by 20% to 30%, two Belgian investigators found no deleterious effect on skeletal muscle in resting animals but biochemical changes (increased creatine phosphokinase and increased urinary excretion of creatine) suggesting a loss of integrity in skeletal muscles of exercised PB-treated rats (Hubert and Lison 1995). The applicability of these findings to humans is unclear.

Orally administered PB decreases rates of response in conditioned operant behavior in rats at doses insufficient to cause overt cholinergic crisis, but the lowest effective dose for performance disruption was 6 mg/kg, far higher than the PB pretreatment dose in humans (Shih et al. 1991). Evidence exists that at low doses in rats decrements in discrimination involve motivational dysfunction rather than motor impairment (Liu 1992). Another study in rats found a no-observed-toxic-level for PB of 5 mg/kg-day, equivalent to four times the daily PB pretreatment dose in humans (Levine et al. 1991b). A more recent study, in rhesus monkeys, concluded that behavioral toxicity from PB becomes significant for primates only at approximately four times the pretreatment dose (Blick et al. 1994b).

Blurred vision may be an effect of cholinergic excess whether from organophosphate (nerve-agent) exposure or from carbamate (PB) administration (Leikin and Paloucek 1995), but an investigation into the effect of three days of PB pretreatment on visual performance revealed no effect on lateral phoria, fusional vergence, or accommodative amplitude and only minimal effects on refractive error and pupil diameter. The conclusion of the investigators was that the use of PB at doctrinal doses for pretreatment would not significantly compromise a military aviator's visual acuity (Wiley et al. 1992). In a separate study, contrast sensitivities to laser interference fringes observed in the Maxwellian view (a technique that assesses the neural component of vision) were unchanged after oral administration 60 mg of PB in man. This study came to the conclusion that prophylactic use of PB could be given without a deleterious effect on stationary visual function (Kay and Morrison 1988).

A well-documented effect of PB when given as a single oral dose of 120 mg (four times the amount in a NAAPS tablet) is an inhibitory effect on somatostatin release by the hypothalamus (Thakore and Dinan 1995) and a resulting increase in growth-hormone (GH) responsiveness to GH-releasing hormone (GHRH) (Cordido et al. 1989; Arvat et al. 1997). The observation that both panic disorder (Cooney et al. 1997a) and depression (Cooney et al. 1997b) exaggerate this PB-mediated increase in GH in response to GHRH forms the basis for the use of PB challenges in the diagnoses of these disorders. The same dose (120 mg orally) of PB also has a stimulatory effect in obese but not in normal subjects on insulin secretion in response to a glucose load (Del Rio et al. 1997). PB given orally as a single 180-mg dose significantly augments secretion of GH, thyroid-stimulating hormone (thyrotropin or TSH), and prolactin in response to thyrotropin-releasing hormone (TRH), but PB doses of 60 or 120 mg do not have this effect (Yang et al. 1995). PB has also been reported to cause an increase in the ratio of serum alanine aminotransferase (ALT or SGPT) to serum aspartate aminotransferase (AST or SGOT) in addition to increasing serum levels of amylase (Leikin and Paloucek 1995). In one study, PB doses sufficient to produce a 25% to 30% inhibition of serum cholinesterase activity and a 15% decrease in heart rate also caused a transient 25% increase in sweating and a transient 12% decrease in metabolic rate in patas monkeys (Avlonitou and Elizondo 1988). PB administration in healthy patients may also lead to false positives in biochemical testing for pheochromocytoma (Hernandez Pascual et al. 1993).

At the currently recommended oral dose of 30 mg every eight hours, PB administered to healthy individuals occasionally causes a slight increase in flatus, occasional looseness of the bowels, and a decrease of approximately five beats per minute in the heart rate (Gall 1981). In a double-blind evaluation begun in November 1994, 90 subjects, equally divided by sex and weight class, took a 21-day course of PB at the pretreatment dosing regimen of 3 tablets every eight hours. Four subjects in the PB group reported diarrhea and abdominal pain; all other symptoms (headaches, dizziness, nausea, rash, and hair loss) occurred in both experimental (PB) and placebo groups. The overall incidence of adverse effects was the same for PB and placebo, and no differences between males and females or between weight groups could be found. One year later, follow-up identified no long-term effects except one rash that was treated and that then resolved (Lasseter and Garg 1996).

Some PB effects in a small number of apparently healthy individuals appear to be mediated by a rare variant of a kind of cholinesterase called plasma cholinesterase, pseudocholinesterase, or butyrylcholinesterase (BuChE). BuChE is not found in synapses or at neuromuscular or neuroglandular junctions, nor is it a component of red blood cells. It circulates in plasma, and its functions have not been completely elucidated. At least two forms of BuChE are known. Individuals who are homozygous for the predominant form, which is susceptible to inhibition not only by nerve agents but also by carbamates such as PB, include approximately 96% of the general population. Heterozygotes who have inherited one gene for the predominant or native form of BuChE and one gene for a variant that is markedly resistant to inhibition by PB represent 4% of the population; homozygosity for the resistant form of BuChE probably occurs no more frequently than 0.03% of the time. BuChE is also responsible for the metabolism of certain anesthetic medications such as succinylcholine and ester local anesthetics, and the PB-resistant form of the enzyme is also relatively incapable of metabolizing these drugs.

Individuals who are homozygous for the resistant form of BuChE generally have red-blood-cell and tissue AChE that is susceptible to PB inhibition. These individuals would therefore be expected to derive the usual benefit from PB pretreatment. However, an Israeli soldier who was homozygous for the PB-resistant form of BuChE and who had a history of prolonged apnea

after succinylcholine premedication for surgery developed cholinergic symptoms after taking PB for nerve-agent pretreatment (Loewenstein-Lichtenstein et al. 1995). Individuals who appear healthy but who report a history of prolonged effects from succinylcholine, ester local anesthetics, or cholinergic medications should therefore probably receive further assessment to determine their deployability to combat theaters where they may be required to take PB pretreatment or where they may be exposed to nerve agents or to cholinergic anesthetic medications (Dunn et al. 1997).

Hypersensitivity reactions are theoretically possible with almost any medication but are rare with PB. Bronchial reactivity may be increased in asthmatic patients but is the result of the muscarinic effects of PB on smooth muscle and glands already sensitized by reactive airway disease rather than a hypersensitivity to the medication *per se*. No photosensitivity or phototoxicity has been reported from PB use. A skin rash to the bromide portion of PB may occur on occasion; this rash usually subsides promptly when the medication is discontinued (Rothenberg et al. 1990; Leikin and Paloucek 1995; Arky 1998). It is unclear whether the bromide moiety or the pyridostigmine moiety of PB is responsible for the allergic contact dermatitis seen in some animals receiving PB from transdermal delivery devices; surfactants in the delivery device appear to potentiate this dermatitis (Harris and Maibach 1989). Because the results of studies of the overall incidence of unusual or idiosyncratic reactions to PB in healthy military-age individuals given one or two 30-mg doses of PB were found to parallel the experience of clinicians using PB in patients with myasthenia gravis, in which the incidence of these reactions is significantly less than 0.1%, no military populations currently receive test doses of PB to screen for adverse reactions (Dunn et al. 1997).

None of the limited number of animal studies performed with PB has yet demonstrated evidence of teratogenicity or mutagenicity from this compound (Dunn et al. 1997). PB was the subject of a group of studies designed to assess its reproductive and developmental toxicity in rats and including separate male and female fertility/reproductive-performance studies, a perinatal/postnatal study, and a teratology study. The high dose in each study was sufficient to induce overt cholinergic tremors. PB had no demonstrable effect on male or female fertility/reproductive performance and did not cause an increase in skeletal or visceral malformations. Pups born to treated dams showed slight, transient decreases in weight gain, but these decreases were presumed secondary to dysfunctional nursing by dams with overt tremors. The slight increase in delayed ossification and early resorption noted at the highest doses tested appear to have resulted from maternal stress or maternal toxicity rather than from direct effects on the fetus (Levine and Parker 1991a). Mention has already been made of the fact that PB can cross the placenta (Leikin and Paloucek 1995) and of the fact that PB concentrations in breast milk of nursing mothers range from 36% to 113% of the concentration in maternal plasma, implying a very low dose (approximately 0.1% of the dose per kilogram taken by the mother) to the nursing infant (Skoglund et al. 1978; Hardell et al. 1982). Because the safety of PB has not yet been fully established during pregnancy or lactation in humans, the Food and Drug Administration (FDA) considers PB a Class C drug (a drug in which risk cannot yet be excluded) (Dunn et al. 1997). Its use in women who may become pregnant requires assessing the potential benefits of the medication compared to its possible hazards to the mother and her child (Arky 1998). Nevertheless, when used in pregnant women with myasthenia gravis in doses far higher than those used for PB pretreatment and for periods of time much longer than those contemplated for wartime use, PB has not been linked to fetal malformations in humans (Briggs et al. 1990). Although the safety and efficacy of PB in children are also not proven (Arky 1998), PB has been used for decades in the treatment of childhood and neonatal myasthenia gravis without untoward effects (Oberklaid and Hopkins 1976; Calderon-Gonzalez et al. 1990). There is no evidence from animal or human studies that PB has any deleterious effects upon sexual desire (libido) or performance (including potency, ejaculation, and orgasmic response).

Long-term effects from anticholinesterases fall into three categories (Ecobichon 1996). The first syndrome includes neurobehavioral, cognitive, and neuromuscular effects that persist for several months to years after exposure to high concentrations of organophosphorus compounds. Evidence for these effects first came from a study (Spiegelberg 1963) of workers exposed to high but sublethal concentrations of nerve agents in German production and handling facilities during World War II. A second distinct category is a paralytic condition called intermediate syndrome and reported in Sri Lankan patients who attempted suicide by drinking the organophosphorus ester insecticides fenthion, dimethoate, monocrotophos, and methamidophos. In the third group, organophosphate-induced delayed neurotoxicity, or OPIDN, patients developed severe polyneuritis (beginning with mild sensory disturbances, ataxia, weakness, ready fatigability of the legs, twitching, and fasciculations and progressing in many cases through flaccid paralysis to a spastic paralysis with hyperreflexia). OPIDN results from exposure to certain phosphate, phosphonate, and phosphoramidate esters; implicated insecticides have included omethoate, trichloronate, trichlorfon, parathion, methamidophos, fenthion, and chlorpyrifos (Abou-Donia and Lapadula 1990). In all reported cases in humans, OPIDN followed accidental or suicidal exposure to massive quantities of insecticides. Biochemical studies of OPIDN showed that all of the involved insecticides were able to inhibit a neuronal carboxylesterase called neuropathic target esterase (NTE), which appears to serve a role in lipid metabolism in neurons (Johnson 1982). However, the fact that OPIDN is not seen after poisoning with nerve agents, which markedly inhibit NTE, suggests that NTE inhibition need not necessarily lead to OPIDN (Johnson et al. 1985; Lotti 1992; Marrs 1993).

Carbamate anticholinesterases such as PB do not inhibit NTE or lead to OPIDN-type neurotoxicity (Ecobichon 1996). A farmer who drenched himself with the carbamate pesticide carbaryl later developed a chronic polyneuropathy (Ecobichon 1994), and an elderly man taking cimetidine (known to inhibit hepatic metabolism of carbaryl) and exposed over eight to ten months to high concentrations of carbaryl dust in his home developed a variety of signs and symptoms including weakness, fasciculations, confusion, a stocking-and-glove peripheral neuropathy, and cerebral atrophy. Symptoms resolved after removal of the patient from the house but reappeared when he returned home. At the same time, inhibition of both plasma and erythrocyte AChE occurred, with slow resolution over two months (Branch and Jacqz 1986). It is important to emphasize that

these cases both involved extremely high carbaryl doses and that prolonged or delayed neurotoxicity is not associated with carbamates except under extremely unusual conditions or with exceedingly high doses. Except for a single report of a PB-rash that resolved after treatment (Lasseter and Garg 1996), there is no convincing evidence of long-term effects of PB in humans even at high doses, and the likelihood of long-term effects from PB at the doses used for pretreatment would appear to be vanishingly small.

☞ 13. What effects does PB have on military performance?

Testing prior to the fielding of PB during Operation Desert Storm in the Persian Gulf in 1991 had already examined the relationship between PB administration and the performance of military tasks. A review of British investigations found no PB-associated changes in memory, manual dexterity, vigilance, day or night driving ability, or psychological testing for cognitive and psychomotor skills (Gall 1981), and mention has already been made of the double-blind study in which Glickson et al. (1991) could not detect any significant neuromuscular dysfunction in healthy subjects demonstrating 20% to 30% inhibition of AChE from PB given 30 mg orally every eight hours for eight days.

The performance of military aviators pretreated with PB represented an active area of research beginning in the 1980s. Despite an early study that noted that PB-pretreated subjects had a slight performance decrement while trying to perform two tasks simultaneously and a slight decrement on a visual probability monitoring task (Graham and Cook 1984), a study reported three years later detected no significant changes in sensorimotor or cognitive functioning at ground level, 800 feet, or 13,000 in twelve healthy subjects after four 30-mg doses of PB (Schiflett et al. 1987a). Another study that was presented at the same conference found no adverse effect of PB on flight performance (Schiflett et al. 1987b), and the conference also included a presentation that showed that PB given in pretreatment doses had no deleterious effects on subjects exposed in a decompression chamber to mild hypoxia and rapid decompression (Krutz et al. 1987). In a subsequent double-blinded study by the same research group, 21 C-130 pilots taking 30 mg of PB every eight hours flew simulated tactical transport airdrop simulation missions. The pilots successfully completed their assigned missions without any airdrop inaccuracies or navigation errors attributable to PB and without any discernible effect on performance or crew coordination. In fact, the pilots and copilots could not discriminate beyond chance between PB and placebo conditions (Gawron et al. 1990). Three months before the Iraqi invasion of Kuwait in August 1990, an Israeli group reported the results of a crossover double-blind placebo-controlled investigation into the effects of 30 mg of PB given by mouth every eight hours on flight skills in ten pilots in actual and simulated A-4 flights. In a series of maneuvers that included rapid ascent, 360-degree turns, and instrument landing, there was no performance decrement in the aviators, whose mean whole-blood AChE inhibition was 29% (Izraeli et al. 1990). A later study used double-blind placebo trials to evaluate the effects of the 90-mg/day PB pretreatment regimen on +Gz acceleration tolerances and performance. Subjects were subjected to gradual-onset-rate accelerations of 0.1 G/s, a series of rapid-onset-rate exposures of 6.0 G/s, and a simulated aerial-combat maneuver of 4.5 to 9.0 +Gz. Despite AChE inhibitions of up to 45%, the study found no significant alteration of +Gz tolerance or performance (Forster et al. 1994). Reference has already been made to the studies (Kay and Morrison, 1988; Wiley et al. 1992) that concluded that PB pretreatment would not significantly compromise visual acuity or other measures of stationary visual function in military aviators.

Military personnel are expected to complete missions under a variety of environmental stressors, including exposure to heat from the environment or generated by strenuous activity. PB pretreatment under these conditions is not expected to have any serious untoward effects. Early investigators expecting to see adverse thermoregulatory effects of PB based upon previous data from intraperitoneal administration were surprised to find a PB-associated increased rate of weight loss but no further effects on thermoregulation or performance (Francesconi et al. 1986). Later, in two separate experiments, the heart rates of healthy males exercising 150 minutes after a single 30-mg dose of PB sufficient to inhibit AChE by approximately 40% were slightly lower (by an average of 9 beats per minute) than those of exercising control subjects who were not treated (at rest, there was a 7-beat-per-minute decrease in the pretreated group). PB did not affect heat production during exercise and did not change blood flow to forearm muscles, but sweating was increased relative to controls. In one study, skin blood flow in the pretreated group was 40% lower than in the exercising control group at 29 C and 30% lower when the ambient temperature was 36 C (Kolka and Stephenson 1990); and in the other study, skin blood flow in PB-pretreated subjects exercising at 29 C was 37% less than in nonpretreated exercising controls (Stephenson and Kolka 1990). The decreased blood flow to the skin was presumed to result from cutaneous vasoconstriction by ACh that had accumulated secondary to the PB-induced inhibition of AChE. The threshold for cutaneous dilatation in one study (Stephenson and Kolka 1990) was 37.0 C (±0.3 C) for the PB-pretreated group and 36.8 C (±0.3 C) for the exercising controls. Two studies by an Israeli group tested subjects four hours after ingestion of the last of four 30-mg PB doses given eight hours apart. Apart from mild bradycardia in the PB-pretreated groups, the first study found no significant PB-associated changes during exercise (Epstein et al. 1990a); the other study (Epstein et al. 1990b) demonstrated higher nonevaporative heat exchange in pretreated subjects exercising in chemical-protective clothing but no significant changes in other physiological responses. In another experiment, PB pretreatment in subjects undergoing heat stress tests in euhydrated and hypohydrated states reduced the rise in rectal temperature during hypohydrated exercise but had no significant effect on sweating, hematocrit, hemoglobin, total plasma protein, osmolality, *ad libitum* drinking, the rate of oxygen uptake, or subject ratings of temperature, discomfort, or exertion (Wenger and Latzka 1992). A more recent study from the same experimental institute subjected participants to four four-hour heat-stress tests (separated by 72 hours) in a hot dry environment (42 C and 20% relative humidity). In the first heat-stress test, there was no difference in heart rate between PB-pretreated participants and controls; but by the fourth test the pretreated exercisers had a mean heart rate 8 beats per minute lower than

controls. PB increased sweating and evaporative water loss by approximately 4% and lowered chest skin temperature during exercise by 0.7 C but had no significant effect on rectal temperature, other skin temperatures, oxygen uptake, or fluid balance (Wenger et al. 1993). In a two-week double-blind placebo-controlled crossover-design study in the desert, PB-pretreated soldiers performed moderate-intensity exercise requiring 40% maximal aerobic power. Except for fewer headaches in pretreated soldiers, there were no differences in symptoms between the experimental and control groups; and soldiers could not distinguish between PB and placebo. PB was associated with lower resting diastolic blood pressure (by 4 mm Hg), smaller pupil diameter (by 0.5 mm), decreased handgrip strength (by 3%), and higher final rectal temperature (by 0.1 C); but none of these effects was judged likely to impair performance (Cook et al. 1992). Finally, in an Israeli study designed to investigate the effects of PB pretreatment, chemical-protective clothing, and heat-exercise exposure on psychomotor performance and subjective sensations, exercise in the heat prolonged reaction time by 4.4% and actually enhanced by 7.3% the performance of vertical addition, but the effects of PB and of protective clothing were not significant, and no significant interactions between the variables were detected. Subjects from both the PB-pretreated and non-pretreated groups reported discomfort from wearing the protective clothing but did not show any major cognitive decrements (Arad et al. 1992b). The results of these experiments suggest that despite the fact that PB pretreatment reduces blood flow to the skin (an effect at least partly counteracted by increased sweating), pretreatment is unlikely to be responsible for important adverse effects upon military performance in environments generating heat stress.

Military personnel may also be exposed to cold. Two hours after taking 30 mg of PB, six men were immersed in cold water (20 C) for up to 180 minutes. AChE inhibition averaged 33% (±12%) ten minutes prior to immersion and 30% (±7%) at the termination of cold-water exposure. In both experimental and control groups, metabolic rate (which also increased with duration of immersion), ventilatory volume, and respiratory rate increased over pre-immersion values; but these values did not differ significantly between pretreated and non-pretreated groups. PB had no significant effect on rectal temperature, mean body temperature, thermal sensations, heart rate, plasma cortisol, or change in plasma volume and was judged not to increase susceptibility to hypothermia. However, abdominal cramping forced termination of the experiment in three of the six PB-pretreated subjects but did not require exposure termination in any of the controls (Prusaczyk and Sawka 1991). In a subsequent double-blind crossover experiment, healthy men receiving 30 mg of PB every eight hours exercised in cold air (5 C) at low and moderate exercise intensities. PB inhibition of AChE averaged 34% on the first day of the experiment and 43% on days 3 through 7. No differences between PB-pretreated and control groups were found in metabolic rate, body temperatures, or in regional-heat-conductance responses; and plasma glucose, glycerol, free fatty acids, lactate, sodium, and potassium were not measurably different between pretreated and control subjects. Moreover, there were no differences between acute and chronic experiments for any thermoregulatory or metabolic responses (Roberts et al. 1994). Therefore, except for its association with abdominal cramping, PB would appear to have no adverse impacts on military performance in cold-weather operations.

Data from actual experience during the Persian Gulf War, during which PB pretreatment was ordered for the first time, comes mainly from two studies, one conducted by investigators from the United States and one by an Israeli team. The U.S. investigation (Keeler et al. 1991) was a retrospective study that asked medical officers of the XVIII Airborne Corps to report signs and symptoms from nearly 42,000 soldiers (6.5% of whom were women) taking PB as pretreatment for nerve-agent exposure. Over 34,000 of these soldiers took PB for six to seven days, and compliance was estimated at well over 99% at the start of hostilities. Although there was no noticeable impairment of mission performance, over 50% of the 41,650 soldiers reported gastrointestinal symptoms such as flatus, loose stools, and abdominal cramps. Another 5% to 30% reported urinary urgency and frequency. Fewer than 5% complained of headaches, rhinorrhea, excess sweating, or tingling of the extremities; rare effects included bad dreams (five cases), worsening of acute bronchitis (three cases), headache (three cases), slurred speech (three cases), rash (two cases), vertigo (one case), bronchospasm (one case in a previously diagnosed asthmatic), and hypertension (two individuals). Only 483 soldiers (fewer than 1%) sought medical advice, and of these, PB was discontinued in only 28 soldiers (fewer than 0.1%). PB pretreatment was discontinued in 20 soldiers with intolerable nausea and vomiting, the three soldiers with exacerbated bronchitis, the two soldiers with rashes, the two hypertensive individuals, and the asthmatic soldier. The role of other stressors in the development of some of these symptoms is unclear. Two women each weighing approximately 45 to 50 kg reported increased salivation, severe abdominal cramps, nausea, sweating, and twitching; but whether these symptoms reflected an effectively increased dose in mg of PB per kg of body weight was not determined.

Apart from the nine PB overdoses reported by Almog et al. (1991) and mentioned previously, the chief source of information about acute effects of PB administration in Israeli forces during the Persian Gulf War is a report from Sharabi et al. (1991), who searched for PB-related signs and symptoms in a sample of 213 soldiers and also examined AChE levels. In this group, 75% of the soldiers reported symptoms, but dry mouth (71.4% incidence in the Israeli study), general malaise (53.4%), fatigue or numbness (37.2%), weakness (36.6%), and other relatively nonspecific symptoms were the most frequent symptoms, whereas the nausea (22.1% in the Israeli study), abdominal pain (20.4%), and frequent urination (11.3%) that were so prevalent in the American study were less frequently reported. No correlation between AChE levels and symptoms was found.

Although the role of stress and other psychological factors remained unclear in both the U.S. study and the Israeli study, it is of interest that the incidence of PB-related complaints in one American infantry battalion in which organic medical staff went from company to company to explain the mechanism of action of nerve agents and PB was significantly less than the incidence of such complaints in the two adjoining battalions in which classes on PB were not held (author's unpublished observation).

The results of the PB-related research from on and off the battlefield strongly suggest that PB used by military units in

wartime may cause a variety of annoying but not disabling signs and symptoms and that its use is unlikely to degrade mission readiness or impair mission performance significantly.

IN SUBJECTS WITH MEDICAL OR SURGICAL CONDITIONS

☞ 14. What are the effects of PB in subjects with medical or surgical conditions?

PB has long been used in patients with *myasthenia gravis* to increase ACh especially at nicotinic sites such as skeletal-muscle neuromuscular junctions; in so doing, PB (in doses usually much larger than those used for nerve-agent pretreatment) at least partially alleviates the weakness associated with this disease. Because of the possible cholinergic effects of PB on the gastrointestinal (GI) and genitourinary (GU) systems, recognized contraindications to PB administration are *mechanical GI or GU obstruction* (Leikin and Paloucek 1995; Arky 1998); and other gastrointestinal conditions such as *reflux esophagitis* and *peptic ulcer* may be exacerbated by PB. Although glucuronidation by the liver is not the major pathway by which PB leaves the body, it has been suggested that *liver disease* may be at least a relative contraindication (Institute of Medicine 1996). In uremic patients, there is no change in the volume of distribution of PB (Benet et al. 1996) but a decrease in plasma clearance (Cronnelly et al. 1980) and an increase in the elimination half-life (Benet et al. 1996), and the dose of PB should be decreased in elderly patients and in patients with significant *renal disease* (Arky 1998).

Hypertension by itself is not a contraindication to PB administration despite the case reports of symptomatic hypertension (with epistaxis in one case and profuse bleeding from a shaving nick in the other case) from Operation Desert Storm (Keeler et al. 1991). However, hypertensive patients may be taking medications that interact with PB, as may glaucoma patients [see Question 15].

Hyperthyroid patients given PB may develop atrial fibrillation, but although caution is recommended in general when giving anticholinesterase medications to patients with *inflammatory, infiltrative, or degenerative conditions involving the conduction system of the heart* (Dukes 1996), these conditions are not listed as specific contraindications to the administration of PB (Arky 1998).

PB should always be used with care in patients with *bronchial asthma*. In a double-blind placebo-controlled crossover study, subjects with mild bronchia asthma took 30 mg of PB orally and underwent pulmonary function testing at rest and after exercise. Although a significant decrease in heart rate was noted, no changes in respiratory function appeared when compared with the effects of the placebo. The effect of postexertional atropine inhalation on pulmonary function was also unchanged, and the investigators concluded that even though they could not exclude the existence of a subpopulation of asthmatic patients more vulnerable to PB effects, PB should be a safe drug for at least the majority of asthmatics (Ram et al. 1991). Similarly, an Army-sponsored randomized double-blind placebo-controlled crossover trial to evaluate the effects of the PB pretreatment dosage regimen on nonspecific bronchial hyperreactivity in ten normal nonsmokers, ten smokers, and ten mild asthmatics found no significant effect of PB in any of the groups (Roach et al. 1993). However, following reports of PB-associated exacerbations of asthma during the Persian Gulf War, 30 mg of PB was given to ten asthmatic and six nonasthmatic soldiers during the war (Gouge et al. 1994). No changes in forced vital capacity occurred in any of the soldiers, but seven of the asthmatics became dyspneic two to six hours after receiving PB (only three of the seven also exhibited wheezing). Because there were no controls and because the testing medical facility lacked the capability of performing more sensitive pulmonary function tests, the significance of these results is debatable and a placebo effect cannot be excluded. Confounders included fear (known to worsen asthma) and fine dust. Nevertheless, caution should still be used when administering PB to an individual with reactive airway disease, especially if other medications active at airway receptors are being used [see Question 15].

A significant proportion of battlefield casualties, including those having been pretreated with PB, will have *penetrating trauma with hypovolemic shock*. However, PB appears not to alter the hemodynamic responses to hypovolemia in PB-pretreated swine and does not appear to affect resuscitation (Wade et al. 1988). Even though up to 75% of ingested PB may be eliminated via the kidneys with the rest undergoing metabolism in the blood and the liver, hemorrhage-induced decreases in renal and hepatic blood flow have not been found to prolong PB elimination. This is despite warnings that because the kidneys are the main route of elimination for PB, lower doses and titration to clinical effect may be required in patients with renal disease (Arky 1998).

IN SUBJECTS RECEIVING OTHER MEDICATIONS

☞ 15. What are the effects of PB in subjects who are taking other medications?

Most of the possible interactions of PB with other drugs have not been investigated in great detail. *Antimuscarinics* such as atropine, glycopyrrolate, and scopolamine antagonize the effects of PB, and *other cholinergic agents* should potentiate the effects of PB, although this potentiation is not of equal magnitude at all cholinergic receptors. It might be expected that *beta-adrenergic antagonists (beta blockers)* such as propranolol might potentiate the decrease in heart rate produced by PB-induced

cholinergic actions on the vagal nerve, but a 1992 randomized double-blind crossover study of hypertensive patients being treated with beta blockers and also taking 30 mg of PB orally every eight hours could find no PB-associated effect on heart rate, plasma catecholamine levels, or resting blood pressure when compared with placebo. The rise in diastolic blood pressure with exercise was lower by 5 mm Hg in PB-pretreated subjects than in controls, but no clinical adverse effects were found (Arad et al. 1992a). These findings were confirmed for the beta-adrenergic antagonists propranolol, nadolol, and acebutolol in a more recent study involving mice injected with the drugs and then given PB (Chaney et al. 1997). However, respiratory effects were not investigated, and it is still possible that PB might interact with adrenergic agents active on the airways. The combination of PB and a nonselective beta blocker active at beta-2 receptors in airways has the potential to precipitate bronchospasm. Timolol is one such compound that may be encountered not only in patients with high blood pressure but also in glaucoma patients, as an agent to reduce intraocular pressure; and in a glaucoma patient with reactive airways, timolol and PB could theoretically cause bronchospasm. (It is of interest that four patients being treated with beta blockers [propanolol in two patients, oxprenolol in a third case, and practolol in the fourth patient] developed myasthenic symptoms of unknown origin and that two of these patients were treated successfully with PB [Herishanu and Rosenberg 1975].)

Chaney et al. (1997) also investigated a variety of other medications that might be used with PB and found that *clonidine*, an alpha-2-adrenoceptor agonist, did not decrease the LD50 (that is, did not increase the toxicity) of PB. However, *epinephrine* and *norepinephrine* (agents with agonist activity at both alpha and beta adrenoceptors) additively increased PB lethality when given before PB; and the following agents strongly potentiated the lethal effect of PB when given prior to PB:

Compound(s)	Class
isoproterenol	beta-adrenergic agonist
salbutamol (albuterol)	selective beta-2-adrenoceptor
terbutaline	selective beta-adrenoceptor (primarily beta-2)
yohimbine	alpha-2-adrenoceptor antagonist
phentolamine	alpha-2-adrenoceptor antagonist
prazosin	selective alpha-1-adrenoceptor antagonist
caffeine	stimulant

Pretreatment with atropine and atropine methyl nitrate abolished the potentiation of PB toxicity caused by these agents. The caution advised in the administration of *atropine* in a patient receiving PB pertains to patients with myasthenia gravis, where suppression of muscarinic side effects from PB may mask signs of overdose and lead to inadvertent induction of cholinergic crisis (Arky 1998); this situation will not obtain on the battlefield.

Antiarrhythmic drugs may interact with PB. The Type Ia antiarrhythmic agents procainamide, quinidine, and disopyramide each have a nondepolarizing blocking effect on skeletal muscle, an effect that PB would diminish. When given with disopyramide, myasthenic doses (180 mg orally every twelve hours) of PB completely prevent the anticholinergic side effects of disopyramide without affecting its antiarrhythmic properties (Teichman et al. 1985, 1987). PB used in combination with quinidine may accentuate atrioventricular block and secondarily lower blood pressure. Antimalarials such as quinine, quinidine, and chloroquine (chloroquine is used for malarial chemoprophylaxis in military units being sent to areas where chloroquine-sensitive malaria is endemic) may also potentiate gastric upset associated with PB and may lead to diarrhea. The type Ic antiarrhythmic agent propafenone opposes the effects of PB in myasthenic patients (Stockley 1994), but no studies of propafenone and PB in healthy subjects are known.

It is at least theoretically possible that PB could intensify the orthostatic hypotension sometimes seen with *calcium-channel blockers* or *direct-acting vasodilators* (Institute of Medicine 1996).

Griffith (1996) warned that *anesthetics*, *antiarrhythmics*, *anticholinergics*, *guanadrel*, *guanethidine*, *mecamylamine*, *nitrates*, *procainanamide*, *quinidine*, and *cocaine* could interact with PB but found no evidence for PB interactions with *alcohol* (in small doses), *marijuana*, *tobacco*, or *foods* (beyond the up-to-90-minute food-induced delay in absorption without a corresponding change in the area under the plasma concentration-time curve or AUC [Aquilonius et al. 1980]). However, some soldiers during the Persian Gulf War noticed a decrease in gastrointestinal side effects when PB was taken with a meal (Keeler et al. 1991).

A single case report exists of a PB-treated patient with myasthenia gravis who developed overwhelming weakness when treated with *methocarbamol* and whose weakness resolved upon withdrawal of the methocarbamol (Podrizki 1968). There is no evidence that methocarbamol has any significant interaction with PB in the majority of myasthenic patients or in healthy subjects.

Antibiotics with the capability of inhibiting the release of ACh at neuromuscular junctions include neomycin, gentamicin, tetracycline, clindamycin, and metronidazole. PB may oppose this effect, which is generally significant only in surgical patients undergoing nondepolarizing neuromuscular block during general anesthesia (Keeler 1990). In a double-blind randomized study involving healthy male volunteers receiving oral doses of PB and oxytetracycline alone and in combination, coadministration of the drugs led to no significant interactions involving the pharmacokinetics of each drug. However, a small

increase in erythrocyte AChE activity occurred when the two drugs were given together (Johnston et al. 1988). Interactions of PB with quinolone antibiotics have been reported only in patients with myasthenia gravis. In one case, a myasthenic woman taking 360 mg of PB daily progressively developed double vision, weakness of the neck and proximal muscle weakness of the arms and legs, dysphagia, weakness of the chest-wall muscles, and shortness of breath after beginning a course of norfloxacin for a urinary-tract infection; these symptoms promptly resolved after discontinuation of the antibiotic but reappeared six months later during another trial of norfloxacin (Rauser et al. 1990). Ciprofloxacin precipitated shortness of breath and weakness of limb and neck muscles in one myasthenic woman being treated with 180 mg of PB every four to six hours and unmasked previously unsuspected myasthenia gravis in a man whose symptoms were then relieved by edrophonium and PB (Stockley 1994).

The carbonic-anhydrase inhibitor *acetazolamide* when given as a diuretic (500 mg intravenously) has exacerbated the muscle weakness of patients with myasthenia gravis who were being treated with the anticholinesterase drug edrophonium (Carmignani et al. 1984). The mechanism of the interaction is unknown. No known cases of acetazolamide-induced weakness in PB-treated myasthenic patients are known, and patients with myasthenia gravis are of course not sent into combat. Nevertheless, given the potential of the use of acetazolamide in troops at high altitudes, this potential interaction should not be forgotten.

Patients taking *birth-control pills* or *corticosteroids* may exhibit plasma cholinesterase levels up to 50% lower than normal, but because plasma cholinesterase is not the form of cholinesterase that exists in the synapses and in the neuromuscular and neuroglandular junctions, depression of plasma cholinesterase will be important only insofar as it affects the metabolism of compounds such as succinylcholine and ester local anesthetics, which depend upon plasma cholinesterase for their metabolism.

The peer-reviewed medical literature generated during or after the Persian Gulf War or in association with illnesses of Gulf War veterans has not documented any interactions of PB with other medications or with caffeine.

☞ 16. What are the effects of PB in subjects undergoing general anesthesia?

A large number of anesthetic agents and adjunctive compounds are used in the practice of modern anesthesia, and the nature of their interactions with PB is an important consideration for anesthesiologists and nurse anesthetists providing care for casualties who may have been pretreated with PB. Keeler (1990) has provided the definitive review of this topic, and unless otherwise indicated the following points are based upon her findings.

Premedicants include cimetidine (a histamine-2 blocker administered preoperatively to raise gastric pH), hydroxyzine (a compound with anxiolytic, antiemetic, and opoid-potentiating actions as well as H1 antihistaminic properties), and promethazine (a phenothiazine with sedative, antihistaminic, anticholinergic, and antiemetic properties). Cimetidine is so specific for the histamine-2 receptor that it is unlikely to interact with PB in any clinically significant way. Hydroxyzine does not share the antinicotinic activity of diphenhydramine (another H1 blocker) and therefore would not be expected to decrease the effects of PB. Although the peripheral interaction with PB at muscarinic receptors should essentially counter the anticholinergic effects of promethazine, occasional cases of prolonged apnea have followed phenothiazine administration in combination with cholinomimetic agents such as organophosphates and succinylcholine. As a phenothiazine, promethazine may also inhibit cholinesterases, so this compound has the theoretical capacity to interact with succinylcholine and PB. Nevertheless, no cases of such an interaction have been reported to date.

Antimuscarinics such as atropine, glycopyrrolate (Robinul), and scopolamine decrease oral secretions, protect against laryngospastic vagal reflexes, and counteract bradydysrhythmias. Because antimuscarinics oppose the action of PB, a PB-pretreated patient will require larger-than-usual doses of these compounds.

The *barbiturate* generally used as an intravenous anesthetic induction agent is sodium thiopental (Pentothal), which because it can induce bronchospasm in asthmatics should be used with care in a PB-pretreated casualty. The occasional occurrence of vagally mediated thiopental-induced excitatory phenomena such as cough, hiccups, or laryngospasm is another reason to avoid thiopental when possible in PB-pretreated casualties or to precede its use with an antimuscarinic compound. Lastly, because thiopental can lower blood pressure at the same time that PB induces bradycardia, soldiers in hemorrhagic shock or with myocardial ischemic disease should be monitored carefully when receiving thiopental.

Benzodiazepines such as diazepam act chiefly in the central rather than the peripheral nervous system, but their mild cardiovascular depressant effects may sum with those of PB.

Droperidol (Inapsine) is a *butyrophenone* used alone as an antiemetic and with opioids to produce neuroleptanalgesia. It probably will not interact with PB.

The prototypical *arylcyclohexylamine* in anesthesia is the dissociative agent ketamine (Ketalar), which supports the cardiovascular system and also causes bronchodilatation. These effects would tend to offset the minimal cardiovascular depression and bronchoconstriction that PB may produce. However, since both ketamine and PB increase oropharyngeal secretions, and since ketamine leaves laryngeal reflexes intact, ketamine anesthesia in a PB-pretreated patients runs the risk of secretion-induced laryngospasm. Of interest but of dubious practical significance is the observation that ketamine offers a degree of protection against sarin (GB) *in vitro* (Puu 1988).

Representative *opioids* include fentanyl (Sublimaze), meperidine (Demerol), morphine, nalbuphine (Nubain), and naloxone (Narcan). Nalbuphine is a partial opioid agonist, whereas naloxone is the opioid antagonist used antidotally in cases of opioid overdose. The major peripheral effect of opioids is gastrointestinal hypomotility, an effect that opposes the action of PB but that is probably of little concern clinically in battlefield surgery. However, morphine-induced vasodilatation and fentanyl-induced bradycardia may be additive with PB effects. Meperidine, which characteristically produces tachycardia rather than bradycardia, may be a better choice of agent in a PB-pretreated casualty.

Neuromuscular blocking agents are used to facilitate intubation and to achieve surgical paralysis. They act at the nicotinic receptor of skeletal muscle and are of two kinds: a) nondepolarizing agents such as pancuronium bromide (Pavulon) and the intermediate-acting vecuronium bromide (Norcuron) and b) depolarizing agents such as succinylcholine (Anectine, Quelicin, Sucostrin, and Sux-Cert). As a competitive antagonist of ACh, pancuronium bromide acts in much the same way as atropine. Because PB increases the concentration of ACh in neuromuscular junctions (and thus at receptors in the motor end plate of the muscle), PB-pretreated patients may require more of a nondepolarizing agent to achieve satisfactory paralysis. The compound 4-aminopyridine, used as a bird repellent in Europe but not approved by the Food and Drug Administration for sale in the United States, has been used to reverse neuromuscular blockade from nondepolarizing neuromuscular blockers and from botulinum toxin, as an antidote in verapamil overdoses, and in the management of patients with incomplete spinal-cord injury, myasthenia gravis, multiple sclerosis, Eaton-Lambert syndrome, Huntington's chorea, and Alzheimer's disease. It potentiates the PB-induced antagonism of neuromuscular blockade caused by pancuronium (Miller et al. 1979).

In contrast, depolarizing agents such as succinylcholine act essentially as long-acting ACh. Because they are not broken down by tissue AChE, they cause continued stimulation of nicotinic receptors and a so-called Phase I block. It is not hard to see that less of a depolarizing agent might be required in a PB-pretreated casualty. Also, because succinylcholine is

metabolized by plasma cholinesterase (butyrylcholinesterase, or BuChE), which PB inhibits, the elimination half-time of succinylcholine may be prolonged in the presence of PB, although the clinical significance of this effect is unclear. Finally, succinylcholine and PB at a neuromuscular junction may convert a Phase I neuromuscular block to a Phase II or desensitization block, which resembles the block caused by a nondepolarizing blocking agent and can be reversed in its advanced stages by an anticholinesterase. The best way of assessing the various responses to neuromuscular blocking agents in a casualty pretreated with PB is to use a peripheral nerve stimulator.

PB-like *anticholinesterases* include edrophonium (Tensilon) and neostigmine (Prostigmin) and find their use in anesthesia as agents to reverse nondepolarizing neuromuscular blockade. In fact, PB itself (as Regonol) is used for this purpose at medical treatment facilities during peacetime, although it is not stockpiled for anesthetic use in wartime. Although in a PB-pretreated casualty more nondepolarizing neuromuscular blocking agent will be needed to achieve a block, reversal of the block should be attempted with a normal dose of anticholinesterase, because the ratio of blocking agent to ACh after establishment of the block is approximately the same as in an untreated patient. A separate use of an anticholinergic agent during anesthesia might be to treat paroxysmal atrial tachycardia (PAT); for this purpose, less of the anticholinergic will probably be required in a casualty treated with PB.

Inhalation agents exert most of their actions in the central nervous system, but although nitrous oxide has no known interactions with PB, the halogenated inhalational agents enflurane (Ethrane), halothane (Fluothane), and isoflurane (Forane) have to varying degrees a direct bronchodilating effect as well as the capacity to relax skeletal muscle by means of a nondepolarizing-like blockade and the ability to potentiate nondepolarizing neuromuscular blocking agents. Some of the halogenated agents can also potentiate succinylcholine. The bronchodilatation might relieve PB-induced bronchoconstriction, and use of isoflurane or enflurane in PB-treated patients should reduce the required doses of nondepolarizing blocking agents as well as the requirement for succinylcholine.

Local anesthetics include the esters or procaine-like compounds cocaine and tetracaine (Pontocaine) and the amide local anesthetics: bupivacaine (Marcaine), lidocaine (Xylocaine), and mepivacaine (Carbocaine). PB interacts with the ester local anesthetics by inhibiting the plasma cholinesterase (butyrylcholinesterase, or BuChE) needed for their metabolism. This BuChE inhibition results in a prolonged duration of action for cocaine and tetracaine but, more importantly, may raise serum concentrations enough to cause cardiovascular and central-nervous-system toxicity. Ester local anesthetics are therefore not recommended in casualties pretreated with PB.

The *miscellaneous agents* dantrolene (Dantrium, which is used to treat malignant hyperthermia and which acts directly on muscle) and doxapram (Dopram, which is used to stimulate ventilation postoperatively and which acts on central and peripheral chemoreceptors) should not interact with PB.

The *antibiotics* neomycin, gentamicin, tetracycline, clindamycin, and metronidazole inhibit ACh release at the neuromuscular junction, have postjunctional effects, and can potentiate the block produced by nondepolarizing neuromuscular blocking agents. PB may diminish all of these effects.

Downregulation of ACh receptors refers to the process in which overstimulation of ACh receptors (such as the nicotinic receptors on the motor end plates of skeletal muscle) leads either to decreased responsiveness of the receptors or to an actual decrease in their number. The consequences of such a process would be the most troublesome with neuromuscular blocking agents and could lead to interactions the opposite of the ones described. Chronic receptor stimulation by PB in PB-pretreated patients could theoretically lead to receptor downregulation, but no experimental confirmation as yet exists. The wisest course of action to take if such a process is suspected is to rely intraoperatively on a peripheral nerve stimulator to estimate the condition of ACh receptors in skeletal muscle.

In a PB-pretreated wartime casualty who presents for surgical intervention, a good understanding of the pharmacology of PB and anesthetic agents dictates that all administered drugs should be carefully titrated to effect. This by itself may be sufficient to prevent or at least to minimize adverse interactions. Secondly, because many of the interactions of PB with anesthetic agents result in overstimulation of nicotinic receptors, all PB-pretreated casualties should be atropinized before and during surgery. When local anesthetics are considered for brachial-plexus block or topical anesthesia, the use of an amide compound instead of an ester local anesthetic will eliminate potential toxicity from nondegraded local agent. Because the probability of an interaction with PB is the greatest for the neuromuscular blocking agents, careful monitoring of neuromuscular function by means of a peripheral nerve stimulator is essential for every procedure requiring general anesthesia and surgical paralysis. The use of vecuronium bromide as an intermediate-action nondepolarizing neuromuscular blocking agent should be considered in lieu of pancuronium bromide. Finally, remembering the possibility of PB-induced downregulation of ACh receptors may explain otherwise baffling reactions to administered anesthetic agents.

IN SUBJECTS EXPOSED TO OTHER SUBSTANCES

☞ 17. What are the effects of PB in subjects exposed to DEET and permethrin?

Because PB-pretreated military personnel during the Persian Gulf War were also potentially exposed to a wide range of other substances, attention has focused on those possible exposures and their relationship, if any, to illness. A 1995 initial report from the Committee to Review the Health Consequences of Service During the Persian Gulf War (Institute of Medicine 1995) contained a list of putative outcomes and a list of putative exposures, one of which was pyridostigmine. Another was pesticides. Military personnel assigned to the Persian Gulf were encouraged to spray their desert battle dress uniforms with the insecticide permethrin (3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid (3-phenoxyphenyl)methyl ester) and to use the insect repellant DEET (N,N-diethyl-*m*-toluamide) on their skin. It has been suggested that exposure to PB at pretreatment doses together with exposure to the insecticide (permethrin) and the insect repellant (DEET) might have caused some of the symptoms (combinations of fatigue, skin rash, muscle and joint pain, headache, loss of memory, shortness of breath, and gastrointestinal and respiratory symptoms) reported in Gulf War veterans, in their spouses, and in children conceived after the war (Dunn et al. 1997).

After the Gulf War, a sublethal dose of DEET was found in one study (U.S. Senate 1994) to exert a fourfold enhancement of PB toxicity; that is, in the presence of the sublethal dose of DEET only one-fourth of the usual amount of PB was required to kill cockroaches. Two years later, the converse was reported: nearly lethal doses of PB were found to potentiate the neurotoxicity of DEET and permethrin in hens (Abou-Donia and Wilmarth 1996a). The investigators suspected that increased competition for liver and plasma esterases by the three compounds led to their decreased breakdown and their eventual transport to nervous tissues. In a separate study, the same authors in collaboration with four other investigators performed more experiments on hens and found a synergistic relationship among the three compounds when administered to hens (Abou-Donia et al. 1996b). When McCain et al. (1997) gave PB, permethrin, and DEET concurrently to rats, they, too, found a significant increase in lethality when compared to simple additive values, suggesting a significant interaction among the three chemicals.

The Presidential Advisory Committee on Gulf War Veterans' Illnesses (1996) noted that the animal studies did not address the issue of morbidity in humans from the combined exposures or the issue of whether the three chemicals in the concentrations used operationally in the Persian Gulf could have resulted in harmful effects to soldiers. The committee pointed out that the doses used in the animal experiments were far greater than exposures that U.S. servicemen could possibly have experienced during the Gulf War and that the routes of exposure to DEET and permethrin (subcutaneous injection in the hens and oral administration in the rats) were not realistic given the circumstances of exposure in the Gulf, where permethrin would have been applied to uniforms and DEET to the skin. Permethrin from treated uniforms can leach onto underlying skin, but at the relatively low rate of 3.2% of the available permethrin per week (0.49% per day) (Snodgrass 1992).

However, Abou-Donia et al. (1996b) postulated that competition for xenobiotic metabolizing enzymes in the liver and blood may have raised blood concentrations of the three compounds and that in some way the high concentrations could have compromised the integrity of the blood-brain barrier in such a way that intracerebral concentrations of toxicants would be many times higher than expected from a given low external dose. At about the same time, Friedman et al. (1996) reported that mice subjected to a forced-swim protocol (previously shown to induce stress) underwent a dramatic increase in permeability of the blood-brain barrier such that the PB dose required to inhibit by 50% the activity of brain AChE was less than one hundredth of the usual dose. Moreover, *in vitro* studies demonstrated that brain slices exposed to PB showed increases in both electrical excitability and c-fos mRNA levels (Friedman et al. 1996).

A recent cross-sectional epidemiological study (Haley and Kurt 1997) investigated the association between self-reported wartime exposures and the development of certain syndromes. Statistically significant high relative risks and other measures of association were demonstrated for three separate exposure-outcome pairs: a) the wearing of flea collars + syndrome 1 ("impaired cognition"); b) advanced adverse effects from PB, belief of involvement in chemical-weapons exposure, and presence in a sector of far northeastern Saudi Arabia during the fourth day of the air war + syndrome 2 ("confusion-ataxia"); and c) exposure to DEET and advanced adverse effects from PB + syndrome 3 ("arthro-myo-neuropathy"). Recognizing the of cross-sectional investigations to prove causation, the study authors nevertheless suggested that some Gulf War veterans might have delayed, chronic neurotoxic syndromes from wartime exposure to chemicals that inhibit butyrylcholinesterase (BuChE) and the brain enzyme neuropathic target esterase (NTE).

Despite the assertions of the investigators who claim that a synergistic mix of chemicals (including PB) could have penetrated the blood-brain barrier and caused long-term chronic neuropathological and neuropsychiatric effects, there is at the present time no evidence that exposure to PB, alone or in concert with other compounds, has any long-term effects, especially in the absence of a severe acute intoxication. The final report of the Presidential Advisory Committee (1996) reiterates that "on-site medical personnel did not observe any immediate and severe effects of OP [organophosphate] poisoning among U.S. service members, and the current scientific knowledge base indicates that long-term health effects do not occur in the absence of immediate poisoning." More recently, a study from the Lawrence Livermore National Laboratory (Buchholz et al. 1997) used accelerator mass spectrometry (AMS) to study the effect of PB on the uptake of radiolabeled permethrin and found that orally administered PB actually lowered the levels of permethrin in the central nervous system. The investigators pointed out that their results were "inconsistent with [the] hypothesized synergy of such compounds as a precursor to 'Gulf War syndrome.'"

It seems prudent to recommend avoidance when possible of untoward levels of simultaneous exposure to incompletely studied combinations such as PB, permethrin, and DEET while at the same time realizing that currently available evidence is insufficient to warrant abandoning current doctrinal guidance concerning the military use of these compounds. It is also

important to realize that it is premature to conclude that PB is involved in any chronic or long-term effects from the Gulf War.

SUMMARY

☞ 18. What is the current state of medical knowledge concerning PB?

Pyridostigmine bromide (PB), a carbamate anticholinesterase, has been used for decades in neurology and anesthesiology. Its usefulness as a pretreatment for nerve-agent intoxication arises from its ability to bind a fraction of the body stores of acetylcholinesterase (AChE) so that the bound fraction is protected from nerve agent during a subsequent exposure. PB later dissociates from the AChE, which is then free to break down the excess acetylcholine (ACh) that has accumulated.

The pharmacokinetics (absorption, distribution, biotransformation, and elimination) of PB is known in some detail, as is the kinetics of the AChE inhibition produced by given doses. The efficacy of PB as a pretreatment against exposure to soman (GD) and tabun (GA) has also been well documented.

PB pretreatment in wartime is founded upon a clear understanding of both its safety and its efficacy, and clear guidelines exist for its use.

The effects of PB in pretreatment doses, high (myasthenic) doses, and overdosage are known and are understandable given a knowledge of the pharmacology of the compound. Many studies of its effects on military populations and on military performance under environmental extremes have demonstrated its safety and efficacy in these situations.

PB given to individuals who have medical conditions or who are taking other medications acts in predictable ways to produce its effects under these circumstances. A special case is combat anesthesia, in which a knowledge of the pharmacology of PB allows modification as appropriate of anesthetic protocol and the administration of the safest anesthetic agents to PB-pretreated surgical candidates.

The relationship of PB exposure in the Persian Gulf War to other possible exposures is not yet clearly delineated, but the weight of decades of pharmacokinetic and pharmacodynamic investigations does not suggest that PB causes chronic or long-term effects, especially in the absence of severe acute intoxication.