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BRAIN CHOLINESTERASE ACTIVITY OF APPARENTLY NORMAL WILD BIRDS

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ABSTRACT: Organophosphorus and carbamate pesticides are potent anticholinesterase substances that have killed large numbers of wild birds of various species. Cause of death is diagnosed by demonstration of depressed brain cholinesterase (ChE) activity in combination with chemical detection of anticholinesterase residue in the affected specimen. ChE depression is determined by comparison of the affected specimen to normal ChE activity for a sample of control specimens of the same species, but timely procurement of controls is not always possible. Therefore, a reference file of normal whole brain ChE activity is provided for 48 species of wild birds from North America representing 11 orders and 23 families for use as emergency substitutes in diagnosis of anticholinesterase poisoning. The ChE values, based on 83 sets of wild control specimens from across the United States, are reproducible provided the described procedures are duplicated. Overall, whole brain ChE activity varied nearly three-fold among the 48 species represented, but it was usually similar for closely related species. However, some species were statistically separable in most families and some species of the same genus differed as much as 50%.

Key words: Wild birds, brain cholinesterase activity, normal values, toxicology, anticholinesterase poisoning, diagnostic methodology, organic phosphorus pesticide, carbamate pesticide.

INTRODUCTION

Organophosphorus and carbamate pesticides are potent anticholinesterase substances that are used extensively in diverse wildlife habitats and have been responsible for the death of large numbers of birds of various species (Seabloom et al., 1973; Mendelsohn and Paz, 1977; Stone, 1979; Hamilton et al., 1981b; Grue et al., 1983; Hardy and Stanley, 1984; Smith, 1987). Cause of death in such kills is best diagnosed by demonstration of depressed brain cholinesterase (ChE) activity in combination with chemical detection of anticholinesterase residue in the ingesta or postabsorptive tissues of affected specimens (Hamilton et al., 1976; Hill and Fleming, 1982). A conservative threshold of about 50% depression in whole brain ChE activity has been proposed as diagnostic of death from anticholinesterase poisoning (Ludke et al., 1975), even though depression of more than 70% is routinely reported for birds killed experimentally with either single or multiple doses of organophosphorus or carbamate pesticide (Bunyan et al., 1968a, b; Shellenberger et al., 1970; Ludke et al., 1975; Westlake et

al., 1981a, b). Exposure levels in the field undoubtedly are more variable than in the laboratory, yet depression of whole brain ChE activity consistently exceeds 80% in birds killed in the field by organophosphorus poisoning (White et al., 1979, 1983; Hill and Fleming, 1982; White and Kolbe, 1985; Henny et al., 1987). In contrast, when birds are killed in the field by carbamates, whole brain ChE activity may vary from near normal to depressions of only 60 or 70% (Hill and Fleming, 1982; Flickinger et al., 1986; E. F. Hill, unpubl. data). These lesser and more variable depressions of brain ChE activity associated with carbamate kills may be due to a high level of exposure resulting in rapid death from neuromuscular blockage which may precede significant penetration of the central nervous system (Westlake et al., 1981b), or may simply reflect various degrees of spontaneous postmortem reactivation of carbamylated ChE (Hill and Fleming, 1982).

Brain ChE activity has been used also as an indirect means of monitoring exposure of wild birds to field applications of anticholinesterase pesticides (Elder and

Henderson, 1969; Hill et al., 1971; McLean et al., 1975; Zinkl et al., 1977, 1980; Busby et al., 1981, 1982, 1983; Hamilton et al., 1981a; Custer et al., 1985; Henny et al., 1985; McEwen et al., 1986). However, because sublethal implications of inhibited whole brain ChE activity are not well understood, it is probably inappropriate to assume a direct correlation exists between the degree of depression and level of toxicity. Instead, ChE depression in apparently healthy specimens obtained by random selection or chance simply indicates recent exposure to anticholinesterase substance, and provides only circumstantial evidence of the presence of active substance in a particular habitat. The degree of depression indicative of exposure is a statistically derived value that must be determined for each species under the specific conditions of each study and may vary from about 10% to over 40% (Ludke et al., 1975; Grue et al., 1983).

The principal requisite for demonstrating a species' exposure to, or death from, an anticholinesterase substance is the determination of the species normal whole brain ChE activity. Ideally, calculation of normal ChE activity is based on a statistically adequate sample of control specimens of the same species, age, sex, and physiological status as the species of interest, and should be collected from a locale where exposure to anticholinesterase substance was not likely. Factors of storage, processing, and biochemical assay should be the same for all specimens. Adherence to the above criteria is a reasonable expectation for planned studies of pesticide application, but may sometimes be too restrictive for the spontaneity required in field diagnosis of unexpected wildlife mortality. In diagnostic cases, the species cannot be anticipated and procurement of optimal controls may not always be possible. Therefore, it has been suggested that development of a reference file of normal brain ChE activity under a standardized protocol for various wildlife species would enhance our diagnostic capability in the

absence of concurrent controls (Westlake et al., 1983; Hill and Murray, 1987). Although use of concurrent controls is always preferred, such a reference file could be used as a substitute in cases when controls are not obtainable. The reference file would provide an estimate of the mean and normal bounds of whole brain ChE activity for each species within the constraints of a set of storage and assay conditions that can be provided in a modestly equipped field laboratory. Although an optimal data set would be based on specimens of the same age, sex, and physiological status, none of these variables or season of the year have been shown to interact with whole brain ChE activity in postfledging birds to the degree that pooling without regard to age or sex would result in a misdiagnosis (Hill and Murray, 1987). Nestling altricial and very young precocious birds may have significantly lower brain ChE activity than older subadults and adults which appear inseparable (White et al., 1979; Grue et al., 1981; Grue and Hunter, 1984).

The purpose of this paper is to (a) present the whole brain ChE activity of 48 species of apparently healthy wild birds, (b) compare data sets of captive and free-ranging populations, (c) discuss factors that may affect results of the assay, and (d) consider the utility of archived data for use in the field diagnosis of wildlife mortality from anticholinesterase poisoning.

MATERIALS AND METHODS

The data reported herein are summarized from 83 sets of control specimens submitted to the Patuxent Wildlife Research Center (PWRC; Laurel, Maryland 20708, USA) between 1978 and 1985 for use as reference samples in the evaluation of possible cases of wildlife poisoning by anticholinesterase pesticides. Because each case was essentially unique and the level of interest varied widely among field investigators, methods of collecting and handling control specimens were irregular among the cases and exact procedures were often poorly documented. Therefore, for the purposes of this evaluation, and probably the strictest test of the utility of archived control data, variations in field han-

dling and shipping procedures were not considered in statistical treatment of data.

Specimen collections

Submitters were requested to collect 5–10 apparently healthy specimens of the affected species from a location where recent exposure to anticholinesterase pesticides was unlikely. If very young and adult specimens were involved in the poisoning, then control specimens from both age groups were requested, but other factors such as sex and reproductive status were not considered critical. Control specimens were then treated exactly the same as the affected specimens. The only mandated procedure was to chill all specimens to near freezing as soon postmortem as possible. Although immediate freezing is the preferred method of preserving tissues for enzymology and residue analysis, prompt refrigeration was usually the method of choice in deference to storage requirements in evaluation of infectious diseases. After procurement of controls, which sometimes took several days, both controls and affected specimens were to be shipped intact on either wet or dry ice to PWRC for final processing and whole brain ChE assay. Between 1982 and 1985, cases other than obvious incidents of poisoning were first examined at the National Wildlife Health Center (NWHC; Madison, Wisconsin 53711, USA) for infectious diseases and then were forwarded to PWRC for toxicological tests. All specimens were stored at -25°C for at least 3 days at PWRC prior to preparation for same day ChE assay under a common protocol. This period of freezing was a conservative procedure used to insure pre-assay consistency in an effort to compensate for any changes of ChE activity that may have occurred during the freezing process. It has been reported that brain ChE activity may decrease somewhat during the first few hours of freezing at moderate temperatures but then it appears to stabilize and remains essentially unchanged for at least 2 wk (Ludke et al., 1975).

Sample preparation and ChE assay

Preparation of samples and determination of whole brain ChE activity were based on the method of Ellman et al. (1961) with several minor modifications for ease of use in a modestly equipped laboratory. With the exception of some variability in the assay temperature of a few pre-1978 data sets, the following summary represents the methods used in development of the baseline ChE activities presented in Table 1. An outline of procedural details in preparation of reagents, conduct of the assay, sources of error, and alternative techniques has been published elsewhere (Hill and Fleming, 1982).

Sample preparation: Specimens were partially thawed to facilitate removal of the cranium. The intact brain was then excised, bisected medially, and each half was weighed. One-half was promptly returned to the freezer for possible future studies in the event that a sample was destroyed or other chemical analysis was desired. The other half, representing “whole” brain, was homogenized in 0.05 M Tris buffer (pH 8.0) at the ratio of 100 mg/ml with a power driven homogenizer. If the half brain weighed more than about 1 g, it was diced, stirred and several subsamples totaling about 0.5 g were pooled for the purpose of reducing the sample to a manageable size for homogenization. After homogenization the sample was refrigerated at $\approx 4^{\circ}\text{C}$ pending same day assay. If the ChE assay could not be performed on the day of dissection, the half brain in buffer was frozen and homogenization deferred until the day of assay.

ChE assay: All ChE assays were conducted in duplicate under strictly linear conditions at 25°C , or prior to 1978 at room temperature and corrected to 25°C (Boehringer Mannheim Corp., 1971). Linearity of the assay was verified by periodic comparison of three to four serially diluted concentrations of sample over time. Reactions were conducted in 4-ml quartz or disposable polystyrene photocells with a 1-cm lightpath, and absorbance was measured at a wavelength of 405 nm with an ACTA II double-beam spectrophotometer (Beckman Instruments, Fullerton, California 92634, USA) equipped with a circulating waterbath and chart recorder. The reagents and steps of the assay were as follows:

1. In sequence the following were added to the photocell:
 - a. 3.0 ml of buffer/chromogen reagent (0.05 M Tris buffer, pH 7.4; 2.5×10^{-4} M 5,5'-dithiobis[2-nitrobenzoic acid]).
 - b. 20 μl of thoroughly mixed whole brain homogenate.
 - c. 100 μl of acetylthiocholine substrate (0.156 M acetylthiocholine iodide).
2. The photocell was then covered with parafilm, gently inverted, the bubbles tapped out, and the solution was allowed to stabilize for 30 to 60 sec.
3. The sample was placed in a spectrophotometer set at a wavelength of 405 nm and change of absorbance was read for 2 to 3 min at 25°C .
4. The average change of absorbance per minute was multiplied by a factor of 130 and ChE activity was reported as “ μmoles of acetylthiocholine iodide hydrolyzed per min per g of tissue (wet weight) at 25°C .”

Estimation of normal bounds of ChE activity

Normal bounds of whole brain ChE activity as presented in Table 1 are defined as two standard deviations above and below the arithmetic mean for a random selection of postfledging birds of a given species, and without regard for their exact age, sex, or physiological status (Copeland, 1974; Hill and Fleming, 1982; Hill and Murray, 1987). Normal bounds were calculated uniformly for all data sets; this involved simple pooling of all relevant observations, but their credibility for diagnostic use was classed on the basis of adherence to the basic assay procedure, number of data sets, and sample size. Class I bounds are pooled estimates based on individuals from at least two sets ($n \geq 3$ per set) of controls assayed at the reaction temperature of 25 ± 1 C, and where the data sets were statistically inseparable (F -test, $\alpha = 0.05$). Class II bounds are estimates based on at least five individuals from a single set of controls assayed at the reaction temperature of 25 ± 1 C. Class III bounds are estimates based on sample sizes of <5 or data sets where assay temperatures were outside 25 ± 1 C. Temperature outliers were adjusted to 25 C by use of standard temperature conversion tables (Boehringer Mannheim Corp., 1971). Statistical comparisons of whole brain ChE activity within and between taxonomic groups were by one-way analysis of variance with mean separations only for significant F -tests by Duncan's (1955) procedure. Statistical significance was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The whole brain ChE activity was determined for 48 species of wild birds representing 11 orders and 23 families during 10 yr of evaluating cases of wildlife mortality from across the United States (Table 1). The presented values apply exclusively to whole brain homogenate assayed by the Ellman et al. (1961) method as described earlier in this paper. The specificity of the assay method is critical because ChE activity may be measured by many methods that are satisfactory for gross diagnostic purposes (Witter, 1963; Aldrich, 1969), but results are not usually interchangeable because of differences in substrates, reaction principles and conditions and basis of expressing ChE activity. Results may even be incompatible for a given method within a laboratory. For example, in the mid-1970's the Ellman et al. (1961) method was

used in a series of studies at PWRC. One study deviated, however, in that supernatant of whole brain homogenate was assayed and the ChE activity based on the protein concentration of the sample and reported as nmoles acetylthiocholine iodide hydrolyzed per min per mg protein (Ludke et al., 1975), while the other studies assayed the homogenate and reported ChE activity as nmoles acetylthiocholine iodide hydrolyzed per min per mg tissue (Dieter and Ludke, 1975) or μ moles acetylthiocholine iodide hydrolyzed per min per g tissue (Dieter and Ludke, 1978). Although it has been determined that whole brain ChE activity is about 2–2.5 times that of its supernatant (E. F. Hill, unpubl. data), the first study was not compatible with the latter studies because enzyme activity was reported on the basis of protein concentration. The latter two studies were conducted essentially the same and the units of enzyme activity are interchangeable, but the buffering systems differed; 0.1 M phosphate versus 0.05 M Tris and the reaction temperatures were not reported. All comparisons of enzyme activities within these studies used internal controls and therefore were valid, but intertest comparisons are inappropriate without knowledge of the exact influence of possible buffer and temperature differences on reported ChE activities.

The reaction temperature is vital to enzymatic activity. It has been recommended that all assays be conducted at the same temperature rather than corrected through standard conversion tables (Hill and Fleming, 1982). Use of such conversion tables requires the improbable assumptions that temperature is the principal variable affecting batches as assays conducted at different times, and that the temperature-ChE activity relationship is the same for whole brain homogenate as for blood serum. The former assumption is an unreasonable expectation, particularly among field laboratories. The latter was not supported by a study which indicated a curvilinear relationship between temperature

and whole brain homogenate (Hill and Fleming, 1982) compared to a linear relationship for plasma (Boehringer Mannheim Corp., 1971). For these reasons, deviant assay temperature of below 24 C or above 26 C was an important criterion in downgrading otherwise sound data sets to Class III.

Because each incident of wildlife mortality is essentially unique, it was believed initially that a set of concurrent controls was required for each case to negate influences of variable storage and assay conditions on ChE activity (Hill and Fleming, 1982). However, multiple sets of controls were received over time for some species, and statistical comparisons indicated whole brain ChE activity was inseparable among the data sets. Six species were selected to demonstrate the consistency among data sets collected at different times and stored under undefined conditions except all had been finally frozen at -25 C for at least 3 days prior to assay (Table 2). The species represented were selected for their phylogenetic diversity or because they were worst case data sets; they were comprised of individuals of different origins drawn from storage at PWRC or NWHC rather than collected and handled as a control package (1982 brown pelican, *Pelecanus occidentalis*; 1981 and 1984 golden eagle, *Aquila chrysaetos*). The difference in means of whole brain ChE activity among intraspecific data sets varied from 0 (mallard, *Anas platyrhynchos*) to 23% (red-winged blackbird, *Agelaius phoeniceus*), but set to set differences were usually <5%. Although sample size varied from 3 to 13, coefficients of variation (CV) for the data sets varied from 9 to 28 and exceeded 15 only in the pelican and eagle sets mentioned above and the 1980 red-winged blackbird data sets. The extreme ChE values within data sets usually differed by 1.3–1.7 times. Both the consistency among data sets and the reliability of any given data set for gross clinical use was indicated by the similarity of the calculated diagnostic threshold which varied <1.5 ChE

activity units between data sets for five of the six species compared in Table 2. The diagnostic threshold for the 1981 golden eagle set was 3.6 ChE activity units lower than the 1984 set. Because the eagles had different histories, this disparity may be the result of unequal within-set storage conditions and duration or some individuals may have experienced sublethal exposure to anticholinesterase substance prior to death from another cause. Avian brain ChE activity may take as long as 1 mo to return to normal, depending on the initial degree of inhibition (Fleming and Grue, 1981).

Although sublethal anticholinesterase exposure could affect the utility of free-ranging birds as controls, this source of error has not proven to be a serious problem. For example, if incidental exposure resulted in 15–30% ChE inhibition in a few individuals of a sample of 5–10, it would increase the variance and reduce the diagnostic threshold but would probably not significantly alter the mean ChE activity of the data set. Since the first step in evaluation of anticholinesterase poisoning is the quantitative demonstration of ChE depression, the inflated variance would simply result in a more conservative diagnostic threshold. The second step of the evaluation is based on the mean ChE activity of the controls which are usually similar within a given species in spite of irregular variances among data sets. These relationships are illustrated by the disparate golden eagle data sets in Table 2. The diagnostic threshold of exposure was 38% lower in 1981 than in 1984 while the means of 13 and 15 ChE activity units differed by a statistically inseparable 13%, and 50% depression which is considered diagnostic of death differed by only one activity unit.

The frequency with which a set of haphazardly collected free-ranging controls includes some individuals with depressed ChE activity is not known. However, when collections are made at one time from a single flock or locale, the likelihood of such within-set disparity is lessened. This con-

TABLE 1. Whole brain cholinesterase activity (μ moles of acetylthiocholine iodide hydrolyzed per min per g of tissue, wet weight) of apparently healthy wild birds.

Family and species	n	Mean	SD	Bounds ^a	Class ^b
Pelecanidae					
Brown pelican (<i>Pelecanus occidentalis</i>)	22	10	1.5	7–13	I
Phalacrocoracidae					
Double-crested cormorant (<i>Phalacrocorax auritus</i>)	3	25	6.1	12–38	III
Ardeidae					
Black-crowned night-heron (<i>Nycticorax nycticorax</i>)	9	14	0.9	12–16	I
Anatidae (Anserinae)^c					
Tundra swan (<i>Cygnus columbianus</i>)	2	17	1.4	14–20	III
Snow goose (<i>Chen caerulescens</i>)	9	18	3.5	11–25	I
Brant (<i>Branta bernicla</i>)	10	14	2.3	9–19	II
Canada goose (<i>Branta canadensis</i>)	19	13	1.6	9–17	I
Anatidae (Anatinae)^c					
American black duck (<i>Anas rubripes</i>)	14	8	0.8	6–10	III
Mallard (<i>Anas platyrhynchos</i>)	11	12	1.3	9–15	I
Gadwall (<i>Anas strepera</i>)	9	15	2.1	10–20	II
American wigeon (<i>Anas americana</i>)	8	10	1.2	7–13	II
Canvasback (<i>Aythya valisineria</i>)	3	9	0.6	7–11	III
Redhead (<i>Aythya americana</i>)	5	10	1.1	7–13	II
Cathartidae					
Black vulture (<i>Coragyps atratus</i>)	4	18	0.8	16–20	III
Accipitridae^c					
Bald eagle (<i>Haliaeetus leucocephalus</i>)	6	16	1.7	12–20	I
Sharp-shinned hawk (<i>Accipiter striatus</i>)	10	21	1.3	18–24	II
Cooper's hawk (<i>Accipiter cooperii</i>)	3	24	3.5	17–31	III
Red-tailed hawk (<i>Buteo jamaicensis</i>)	15	19	3.2	12–26	I
Golden eagle (<i>Aquila chrysaetos</i>)	16	14	3.2	7–21	I
Falconidae					
American kestrel (<i>Falco sparverius</i>)	11	27	2.8	21–33	I
Phasianidae^c					
Ring-necked pheasant (<i>Phasianus colchicus</i>)	10	14	1.0	12–16	II
Sage grouse (<i>Centrocercus urophasianus</i>)	8	11	0.7	9–13	I
Northern bobwhite (<i>Colinus virginianus</i>)	14	13	1.2	10–16	I
Rallidae					
American coot (<i>Fulica americana</i>)	5	18	1.5	15–21	II
Gruidae					
Sandhill crane (<i>Grus canadensis</i>)	8	17	1.5	14–20	II
Scolopacidae					
Willet (<i>Catoptrophorus semipalmatus</i>)	17	14	2.5	9–19	III
Least sandpiper (<i>Calidris minutilla</i>)	2	22	3.5	15–29	III
Dunlin (<i>Calidris alpina</i>)	15	15	3.1	8–23	III
Laridae^c					
Laughing gull (<i>Larus atricilla</i>)	10	16	3.9	8–24	III
Ring-billed gull (<i>Larus delawarensis</i>)	9	18	2.6	12–24	I
California gull (<i>Larus californicus</i>)	2	20	3.0	14–26	III
Herring gull (<i>Larus argentatus</i>)	5	23	3.0	17–29	II

TABLE 1. Continued.

Family and species	n	Mean	SD	Bounds ^a	Class ^b
Columbidae ^c					
White-winged dove (<i>Zenaida asiatica</i>)	5	21	1.6	17–25	II
Mourning dove (<i>Zenaida macroura</i>)	7	16	1.0	14–18	I
Tytonidae					
Common barn owl (<i>Tyto alba</i>)	11	20	3.4	13–27	I
Strigidae ^c					
Eastern screech owl (<i>Otus asio</i>)	11	19	2.5	14–24	I
Great horned owl (<i>Bubo virginianus</i>)	19	16	2.5	11–21	I
Trochilidae					
Ruby-throated hummingbird (<i>Archilochus colubris</i>)	2	20	0.7	18–22	III
Hirundinidae					
Barn swallow (<i>Hirundo rustica</i>)	19	12	1.4	9–15	I
Corvidae					
American crow (<i>Corvus brachyrhynchos</i>)	8	20	3.4	13–27	II
Muscicapidae					
American robin (<i>Turdus migratorius</i>)	7	18	1.1	15–21	II
Bombycillidae					
Cedar waxwing (<i>Bombycilla cedrorum</i>)	8	22	3.1	15–29	I
Sturnidae					
European starling (<i>Sturnus vulgaris</i>)	12	22	2.0	18–26	I
Emberizidae					
Sharp-tailed sparrow (<i>Ammodramus caudacutus</i>)	15	22	2.2	17–27	I
Red-winged blackbird (<i>Agelaius phoeniceus</i>)	19	21	3.3	14–28	I
Common grackle (<i>Quiscalus quiscula</i>)	20	20	3.0	14–26	I
Brown-headed cowbird (<i>Molothrus ater</i>)	4	21	4.2	12–30	III
Passeridae					
House sparrow (<i>Passer domesticus</i>)	4	20	4.2	11–29	III

^a Normal bounds are defined as two standard deviations above and below the arithmetic mean (Copeland, 1974).

^b Status: I, pooled value for multiple data sets (F -test, $NS = 0.05$) assayed at 25 ± 1 C; II, single data set of $n > 5$ assayed at 25 ± 1 C; and III, single data set of $n < 5$ or assay temperature was $>25 \pm 1$ C but corrected to 25 C (Boehringer Mannheim Corp., 1971).

^c Species differ significantly within taxonomic family or subfamily as determined by one way ANOVA ($P < 0.01$) for species in status classes I and II.

clusion is supported by the similarity of the level of ChE activity and its coefficient of variation for wild birds maintained in captivity and their free-ranging counterparts, and by the general consistency of the coefficient of variation among the various data sets collected over the years. The similarity of brain ChE activity in free-ranging birds of unknown histories and “clean” specimens from PWRC pen studies is illustrated by comparison of these two

groups of controls for five species (Table 3). All PWRC controls were in captivity at least 4 mo before being asphyxiated by carbon dioxide and immediately frozen intact at -25 C pending assay for brain ChE activity. Because the duration of captivity insured recovery from any pre-capture ChE inhibition (Fleming and Grue, 1981), all specimens were processed promptly postmortem and all assays were performed in one morning at 25 C, the PWRC captive

TABLE 2. Whole brain ChE activity (μ moles of acetylthiocholine iodide hydrolyzed/min/g of tissue, wet weight, 25 C) of multiple sets of apparently healthy wild birds collected at different times by various methods and stored under various undefined conditions of refrigeration.

Species	Year	n	Mean	SD	Extremes	DT*
Brown pelican	1982	7	10.4	2.0	7.9, 13.3	6.4
	1982	7	10.0	1.3	8.5, 12.0	7.4
	1983	8	9.6	1.1	8.1, 11.7	7.4
Mallard	1978	13	11.7	1.7	8.9, 15.0	8.3
	1982	7	11.7	1.2	10.4, 13.8	9.3
	1984	4	11.7	1.6	10.1, 14.0	8.5
Golden eagle	1981	7	13.0	3.6	9.1, 19.6	5.8
	1984	8	15.0	2.8	11.2, 19.1	9.4
Ring-billed gull	1978	6	17.6	2.7	13.6, 21.5	12.2
	1981	3	18.4	2.8	15.2, 20.2	12.8
Cedar waxwing	1980	4	22.8	3.5	18.0, 26.0	15.8
	1984	4	20.5	2.5	17.8, 23.5	15.5
Red-winged blackbird	1980	5	23.5	4.5	18.0, 28.9	14.5
	1980	4	19.9	2.8	16.2, 22.8	14.3
	1984	10	19.1	1.8	16.4, 22.0	15.5

* DT, diagnostic threshold, defined as two standard deviations below the arithmetic mean.

controls were believed to have provided optimized estimates of normal brain ChE activity and within-species variance. This presumption was corroborated in part by comparison of coefficients of variation among the five captive species which varied only from 11.6 to 13.9 (\bar{x} = 12.5, SD = 0.9) and were based on sample sizes of 13–20 birds (Table 3). In contrast, this statistic varied from 4.6 to 18.7 (\bar{x} = 11.3, SD = 4.3) for field samples of 3–10 specimens of unknown history. Class II data sets in Table 1 are analogous to the aforementioned field samples and their mean and extreme coefficients of variation of 10.6 (SD = 3.8) and 6.1 and 17.0 for 12 species were in close agreement. Class I data sets, the preferred reference values for normal brain ChE activity, had more erratic within-species variances than Class II sets as indicated by coefficients of variation of 6.2–22.9 and a mean of 12.8 (SD = 4.4). This Class I variability was a function of pooling independent data sets which, although statistically inseparable, probably had subtle differences that were not detectable by the power of the statistical analysis. The principal value of Class I data sets over Class II was that apparently normal brain ChE

activity was verified by replication in both space and time. Class III data sets were considered provisional because of an assay irregularity or small sample size and accordingly were most variable with coefficients of variation ranging from 6.1 to 24.4 (\bar{x} = 14.7, SD = 7.1). The frequency of coefficients of variation exceeding 15, the upper bound in studies of caged wild captured birds (Hill and Murray, 1987), was 6/22, 2/12, and 7/14 for Class I, II, and III data sets, respectively (Table 1).

Overall, whole brain ChE activity varied nearly three-fold among 34 Class I and Class II species of birds, but it was usually similar for close relatives although some species were statistically separable in every family except Emberizidae (Table 1). No convincing correlation was detected between the level of ChE activity and phylogenetic relationship except most Anseriformes and Galliformes and a few other large species had means of less than 15 ChE activity units while activity was usually above 20 units in small species such as passerines. This general relationship was contradicted by the small barn swallow (*Hirundo rustica*) which depends on its extreme agility for catching insects in flight,

TABLE 3. Whole brain ChE activity (μ moles of acetylthiocholine iodide hydrolyzed/min/g of tissue, wet weight, 25 C) of apparently healthy wild birds maintained in captivity and their free-ranging counterparts submitted as controls for toxicologic evaluations.

Species	Source*	n	Mean	SD	Extremes	CV ^b
Mallard	PWRC	13	11.5	1.6	8.9, 15.0	13.9
	1982	7	11.7	1.2	10.4, 13.8	10.3
	1984	4	11.7	1.6	10.1, 14.0	13.7
Northern bobwhite	PWRC	17	12.6	1.5	9.2, 14.6	11.9
	1980	4	14.2	1.8	11.6, 15.6	12.7
	1982	10	13.1	0.9	11.6, 14.3	6.9
European starling	PWRC	20	22.4	2.8	18.1, 29.1	12.5
	1977	9	22.4	2.2	18.8, 26.8	9.8
	1980	3	22.1	1.6	21.2, 23.9	7.2
Red-winged blackbird	PWRC	20	22.6	2.9	18.7, 28.1	12.8
	1980a	5	23.5	4.4	18.0, 28.9	18.7
	1980b	4	19.9	2.8	16.2, 22.8	14.1
Common grackle	PWRC	20	19.8	2.3	15.6, 24.2	11.6
	1978	7	21.7	1.0	20.3, 23.2	4.6
	1980	10	18.6	2.8	13.0, 22.6	15.1

* Source of data: PWRC, captive colonies; 1982, etc., unpublished PWRC analytical reports.

^b Coefficient of variation.

but its mean ChE activity of 12 units was less than all but five of the 34 Class I and II species. The mean ChE activity of the other nine passerines varied from 18 to 22 activity units. Sometimes ChE activity in birds of the same genus and similar size differed as much as 25–50% (*Larus delawarensis* versus *L. argentatus*, *Anas americana* versus *A. strepera*); whereas, others such as the two species of dark geese (*Brantha bernicla* and *B. canadensis*) had about the same ChE activity although the latter species may weigh three times the former. This failure to demonstrate any consistent phylogenetic relationship between avian species and whole brain ChE activity was also reported in a study of 47 species of European birds (Westlake et al., 1983). In that study, the 23 species with sample sizes of 5–20, which are equivalent to Class II data sets, showed an overall variation in ChE activity of 3.6-fold and the within- and between-species relationships were similar to the present study.

In conclusion, brain ChE activity is routinely evaluated in diagnosis of anticholinesterase poisoning of wildlife, timely procurement of concurrent controls is not always feasible, and development of a ref-

erence file of normal ChE activities for wildlife species has been suggested (Westlake et al., 1983; Hill and Murray, 1987). The estimated normal whole brain ChE activities presented in Table 1 are offered as the beginning of such a file with the recommendation that the data base initially be confirmed and expanded rather than used exclusively in lieu of concurrent controls. I encourage the use of presented values as emergency substitutes in diagnosis of lethal anticholinesterase poisoning when concurrent controls cannot be obtained. The ChE values as represented are conservative because they were based on wild control specimens of unknown histories including variable postmortem storage conditions. However, the values are reproducible (Table 2), provided the described procedures including reaction temperature are duplicated and the assay can be easily performed in a modestly equipped field laboratory.

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