PB has been widely used in treatment of myasthenia gravis since it was licensed for this purpose by the FDA in the 1950s. No chronic adverse effects have been widely reported. However, there are two significant limitations to the evidence for safety in normal subjects: first, data from patients with myasthenia may not apply to normal subjects, or more specifically to PGW veterans; second, evidence of lack of chronic effects in patients with myasthenia is itself limited. Chronic adverse effects of time-limited administration of PB in large groups of normal individuals have not been studied, complicating the ability to comment on PB’s safety for this group.

EXTRAPOLATION OF DATA FROM MYASTHENIA GRAVIS

Chapter Three included some discussion regarding potential limitations in extrapolation of data on use of PB from myasthenia to those without myasthenia. This chapter reprises and extends comments regarding why information regarding effects of PB use in myasthenia may not ensure safety in PGW veterans.

First, use of PB in myasthenia helps normalize nicotinic cholinergic activity, while use of PB in nonmyasthenics drives nicotinic cholinergic activity away from normal and might therefore have different effects.¹

¹There is precedent for such regulatory changes with drugs affecting many neurotransmitter systems, and the time-courses of the consequent effects are variable. Some are short lived, while others are long-lasting. Beta-blocker withdrawal leads to rebound hyperactivity—a short-lived but clinically important effect. Administration of hypnotic drugs to assist with sleep is problematic because tolerance develops quickly—within a couple of weeks—followed by rebound worsening of insomnia or withdrawal of the drug, an effect of ill-defined time-course. Narcotics, such as heroin, have well-known acute withdrawal effects in addicts, but many patients and physicians are familiar with additional long-term—perhaps lifelong—effects in the form of increased pain sensitivity (reduced pain threshold) experienced by prior addicts, presumably a consequence of down-regulation of the opiate neurotransmission system. Administration of exogenous corticosteroids for as little as a week is known by clinicians to lead to relatively prolonged (potentially one year) down-regulation in the form of suppression of the adrenal response to stress. (Such patients must then be
Second, patients with myasthenia continue to receive PB for life. If down-regulation of the cholinergic system occurs, initially in response to excessive cholinergic activity (see Chapter Thirteen, “Neurotransmitter Dysregulation”), the consequences might not be detected in persons continuing to receive cholinergic augmentation. In myasthenia, the body is responding to high levels of ACh (and high ACh activity at muscarinic receptors, though activity may be normal or low at skeletal muscle nicotinic receptors) by driving ACh response down. As long as PB administration continues—and doses must often be increased as “tolerance” to PB occurs in myasthenia, suggesting development of downregulation—effects of low ACh response will be masked by the presence of pharmacologically induced high ACh. If PB were discontinued, low ACh effects would be unopposed by augmented ACh, and symptoms could result; however, it is seldom possible to discontinue use of PB in patients with myasthenia.

Third, several critical PB interactions further complicate the extrapolation of “safety” (lack of identified long-term adverse effects) from myasthenia to PGW veterans. For instance, under ordinary circumstances, based on animal studies (and to some degree supported by reports of symptoms in people), little PB will cross the blood-brain barrier because of PB’s charged quaternary ammonium structure. However, evidence (also from animal studies) suggests that more PB may cross the blood-brain barrier in the context of some forms of stress or severe chemical exposure (see Chapter Seven, “Blood-Brain Barrier”); stresses of this kind might more likely have been present for PGW veterans than for patients with myasthenia gravis (though whether stresses in the PGW were sufficient to induce similar effects is unknown). Thus, the de facto “exposure” from PB in PGW veterans may differ from the de facto exposure associated with PB use in myasthenics. Moreover, since PB may interact with other chemical exposures, for example by enhancing access of other chemicals to the CNS and by other chemical effects through competition for scavenging and metabolizing enzymes, chronic symptoms may result from the use of PB concomitantly with exposure to other chemicals, such as a low-level nerve agents, pesticides, or solvents. (See Chapter Nine, “Interactions Between PB and Other Exposures.”) For these reasons, effects of PB in the PGW context might be presumed to differ from effects in patients with myasthenia gravis. Moreover, while a number of

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given “stress doses” of corticosteroids when major stressors, such as surgery, are encountered.) Fenfluramine, a drug until recently given to enhance serotonergic function (until long-term side effects on heart valves were discovered) has been shown to be toxic to serotonergic neurons—an effect that is presumably long-lasting or permanent. Clearly, compensatory changes are made in regulation of many or most chemical-signaling systems in the body in response to pharmacological alteration of those signaling systems. The time-courses of these effects are highly variable. Moreover, different compensatory changes in response to the same pharmacological challenge may have different time-courses. As mentioned in previous chapters, the time-course of such effects for the acetylcholinergic systems have not been well defined.
studies in small samples of military volunteers followed generally for short time periods provide confidence that acute effects of PB in these populations are modest (see the discussion of side effects and associated table in Chapter Three), these studies also would be expected to miss effects of PB in the context of stress or interactions. (Such studies have typically evaluated such physiological parameters as heart rate and temperature, side effects, and effects on performance. Although some such studies have been done in the context of “basic training” for the Israeli military, such basic training is “routine” and is undergone by the whole population, unlike in the United States. Persons in the Israeli military, queried during a fact-finding mission to the Middle East, did not regard basic training as particularly stressful, but likened it to “camp.”) Indeed, evidence suggests that effects of PB in the context of war may indeed have differed from effects reported in military volunteers. In contrast to low rates of reported side effects in the latter group, a cross-sectional survey of Israeli soldiers who took PB during the PGW indicated quite high rate of symptom reporting (see Table 3.8) (Sharabi, Danon, et al., 1991).

Thus, differences in the circumstances of use between myasthenia patients and PGW veterans make it difficult to extrapolate evidence of long-term safety from myasthenic to nonmyasthenic subjects. Moreover, systematic evaluation of chronic effects of PB has seldom been performed for myasthenic PB users. The benefits of PB treatment in myasthenics (including maintenance of “activities of daily living” and prevention of death) so clearly outweigh the risks of use that subtle or even less-subtle adverse effects, if present, might fail to excite concern in patients or physicians, diminishing the ability to conclude that no long-term adverse effects are present. Furthermore, myasthenics have an identified neurological disorder, and evolving or chronic symptoms, if they occur, might be ascribed to the disease process or psychological response to this process, rather than to the treatment. The clear preponderance of benefit with PB treatment for myasthenics would render attempts to compare chronic consequences of active treatment to those of placebo in this group impractical and perhaps unethical. Nonetheless, if symptom-reporting serves as a guide, in fact more than 50 percent of myasthenia patients receiving PB report side effects they attribute to medication, including diarrhea and cognitive symptoms (Hood, 1990). Of particular note, myasthenia patients have also reported that adverse effects of anticholinesterase medications were aggravated by stress and by extreme hot weather or sun exposure—both factors reported in animals to enhance permeability of the blood-brain barrier to PB in some conditions, by taking the medication on an empty stomach, by lack of adequate sleep, and by exercising immediately after a dose, among other factors that may have occurred in the PGW setting (Hood, 1990). Other factors to which PGW veterans may have been exposed that were also reported by patients with myasthenia gravis to aggravate symptoms included use of aspirin and other unspecified
over-the-counter analgesics, use of Maalox and certain antibiotics, and consumption of dairy products, alcohol, and carbonated beverages (Hood, 1990).

Thus, many factors complicate the ability to compare chronic adverse effects in myasthenics and in normals. One class of chronic adverse effects from PB in myasthenics has been postulated. Specifically, research suggests that ultrastructural damage at the motor endplate resulting from use of PB is similar to ultrastructural damage at the motor endplate seen in myasthenics. It has been suggested that PB may contribute to these pathological changes, and thereby to the apparently reduced rate of cure seen in modern myasthenics. (The prevalence of myasthenia has risen significantly, despite no significant change in incidence or mortality (Phillips and Torner, 1996).) Evidence from animal studies clearly shows alterations in the motor endplates with use of PB—alterations that abate with continued use but may not disappear. Whether such alterations have clinical implications in humans is unknown.

In short, there exists “evidence” of the long-term safety of PB use by virtue of long-term use in myasthenics. But this “evidence” of safety consists primarily of absence of evidence of harm, in the context of absence of testing for harm, in persons in whom the evolution of some symptoms might be attributed to the underlying disease and in whom indefinitely continued use of PB might mask effects of cholinergic downregulation initially produced to help “normalize” function in the face of such use. Tests for chronic adverse effects of PB would be difficult in myasthenics because of the problems associated with generating adequate controls. Moreover, little data exists on what becomes of myasthenics who have taken PB and then ceased to use it, because this is seldom possible. Although controlled studies of the effects of PB in myasthenia are difficult for the reasons noted, observational studies do in fact suggest a high rate of side effect reporting in subjects with myasthenia gravis who receive PB (Hood, 1990). (However, they continue to receive it.) Meanwhile, chronic follow-up of large samples of military volunteers following short-term PB exposure, with neuropsychological tests designed to be sensitive for problems reported by ill PGW veterans or designed to sensitively test cholinergic function, have not been performed. Such factors render it difficult to exclude the possibility of chronic effects of short-term PB administration. Because PB may interact with other PGW exposures, for example enhancing access of other neurotoxic agents to the CNS, a contributory role for PB in long-term adverse effects due in part to

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2Similarly, use of corticosteroids leads to a hypoadrenergic state—seen primarily in the face of adrenal challenge—that is unmasked only when the agent is discontinued.

3Anecdotally, many patients with myasthenia do experience central symptoms that are incorrectly attributed by their physicians to effects of the myasthenia (Haley, 1988).
other agents must also be considered when the issue of chronic effects is approached.

The aim in this chapter is to evaluate the plausibility of chronic effects by examining the evidence of long-term effects associated with PB and with other AChE inhibitors, where such evidence is available. This evidence is by its nature hypothesis-generating and is not intended to prove or disprove a relationship between PB and chronic effects. However, it can be used to direct further inquiry. It should be noted that interactions with many other exposures may play a role in determining susceptibility to chronic, as well as to acute, adverse effects; and individual differences in drug absorption, metabolism, elimination, cholinergic regulation, and levels of scavenging enzymes may condition which individuals (if any) experience long-term deleterious consequences when receiving PB alone or in combination with other exposures.

In an effort to evaluate the plausibility of chronic effects from administration of PB, this chapter will focus on the following potentially pertinent areas of investigation: Chronic EEG changes with AChE inhibitors, chronic changes in regional cerebral blood flow, other alterations seen in imaging studies following exposure to AChE inhibitors, chronic neuropsychiatric effects following exposure to AChE inhibitors and pesticides, chronic neurologic effects following exposure to AChE inhibitors, chronic neurochemical changes following exposure to AChE inhibitors, and the relation of acute exposure to chronic symptoms with AChE inhibitors. The implications of these findings for possible chronic effects of PB in PGW veterans will then be discussed.

**EEG**

Studies have evaluated effects of exposure to AChE inhibitors (predominantly OP pesticides and nerve agents) on the EEG. Acute and chronic EEG alterations have been reported in several but not all such studies.

**Positive Findings**

EEG changes were seen in primates with chronic low dose administration of pesticides (given for 18 months) without clinical signs (Santolucito and Morrison, 1971). Changes were seen for all four pesticides tested, including the carbamate pesticide carbaryl. (The other tested agents were the organochlorines DDT and dieldrin and the OP parathion.) Doses were “1,000 times” estimated consumption by humans based on market basket surveys (estimates of per capita use based on pesticide purchases); carbaryl was tested at 0.01 mg/kg/d, and 1.0 mg/kg/d. EEG changes included increased bilateral synchrony, reduction in high-amplitude slow waves, and reduction in low amp-
Pesticide exposure may cause temporary fast waves in pesticide-exposed monkeys (Santolucito and Morrison, 1971). This study evaluated the effect of chronic exposure but did not in fact evaluate for chronic effects following termination of the exposure.

Such an evaluation has been done for the cholinesterase-inhibiting nerve agent sarin. Rhesus monkeys were exposed to a single high dose of sarin (5 µg/kg) or to 10 low doses, which did not produce any “major” clinical symptoms (1 µg/kg at one week intervals) or to a placebo. EEGs were recorded before exposure and at 24 hours and one year after. Both large and small doses of sarin produced significant and persistent increases in the relative amount of high-frequency beta activity (13–50 Hz) in the EEG, changes that did not occur in control animals (Burchfiel and Duffy, 1982). These authors also conducted a retrospective study in 77 humans with past industrial exposure to sarin, occurring more than 1 year previously, comparing EEG findings to those in 38 unexposed controls from the same plant. Increased beta activity was again seen in the sarin-exposed population, as was increased REM sleep. In addition, increased slow activity (0–8 Hz, delta and theta) and reduced alpha (9–12 Hz) were reported. EEG findings were more pronounced in the “maximally exposed” group (n = 41), who had experienced at least three separate sarin exposures over the previous six years, than in the overall exposed group, suggesting a dose-response effect. Results appeared bimodal, with one mode corresponding to the mode of the control population, suggesting that differences in exposure or susceptibility may condition which individuals experience these changes (Burchfiel and Duffy, 1982). Of note: increased beta is also seen during drowsiness and sleep, aging, organic brain lesions, and with many drugs which produce beta as part of generalized desynchronization (Burchfiel and Duffy, 1982).

For more on possible long-term effects of nerve agents, see A Review of the Scientific Literature As It Pertains to Gulf War Illnesses, Vol. 5: Chemical and Biological Warfare Agents (Augerson, forthcoming). Increased REM is a relatively uncommon pharmacological effect; many drugs, including alcohol, mood elevators, barbiturates, common tranquilizers, and amphetamine produce reductions in REM, but few produce increased REM. Indeed, REM sleep mechanisms are believed to be under cholinergic control, with procholinergic drugs reducing latency to REM and increasing the duration of REM (Sitaram and Gillin, 1979).

In a study of 300 persons industrially exposed to pesticides requiring medical care and 300 controls, 15 percent of pesticide exposed versus 1 percent of controls showed (undefined) EEG abnormalities (Amr, Allam, et al., 1993). Abnormal EEGs have been reported by several other investigators, both following acute OP toxicity (Holmes, 1964; Metcalf and Holmes, 1969; Brown, 1971; Grob and Harvey, 1953; Korsak and Sato, 1977) and following chronic exposures to
pesticides (Metcalf and Holmes, 1969; Korsak and Sato, 1977) or industrial exposure to sarin (Duffy, Burchfiel, et al., 1979).

Blinded assessment of EEGs in ill PGW veterans and controls, in sleep and awake, including assessment of beta-activity, is under way (Haley, 1998).

**Negative Findings**

No chronic EEG changes were reported in 100 OP-exposed cases compared to age, sex, SES, occupational level, and education-matched controls, despite pervasive differences in neuropsychological test results. However, this study does not discuss which EEG parameters were reviewed and compared (Savage, Keefe, et al., 1988). In one study of 20 ill PGW veterans, EEGs were performed as part of a battery of tests, and were stated not to demonstrate abnormalities. No control group was used, and there was no discussion of whether quantitative evaluation took place or of what EEG parameters were evaluated (Amato, McVey, et al., 1997).

Evidence supports the possibility that long-term changes in the EEG may occur following exposure to some AChE inhibitors, including the nerve agent sarin and the carbamate pesticides carbaryl. Some evidence involves relatively brief exposures or relatively low doses that do not produce overt chemical signs, although none involve long-term follow-up following a single low-dose exposure.

The EEG is a crude measure that is difficult to interpret. Chronic changes in this measure suggest widespread if subtle alterations in brain activity, but the relation to functional change is ill-defined. Moreover, the degree to which observed EEG abnormalities are agent-specific or represent the effect of exposure to AChE inhibition is unknown. Therefore, it cannot be stated that similar effects would be seen with PB exposure (although this is testable). PB exposure occurring together with pesticide or a low dose of a nerve agent might, by competing for scavenging and metabolizing enzymes, increase access of these other agents to the CNS and potentiate their ability to produce EEG effects, presumably resulting from alterations in underlying brain signaling.

**EFFECTS ON CEREBRAL BLOOD FLOW**

SPECT imaging has revealed abnormal regional cerebral blood flow in pesticide-exposed subjects. In one study, 40 cases (16 young, age 34±8, and 225 elderly, age 55±7) exposed to neurotoxic chemicals, including pesticides, glues, and solvents, and 30 controls (10 young and 20 elderly) underwent SPECT imaging. Results of Xenon regional cerebral blood flow showed diminished
cerebral blood flow in cases compared to controls, which were worse in the right hemisphere, with random presentation of areas of hypoperfusion more prevalent in the dorsal frontal and parietal lobes (Heuser, Mena, et al., 1994). The findings were reported to be significantly different from those in patients with chronic fatigue and depression. The authors suggest a primary cortical effect (Heuser, Mena, et al., 1994).

It has been suggested that SPECT might be useful for identifying disease and tracking the response to treatment in neurotoxin-exposed subjects. In one case report, a woman exposed to an insecticide mixture (pyrethrin, phosphorothioate, piperonyl butoxide, and petroleum distillates), who had delayed chronic symptoms (including coarse tremor, hemiballistic movements of the right arm and leg, flaccid muscular tone, fasciculations of muscle groups, muscle cramps, and sensory disturbances) underwent SPECT imaging 34 months after exposure. SPECT revealed significantly reduced blood flow to the left temporal lobe and the right and left basal ganglia. A trial of medication (amantadine and selegiline) resulted in dramatic reduction in dysfunctional movements and ataxia, and posttherapy SPECT revealed significantly improved blood flow, suggesting that SPECT may both reveal abnormalities and their reversal with treatment (Callender, Morrow, et al., 1994). This study is severely limited in that it is a single case report with single SPECT scans before and after treatment. Larger trials examining response of SPECT to treatment are desirable, but depend on the existence of a treatment modality.

The role of SPECT (or alternatively, functional MRI, which allows a view of regional cerebral blood flow over time in the course of specific tasks and activities) in evaluation of toxin-exposed individuals remains to be defined. Alterations in regional cerebral blood flow are seen in many conditions (Schwartz, Komaroff, et al., 1994; Simon, Hickey, et al., 1994; Ito, Kawashima, et al., 1996; Krausz, Bonne, et al., 1996; Galynker, Weiss, et al., 1997; Iidaka, Nakajima, et al., 1997; Schmitz, Moriarty, et al., 1997). Distinctions in regional cerebral blood flow have been reported between conditions in some instances. For example, major depression produces a different profile than that seen in chronic fatigue and healthy controls (Fischler, D’Haenen, et al., 1996). Additional work is needed to more clearly establish whether distinct patterns of deficit can be discriminated following neurotoxic exposures, and care will be needed to ensure blinded reading of SPECT images.

**Recommendations**

SPECT is a potentially important technique that may be sensitive to deficits produced with neurotoxins. This technique has been applied only to a very small group of ill veterans (see Chapter Eleven, “Multiple Chemical Sensi-
tivity”). Evaluation of a larger sample of ill PGW veterans, who may be compared to healthy controls in blinded studies, may be desirable. If abnormalities are found, these can be compared to observed SPECT abnormalities in patients with depression, pesticide exposure, and chronic fatigue. There is no consensus regarding the source of illnesses in these veterans, and hypotheses include neurotoxic exposure and infection-related chronic fatigue, as well as psychiatric etiologies and “exaggeration” (Kotler-Cope, Milby, et al., 1996), among others. Therefore, SPECT should be evaluated for sensitivity and specificity regarding these diagnoses. (Absence of ability of SPECT, in isolation, to discriminate among these diagnoses does not necessarily preclude the utility of this test. Just as an elevated white blood count is compatible with several causes, which are distinguished by other tests, so supplementary testing may be needed to clarify the source of abnormal regional blood flow on SPECT imaging.) Moreover, ill PGW veterans should perhaps be compared to controls to establish whether objective findings, in the form of differences in regional cerebral blood flow compared to controls, characterize ill PGW veterans. If SPECT imaging is found to correlate with self-reported symptoms, it might offer an objective technique, as an adjunct to self-report, to track the stability, progression, or regression of CNS dysfunction in ill PGW veterans who report cognitive defects.

NEUROPSYCHIATRIC EFFECTS

The existing literature is mixed regarding whether chronic effects ensue following acute or low-dose chronic exposure to AChE inhibitors, including OP pesticides, nerve agents, and PB—although on balance the literature may be viewed as favoring such long-term effects (Jamal, 1995a; Jamal, 1995b). Short-term low-dose exposures, such as those likely to have been experienced during the PGW, may be less likely to lead to chronic sequelae, though little testing of this possibility has been performed.

Pesticides

Some studies have failed to find increased incidence of psychiatric disorders (Stoller, Krupinski, et al., 1965) or serious sequelae in humans (Tabershaw and Cooper, 1966), or abnormal behavior in animals (Clark, 1971) exposed to OP pesticides. For example, one study of 49 pesticide applicators and 40 comparison subjects (not well matched on education or language preference) found that after controlling for baseline performance, only one subtest showed worse adjusted postseason performance in cases than controls after one six-month season of pesticide application, in a group with “generally low, intermittent, and well-controlled OP exposure” (Daniell, Barnhart, et al., 1992). (In Chapter Eight, it was noted that in another study of pesticide applicators, all had the
“resistant” PON phenotype, suggesting possible self-selection for the job; pesticide-sensitive individuals may selectively drop out and not undergo postseason testing. Something more akin to an “intention to treat” type of analysis, testing subjects before their first pesticide application and again later, would provide more persuasive evidence.)

In other studies, neuropsychiatric disturbances have been reported following acute (Rosenstock, Keifer, et al., 1991; Tabershaw and Cooper, 1966) or chronic low-dose (Richter, Chuwers, et al., 1992; Gershon and Shaw, 1961; Diille and Smith, 1964; Metcalf and Holmes, 1969; Rodnitzky, Levin, et al., 1975; Levin, Rodnitzky, et al., 1976; Maizlish, 1987; Korsak and Sato, 1977) OP exposure. For instance, EMG abnormalities have been reported in workers in OP production plants (Drenth, Ensberg, et al., 1972). In one study, of 300 persons industrially exposed to pesticides, only 30 percent were free of a set of neurological and behavioral symptoms compared to 80 percent of a group of 300 controls; and only 30 percent were free of a set of signs, versus 76 percent of controls (Amr, Allam, et al., 1993).

Chronic neuropsychiatric effects, including blurred vision, headache, weakness, and anorexia have been reported to persist in some workers months after exposure, when cholinesterase activity has returned to normal (Midtling, Barnett, et al., 1985). In one study, “poisoned” pesticide workers exhibited long-term deficits in memory, attention, visuomotor function, and motor skills (Rosenstock, Keifer, et al., 1991); in another, workers with chronic exposure to the OP pesticide fenthion showed significant changes in several neuropsychiatric tests (such as memory tests) suggestive of subclinical effects on cognitive function (Misra, Prasad, et al., 1994); exposed subjects performed worse on 15 of 17 tests, with equal scores on the remaining two. The differences were statistically significant in 7 of 17 tests.

Vibration sensitivity has been used as a model of sensory assessment in neurotoxicology (Maurissen, 1985). In one study, vibration thresholds in both hands (but not in feet) were significantly higher for 90 male pesticide “applicators” than for age-, sex-, and country-matched controls (Stokes, Stark, et al., 1995). Moreover, in pesticide applicators but not in controls, vibration thresholds increased dramatically with increasing age (Stokes, Stark, et al., 1995). Another study reports higher vibration thresholds, in hands and feet, in 36 OP-poisoned workers (McConnell, Keifer, et al., 1994).

Studies examining chronic effects following exposure to AChE inhibitors, particularly OP pesticides, have been criticized for not having one or more of the following: matched controls, complete documentation of acute exposures, sufficient quantitative neurologic and behavioral measures, and complete statistical analyses (Savage, Keefe, et al., 1988). A study that purports to correct these
defects, by comparing results on a large neuropsychiatric battery in 100 OP-exposed cases and 100 controls matched to cases on age, sex, education, SES, occupational station, and other factors, found pervasive but subtle differences between cases and controls on many outcome measures (Savage, Keefe, et al., 1988). For instance, cases were significantly worse than controls on four of five neuropsychological summary measures, including the Halstead Impairment index, Average Impairment Rating, Wechsler Adult Intelligence Scale Verbal IQ (p < .001), and WAIS Full Scale IQ (p < .001) but not the WAIS Performance IQ (p = .242). Cases performed worse on 18 of 34 subtest scores for the Halstead Impairment Index. Twenty-four percent of cases versus 12 percent of controls performed in the range characteristic of documented cerebral damage or dysfunction. Subjects' subjective assessment of functioning were significantly worse in cases than in controls in 10 of 32 aspects of language and communication, memory, cognitive intellectual functioning, and perceptual function; while relatives' assessments of patient functioning were lower on four of 31 items as well as in four of 22 personality scale items (depression, irritability, confusion, and social withdrawal). No differences were found on basic neurological examination or EEG (Savage, Keefe, et al., 1988). This study is limited by a lack of data confirming baseline comparability on these measures in cases and controls prior to OP intoxication—that is, cases could have had test scores significantly different from controls in these many measures prior to OP exposure.

The findings of impaired functioning in OP-exposed cases, with diminished functioning as rated by patients and relatives despite technically “normal” performance on many tests, recalls the relatively subtle (as regards test outcomes, though not necessarily subtle to patients) deficits reported by PGW veterans across a variety of functions despite normal performance on tests. While loss of function within the “normal” range may seem unimportant to an observer, it may critically affect functioning of the individual, whose cognitive strategies evolved to capitalize on a set of strengths and skills that may have become impaired. On reflection, the potential for loss of function is obvious: most people test in the “normal” range on most tests, yet an extraordinary range of differences in skills and in ability to perform selected tasks is seen from one “normal” individual to another. Performance on tests that is reduced, even if still “normal,” can clearly coincide with decrements in performance in daily tasks. (See Vol. 8: Pesticides (Geschwind and Golomb, forthcoming) for more detail on pesticide effects).

PB

One animal study, published in abstract form only, has evaluated persistent neurobehavioral effects of PB in rats. PB was given orally at a dose of 2 mg/kg
(about 4.7 Gulf War doses) for seven days. Startle responses to noise were assessed using whole body and eyelid EMG responses. These responses were unaffected 24 hours after the end of PB treatment, but enhanced startle responses emerged one to two weeks after treatment had ended (Natelson, Ottenweller, et al., 1996). These changes, though still present at 21 days, disappeared in 28 days (Ottenweller, 1998).

**Chronic Symptoms in Ill PGW Veterans**

If AChE inhibitors are capable of producing chronic neuropsychological or other deficits, this is relevant to ill PGW veterans only if some of these veterans demonstrate these deficits. Several studies using selected groups of veterans have shown such deficits, though results are not uniform. This section will review some studies that have evaluated deficits in ill PGW veterans, including both neuropsychiatric deficits and, in some instances, other deficits, such as fatigue. Before reviewing the positive studies, it should be noted that some studies have failed to support neuropsychological or muscular deficits in ill PGW veterans—or have attributed those deficits to psychological factors (Goldstein, Beers, et al., 1996; Amato, McVey, et al., 1997; Sillanpaa, Agar, et al., 1997; Vasterling, Brailly, et al., 1998). Unfortunately, failure to show significant deficits does not necessarily signify absence of true deficits. Such negative evidence is persuasive only if factors that favor high variance or bias toward the null (factors that will produce false negative results) have been eliminated and only if a persuasive case can be made that the outcome measures employed constitute sufficiently sensitive measures of any potential deficits. (This a more difficult case to make persuasively in the presence of studies using other measures that show positive evidence of deficits.) Specifically, spurious null results may result from use of a poor case definition, leading to misclassification bias (producing bias to the null); inadequate power (from too small a sample for the amount of variance) to show a meaningful difference (virtually no such study performed power calculations); use of outcome measures that are insensitive to the deficits of ill PGW veterans; and design flaws, such as lack of actual statistical comparison to a control group or inferences not supported by the evidence. None of the negative studies is free of these factors. Although positive studies have had their own design imperfections, future research endeavoring to understand illness in PGW veterans must follow the trail defined by apparent positive findings, seeking to replicate and extend these findings or to refute them. Less attention is accorded negative studies because their failure to

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4Although time-equivalents between animal species are function-specific, for many biological functions time periods in rats correspond to substantially longer periods in humans. However, neither the biological mechanisms for these particular changes, nor the correct time correspondence in humans—presuming these findings are preserved at all—have been elucidated.
demonstrate deficits does not preclude existence of deficits defined by other measures or using other case definitions or in larger samples.

A study (published only as a several-sentence abstract) of 55 PGW veterans complaining of cognitive dysfunction found relative weaknesses in California Verbal Learning Test, Boston Naming Test, and Category Test performance in veterans compared to standard scores (Kotler-Cope, Milby, et al., 1996). Only two subjects had “a significant number” of scores more than two standard deviations below the standard score mean. However, the abstract does not state what constitutes “a significant number,” in what group standard scores were derived, and whether the standard score group constitutes an appropriate comparison. The authors conclude that results are not consistent with a neuropathological process and suggest the influence of other factors, such as exaggeration, low premorbid functioning, and emotional distress. It is difficult to evaluate this study or these conclusions without more details regarding the study.

One survey of more than 1,000 (self-selected) PGW registry veterans is under way; preliminary evidence suggests a link between reported illnesses or symptoms and self-reported PB exposure. Moreover, a dose-response effect is suggested by greater reports of symptoms in those with reports of longer duration of PB exposure (Ottenweller, 1997). Although studies of this type are necessarily limited by self-selection and possible recall bias (at least some recollected doses are likely to be in error, since subjects have rarely reported taking PB tablets for as long as six months), self-report remains the best available measure for exposures in the PGW.

Several published peer-reviewed studies of selected PGW veterans find evidence of impaired neuropsychological function compared to controls, at least in the included subset. A pair of studies by Haley et al. found that report of side effects with PB use was associated with two of three primary factor-analysis derived syndromes in PGW veterans (Haley and Kurt, 1997), syndromes termed confusion-ataxia and arthro-myo-neuropathy (Haley, Kurt, and Hom, 1997). This study is limited by self-report of PB symptoms.

A third study from this group examines 23 veteran cases and 20 veteran controls (10 deployed and 10 not, from the same Naval Mobile Construction Battalion) in a nested case control study. The study found impairment in ill PGW veterans compared to controls on both of two global measures of brain dysfunction, the Halstead Impairment Index (p < .01), and the General Neuropsychological Deficit Scale (p < .05). Performance was worse on an assortment of other tests. Of 35 tests out of 165 on which cases and controls differed significantly or borderline significantly (p < .01 by Mann-Whitney U test), cases were more impaired than controls in 27 (p < .001 by binomial test). There were significant
differences on 20 of 89 tests with endpoints that did not depend on volitional action by subjects (such as evoked potentials); cases were more impaired than controls on 18 of these. There were significant differences on 15 of the 76 tests with endpoints depending on volitional action; cases were more impaired on nine and controls on six (Haley, Hom, et al., 1997); findings were similar for those with each of the three factor-derived syndromes. Worse performance in ill PGW veterans was thus more rather then less evident in tests in which volition did not play a role, lending support to the substantive nature of the findings.

A fourth study from this group tested 26 cases and 20 controls (half deployed and half not, from the same battalion) on a different set of neuropsychological studies. The results were similar. A trend or effect toward greater impairment in cases than control was found in nine of nine measures of global brain function and 16 of 26 measures of specific brain function (in six there was no difference, in three a trend toward better performance, and in one significantly better performance). Of 71 total measures, PGW veterans performed worse on 59, significant by the binomial test at p < 10^{-8} (Hom, Haley, et al., 1997). Of note: psychological profiles in ill veterans were found to be similar to those in general medical patients. The authors express the view that neuropsychological abnormalities seen in PGW veterans are likely to result from neurotoxic exposures in the PGW.

A study by a different set of investigators, with no control population, is potentially consistent with the construct of “confusion ataxia” syndrome described by Haley et al. (1997), in that, in a group of tested PGW veterans, motor incoordination (“ataxia”) was significantly correlated with general cognitive functioning (p < .01) and executive functioning (p < .01) (Sillanpaa, Agar, et al., 1997). (There were few other correlations among assessed measures.) It is difficult to draw conclusions regarding either chronic symptoms or a relation to PB from this study: no control group was tested, and the measure of “exposure” was an averaged measure derived from self-report of exposure to each of the following: oil fire smoke, armored vehicles, shells, shell explosions, land mine explosions, battlefield sites, and burning enemy vehicles, sandfly bites, eating inadequately refrigerated food, and contact with reptiles, warm-blooded animals, prisoners, and corpses, as well as contact with unpurified water. (PB was not included as an exposure.) Obviously an averaged exposure measure of this type is inappropriate for assessing exposure-outcome links. However, such a measure may be useful for evaluating recall bias. The fact that this measure of exposure did not significantly predict neuropsychological variables and subjective complaints suggests against an important role for recall bias in exposure-outcome links for PGW veterans.
One study compared findings for 14 ill PGW veterans and 13 healthy civilian controls (matched on age, sex, physical activity, and handedness) on predefined outcome measures. The mean score for symptoms was substantially greater in the PGW veterans, and the mean score for clinical signs was also statistically significantly greater ($p < .02$). Of 22 individual tests performed, two were statistically significant after Bonferroni adjustment for multiple comparisons—namely, cold threshold and median nerve amplitude (Jamal, Hansen, et al., 1996).

Some studies have failed to demonstrate differences in neuropsychological function between ill veterans and controls. These differences in result may reflect subject selection and/or test sensitivity. Nonetheless, the existence of several studies finding objective evidence of impairment, albeit in selected subgroups of ill veterans, merits concern. Because there need be no single “Gulf War Syndrome,” but rather there may exist several or many illnesses with distinct causes, it is appropriate to select those individuals with subjective neuropsychological complaints to undergo objective testing, just as would occur in the civilian population. Then it remains to be addressed whether frequency of these deficits is greater in ill PGW veterans than in nondeployed individuals, in those who received PB, or in those who responded adversely to PB.

Recently (since this report was initially sent for review), additional studies have found statistically significant alterations in neuropsychological function in defined sets of ill PGW veterans. These data are briefly reviewed here.

One study examined neuropsychological function in three cohorts of veterans including two followed since their return from the Gulf (abstract only) (White, Krengel, et al., 1998). These included the Fort Devens Reunion Survey sample, n~3000; a similar sample in New Orleans; and a cohort of National Guard members from Maine deployed to Germany rather than the Gulf. Deployment to the Gulf was reportedly associated with lower neuropsychological test scores on a number of tasks, which could not be explained on the basis of mood results or stress. The authors found relationships between self-reported exposures “to certain chemicals” and lower scores on specific neuropsychological tests, although the chemicals and tests are not named, and the results must in any event be viewed as exploratory and requiring subsequent confirmation.

Fatiguing symptoms have been considered a part of a Gulf War “syndrome” in ill PGW veterans by several investigators (Nicolson and Nicolson, 1995, 1997), and a case definition highlighting symptoms of fatigue, along with cognitive alterations, showed strikingly differential prevalence between deployed and nondeployed veterans (Fukuda, Nisenbaum, et al., 1998). In a study (abstract only) comparing performance on neuropsychological tests in registry veterans with fatigue as a major complaint who fulfilled clinical case definitions for
chronic fatigue syndrome, idiopathic chronic fatigue, and/or multiple chemical sensitivity (n = 44) to performance of healthy PGW veteran controls (n = 23), group performance was found to differ on several tests, including the WAIS-R (Wechsler Adult Intelligence Scale) Digit Span Forward task, a simple Backwards subtest, the NES Complex Performance Test, and the PASAT (Paced Auditory Serial Addition Task) (Lange, Tiersky, et al., 1998a). Scores on a test of executive function, the Trail Making Test, were also significantly worse in fatigued than healthy PGW veterans. A strong trend toward significance was observed on all measures assessing visual-perceptual function. No verbal or visual learning or memory problems, or fine motor function problems (assessed by the Grooved Pegboard Test) were identified between the groups. There were no significant differences in alcohol consumption between the groups, with a trend toward lower consumption in fatigued veterans (Lange, Tiersky, et al., 1998a). Difficulties were believed similar to those in civilians with fatiguing illness.

Not unexpectedly, cognitive performance in another study was found to be worse in persons diagnosed with PTSD than in controls (Storzbach, Binder, et al., 1998). However, other studies have found that psychiatric disorders cannot explain symptoms in PGW veterans with fatiguing illness.

**PSYCHIATRIC DISORDERS CANNOT EXPLAIN SYMPTOMS IN PGW VETERANS WITH FATIGUING ILLNESS**

Registry veterans with fatigue as a major complaint who fulfilled case definitions for chronic fatigue syndrome, idiopathic chronic fatigue, and/or multiple chemical sensitivity (n = 53) were compared in DSMIII-R diagnoses to healthy PGW veterans (n = 42), who did not report health problems since the war. Forty-nine percent of fatigued PGW veterans had psychiatric diagnoses similarly distributed to those in healthy veterans, while the profile was significantly different in the remaining 51 percent (abstract) (Lange, Tiersky, et al., 1998b). Thus, psychiatric problems were present in some ill PGW veterans, but were not required for development of fatiguing illness.

Moreover, although PGW veterans who had psychiatric illness (in addition to fatigue, n = 29) were, as might be expected, the most impaired on measures of psychological well-being, they did not demonstrate reduced physical functioning compared to other fatigued veterans (n = 19), in whom reduced function and well-being were observed relative to healthy PGW veterans (abstract) (Tiersky, Tiersky, et al., 1998b). Thus, psychiatric disturbance did not appear to be responsible for functional decline in fatigued PGW veterans.

Consonant with these results, another study in a stratified subset of PGW veterans (n = 198) reporting high or low rates of health symptoms related to their
Gulf War deployment found that although PTSD and major depression were each significantly linked to report of health problems, nearly two-thirds of those reporting moderate health problems and one-third with severe health problems had no psychiatric diagnosis (Wolfe, Proctor, et al., 1998c). Moreover, comparison to personnel deployed to Germany rather than the Gulf showed that health symptom rates for PGW veterans were higher regardless of psychiatric status. This study also reported that lifetime PTSD and major depression (7 percent and 23 percent respectively) were “comparable to those provided by recent national comorbidity studies, suggesting no appreciable elevation of psychiatric disorders” (Wolfe, Proctor, et al., 1998c). In fact, however, unless age adjustment was made, an increase may have been present. It is crucial to reinforce that psychiatric symptoms can be sequelae of physical exposures and illness that may be part of or distinct from the causal pathway for other symptoms and that coexistence of psychiatric conditions must be considered as a possible concomitant and not exclusively as a possible cause.

**NEUROLOGIC EFFECTS: OP-INDUCED DELAYED POLYNEUROPATHY AND INTERMEDIATE SYNDROME**

Intermediate Syndrome and OPIDN represent examples of delayed and long-lasting or permanent clinical conditions that arise following exposure to selected OP AChE inhibitors. (For details, see Vol. 8: Pesticides (Geschwind and Golomb, forthcoming).) A brief discussion of these conditions is included to show instances of identified conditions in which delayed neurological illness follows neurotoxic exposure.

**OPIDN**

Some investigators perceive symptoms in PGW veterans to be consistent with OPIDN (Hom, Haley, et al., 1997). OPIDN illustrates that delayed and long-lasting clinically significant effects of AChE-inhibiting agents may occur. Classic OPIDN is not classically associated with PB or other carbamates in isolation (though the view has been put forward that all agents capable of inhibiting the enzyme “neurotoxic esterase” may have the potential to do so (Moretto, Bertolazzi, et al., 1992)). Indeed, carbamates given before some OPIDN-producing OPs may confer protection against OPIDN; however, carbamates given after OPs may enhance the severity of OPIDN or even induce OPIDN that would otherwise not have occurred (Lotti, 1991; Lotti, Caroldi, et al., 1991; Lotti and Moretto, 1993; Moretto, Bertolazzi, et al., 1994). Moreover, carbamates may induce OPIDN when potentiated by other agents (Moretto, Bertolazzi, et al., 1992). Symptoms of classic OPIDN are not consistent with symptoms reported
by most PGW veterans. Similar or unrelated mechanisms of delayed neurotoxicity could conceivably play a role in these conditions.

Briefly, OPIDN is a condition in which chronic combined central and peripheral axonopathy with secondary myelinopathy, leading to combined sensory and motor deficits, occurs one to several weeks following exposure to certain OPs and lasts for months or sometimes permanently. Clinical symptoms of OPIDN are divided into a progressive phase (primarily a peripheral neuropathy) lasting three to six months after symptom onset, first involving pain, burning, tingling, or tightness in the lower extremities followed later by hypoesthesia in a “stocking and glove” (or “stocking” only) distribution; weakness of legs possibly spreading to the hands; foot drop, with steppage gait, positive Romberg, and in severe cases bilateral flaccid paralysis (paraplegia or quadriplegia) (Abou-Donia and Lapadula, 1990). This is followed by a stationary phase, lasting from three to 12 months after symptom onset, in which sensory symptoms disappear but paraplegia or quadriplegia persists, and an improvement phase, from six to 24 months after onset of neurological deficits, with improvement occurring in reverse order to symptom onset. In mild cases, recovery is complete. In severe cases, hands show great improvement but paralysis remains below the knees. Later stages of neurologic deficits involve central, not peripheral lesions, in the spinal cord. These persisting lesions are unmasked as peripheral neuropathy is diminished, and are characterized by spasticity (excessive muscle tone) and exaggerated knee jerk (Abou-Donia and Lapadula, 1990).

OPIDN also occurs preferentially in some species (e.g., rats are relatively refractory; cats, primates, and hens develop OPIDN more readily). It is thought that induction of OPIDN requires at least 70 percent inhibition of the enzyme neurotoxic esterase (NTE, also called neuropathy target esterase), a membrane-bound carboxylesterase. Not all agents that inhibit NTE equally produce OPIDN, and, like carbamates, those that do not typically produce it protect against it if administered before a neurotoxic OP (Pope, Tanaka, et al., 1993; Pope and Padilla, 1990). Of note, inhaled sarin (5 mg per cubic meter for 20 minutes a day for 10 days) produced OPIDN (first symptoms occurring on day 14) in female mice. The report does not cite the presence of clinical symptoms prior to development of delayed neurotoxicity (Husain, Vijayaraghavan, et al., 1993). “Nonneuropathic” OPs, as described above for carbamates, may actually potentiate OPIDN if administered after the neuropathic OP (Pope, Tanaka, et al., 1993; Pope and Padilla, 1990); or may even produce it in animals who would not have been susceptible from the neuropathic OP exposure alone (Pope, Tanaka, et al., 1993). The mechanism of potentiation is unknown but has been proposed to be independent of NTE (Moretto and Lotti, 1993).
Intermediate Syndrome

While we naturally perceive the state of science and medicine to be advanced (perhaps because the only comparison we have is the past, in which science was, almost by definition, less advanced), many medical conditions and biochemical factors, even critical ones, continue to be defined. Thus, *Helicobacter pylori* was recently discovered as the major cause of peptic ulcers;\(^5\) leptin was recently identified as a hormone;\(^6\) and human pheromones were only just shown to exist (Stern and McClintock, 1998). Likewise, neurotoxic effects, like other medical conditions, are continuing to be defined.

Intermediate syndrome is an instance of a condition of delayed neurotoxicity that was described relatively recently. Like OPIDN, it is a condition in which delayed symptoms occur following AChE inhibitor exposure. Although the symptoms and time-course are not consistent with illnesses commonly described in PGW veterans, intermediate syndrome will be reviewed as additional example of (mildly) delayed clinical effects that may occur with AChE-inhibiting compounds.

Intermediate syndrome (also called type II paralysis) results from an OP exposure and causes an associated muscle-weakening condition which occurs 24 to 96 hours after exposure, occurring subsequent to an acute OP cholinergic crisis (which typically happens within the first 24 hours) but before the development of OPIDN (two to three weeks later). It is characterized by acute onset of muscle weakness or paralysis affecting neck flexors, motor cranial nerves, proximal muscles of the limbs, and respiratory muscles (Leon-S, Pradilla, and Vezga, 1996; Mani, Thomas, et al., 1992). Deep tendon reflexes are commonly absent. It is often associated with respiratory insufficiency, and artificial ventilation may be required. It may occur with many OPs including fenthion, methylparathion, parathion, dimethoate; though it may occur more commonly with certain compounds (perhaps those with high lipid solubility), it is not confined to a few distinct compounds (De Bleecker, 1993). Prognosis for recovery is good, and occurs within four to 18 days (Leon-S, Pradilla, et al., 1996b; Mani, Thomas, et al., 1992). The necrotizing myopathy seen with AChE inhibitors (including PB) had been suggested as a cause (Senanayake and Karielde, 1987), but some data suggest against this hypothesis, and some have suggested persistent AChE inhibition as the mechanism (De Bleecker, 1993), possibly due to toxicokinetic properties of the OP ester involved (e.g., fat solubility).

\(^5\)There were four citations in the MED85 MEDLINE database (extending from 1985–1990), compared to 985 in the MED90 database (from 1990–1993) with the key words “*helicobacter*” and “peptic.”

\(^6\)As of August 1, 1998, there were 878 citations in the latest MEDLINE database with the keyword “leptin”; there are 0 in the MED90 database, which extends from 1990 to 1993.
individual variation in the safety factor of neuromuscular transmission, and delayed metabolism and clearance (De Bleecker, 1993). Of note: One study found that biochemical, electromyographic, and morphological data were unable to discriminate between patients with and without a symptom-free episode (De Bleecker, 1993).

**NEUROCHEMICAL EFFECTS**

Little work has been done to determine whether chronic neurochemical effects ensue following cholinergic manipulation with carbamates, and particularly with PB, though work is currently in progress (Albuquerque, 1997). As seen below, examination of such effects will be complicated by the potential for regional and measure-specific differences in effect and by differences in the time-course of an effect. In this section, evidence regarding the existence of delayed and chronic neurochemical changes with AChE inhibitors (usually AChE inhibitors distinct from PB, because little literature exists on chronic effects of PB) will be examined. Emphasis will be given to the emerging complexity of our understanding of the cholinergic system, with regional differences, differences in time-course of different effects, and differences in receptor characteristics complicating the process of extrapolating evidence on ACh and AChE function to new settings.

**Delayed Effects**

Effects associated with the delivery of AChE-inhibiting drugs may occur with differing temporal characteristics. In particular, not all drug effects of cholinesterase inhibitors quantitatively or even qualitatively parallel AChE inhibition in their time-courses. The existence of OPIDN and Intermediate Syndrome, among other effects, supports this point. For example, muscarinic receptor numbers fall in rats (receptor downregulation) following exposure to AChE inhibitors, an effect that does not show up at three days postexposure, but will by six days in rat pups exposed to chlorpyrifos at age 21 days (Stanton, Mundy, et al., 1994).

Even within a selected area of the brain, the time-course of effect of an AChE-inhibiting agent may differ depending on which measure of effect is evaluated. In OP-exposed rats, a shift was seen from greater AChE inhibition early, to greater BChE inhibition later, as tolerance to the behavioral effects emerged. (From this finding it was surmised that pseudocholinesterases may be involved in the development of tolerance and the reduction of toxicity (Swamy, Ravikumar, et al., 1992). The time-course, therefore, differed for AChE and for BChE inhibition. This finding reflects the general principle that distinct effects of the same drug may have distinct time-courses. Thus, demonstrations that one or
several effects of a drug are constrained to a specific temporal framework is not necessarily an assurance that other effects will be similarly circumscribed in time.

Moreover, changes in neurochemistry with cholinesterase inhibition occur differentially in different brain regions, a finding determined primarily from studies in rodents. For example, while cholinesterase inhibition occurred in both the cortex and hippocampus in one study, only the cortex showed muscarinic receptor downregulation (Stanton, Mundy, et al., 1994). In rat studies using OPs, the cerebral cortex was affected more initially, but striatum was affected more during later dosings (Swamy, Ravikumar, et al., 1992). This study performed measurement while OP administration continued. Such regional differences in effect complicate efforts to extrapolate effects, or the lack of effects, determined from one area of the brain to other areas. Even within an area, different effects may follow a different time-course.

Differences may also occur based on receptor type. Some studies have shown evidence of muscarinic receptor downregulation (Russell, Booth, et al., 1989). However, central nicotinic receptors may behave differently, demonstrating increased receptor number in some areas of the brain following AChE inhibition, possibly as secondary compensation to reduced receptor affinity (see Chapter Thirteen, “Neurotransmitter Dysregulation”). Further complicating the picture, there exist different structural and perhaps functional forms of nicotinic receptors. These may have distinct regulatory properties, perhaps accounting for some regional differences in nicotinic receptor response.

Evidence of Long-Standing Alteration in Neurochemistry After Brief Chemical Exposure. The question remains whether all effects of cholinergic dysregulation are relatively transitory and fully reversible, or if there exist long-term changes that coincide with the time-course of illnesses in PGW veterans. For most phenomena examined thus far, substantial reversibility occurs early, though in some instances full reversibility has not been demonstrated even with prolonged follow-up. There exist additional reports indicating that the neurochemistry of animals may be permanently altered after a single exposure to AChE inhibitors. A single subcutaneous dose of chlorpyrifos led to long-lasting enhanced sensitivity to cholinergic antagonists (evidenced by exaggerated hyperactivity in response to scopolamine) in rats, which persisted for months although muscarinic receptor density and cholinesterase activity had returned to normal levels (Pope, Chakraborti, et al., 1992). (See Chapter Thirteen.) Similarly, a single dose of fenthion led to apparently permanent alterations in intracellular communication of muscarinic receptors studied in rats (Tandon, Padilla, et al., 1994; Tandon, Willig, et al., 1994).
The existence of long-term neurochemical and behavioral consequences to temporally defined exposures is well known in other systems and is manifested in such other widely recognized phenomena as addiction, sensitization, and habituation. The above studies suggest that long-term changes in neurochemical properties may occur with AChE inhibitors. It is not known whether these or other changes occur with subacute administration of low doses of PB in humans. Neither is it known whether such changes, if they occur, provide the substrate for cognitive and behavioral changes reported following exposure to AChE inhibitors.

**RELATIONS OF SYMPTOMS TO DRUG LEVEL OR MEASURE OF “DRUG EFFECT”**

It is often assumed that symptoms resulting from AChE inhibitors require concurrent AChE inhibition to be manifest. Indeed, it is commonly assumed that drug levels determine drug effects. Yet there are many examples of drugs in which a time lag exists between the plasma or biophase drug concentrations and the time-course of pharmacodynamic response (Jusko and Ko, 1994). One example is corticosteroids, for which adrenal suppression may persist long after cessation of the drug.

As noted elsewhere, symptoms have been shown to persist beyond the time of AChE inhibition when AChE-inhibiting drugs are administered. Effects of carbamate insecticides (using screening tests recommended by the U.S. EPA, including a Functional Observational Battery and motor activity) were shown to last for days after cholinesterase inhibition returned to normal (Padilla, 1995; Moser, 1994). Adverse effects of both OP and carbamate insecticides have been reported weeks after an initial dose in rats (Ehrich, Shell, et al., 1993) and longer-term clinical and behavioral effects have been reported to differ from short-term effects (Padilla, 1995). (Clinical aspects are reviewed in the section on “Chronic Neuropsychiatric Effects” findings.)

**RELATION OF ACUTE TO CHRONIC SYMPTOMS**

It is widely thought that chronic symptoms following AChE inhibitor exposure require the presence of antecedent acute symptoms. However, this assumption may not be valid.

One study examined 77 16–65-year-old male British sheep-dippers. This study failed to find a relation between acute symptoms of OP toxicity, determined by a change from baseline to 24 hours after sheep-dipping exposure, and chronic neuropsychiatric symptoms, ascertained a minimum of two months following exposure (Stephens, Spurgeon, et al., 1996). The OP (AChE-inhibiting) drugs to
which the sheep-dippers were exposed included Diazinon, a mixture of Diazinon and chlorfenvinphos, or propetamphos. This study is limited by investigation of acute symptoms on only one sheep-dipping occasion, which may miss more marked effects from prior sheep-dipping events; lack of baseline data on the chronic symptom profile; lack of usable comparison data on cases and controls; and a control group (quarry workers) of uncertain “baseline” comparability on the chronic effect profile. The study concludes that “the chronic effects found in this group . . . appear to occur independently of symptoms that might immediately follow acute OP exposure,” though this conclusion is not necessarily justified by the data.

To the contrary, another study found that 45 California pesticide workers who had been temporarily pulled from carbamate and OP pesticide application work because of red blood cell cholinesterase values under 70 percent of normal, or plasma cholinesterase values under 60 percent of normal but who had no evidence of toxicity, did no worse on a battery of neuropsychological tests than 90 non-age-matched controls (friends brought in by pesticide workers), using regression analysis. Limitations of this study include aborting of subtoxic exposure, which may reduce chronic toxicity compared to more subchronic or chronic AChE inhibition exposure; strong differences in age between cases and controls (with younger controls), which may produce collinearity of pesticide exposure with age in the regression analysis, leading to the spurious appearance of no association following age adjustment; and a small sample, in which toxicity to a subset, or subtle effects to all, could be missed. The authors conclude that this study gives some support to the notion that protection from acute effects also confers protection against chronic neuropsychological sequelae. Of note: The Haley and Kurt study (1997) found that self-reported experience of acute effects with PB (reported retrospectively) was associated with subsequent illnesses in PGW veterans, a finding that, if accepted, may be consistent with this report.

**Interactions**

In a slightly different vein, chronic neurochemical and behavioral effects have been seen in rodents in the absence of acute symptoms of toxicity, when pyrethroid insecticide exposure (bioallethrin) as adults occurred following organochlorine exposure (DDT) as neonates (Johansson, Fredriksson, et al., 1995). (Pyrethroid insecticides have some esterase-inhibiting activity.) Those exposed to DDT alone as neonates had an apparently permanent decrease in muscarinic receptor density, with increased low- compared to high-affinity muscarinic binding sites. Those exposed to DDT also had an (apparently permanent) increase in susceptibility to bioallethrin-induced increase in muscarinic receptor density, also accompanied by increased low- to high-affinity
binding sites. (No changes in nicotinic receptors were observed in these groups.) Animals receiving bioallethrin showed difficulty learning a skill, the swim maze of the Morris water maze type. While some behavioral effects occurred in animals not receiving prior DDT, these abated with time, whereas permanent changes in muscarinic receptor density and behavioral variables were seen in the animals with prior DDT exposure. No studies have been identified that assess whether similar findings ensue with pyrethroid use following adult exposure to organochlorines or whether similar findings occur when the first exposure is to an AChE inhibitor, such as an OP (or carbamate). This study makes the point that complex interactions between exposures may occur despite separation in time between such exposures, prior exposures may serve to influence individual differences in susceptibility or response to drugs, and permanent alterations in neurochemical and behavioral susceptibility or permanent neurochemical and behavioral effects can occur following time-limited exposure to drugs. This may occur specifically within the muscarinic cholinergic system, at least in animal studies. No data were identified to assess whether analogous phenomena may occur with adult exposures in animals, in humans, or with exposures to AChE inhibitors, such as those experienced during the PGW.

OTHER CHRONIC EFFECTS

Porphyria. Porphyria refers to a set of genetic and acquired disorders in the synthesis of heme, an iron complex critical to many body functions that serves as a prosthetic group to proteins, including hemoglobin and mitochondrial proteins essential to life (Wilson, Braunwald, et al., 1991). Carbamates have been found to cause experimental porphyria in liver cell cultures (Matters, 1967). More relevant is the finding that carbamate poisoning precipitated clinical porphyria in a 17-year-old Caucasian woman who accidentally drank a glass of carbaryl liquid. Symptoms included abdominal cramping, nausea, and vomiting (commencing two days after ingestion); limb weakness (commencing four days after ingestion and progressing to quadriplegia with hypoactive tendon reflexes); and behavioral changes (beginning 21 days after ingestion). Porphyria was confirmed by elevated levels of uroporphyrin and coproporphyrin (Sargin, Cirak, et al., 1992). The high carbamate exposure greatly exceeded that experienced by PGW veterans, and the symptomatology is not strongly reminiscent of symptom reports by most ill PGW veterans. However, the finding of behavioral changes that commenced 21 days after exposure reinforces the observation that delayed neuropsychiatric changes in response to drugs including carbamates may occur through a variety of identified and unidentified mechanisms. Though one ill veteran has testified that porphyria was among the new diagnoses he received following the PGW (Zeller, 1997), it is difficult to
draw conclusions regarding causality from a single case. Some researchers state that abnormalities in porphyrin metabolism are present in many ill PGW veterans, with measured values outside of normal ranges although the findings do not meet clinical criteria for porphyria (Donnay, 1999). It would be reasonable for these findings (presently unpublished) to undergo replication or refutation. As noted elsewhere (Chapter Fifteen, “Other Considerations”), identification of specific objective findings in ill PGW veterans constitutes an important priority, as candidate etiologies—including PB and others—can then be tested in animal studies to assess for production of corollary findings, thus abetting the process of understanding the possible causes and contributors of illnesses in PGW veterans, which may in turn assist with identification of potential treatments and prevention of similar problems in the future.

**Conditioned Taste Aversion.** PB as well as other carbamates and anticholinesterase nerve agents have been found to produce conditioned taste aversion in rats (McPhail, 1981; Romano and Landauer, 1986; Romano, King, et al., 1985; Romano and King, 1987), in doses that did not affect response rates in a rat operant paradigm (Modrow and McDonough, 1985). An animal may develop an enduring aversion to a “conditioned stimulus” (such as a taste, when it has been paired with an unconditioned stimulus such as PB) following a single association of the two—another instance in which a brief exposure may produce lasting effects.

Whether such enduring aversions relate to new chemical sensitivities, such as those reportedly experienced by some PGW veterans, is open to speculation.7

**SUMMARY AND CONCLUSION**

Although PB has been given to myasthenics for many years with few concerns regarding long-term adverse effects, the circumstances of use are quite different for patients with myasthenia and for PGW veterans. Despite use of a lower dose of PB in PGW veterans than in myasthenics, the possibility that PB could produce chronic effects in some PGW veterans cannot be excluded: PGW veterans differ from myasthenics in their cholinergic state, in short-term rather than lifelong PB use, and in the presence of concomitant stress and a diverse assortment of potential chemical and environmental exposures that have been demonstrated in animal studies to interact with PB.

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7Perhaps surprisingly, not only do PB and physostigmine produce conditioned taste aversion, but so also does the anticholinergic agent atropine (Romano and King, 1987). Thus, dysregulation of the cholinergic system—perhaps in either direction—may produce lasting aversion to coadministered chemicals.
Few studies examine chronic effects of PB, perhaps in part because under normal unstressed conditions little PB enters the CNS. (Under conditions of stress and chemical coexposure, however, PB does penetrate the blood-brain barrier and produce central cholinesterase inhibition in animal studies.) Though few studies look at the chronic effects of PB, many studies examine other AChE inhibitors, and these were briefly reviewed. Additional information on effects of pesticides and nerve agents can be found in Vol. 5: Chemical and Biological Warfare, and Vol. 8: Pesticides (Augerson, forthcoming; Geschwind and Golomb, forthcoming).

The neurochemical effects of AChE inhibitors, including PB, on the cholinergic system are complex. The precise nature of these changes—and regional differences in their character or time-course—remain to be fully elucidated. At present, we can neither confirm nor exclude the theory that PB (assuming circumstances that allow it to cross the blood-brain barrier) precipitates long-lasting or permanent changes in CNS neurochemistry or that PB in combination with other exposure facilitates the ability of these coexposures to do so. Nonetheless, some evidence indicates that long-term or permanent changes may occur in the cholinergic system and in other neurotransmitter systems, following time-limited exposures to agents. Lasting alterations in muscarinic receptor number and affinity are seen with exposure to AChE inhibitors, and interactions between early and later exposures have been demonstrated—one exposure may evidently prime an animal to experience a marked effect with a much later exposure. Regional brain and temporal differences arising from different effects of the same AChE inhibitor have been demonstrated. A single exposure to AChE inhibitors can produce a lasting aversion to coadministered chemicals in rats, perhaps suggesting a mechanism for chemical sensitivities such as have been reported by some veterans. The possibility of chronic effects on the motor endplate are discussed in a separate chapter (see Chapter Twelve, “Neuromuscular Junction Effects”). In the absence of entry into the CNS, there remain mechanisms by which changes in some elements of neuropsychiatric function could take place, involving dysregulation of release of catecholamines from the adrenal medulla, a process regulated by ACh.

In humans, AChE-inhibiting drugs have been shown to produce delayed and chronic effects. Chronic EEG abnormalities have been demonstrated with pesticides and nerve agents; chronic changes have been isolated in SPECT brain scans in subjects exposed to pesticides; and neuropsychiatric tests have demonstrated variable deficits in such subjects. Many of these findings are preliminary and require replication. Moreover, whether PB may have contributed to similar chronic effects in PGW veterans, alone or through potentiation of actions of pesticides or other exposures, is uncertain. Subjects exposed to pesticides, and relatives of these subjects, report development of
functionally significant chronic changes that may occur despite relatively subtle deficits on neuropsychological testing. This appears to parallel the experience of PGW veterans and suggests that more targeted tests sensitive to the deficits perceived by veterans need to be identified. It is critical to note that reductions in function that occur within the “normal” range may still produce marked functional impairment in real-world tasks. For instance, two individuals may both score “normal”—although one excels in a challenging job (or school) and another struggles at a substantially less challenging enterprise. Development of tests sensitive to deficits experienced by individuals with pesticide exposures may prove useful in evaluating the characteristics and possible etiology of illnesses in ill PGW veterans.

Some studies in PGW veterans indeed report (modest) neuropsychiatric abnormalities that appear to be widespread rather than confined to one or two specific functions, potentially consistent with the pervasive influence of ACh in the brain and body. (Nonetheless, efforts should be made to develop neuropsychiatric tests sensitive to functions likely to be potently regulated by ACh, as discussed in Chapter Thirteen.)

These findings, taken together, suggest that chronic effects from PB cannot be excluded. Such effects could occur from PB exposure alone (in the context of stress) or perhaps more likely in combination with other chemical exposures. Chronic biological and clinical effects have been demonstrated with related agents that share the capacity to inhibit AChE. Some, but not all, of these effects have been shown to be restricted to a subset of AChE-inhibiting agents. Preliminary neuropsychiatric studies offer support, in some cases, for chronic neuropsychiatric effects in subsets of ill PGW veterans, and additional studies continue.

In short, it remains undetermined whether PB produces chronic effects that in turn contribute to illnesses in PGW veterans. However, findings from PB and related agents suggest that chronic neurochemical and neuropsychological effects are possible following exposure to AChE inhibitors under certain conditions.

**SCIENTIFIC RECOMMENDATIONS**

- Efforts to evaluate the connection between PB exposure or adverse response to PB and subsequent chronic neuropsychiatric symptoms should continue.
- Sensitive testing techniques, able to capture the deficits reported by PGW veterans, are imperative to adequate evaluation of their chronic cognitive and neuropsychiatric complaints. Continued efforts should be made to
find neuropsychiatric tests sensitive to the functional level and to the possibly “mild” cognitive deficits reported by some ill PGW veterans (which may nonetheless produce marked changes in functional ability for those in more-demanding occupations). A test of executive decisionmaking has been shown to correlate with job success with a correlation coefficient of 0.6, compared to 0.3 for IQ tests, and has been shown to identify functional impairment in patients with mild head injury when other tests fail to do so (Streufert, Satish, et al., 1997). This test is quite costly and time-intensive, but consideration should be given to devising similar tests to supplement current testing in a trial of ill PGW veterans and controls.

• Prior to future deployments in which PB may be given, at least some personnel and some controls who will not be deployed should receive baseline neuropsychological testing using sensitive instruments (preferably instruments that preliminary data suggests may differ between ill PGW veterans and controls), to allow comparison of pre- and postresults if PB exposure occurs as part of deployment. Although delicate ethical issues are associated with conducting research on military personnel, the greater ethical breach is to fail to perform such testing of an intervention and then administer it to hundreds of thousands of individuals.

• Consistent with recommendations in Chapter Thirteen, a trial of (blinded) SPECT imaging might be appropriate in PGW veterans with chronic cognitive complaints, particularly those who report neurotoxic exposures. SPECT has been reported to identify abnormalities in individuals exposed to pesticides that are distinct from those of individuals suffering from depression and chronic fatigue. Thus, objective differences on quantitative SPECT, if present, might distinguish among several possible etiologies of illnesses in PGW veterans. Challenge studies in which pro- and anti-cholinergic agents are given and regional blood flow evaluated by SPECT may help to clarify short-term and chronic effects. Moreover, SPECT might provide an objective marker of cerebral dysfunction in subjectively impaired individuals and may conceivably offer a marker for treatment effect.

• Basic research in cholinergic function in the CNS should continue (see recommendations from Chapter Thirteen), with emphasis on the character and time-course of effects, and particularly on identifying and assessing chronic effects.