DO PHYSIOLOGIC DIFFERENCES INFLUENCE SUSCEPTIBILITY TO PB?

This chapter addresses evidence about the existence of individual differences and the possible relation of individual differences to illnesses in PGW veterans. Differences have been demonstrated in side effects with PB administration, in chemical response to PB, and in the relation of chemical response to side effects. Some evidence suggests a connection between differences in response to PB and subsequent development of illnesses in PGW veterans. Possible sources of individual differences are discussed.

Individual differences in properties that may influence PB metabolism and effects have been identified (such as quantitative differences in BChE levels), and others are likely. The existence of individual differences in response to PB in the short term is illustrated by the wide variation in symptoms (symptom character and severity) reported in response to PB in the PGW. One study suggests a correlation between these short-term differences (gauged by self-report of adverse response to PB administered during the PGW) and symptom reporting in PGW veterans (Haley and Kurt, 1997), although this result remains to be confirmed and, if confirmed, to be understood. Differences in levels of such enzymes as BChE that are thought to scavenge PB have also been found and have been reported to correlate with symptoms (not confined to acute symptoms) following exposure to carbamates.

The potential for individual differences in response to an exposure to such carbamates as PB is great. Empirically, differences in response to PB are known to occur; and biological differences that may contribute to this individual variability are beginning to be understood (though current understanding remains limited). A great variety of classes of factors have been identified that have potential for contributing to individual differences. Whether individual differences in sensitivity to PB contribute meaningfully to differences in long-term clinical outcomes in PGW veterans remains to be determined. However, evi-
evidence can be cited that suggests a possible role for such differences, supporting the need for further scientific inquiry. Because of these factors, the same oral dose of PB may lead to different blood levels of PB and to different “therapeutic” and toxic effects.

**CHEMICAL FINDINGS**

Differences in the inhibition of AChE in response to PB are marked. First, absorption of PB may be affected by clinical differences in response to PB itself (see below). For instance, PB itself may increase gastrointestinal peristalsis, speeding transit of PB from the gut to the feces and limiting absorption of PB into the bloodstream. This process may occur at different rates in different individuals because of the variability in effect of PB (discussed below)—a variability which this effect will amplify. Second, once PB is absorbed, there may be differences in rates of clearance from the bloodstream due in part to differences in enzymes (different types and different amounts) that assist in clearance of PB. This may help account for the approximately threefold differences in plasma PB levels measured at comparable times after a single 30 mg dose of PB—values of about 10 ng/ml to about 30 ng/ml at two hours after the dose (Parker, Barber, et al., 1986)—and even larger differences reported in other studies (see Chapter Three for more data).

Moreover, the same plasma level of PB may be associated with substantially different degrees of RBC AChE inhibition; the correlation in several studies has been rather low, with 2 percent inhibition associated with 14 ng/ml PB in one subject, compared to 24 percent AChE inhibition in another, despite a lower plasma level of 11 ng/ml (Parker, Barber, et al., 1986). Because of these various factors, the same oral dose of PB has been associated with quite different degrees of AChE inhibition—with AChE inhibition in one study ranging from 18 percent to 57 percent (Kolka, Burgoon, et al., 1991); and in another study of 19 subjects, ranging from 0 percent to 49 percent at two to three hours after a single 30 mg dose (Parker, Barber, et al., 1986). (For the three subjects measured at exactly two hours after dosing, the figures ranged from 2 percent to 49 percent inhibition. Plasma PB levels were not even monotonically related to RBC AChE inhibition among these three cases.) Presumably even larger ranges would be uncovered if larger populations were tested. (In the study cited, subjects with greater than 40 percent inhibition were excluded from further evaluation—including further use of the drug. In the PGW, personnel who may have had excessive AChE inhibition would not have had access to this information, because testing was not performed, and they would therefore not have been told to terminate or modify PB use.)
Moreover, in another study, the duration of effect in which AChE inhibition exceeded 20 percent ranged from 0.33 to 5.0 hours, a fifteenfold difference (Sidell, 1990). The range of individual differences might be seen to be even greater if larger samples were tested. (Quite large standard deviations were seen in some subgroups in a larger study of 90 volunteers who took PB (Lasseter and Garg, 1996).) These differences may be in part the consequence of differences in absorption leading to different blood levels of PB and may in part relate to differences in sensitivity of AChE to PB, because the correlation between PB and AChE inhibition has been as low as −0.61 (signifying that only about 37 percent of the variance in AChE inhibition is accounted for by the blood level of PB) and because marked individual differences were not highly correlated with weight, height, or body surface area (Kolka, Burgoon, et al., 1991; Lasseter and Garg, 1996). Moreover, differences may arise from different AChE levels on RBCs or even different RBC counts. Differences in the rate of elimination of absorbed PB may also play a role (Sidell, 1990). (These differences are also described in Chapter Three, “Characteristics of PB.”) Another potential issue is that of accurate measurement of AChE inhibition, potentially producing erroneous results—perhaps accentuating apparent variability.

**CLINICAL FINDINGS**

Differences in acute response to PB are reflected in differences in side effect profiles noted at the same dose of PB. Different subjects develop different side effects with the same dose of PB, and many develop no side effects. The side effects of PB are reviewed in Chapter Three, “Characteristics of PB.” Baseline differences in personality predicted side effects in response to the related chemical physostigmine (Janowsky, 1997). Since “personality” is substantially influenced by neurochemistry, this is consistent with the possibility that baseline neurochemical profile may in part determine acute effects.

**RELATIONAL FINDINGS**

There are differences in clinical symptoms in response to PB and differences in AChE inhibition in response to PB. Differences in AChE inhibition might be presumed to directly produce the differences in clinical symptoms. However, differences in AChE inhibition do not explain the differences in clinical effect, although they may participate in the production of these differences. Cholinergic side effects from PB do not correlate well with AChE inhibition. For instance, in one clinical report of nine PGW veterans who attempted to overdose on PB, consuming 390 to 900 mg of PB (13 to 30 tablets), no relation was found between AChE inhibition and cholinergic symptoms (Almog,
Winkler, et al., 1991). This clinical report suggests that variation in factors other than AChE inhibition play a role in the effects of PB. These variable factors remain to be fully elucidated (although potentially contributory factors, such as individual susceptibility to psychological stress and disruption of the blood-brain barrier, are discussed elsewhere). It should be remembered that PB participates in numerous effects distinct from AChE inhibition: For example, PB acts as an ACh “partial agonist,” binding to the site on AChE to which serotonin normally binds; PB influences other neurotransmitter systems, such as the GABA and glutamate systems; PB participates in “autoregulation” of ACh release from the nerve terminals; and PB enhances the desensitization of ACh receptors. (See Chapter Three, “Characteristics of PB.”) Individual differences in these additional effects have not been explored, but they could further explain the differences in symptoms in persons with comparable levels of AChE inhibition.

**INDIVIDUAL DIFFERENCES IN BASELINE CHOLINERGIC STATUS**

Substantial evidence supports the existence of baseline differences in acetylcholinergic “status” and responsiveness, which could influence response to PB. The imaged brains of some individuals demonstrate increases in choline, the rate-limiting factor in ACh synthesis (Janowsky and Overstreet, 1995). Moreover, some individuals show heightened behavioral effects (for instance, depression, which commonly occurs acutely after being given AChE inhibitors, or other ACh-promoting agents (Tammings, Smith, et al., 1976)) in response to ACh “agonists” (agents that promote the action of the ACh system) (Janowsky and Overstreet, 1995). Perhaps not surprisingly, ACh stimulated depressive effects occur more readily and more commonly in persons with depression (75 percent)—who have been shown to have cholinergic (muscarinic) “supersensitivity” in many studies (Risch, Janowski, et al., 1981; Sitaram, Nurnberger, et al., 1982; Risch, Kalin, et al., 1983; Janowsky and Risch, 1984; Sitaram, Jones, et al., 1985; Sitaram, Jones, et al., 1987; O’Keane, O’Flynn, et al., 1992; Steinberg, Weston, et al., 1993). However, they also occur in many normal individuals (25 percent) (Risch, Cohen, et al., 1981; Janowsky and Overstreet, 1995). Exemplifying the increased effect of cholinergic agents in groups with baseline cholinergic supersensitivity, administration of the PB-like agent physostigmine led to increased changes in rater-evaluated “behavioral inhibition” and in self-rated anxiety, depression, hostility, and confusion in patients with mood disorders—the class of patients shown to more commonly have the cholinergic supersensitivity—compared to other psychiatric groups or normals (Janowsky, Risch, et al., 1980; Janowsky, Risch, et al., 1981).

“Altered” responsiveness of the ACh system is common not only with depression but in association with many other conditions. For instance, the surge in
growth hormone that occurs following delivery of PB or physostigmine is more marked than in normals not only in those with mood disturbances but in people with high cortisol levels, either naturally or through drugs; Alzheimer’s patients, those with obsessive-compulsive disorder or panic disorder, and schizophrenics; and age may also influence the responsiveness (Raskind, Peskind, et al., 1989; Corsello, Tofani, et al., 1991; Rapaport, Risch, et al. 1991; O’Keane and Dinan, 1992; O’Keane, O’Flynn, et al., 1992; Borges, Castro, et al., 1993; Ghigo, Nicolosi, et al., 1993; Lucey, Butcher, et al., 1993; O’Keane, Abel, et al., 1994; Thakore and Dinan, 1995). Thus, many lines of evidence confirm strongly that the effects of AChE-inhibiting agents (or ACh-stimulating agents) may relate in part to the underlying state of the ACh system, which has been shown to be highly variable. Although this may in part reflect genetic differences (Janowsky, Overstreet, et al., 1994), it is also possible that past exposures to chemicals that affect the ACh system may play a role (see Chapter Thirteen, “Neurotransmitter Dysregulation”). Chronic effects of PB, if PB gains central access, may be more likely in those whose ACh sensitivity is high to begin with. This would be compatible with the report that those with self-reported acute response to PB are more likely to have developed chronic symptoms. (It may be only in these individuals that the heightened ACh action was sufficiently “out of range” of normal to induce alterations in regulation or that those individuals reflect a susceptible subset in some other way.)

INDIVIDUAL DIFFERENCES AND ILLNESSES IN PGW VETERANS

Haley, Kurt, and Horn (1997) examined a set of ill PGW veterans and conducted a factor analysis in which six clinical “syndromes” (three they view as predominant) were identified in ill persons who served in the PGW. Two of the three main syndromes, termed by the authors “confusion ataxia” and “arthro-myo-neuropathy,” were significantly associated with self-report of adverse response to PB at the time of PB administration during the PGW. (These syndromes were derived by Haley et al. using factor analysis, and the symptoms included—as the labels suggest—confusion and gait disturbance in the former case, and joint/muscle/nerve pain in the latter.) The “exposure” here is not use of PB but adverse experience associated with use of PB. These findings suggest that individual differences in tolerance may be linked to long-term adverse consequences. These could, for instance, reflect individual differences: in PB absorption, metabolism, or action; in cholinergic regulation; or in concomitant exposures that influence the effect of PB.
Limitations

There are several limitations to the analysis by Haley, Kurt, and Hom (1997). First, factor analysis has the potential to create “syndromes” or factors that may not be reproduced when a new sample is examined. Second, Haley’s sample was small, exacerbating the potential to produce irreproducible factors. Third, determination of adverse response to PB was made by self-report and thus may be subject to recall bias (Lees-Haley and Brown, 1992). Recall bias has the potential to produce the spurious appearance of a relationship between an exposure and an adverse outcome due to the selective recollection of the exposure in an affected group.

Regarding the first limitation, there is a strong need to determine factors using one sample and to validate them with another (cross validation). (This process is rendered more difficult by the small sample size.) Moreover, other methods for identifying natural groupings of data (in this case “syndromes” or symptom complexes) can also be employed, such as unsupervised neural networks (Bishop, 1995), and it would be instructive to see whether these alternative methods would replicate the findings of Haley’s factor analysis on the same and on new databases of ill PGW veterans. If individuals identified as having one factor-analyzed “syndrome” are found to share common objective neurological findings, particularly findings that distinguish them from those with other factor analysis–derived syndromes, it may enhance confidence in the legitimacy of the syndromes. Such work is currently being done using sensitive neurological tests (Haley, 1998).

Because records of who received PB in the theater were not made and maintained, self-report is the best method available for determining exposure to PB (and to many other putative exposures) in PGW veterans. Moreover, while recall bias might influence recall of exposure in persons with and without illness (Lees-Haley and Brown, 1992), there is no compelling reason to suppose that recall bias would result in differential recall of exposures for persons with one symptom complex compared to another (though such differential recall cannot be excluded). Therefore, if the differential association between these syndromes and exposures were replicated in another sample, it might favor a true association. Moreover, a study in PGW veterans (discussed in Chapter Fourteen, “Chronic Effects”) does not support a connection between symptom reporting and an overall exposure index (in which PB is not included) in PGW veterans (Sillanpaa, Agar, et al., 1997), suggesting that biased recall of exposures may not be prominent.
SOURCEs OF INDIVIDUAL DIFFERENCES IN RESPONSE TO PB

If experiencing an adverse, acute response to PB is indeed associated with subjects’ likelihood of developing subsequent illnesses, it remains to be determined which factors contribute to individual susceptibility. Differences in susceptibility to longer-term consequences of PB (if longer-term consequences exist) could occur in the absence of differences in acute symptoms, if different mechanisms are at play. Similarly, as will be shown in the case of bromism (Chapter Ten), persons who apparently develop chronic sequelae of bromide intoxication could not be predicted on the basis of initial blood levels of bromide or on the basis of acute symptoms.

Several sources for differences in response to PB have been, or could be considered. Butyrylcholinesterase (BChE) polymorphism (differences in structure of the chemical BChE) has been particularly emphasized as a source of possible individual differences, though the numbers of persons who are either homozygous (<1 percent) or heterozygous (<5 percent) for the “atypical” variety of BChE—the variety that has been looked at in this context—are probably too small to account for the numbers of ill veterans. These may constitute 5 to 20 percent of PGW veterans, employing very crude estimates derived from numbers of registry participants with unexplained illness (lower number) and all registry participants assuming additionally some unregistered ill persons (higher number); both figures potentially omit some ill PGW veterans and may include veterans with illness unrelated to PGW service. Other sources of individual differences include other polymorphisms, such as the far more common K-variant (~20 percent of the population); quantitative (rather than qualitative) differences in BChE (that is, differences in amount rather than type of BChE); differences in other “esterases” (enzymes that participate in breakdown of ACh and AChE inhibitors); native differences in cholinergic responsiveness; differential hormonal effects; and differences induced by concurrent, antecedent, or subsequent exposures. Other chapters (such as “Characteristics of PB,” “Chronic Effects,” and “Interactions Between PB and Other Exposures”) make reference to additional sources of individual differences.

The possible contribution of several of these sources of individual differences are discussed here—particularly the contribution of enzyme polymorphism and of differences in enzyme levels. Because there is a moderate amount of literature regarding BChE polymorphism and the effect of PB, a moderate amount of text will be devoted to BChE polymorphism, though it will be concluded that the most commonly discussed polymorphism—relating to the “atypical” BChE—is unlikely to play a major role in explaining selective susceptibility to illnesses in PGW veterans. Quantitative differences in levels of enzymes, and perhaps other polymorphisms of BChE, or polymorphisms related to other enzymes, may play a more important role.
**BChE Polymorphism**

**Terminology.** BChE “polymorphism” refers to the presence of different forms of the chemical BChE in different individuals, resulting from genetic differences in coding for BChE. To understand polymorphism more fully, it is helpful to distinguish between “genotype” and “phenotype” and to understand the difference between “homozygotes” and “heterozygotes. The “genotype” is determined by which genes are present in an individual to code for the enzyme. There is a most-common form of the gene, termed “U” or “usual” and less-common forms of the gene, one of which is termed “A” or “atypical.” Because people have two copies of each gene, that is two “alleles” (one from each parent), the genes may both be the same, or they may be different. A “homozygote” is a person for whom the genes encoding the enzyme, one gene obtained from mother and one from the father, are the same. Thus, a homozygous “usual” person would be designated as “UU.” Persons who are homozygous for the atypical allele (“AA”) are quite rare. A “heterozygote” is a person for whom the genes coding the enzyme, obtained from the mother and father, differ; thus, a person carrying a U allele from one parent and an A (or other) allele from the other parent would be a “heterozygote.” The “genotype” for BChE refers to which genes a person has to encode the enzyme. In some instances, different “genotypes” may nonetheless result in the same “phenotype,” or appearance and amount of the chemical—because some DNA mutations do not affect which amino acids make up the enzyme. In other instances, individuals with the same “genotype” may nonetheless have differences in expression or “phenotype”—most commonly in the amount of the enzyme (presumably due to environmental or other genetic factors), though generally the form of the enzyme will be the same.

**BChE.** BChE is also called pseudocholinesterase or plasma cholinesterase, as opposed to acetylcholinesterase (AChE), also called red blood cell cholinesterase, or true cholinesterase. BChE is a plasma enzyme thought by some to be involved in the scavenging of—and removal of—PB (among other chemicals) from the circulation (Schwarz, Glick, et al., 1995; Soreq, 1995), and more generally in protecting AChE at brain synapses and neuromuscular junctions from anticholinesterases (Schwarz, Glick, et al., 1995).

It has been suggested that pralidoxime (2-PAM), used in the treatment of nerve agent poisoning after exposure, may act by regenerating BChE and allowing it to react with more of the organophosphate (OP) nerve agent before the OP has a chance to inactivate neuromuscular AChE; this reaction effectively turns BChE into an organophosphatase (or agent that metabolizes OPs) (Schwarz, Glick, et al., 1995). Because of BChE’s ability to scavenge PB and assist in its elimination, BChE polymorphism has been postulated to be involved in the large differences in PB dose required by patients with myasthenia gravis.
Although Chapter Three shows evidence that differences in absorption of PB play a major role. In a report of one PB-exposed soldier, although BChE has less affinity for PB than does true AChE, differences in BChE appeared to lead to differences in the clinical effect of PB (that is, to cholinergic toxicity) (Loewenstein-Lichtenstein, Schwarz, et al., 1995).

**BChE Polymorphism: Qualitative Differences in BChE.** “Atypical BChE,” produced by several alternative genetic mutations, has been the focus of some interest following the PGW. BChE (an enzyme) is a protein and as such consists of a chain of amino acids. Atypical BChE has a substitution of the amino acid “aspartate” (D) at position 70 in the chain of amino acids that comprises the BChE protein, by the amino acid “glycine” (G). The result is termed D70G. This enzyme is much less sensitive than the normal enzyme to several inhibitors, particularly to positively charged compounds such as PB (Gentry, Bitsko, et al., 1996). “Atypical” BChE has received more attention than have other genetic polymorphisms of BChE (Loewenstein-Lichtenstein, Schwarz, et al., 1995; Leon-S, Pradilla, and Vezga, 1996).

Altogether, since the gene was cloned, 22 different mutations have been identified in the coding region of the human BChE gene (Soreq, Ehrlich, et al., 1994). Some produce mutations that do not influence the amino acid sequence or the BChE effect. “Nonexpressing” BChE mutations—in which no BChE is produced—occur rarely (Taylor and Brown, 1994). Still other mutations alter amino acids and produce phenotypic changes in the resultant enzyme, in some cases producing BChE that is incapable of metabolizing (“hydrolyzing”) the muscle relaxant succinylcholine, so that patients may develop failure to breath (“apnea”) when given succinylcholine during anesthesia (e.g., Loewenstein-Lichtenstein, Schwarz, et al., 1995).

**Estimates of BChE Variants in the Population.** It has been estimated that approximately 96 percent of the population has the normal genotype (UU, or homozygous “usual”) and that four to five percent are heterozygous for the atypical form (UA) (Gentry, Bitsko, et al., 1996). This information suggests that approximately 0.2–0.25 percent are homozygous atypical (AA). Other reports cite a rate of 0.04 percent homozygous carriers in Europe overall, but cite rates of up to 0.6 percent in certain subpopulations (Loewenstein-Lichtenstein, Schwarz, et al., 1995). In fact, however, recent research suggests that a much more common variant, the “K” variant, occurs in about 20 percent of the population and is, like atypical BChE, associated with decreased BChE activity (Jen-son, Nielsen, et al., 1996; Lehmann, Johnston, et al., 1997; Russ, Powell, et al., 1998). No efforts have been made to examine the frequency of the K-variant in ill Gulf War veterans.
**Effect of Atypical BChE.** Differences in BChE associated with different genotypes not only are structural, but also affect persons’ response to certain chemicals. The clinical effects of homozygous atypical BChE include postanesthesia apnea (for instance, induced by succinylcholine), hypersensitivity to the anticholinesterase insecticide parathion, and reportedly severe effects with administration of PB in one soldier (Loewenstein-Lichtenstein, Schwarz, et al., 1995). Here, differences in hydrolysis of certain chemicals with the atypical as compared to the typical form are shown. These chemicals include the test chemical butyrylthiocholine, PB and carbamates, and the drug tacrine.1

Biochemical effects of atypical BChE (whether native or “recombinant”) include low rates of hydrolysis of the test chemical butyrylthiocholine.2

Atypical BChE, in those homozygous for the atypical form, has been found to react much more slowly with PB and other carbamates (based on differences in the rate of inactivation of PB); heterozygotes demonstrated intermediate rates of reaction (Loewenstein-Lichtenstein, Schwarz, et al., 1995), between those of homozygous usual and homozygous atypical individuals. It has been suggested that cholinergic symptoms in patients receiving treatment with tacrine may occur in up to 15 percent due to BChE heterozygotes and to patients with liver abnormalities who consequently have low BChE levels (Loewenstein-Lichtenstein, Schwarz, et al., 1995). While a recommended dose of PB (for myasthenia gravis, for example) may be 90 percent hydrolyzed by “wild-type” (UU) BChE in the blood, the same dose may not be appreciably hydrolyzed by homozygous atypical BChE. This difference could result in higher levels of PB in the circulation and hence to increased likelihood of symptoms from PB, or increased likelihood of PB “overdose” (Gentry, Bitsko, et al., 1996). Thus, BChE may have an important role in elimination of PB from the circulation, and differences in BChE genotype and phenotype could lead to substantial differences

---

1Atypical BChE had one two hundredth the affinity for tacrine. (Tacrine is a reversible cholinesterase inhibitor used to improve cognitive function in Alzheimer’s disease.) Heterozygotes showed intermediate inhibition curves with tacrine (Loewenstein-Lichtenstein, Schwarz, et al., 1995). A two orders of magnitude increase was found in the 50 percent inhibitory concentration (IC50) for tacrine with atypical BChE—that is, in the amount of tacrine required to inhibit 50 percent of BChE. The concentration of cholinesterase in the blood is approximately 50 nM; 75 percent is due to soluble BChE and 25 percent to RBC AChE (“true” cholinesterase); plasma tacrine levels are 21 nM in patients under treatment. Dissociation constants of 40 nM for normal BChE and 8000 nM for atypical BChE, calculated from this study (Loewenstein-Lichtenstein, Schwarz, et al., 1995), suggest that while 40 percent of tacrine is bound to BChE in persons with the normal allele, only 1 percent is bound in atypical homozygotes (Loewenstein-Lichtenstein, Schwarz, et al., 1995).

2The rate of hydrolysis of butyrylthiocholine in those with the homozygous atypical BChE was approximately one-third the “normal” rate found by averaging the results for 20 normal individuals. (This rate was 81 ± 23 nmol/hour/microliter serum, assayed at 2 mM substrate.) Heterozygotes (average of three cases) had activity approximately 60 to 70 percent of normal (intermediate between homozygous atypical and homozygous normal) (Loewenstein-Lichtenstein, Schwarz, et al., 1995).
in PB concentrations in the blood. See Table 8.1 for different effects of usual and atypical BChE on PB. As this table shows, the inactivation rate for PB is substantially lower with atypical BChE than with normal BChE—about a seventh; although both rates are considerably slower than inactivation rates with either RBC or brain-type AChE, clinical evidence suggests that BChE still plays an important role in PB inactivation.

**Persian Gulf Veteran with Atypical BChE.** A case report has described an Israeli soldier homozygous for “atypical” BChE who suffered severe symptoms following PB prophylaxis during the PGW (Loewenstein-Lichtenstein, Schwarz, et al., 1995). His symptoms included nausea, insomnia, weight loss, and fatigue, which worsened consistently with continued PB administration and were accompanied by a deep depression. These symptoms improved gradually over the weeks following discontinuation of PB (Loewenstein-Lichtenstein, Schwarz, et al., 1995). Serum BChE from the patient, as well as recombinant “atypical” BChE were far less sensitive than normal BChE to PB and several other carbamate cholinesterases. Heterozygotes for BChE were determined to have intermediate activity, as noted above.

**Comparison of Atypical BChE in PGW Veterans Versus Controls.** A comparison of rates of “atypical” versus usual BChE in (only) 20 PGW veterans enrolled in the Comprehensive Clinical Evaluation Program (CCEP) who had received PB, compared to 20 volunteer controls (of unstated origin) matched for sex and age (within five years) did not reveal differences in BChE phenotype between

<table>
<thead>
<tr>
<th>Table 8.1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effects of Different Esterases on PB Inactivation</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PB second-order inactivation rate constant (per M per minute times 1/1,000)</td>
</tr>
<tr>
<td>PB: (10^{-5}) M</td>
</tr>
<tr>
<td>Time-dependent reactivation (percentage original activity after 30 minutes), PB</td>
</tr>
</tbody>
</table>

**SOURCE:** Loewenstein-Lichtenstein, Schwarz, et al., 1995).
the two groups or in blood cholinesterase levels or spontaneous reactivation times for AChE after PB administration. Fourteen veterans recalled symptoms while taking PB; seven felt ill; six sought medical attention. Seventeen complained of chronic symptoms during the CCEP, including fatigue, rash, joint pain, memory problems, sleep disturbance, depressed mood, diarrhea, shortness of breath, headache, hair loss, difficulty concentrating, muscle pain and others (Gentry, Powell, et al., 1992; Gentry, Bitsko, et al., 1996).

Prior to this study, it was possible to surmise that the interaction of PB with atypical BChE would be unlikely to be responsible for illnesses in most ill PGW veterans because of the low frequency of the atypical BChE allele (~5 percent) and the high rate of reported illnesses in PGW veterans (perhaps 4–20 percent of all PGW veterans, or if all illnesses were in those who had received PB, then perhaps 14–70 percent of those). This study corroborates that prior knowledge, albeit in a small sample. To extend this knowledge, to determine whether a small (but still increased) fraction of ill veterans might have one or more atypical BChE allele, would require a larger study, perhaps confined to confirmed ill PGW veterans with self-reported exposure to PB versus healthy controls who also report PB use. (Only 17 of these 20 CCEP cases complained of chronic symptoms.) Additional subjects have been recruited for the study noted above.

It remains possible that selected instances of illness in PGW veterans are associated with atypical BChE genotype (heterozygous or homozygous). Moreover, it remains possible that substantial phenotypic variability in BChE activity within a genotype, specifically in regard to the amount of BChE, could influence susceptibility to effects of PB.

**Other BChE Mutations.** As mentioned previously, another relatively inefficient variant of BChE termed the K variant—with 30 percent reduced scavenging activity relative to the UU variant (Soreq, 1994; Bartels, Jensen, et al., 1992)—has been identified and shown to be common—e.g., 20 percent frequency—in some populations (Russ, Powell, et al., 1998). There is conflicting information regarding whether the presence of this variant contributes to risk of Alzheimer's disease. This variant has a higher prior probability of contributing to susceptibility to PB effects in PGW veterans, because of its higher prevalence in the population. But to date there is no information regarding the relative frequency of this gene in ill versus well veterans exposed to PB in the PGW; testing for this variant in Gulf War veterans has not been performed.

**Other Putative Links Between BChE Variability and Illness.** A syndrome termed "Intermediate Syndrome" has been described (Senanayake and Kariel, 1987), which generally occurs 24–96 hours after exposure to OPs, and which may also be seen with carbamate intoxication (Leon-S, Pradilla, et
Individual Differences 95

It is characterized by cranial nerve palsies, neck and proximal limb weakness, and respiratory paralysis, and its frequency, mechanism, and long-term deleterious effects remain unknown (Leon-S, Pradilla, et al., 1996). It has been suggested, based on published reports from 1965 to 1995, that intermediate syndrome “and the like” occurred in “between 20 percent and 68 percent” of patients with insecticide intoxication (Leon-S, Pradilla, et al., 1996); and, moreover, that BChE activity was usually more depressed than AChE and that “the lower the BChE concentration (usually less than 10 percent of normal values), the stronger the possibility of developing the intermediate neurotoxic syndrome” (Leon-S, Pradilla, et al., 1996). Although the data have certain limitations (see below), differences in ability to metabolize carbamates and OPs (through variability in BChE, toxin interactions, and variability in other detoxifying enzymes) serve as a plausible factors that could influence why only some persons with apparently similar AChE-inhibitor exposure develop intermediate syndrome and others do not. (Of note: OPs are themselves believed to reduce BChE activity, so that cause and effect are difficult to sort out after the fact.)

Although PB is a carbamate, intermediate syndrome has not been reported with use of PB. In addition, it is not possible to distinguish whether low BChE was a cause or effect in this finding, presuming that the finding of depressed BChE in those with intermediate syndrome is valid (data were not given; the source was a non-peer-reviewed letter). Moreover, illnesses in PGW veterans do not strongly correspond in either time-course or symptomatology to intermediate syndrome, a finding that suggests against a role of BChE variability acting through intermediate syndrome as a cause of illnesses in PGW veterans. However, this does not preclude a role for BChE variability influencing the toxicity of PB, nerve agent, and pesticide exposures, individually or in combination, in PGW veterans.

Quantitative Differences in BChE. Although functionally important BChE polymorphisms (differences in form of BChE) have been presumed to be uncommon, based on data regarding “atypical BChE” without more-recent knowledge of the “K variant,” differences in the quantity of BChE (BChE levels) are common (Loewenstein-Lichtenstein, Schwarz, et al., 1995). Because BChE may serve in a scavenger role, quantitative differences in BChE might result in differential susceptibility to cholinesterase inhibitors, including PB. BChE levels in serum of people homozygous for the “usual” BChE allele have been shown to vary with a standard deviation of 25 percent in the normal non-exposed population (Soreq, Ehrlich, et al., 1994; Altland, Goedde, et al., 1971). Decreased BChE levels have been attributed to burn injuries, and kidney or liver dysfunction, while increases have been seen in obesity, asthma, and alcoholism, suggesting variable sensitivity of individuals affected with these and other conditions to PB (or to OP poisoning) (Soreq, Ehrlich, et al., 1994).
Such variability may contribute to differences in results, and consequently to dispute regarding long-term neurological and psychiatric effects of OP poisoning in humans (Soreq, Ehrlich, et al., 1994). Some studies report abnormalities in memory, abstraction, mood, and motor reflexes (Savage, Keefe, et al., 1988), EMG changes (Grob and Harvey, 1953), paralysis (Drenth, Ensberg, et al., 1972), or chronic neuropsychiatric sequelae (Gershon and Shaw, 1961; Dille and Smith, 1964) in OP exposed individuals. There have also been reports of abnormal EEGs on long-term follow-up (Stoller, Krupinsky, et al., 1965; Metcalf and Holmes, 1969; Brown, 1971; Duffy, Burchfiel, et al., 1979), and of aggressive behavior following exposure to cholinesterase inhibitors in four individuals with no history of violence (Devinsky, Kernan, et al., 1992). Meanwhile, others have failed to observe defects in memory or language abilities (Rodnitzky, Levine, et al., 1975) or defects in more overt long-term psychiatric (Tabershaw and Cooper, 1966) and/or neurological (Clark, 1971) measures in man or experimental animals. Because the sample size is small in many of these studies, individual differences could play a role in generation of discrepant findings. However, other factors, such as study design and subject selection, most likely play a major role.

Atypical BChE Is Unlikely to Account for Illnesses in Persian Gulf Veterans. Other polymorphisms and quantitative differences in BChE activity have not been excluded as a participating factor. Homozygous BChE atypia is rare, occurring in less than 1 percent of the population. It has been said that heterozygous individuals “almost never” have a problem with PB or succinylcholine, as the amount of wild-type BChE in their plasma is “adequate” to handle the “standard” dose (Gentry, Bitsko, et al., 1996). Nonetheless, it is possible that this 4–5 percent of the population (homozygotes plus heterozygotes) could be at heightened risk for adverse effects from PB—and that, therefore, atypical BChE genotype, heterozygous or homozygous, could play a role in selected cases. The more common “K variant” of BChE that has reduced scavenging activity provide a more promising avenue for evaluation of genetic differences. Moreover, variation in BChE levels in persons with the “usual” phenotype have not been excluded as a source of differential susceptibility to PB or to illnesses in PGW veterans.

Other Esterases

AChE, which also interacts with esterase inhibitors such as PB (and indeed shows stronger affinity for PB) does not appear to be subject to mutations to the degree that BChE is. Records of phenotypically relevant variants in the AChE gene (that is, variants that actually affect the structure) are limited to a single point mutation (histidine 322 to asparagine 322). This change has been identified as the basis of the Yth blood group, with an incidence of 5 percent in the
“general Caucasian population” and a substantially higher incidence in Israel. However, the functional effects of this mutation have not been described (Soreq, Ehrlich, 1994).

Others esterases (enzymes, such as AChE and BChE, involved in breakdown of chemicals such as ACh and PB) also involved in detoxification, have been evaluated and found to have moderate to striking individual variability. Table 8.2 illustrates individual variability in esterases for several enzymes involved in OP toxicity in a group of Caucasian males aged 17–62. OPs, like PB, are AChE inhibitors. Some veterans report exposure to OP pesticides; moreover recent estimates based on plume modeling suggest that up to 100,000 veterans may have been exposed to very low levels of OP nerve agents (sarin) following demolition of the Iraqi munitions depot at Khamisiyah (Gulflink, 1997). (This may represent a substantial overestimate, because it is based on the union of several different modeling efforts in order not to understate possible effects.) Exposures to OPs may interact with PB exposure, producing synergistic toxicity. (See Chapter Nine, “Interactions Between PB and Other Exposures.”) Not all listed enzymes play a role in PB binding detoxification. For example, paraoxonase does not (Furlong, Richter, et al., 1989), although it may influence levels of sarin and of pesticides, thus influencing synergistic toxicity of PB. (However, it is unlikely that personnel would have continued to receive PB at the time of the Khamisiyah demolition, so that synergistic toxicity with sarin from this event is unlikely.) Moreover, carbamates may play a frankly protective role for the enzyme neurotoxic esterase (NTE). Differences in paraoxonase and other enzymes are discussed below. There may exist qualitative or quantitative individual variation in other unlisted enzymes that influence the metabolism of PB or its interactants.

**Paraoxonase and Other Enzymes.** Paraoxonase (PON) is an HDL-cholesterol associated enzyme that contributes significantly to the metabolism of certain cholinesterase inhibitors; individual variability in this enzyme could contribute to differences in susceptibility to AChE-inhibitor combinations, such as PB with a nerve agent or with a pesticide. PON contributes to the metabolism of such nerve agents as sarin and soman, as well as insecticides. Parathion, chlorpyrifos, and Diazinon are bioactivated by “cytochrome P-450” systems in the liver to become potent cholinesterase inhibitors; the resulting toxic oxon forms can be hydrolyzed by PON1 (Davies, Richter, et al., 1996). Injecting PON protects against OP poisoning in rodent model systems (Li, Costa, et al., 1993; Costa et al., 1990), and interspecies differences in PON activity correlate well with observed median lethal dose (LD₉₀) values (Costa et al., 1987), so that PON is believed to have a physiologically significant role in OP detoxification.
### Table 8.2

**Individual Variation in Esterases in Humans**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sample Size</th>
<th>Measure</th>
<th>Range</th>
<th>Median/ Mean</th>
<th>Deviation from Normality&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC AChE</td>
<td>127</td>
<td>Acetylthiocholine (nmol/mg Hb/min)</td>
<td>22.7–49.3 (2.2-fold)</td>
<td>36 (median)</td>
<td>p &gt; .05</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>127</td>
<td>Benzoylcholine (nmol/ml/min)</td>
<td>659–1,628 (2.5-fold)</td>
<td>1,085 (median)</td>
<td>p &lt; .05</td>
</tr>
<tr>
<td>NTE</td>
<td>113</td>
<td>Phenylvalerate (nmol/mg/min)</td>
<td>2.5–6.2 (6.0-fold)</td>
<td>7.0 (median)</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>PON</td>
<td>127</td>
<td>Paraoxon (nmol/ml/min)</td>
<td>38–237 (6.2-fold)</td>
<td>116 (median)</td>
<td>p &gt; .05</td>
</tr>
<tr>
<td>NaCl-stimulated PON</td>
<td>123</td>
<td>Paraoxon +1 M NaCl (nmol/ml/min)</td>
<td>67–468 (7.0-fold)</td>
<td>176.4 (median)</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Arylesterase</td>
<td>124</td>
<td>Phenylacetate (µmol/ml/min)</td>
<td>38–126 (3.3-fold)</td>
<td>1.41 (mean)</td>
<td>p &gt; .05</td>
</tr>
<tr>
<td>PON:arylesterase ratio</td>
<td>123</td>
<td></td>
<td>0.8–2.2 (2.8-fold)</td>
<td>1.41 (mean)</td>
<td>p &gt; .05</td>
</tr>
<tr>
<td>NaCl-stimulated PON:arylesterase ratio</td>
<td>123</td>
<td></td>
<td>0.9–4.4 (4.9-fold)</td>
<td>2.03 (mean)</td>
<td>p &lt; .001</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < .05 signifies a significant deviation from a normal or Gaussian distribution, using the Shapiro-Wilks W test.

**SOURCE**: Adapted from Mutch, Blain, et al., 1992.

PON is polymorphic in human populations. Moreover, in addition to qualitative (amino acid structure) differences in PON, people express wide quantitative variation in this enzyme or in its activity (Davies, Richter, et al., 1996). While one isoform, the Arg<sub>192</sub> (R<sub>192</sub>) isoform, hydrolyzes paraoxon rapidly, the Gln<sub>192</sub> (Q<sub>192</sub>) isoform hydrolyzes paraoxon slowly. This difference suggests a greater protective effect for the former. However, this enhanced protection is chemical-specific: the enzyme isoforms hydrolyze some chemicals at approximately the same rate (such as chlorpyrifos-oxon and phenylacetate); the apparently less protective isoform (Gln<sub>192</sub> or Q<sub>192</sub> isoform) hydrolyzes nerve agents sarin and soman more rapidly, actually conferring greater protection against these nerve agents and reversing the perspective on which isoform is considered more protective (Davies, Richter, et al., 1996). This finding serves as a caution that persons relatively robust to one exposure may be relatively susceptible to another and that susceptibility to a specific agent does not imply an implicitly “weaker” or more vulnerable trait. Different populations have different frequencies of these isoforms, with Hispanic persons showing a frequency of 0.41 for the R<sub>192</sub> allele and those of Northern European origin showing a 0.31 frequency (corresponding to around 16 percent versus 9 percent homozygotes, respectively) (Furlong, Richter, et al., 1989; Geldmacher-von
Mallinckrodt and Diepgen, 1988). The mean value for sarin hydrolysis for the R<sub>192</sub> homozygotes was only 38 ± 47 U/l, compared to 355 U/l for the Q<sub>192</sub> homozygotes (Davies, Richter, et al., 1996)⁴ (see Table 8.3).

While average differences in detoxification ability between PON alleles are marked, actual ability to hydrolyze substrates varies substantially for persons with a given isoform, dictating the need to identify phenotype as well as genotype, by enzymatic analysis, to determine the level of risk (Davies, Richter, et al., 1996). (See Table 8.3 for mean values and range for different isoforms.) Of note, exposures to OPs themselves may result in reduced levels of PON (Dwyer, 1998), which may be important not only for detoxification but also because PON may determine whether HDL-cholesterol confers protection or harm for atherosclerosis. Thus it may be instructive to follow exposed veterans and controls for atherosclerotic development.

Polymorphisms that influence susceptibility to nerve agents and pesticides modify the effect of PB, potentially rendering it more toxic; and PB administration may enhance the neurotoxicity of the other agents, an effect that could interact with PON type and quantity. Thus far the influence of PON isoforms has been discussed, not in regard to PB but in relation to susceptibility to pesticide and nerve agent effects. In fact, there is no role for PON in breakdown of PB (Furlong, Richter, et al., 1989). However, “chemical stress” in the form of nerve agent exposure may, in animal models, lead to enhanced ability of PB to penetrate the blood-brain barrier (see Chapter Seven, “Blood-Brain Barrier Passage”; and Chapter Nine, “Interactions Between PB and Other Exposures”). Moreover, the toxicity of PB has been shown to be synergistic with pesticides in

### Table 8.3

<table>
<thead>
<tr>
<th>PON Isoforms: Substrate Activities in Human Sera for Sarin, Soman, and PON⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sarin</strong></td>
</tr>
<tr>
<td>Mean±SD</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>QQ isoform</td>
</tr>
<tr>
<td>QR isoform</td>
</tr>
<tr>
<td>RR isoform</td>
</tr>
</tbody>
</table>

SOURCE: Davies, Richter, et al., 1996. Note that the isoforms that are most active against PON are least active against sarin and soman.

⁶Sample sizes range from 26–33 (QQ), 38–41 (QR), 11–18 (RR), 75–92 (all).

⁴Of peripheral relevance, the Q<sub>192</sub> allele destroys biologically oxidized phospholipids, which contribute to heart disease, while the R<sub>192</sub> allele represents instead a risk factor for CAD, so that PON polymorphism is associated with other health-related effects.
animal studies (see Chapter Nine). Many veterans report exposure to personal pesticides; and the degree of exposure to nerve agents in the PGW is a matter of continued investigation. For this and other reasons, PON polymorphisms could contribute to neurotoxic effects of exposures in ill PGW veterans, and this effect could be amplified by administration of PB. Additionally, PON is acutely reduced following exposure to OPs (Dwyer, 1998), and this could additionally burden nonspecific scavengers, contributing to interactive toxicity.

**Other Enzymes.** While no literature suggesting a specific role for other enzymes has been evaluated in the present literature review, there are likely (“almost certainly”) other enzymes not yet known that have a role in metabolism of PB, which may contribute to individual differences in response to PB (Patrick, 1997).

**Effects of Native Differences in Brain Chemistry**

PB is used by scientists to assess function of growth hormone. Ordinarily, administration of PB leads to a growth hormone surge. However, the influence of PB on growth hormones is altered in a variety of conditions, conditions that are presumed to differ in cholinergic responsiveness for reasons that, in many cases, have not been elucidated. Among the variables and conditions known to modify the growth hormone response to PB are: age (Arvat, Gianotti, et al., 1996; Corsello, Tofani, et al., 1991); sex (Corsello, Tofani, et al., 1991), perhaps mediated through effects of sex steroids estrogen and testosterone (O’Keane, O’Flynn, et al., 1992; Eakman, Dallas, et al., 1996); dementia (Arvat, Gianotti, et al., 1996; Ghigo, Goffi, et al., 1993; Murialdo, Fonzi, et al., 1993); obsessive compulsive disorder (Lucey, Butcher, et al., 1993); affective disorder including depression (Thakore and Dinan, 1995) and mania (Dinan, O’Keane, et al., 1994); obesity (Procopio, Invitti, et al., 1995); hypercortisolism (Procopio, Invitti, et al., 1995; Borges, Castro, et al., 1993); and cognitive disorders (such as schizophrenia) (O’Keane, Abel, et al., 1994).

Thus, identified individual differences alter the effect of PB on one outcome—growth hormone response—that is commonly measured. Differences in cholinergic responsiveness as measured by a growth hormone response may or may not reflect more general differences in cholinergic responsiveness.

**Concurrent, Antecedent, and Subsequent Exposures**

Exposures to chemicals with which PB interacts, such as pesticides (use of personal pesticides was reported by 17 of the 20 CCEP-registered PGW cases in the BCHE trial cited above (Gentry, Bitsko, et al., 1996)), nerve agents, cocaine (Loewenstein-Lichtenstein, Schwarz, et al., 1996; Kaufer, Friedman, et al., 1998),
and other medications such as antihistamines, may provide a basis for apparent individual differences in response to PB, even in the absence of native differences in biochemistry or susceptibility. (See also Chapter Nine.) Moreover, differences in biochemical susceptibility to these exposures may amplify the potential for individual differences in response to PB. Nonetheless, the role of such differences in biochemical susceptibility to PB and subsequent development of chronic illness, such as that seen in PGW veterans, remains undefined.

**Circadian Effects**

Circadian differences may play a role in individual differences, since individuals may have received PB at different times of day.

The role of circadian variation in drug effects is increasingly appreciated: “In humans, variations during the 24 h day in pharmacokinetics (chronopharmacokinetics) have been shown for cardiovascularly active drugs (propranolol, nifedipine, verapamil, enalapril, isosorbide 5-mononitrate and digoxin), antiasthmatics (theophylline and terbutaline), anticancer drugs, psychotropics, analgesics, local anesthetics and antibiotics, to mention but a few. Even more drugs have been shown to display significant variations in their effects throughout the day (chronopharmacodynamics and chronotoxicology) even after chronic application or constant infusion. Moreover, there is clear evidence that even dose/concentration-response relationships can be significantly modified by the time of day. Thus, circadian time has to be taken into account as an important variable influencing a drug’s pharmacokinetics and its effects or side-effects” (Lemmer, 1995). Susceptibility to illness is also influenced by time of day (Lemmer, 1995).

These effects have been capitalized on in treatment protocols. For example, one European multicenter randomized trial found the a chemotherapy regimen for colorectal cancer that was sensitive to circadian timing of delivery resulted in “2 to 10 times fewer” severe toxic effects and a 70 percent increase in objective response rate compared to non-circadian-sensitive treatment (51 percent response with “chono” treatment and 30 percent with other treatment; p < 0.001) (Levi, Giacchetti, et al., 1995). Otherwise phrased, wrongly timed drug delivery could result in 2 to 10 times more toxic effects (or more, if the “bad” delivery was not selected to be worst-case) and a 41 percent reduction in efficacy for these cancer chemotherapeutic agents.

Circadian effects have also been shown for drugs that influence the cholinergic system. In mice, the lethality of the drug lithium depends on what time of day the injection is given (Hawkins, Kripke, et al., 1978). Lithium appears to antagonize cholinergic effects in rodents, including effects on behavior (Janowsky, Abrams, et al., 1979) and on REM sleep (Campbell, Gillin, et al., 1989), and may
do so by acting on the central cholinergic system (Janowsky, Abrams, et al., 1979). More directly pertinent is evidence that the effects of ACh inhibitors, such as soman and OP pesticides, are modified in a circadian fashion (von Mayersbach, 1974; Fatranska, Vargova, et al., 1978; Elsmore, 1981; Augerson, 1986). Moreover, the cholinergic system itself modulates circadian variation of hypothalamic pituitary adrenal activity (Llorente, Lizcano, et al., 1996), suggesting the possibility that PB effects may modify or amplify stress hormone (and other hormone) responses depending on circadian timing of drug delivery.

Indirect evidence further suggests circadian-related effects on response to acetylcholinergic drugs. Bright light prevents the development of supersensitivity to an anticholinesterase (oxotremorine) occurring as a result of forced stress or treatment with a muscarinic receptor antagonist in the rat (Overstreet, Dilsaver, et al., 1990). Treatment with bright light during the regular photoperiod (a time that does not produce a phase-shift or free-running) differentially affects the hypothermic response and activity suppressing effect of both Flinders Sensitive Line (FSL), sensitive to an anticholinesterase) and the Flinders Resistant Line (FRL) rats (Overstreet, Dilsaver, et al., 1990). Both lines exhibit decreased hypothermia without reduction in motor activity in response to oxotremorine following six days of treatment with bright light. The magnitude of blunting of the hypothermic response was greater in the FSL than the FRL rats (Overstreet, Dilsaver, et al., 1990). Thus, the effects of bright light are contingent on the endpoint measured, and the capacity of bright light to blunt the hypothermic response to a muscarinic agonist is greater in animals with an endogenously hyperactive muscarinic cholinergic system (Overstreet, Dilsaver, et al., 1990). Thus, cholinergic stimulation appears to have less of at least some effects when there is exposure to bright light. The possibility cannot be excluded that this factor could have contributed to relatively greater susceptibility to effects of PB in security personnel who worked at night.

**Age, Sex, and Weight**

Sex differences in mean AChE and BChE have been reported in a case control study of 20 veterans and 20 “normal volunteers.” Spontaneous reactivation half-times, in minutes, were 45.2±5.6 minutes in men, and 38.6±5.0 minutes in women (p < .05). The effect on the population as a whole was attributed largely to the difference seen between male and female veterans (Gentry, Bitsko, et al., 1996).

Although one study failed to find sex or weight differences in AChE inhibition with PB (Lasseter and Garg, 1996), large individual differences in AChE inhibition were present; the variability due to individual differences from other factors is likely to have obscured statistical differences based on weight and per-
haps sex. Alternatively, if one accepts that weight truly has no bearing on the effect of the drug, then it becomes unnecessary to correct doses when extrapolating from animal studies using smaller species, such as rodents. “High” doses of PB given in animal studies are generally higher than PGW doses on a per-weight basis—but not necessarily higher in terms of actual mg given. Thus, if one credits the view that weight is immaterial, toxicity findings from such animal studies might be more directly extrapolated to what might be expected in PGW veterans. It is far more likely however, that inability to show differences in AChE inhibition as a function of weight merely reflects the many other sources of individual variation that add to the variance. In that case, a large sample size would be needed to show the independent effect of weight.

SUMMARY ANALYSIS

Likelihood of “Exposure” (i.e., of PGW Veterans Experiencing Individual Differences in PB Susceptibility)

Individual differences occur in response to administration of any chemical agent. For example, for virtually any chemical an “LD$_{50}$” can be identified—a dose at which 50 percent of the animals will die while 50 percent will not—even in highly genetically inbred strains, although the animals are presumed genetically homogeneous and the exposure was the same for all. Moreover, small differences in susceptibility can lead to large differences in clinical outcome in selected instances. For instance, a study examining the effect of the breach of the blood-brain barrier on penetration to the CNS by a virus found that those animals that did not die were entirely healthy, though all received the same dose (see Chapter Seven, “Blood-Brain Barrier Passage”). Therefore, “exposure” to individual differences in susceptibility to PB was certainly present in PGW veterans. Data demonstrating major differences in absorption of PB and rate of choline inhibition for a given blood level of PB (Chapter Three) further support the certainty that individual differences in effective exposure (gauged by measures of PB in the blood, or by consequences of PB in the body), and in response to PB, occurred.

Symptoms Consistent With “Exposure” (Individual Differences)

Individual differences in acute response to PB are reflected in differences in side effects to PB that have been reported (Chapter Three). Character and severity of chronic symptoms in PGW veterans differ from one individual to the another, compatible with (though not persuasive for, or even necessarily suggestive of) individual differences in susceptibility to PB.
Evidence Relating Symptoms to Individual Differences

One relatively weak line of evidence suggests a relationship between chronic illnesses in PGW veterans and individual differences in acute response to PB—differences that might be presumed to reflect differences in susceptibility to toxic effects of PB, including chronic effects if any exist. This stems from the previously cited study that found a relationship between two of three factor-analysis derived syndromes in ill PGW veterans and self-reported adverse response to administration of PB during the PGW (Haley and Kurt, 1997).

No evidence has demonstrated a correlation between identified biochemical differences and chronic illnesses. (Although evidence has failed to show a relation between atypical BChE and CCEP registration in a small sample, a role for atypical BChE appears unlikely in any case, based on its very low prevalence in the population.)

Scientific Recommendations

1. An attempt should be made to replicate the syndromes identified by Haley, et al. (1997a), and particularly to determine whether self-reported adverse response to administration of PB is linked to chronic illnesses in PGW veterans in a second, preferably larger, sample of ill PGW veterans. Moreover, an attempt should be made to replicate the results using different techniques such as unsupervised neural networks, and/or employing cross validation, on the same pool of veterans. Potential confounding factors will be an issue because subjects may be concerned about long-term effects of PB, and recall bias remains an issue. Nonetheless, the information provided will add or lessen support for a connection between individual adverse response to PB and illnesses.\(^5\)

2. Consideration should be given to testing for mutations—such as the K variant of BChE—that occur more commonly than atypical BChE, and that have also been shown to be less active.

3. Studies should be performed to assess the impact of phenotype (quantity and activity, as well as type of enzyme), rather than exclusively for genotype, for BChE, PON, and possibly for other esterases. Phenotypes should be compared in PGW veterans with and without illness. Phenotype (amount and activity and consequent ability to hydrolyze PB) varies greatly

---

\(^5\)Since the draft of this report was originally circulated, other factor analyses have been published, as has another study linking self-reported PB to illness in PGW veterans (Unwin, Blatchley, et al., 1999). These findings are discussed in limited fashion elsewhere in the report.
within those with a normal genotype, and could contribute (along with other factors, such as pesticide and possible nerve agent exposure) to individual differences in response to PB. (If a highly susceptible group is identified by genotype or phenotype, future testing of military personnel might be performed to identify susceptible individuals, who might be debarred from deployment to high chemical warfare threat areas in which military use of PB might occur.)

4. Prior to future deployment, it would be prudent to ascertain the effect of phenotypic differences in AChE and BChE on the effect of PB measured by AChE inhibition.

5. A sample of PGW veterans with and without illnesses, of adequate size to permit testing for modest effect sizes with high power, should be developed as a study group. Careful delineation of symptoms should be made, and blood should be collected and processed for storage for future genetic and blood analyses as new candidate explanatory factors are uncovered.