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AGE-DEPENDENT CHANGES IN PLASMA AND BRAIN CHOLINESTERASE ACTIVITIES OF HOUSE WRENS AND EUROPEAN STARLINGS

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ABSTRACT: We determined age-dependent changes in plasma and brain cholinesterase (ChE) activity for two species of passerines: house wren (*Troglodytes aedon*) and European starling (*Sturnus vulgaris,* starling). In plasma from nestlings of both species, total ChE activity increased with age, acetylcholinesterase (AChE, EC 3.1.1.7) activity declined rapidly immediately after hatching, and butyrylcholinesterase (BChE, EC 3.1.1.8) activity increased steadily. For both species, total ChE and BChE activities and the BChE:AChE ratio in plasma were significantly greater in adults than nestlings suggesting trends observed in nestlings continue post fledging. In older nestlings and adults, AChE activity in plasma was significantly greater and BChE:AChE ratio less in house wrens than starlings. For house wrens as compared with starlings, ChE activity in brain increased at a significantly greater rate with age in nestlings and was significantly greater in adults. However, ChE activity in brain was similar at fledging for both species suggesting that the increase in ChE in brain is more directly related to ontogeny than chronologic age in nestlings of passerines. For both species, ChE activity increased significantly with brain weight of nestlings but not adults. House wrens hold similar patterns of age-dependent change in ChE activity in common with starlings but also exhibit differences in AChE activity in plasma that should be considered as a factor potentially affecting their relative toxicologic response to ChE inhibitors.

Key words: Acetylcholinesterase, butyrylcholinesterase, cholinesterase, European starling, house wren, organophosphorus pesticide, passerine, *Sturnus vulgaris, Troglodytes aedon.*

INTRODUCTION

Hooper et al. (1990) included the European starling (*Sturnus vulgaris,* starling) in a proposed method for standardized monitoring of reproductive toxicity from cholinesterase (ChE) inhibitors because it is widespread, occupies many habitat types, has a diet that includes a variety of terrestrial invertebrates, and has large, easily manipulated, synchronous breeding populations. The house wren (*Troglodytes aedon*) is an insectivorous, cavity-nesting passerine that has a small foraging range centered at nest sites in early successional woodland, agricultural, and suburban environments (Kendeigh, 1941). Although not a subject in past studies of the effects of ChE inhibitors on passerines, the ecologic characteristics of the house wren suggest that it is likely exposed to ChE-inhibiting pesticides used in a variety of natural and human-altered environments. The starling is well studied and a potentially useful passerine model; however, comparisons with other passerines, such as the house wren, relative to factors affecting their toxicologic response to ChE inhibitors are necessary to generalize the starling as a passerine model.

Age-dependent change in ChE activity is common in brain and plasma of altricial species. Custer and Ohlendorf (1989) demonstrated an increase in ChE activity with age in brain for altricial nestlings of colonial waterbirds: black-crowned night-herons (*Nyctiocorax nyctiocorax*), great egrets (*Ardea alba*), and snowy egrets (*Egretta thula*). For passerines, an age-related increase in ChE activity was observed in brain of starlings (Grue et al., 1981; Grue and Hunter, 1984). Gard and Hooper (1993) demonstrated similar patterns of age-dependent change in acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) activities in brain and plasma of eastern bluebirds (*Sialia sialis,* bluebird) and starlings. Butyrylcholinesterase activity in plas-

ma increases steadily with age in nestlings; activities are greater in adults than nestlings. In contrast, AChE activity declines rapidly after hatching. Butyrylcholinesterase is apparently synthesized in the liver (Lyles et al., 1980) and an increase in BChE activity in plasma of newly hatched chicks is related to a decline of BChE activity in liver suggesting mobilization and release of BChE from the liver (Smucker and Wilson, 1990). The origin of AChE in plasma is not known but is likely extrahepatic, possibly originating from the central nervous system or developing muscle fibers (Smucker and Wilson, 1990). The similarity in age-dependent patterns of ChE activity is not surprising given that altricial species share a common early ontogenetic pattern.

Birds are thought to have only limited A-esterase and hepatic microsomal monooxygenase activities for detoxifying ChE-inhibiting compounds (Walker, 1983). Consequently, B-esterases, such as AChE, BchE, and carboxylesterase (CaE, EC 3.1.1.1), are likely important determinants of toxicologic response to ChE-inhibitors in passerines (Leopold, 1996; Parker and Goldstein, 2000). Gard and Hooper (1993) hypothesized that age-related differences in sensitivity to ChE inhibitors in passerines might be explained by age-dependent patterns in ChE activity in brain and BChE activity in plasma. Wolfe and Kendall (1998) compared the sensitivity of red-winged blackbirds (*Agelaius phoeniceus,* blackbirds) and starlings to ChE inhibitors relative to differences in ChE activity but could not explain the interspecies relationship in age-related sensitivity based solely on those age-dependent changes in ChE activity hypothesized to affect sensitivity. Although an age-related sensitivity to ChE inhibitors in passerines may be partially explained by age-dependent patterns identified in passerines, determining patterns of age-dependent change in ChE activity in additional species may provide information on ChE dynamics in passerines necessary to understand interspecies relationships in sensitivity.

Our objective was to determine if the house wren, a passerine having a developmental pattern similar to that of the starling, has patterns of age-dependent change in ChE activities in plasma and brain similar to those in the starling. We measured ChE activities (AChE and BChE) in plasma and brain from known-age nestlings and adults of each species.

MATERIALS AND METHODS

We collected nestling starlings and house wrens in 1994 and 1995 from nest boxes on two field sites free of commercial agriculture located in the mid-Hudson Valley (Dutchess [41°47′N, 73°44′W] and Ulster [41°50′N, 73°57′W] Counties), New York (USA). At 2 day intervals in age from hatching to fledging (approximately 20 days), eight starlings were bled and then euthanized by carbon dioxide asphyxiation for subsequent dissection of brains. Similarly for house wrens older than day zero (day of hatching), nine to 11 birds were bled, euthanized, and dissected at 2 day intervals in age from hatching to fledging (approximately 15 days). For day-zero house wrens, 21 birds were collected. Adults of starlings (two male, four female) and house wrens (two male, five female) were also collected from nest boxes and sampled. No more than one starling or house wren per nest was used at each age and no bird was bled more than once.

Blood and brain samples were collected between 09:00 and 12:00 AM to minimize potential diurnal variation in enzyme activities (Thompson et al., 1988) and processed immediately in the field. For birds older than 2 days, blood samples (200–300 μl) were obtained by jugular venipuncture using a heparinized syringe (0.5 ml) with a 28 gauge needle. For nestlings younger than 4 days, blood samples (100–200 μl) were obtained by drawing blood with heparinized capillary tubes from a transverse incision at the neck immediately posterior to the skull. Samples were kept on wet ice until centrifugation. Blood was transferred from syringe to heparinized capillary tubes and centrifuged. Plasma was separated from the cell pack and stored in microcentrifuge tubes (1.5 ml). The brain was dissected intact from the cranium and placed in polyethylene bottles (5 ml). All samples were transported on dry ice and stored over liquid nitrogen. Plasma was thawed and immediately assayed for ChE activity without dilution. Large brains were divided sagit-

tally and a half used as a sample. Brains were thawed, weighed, diluted in a volume of 5×10^{-2} M Tris buffer (pH 7.4) (Sigma Chemical Company, St. Louis, Missouri, USA) equivalent in milliliters to 10 times the brain weight in grams, and homogenized in glass beakers with a homogenizer equipped with a stainless-steel rotor-stator (Tissue Tearor, Biospec Products, Bartlesville, Oklahoma, USA). Brain homogenate was maintained in suspension and immediately assayed for ChE activity after preparation.

Plasma and brain samples were analyzed for total ChE and AChE activities. Total ChE activity was determined according to methods developed by Ellman et al. (1961) and adapted by Hill (1988). Total ChE activity was measured using a cuvette with a light path of 1 cm in a continuously recording spectophotometer (Beckman Model 25, Fullerton, California, USA) set at a wavelength of 405 nm with a run time of 2 min and a time lag of 30 sec. Reagent volumes were reduced to half of those employed by Hill (1988). The aliquot volume for plasma and brain homogenate was reduced to 7 and 10 μl, respectively. The final assay molarity (FAM) was 2.41×10^{-4} M for 5,5′-dithiobis-(2-nitrobenzoic acid) (Sigma Chemical Company) for all assays. All samples were run at 24.5 to 25.5 C in duplicate with the exception of plasma samples from day-zero house wrens, which were run without duplication due to limited sample volume. Additional assays of plasma and brain for AChE activity were conducted under identical conditions for total ChE with the addition of the specific BChE inhibitor tetraisopropylpyrophosphoramide (iso-OMPA) (Sigma Chemical Company) at an FAM of 1×10^{-4} M (Fairbrother et al., 1991). To ensure complete BChE inhibition without AChE inhibition, an optimal iso-OMPA concentration was determined using non-study plasma samples before analysis. Activities were calculated from tracings of the change in reaction rate with time and converted from absorbance units/min to μmoles of acetylthiocholine iodide (AThCh) hydrolyzed/min/ml plasma or g brain. Butyrylcholinesterase activity was computed as the difference between total ChE and AChE activities.

Acetylthiocholine iodide (Sigma Chemical Company) was used as a substrate. All plasma samples were assayed with an FAM of 5.0×10^{-3} M for substrate as recommended by Marden et al. (1994). Total ChE and AChE activities were assayed in plasma samples from seven to 18 house wren and six to eight starling samples at each age. For assays of brain, optimal substrate concentrations were determined using non-study samples for each species before analysis (Garry and Routh, 1965); an FAM of 8.0×10^{-4} M for substrate produced optimal total ChE activity in brain for both species. Assays for total ChE activity were run with an FAM of 8.0×10^{-4} M for substrate for five to nine house wren and four to six starling brain samples at each age. For AChE activity, assays were run for four to six house wren and four to five starling brain samples at each age. To measure potential substrate inhibition, assays for total ChE activity with an FAM of 5.0×10^{-3} M for substrate were run for nine to 21 house wren and six to eight starling brain samples at each age. Likewise, assays for AChE activity were run for four to seven house wren and four to six starling brain samples at each age.

A standard with a nominal ChE activity of 1.0 μmoles of AThCh hydrolyzed/min/ml plasma was prepared to evaluate daily analytical performance. Plasma samples from seven starlings were pooled and divided into 50 aliquots (30 μl), each stored in a microcentrifuge tube (1.5 ml) over liquid nitrogen. Assays (with and without iso-OMPA inhibitor) using plasma from a single aliquot of standard were run daily at the beginning and end of a set of assays; blanks (7.0 μl of 7.4 buffer) for non-enzymatic activity were assayed similarly. No consistent trend in the activity of standards or blanks was observed over the study. Sample assays were corrected for non-enzymatic activity. The coefficient of difference for sample analyses was $<5\%$ for plasma or brain assays.

Linear regression was used to establish relationships between ChE activity in brain, nestling age, and brain weight (Sokal and Rohlf, 1969). A two-tailed *t*-test was used to compare enzyme activity between ages or developmental stages and a one-tailed *t*-test was used to evaluate brain for BChE activity significantly greater than zero at each age (Sokal and Rohlf, 1969). Programs within the Statistical Analysis System (SAS Institute, 1985) were used to calculate means, standard deviations (SD), and standard errors (SE); to perform *t*-tests, linear regressions, and lack of fit tests; and to compare regressions. A level of significance of $\alpha=0.05$ was used for all tests; the null hypothesis was not rejected unless $P<0.05$ and <0.10 for non-directional and directional alternative hypotheses, respectively.

RESULTS

For house wrens and starlings, patterns of age-dependent change in AChE activity were similar in plasma; however, levels of activity were significantly different between species at several stages in devel-

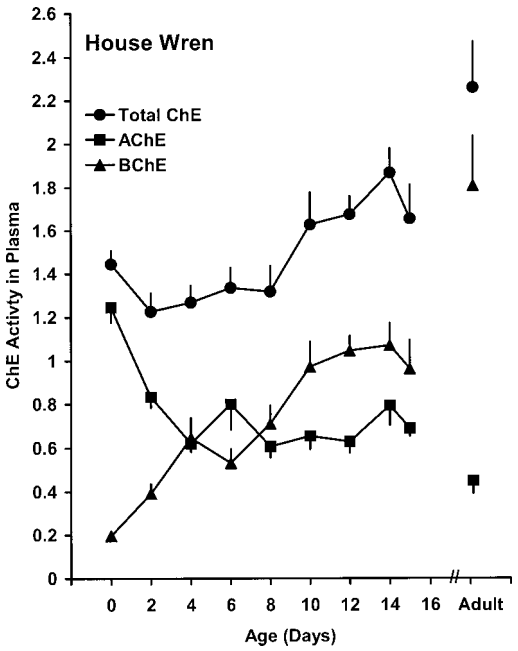

FIGURE 1. Cholinesterase activity in plasma (μmoles acetylthiocholine iodide hydrolyzed/min/ml plasma±SE) of house wren nestlings (n=8–10 birds/age for ages >day zero, n=18 for day zero birds) and adults (n=7) in relation to age.

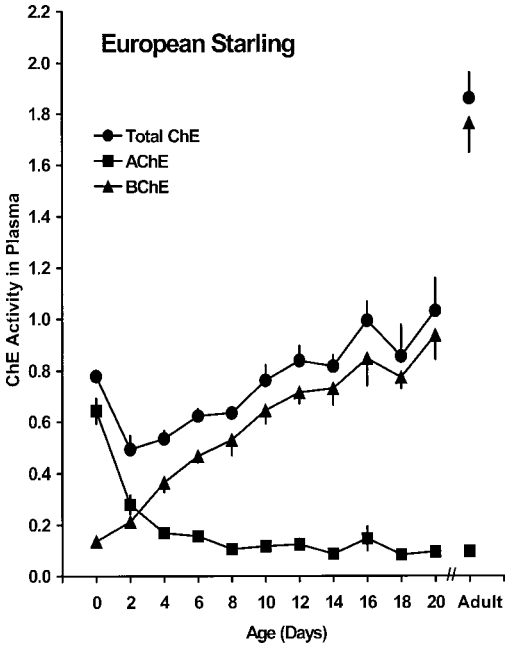

FIGURE 2. Cholinesterase activity in plasma (μmoles acetylthiocholine iodide hydrolyzed/min/ml plasma±SE) of European starling nestlings (n=8 birds/age) and adults (n=6) in relation to age.

opment (Figs. 1–2). Acetylcholinesterase activity produced approximately 85% of the total ChE activity in plasma immediately after hatching (day zero), decreased sharply within 2–5 days after hatching, and then remained constant to fledging in both species. For nestlings at fledging age, AChE activity comprised 42 and 9% of the total ChE activity in plasma of house wrens and starlings, respectively. Relative AChE activity decreased further in adults, comprising 20 and 5% of the total ChE activity in plasma of house wrens and starlings, respectively. In older nestlings and adults, AChE activity in plasma was significantly greater in house wrens than starlings (P<0.001 and <0.001, for nestlings at fledging age and adults, respectively). In house wrens, AChE activity in plasma declined significantly (P<0.01) in adults as compared with nestlings at fledging age. In contrast, AChE activity in plasma of adult starlings was not significantly different (P=0.85) from that of nestlings at fledging age.

For both species, BChE activity in plasma increased throughout the nesting period and was significantly greater in adults than nestlings at fledging age (P<0.01 and <0.001, for house wrens and starlings, respectively; Figs. 1–2). As a consequence, the greater BChE activity in adults resulted in a significantly greater total ChE activity in plasma of adults than nestlings at fledging age for both species (P<0.05 and <0.001 for house wrens and starlings, respectively). For adults, mean (SD) for total ChE activity in plasma was 2.26 (0.56) and 1.86 (0.28) μmoles AThCh hydrolyzed/min/ml plasma for house wrens and starlings, respectively. For nestlings at fledging age, total ChE activity in plasma was 73 and 55% of that in adults of house wrens and starlings, respectively. In contrast to AChE activity in older nestlings and adults, BChE activity in plasma was not significantly different between species (P=0.86 and =0.86, for nestlings at fledg-

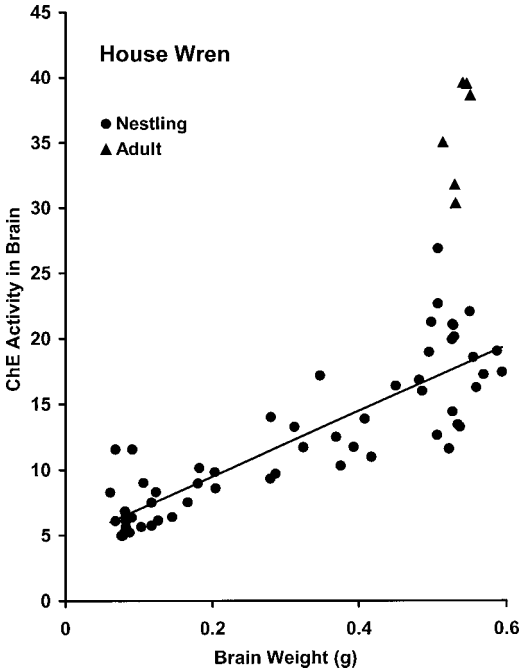

FIGURE 3. Cholinesterase activity (Y) in brain (μmoles acetylthiocholine iodide hydrolyzed/min/g brain) of house wren nestlings (Y=4.45+25.29X, r^2=0.73, n=53, P<0.001, regression includes only nestlings) and adults (n=6) in relation to brain weight (X).

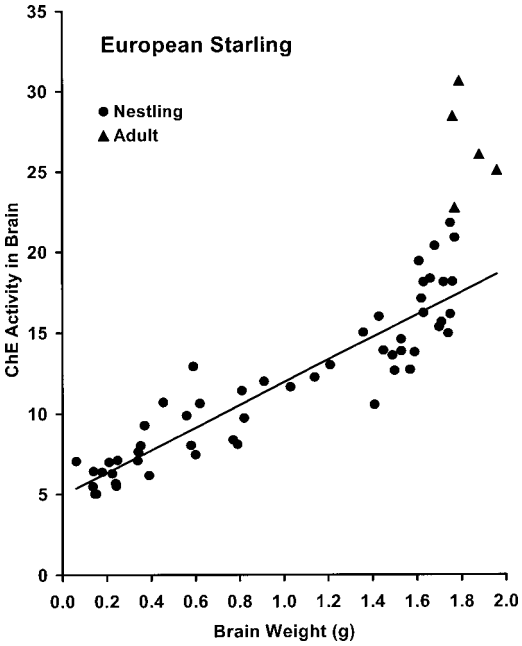

FIGURE 4. Cholinesterase activity (Y) in brain (μmoles acetylthiocholine iodide hydrolyzed/min/g brain) of European starling nestlings (Y=4.93+7.02X, r^2=0.84, n=55, P<0.001, regression includes only nestlings) and adults (n=5) in relation to brain weight (X).

ing age and adults, respectively). For nestlings at fledging age, the greater AChE activity in plasma of house wrens as compared with starlings resulted in a total ChE activity in plasma significantly greater (P<0.01) for house wrens than starlings. For adults, total ChE activity in plasma was greater in house wrens than starlings, but not significantly (P=0.14).

In brain of nestlings, total ChE activity (Y) increased directly in relation to age (X) for house wrens (Y=5.83+0.93X, r^2=0.84, n=53, P<0.001, FAM=8×10^{-4} M for substrate, regression includes only nestlings) and starlings (Y=5.07+0.69X, r^2=0.90, n=55, P<0.001, FAM=8×10^{-4} M for substrate, regression includes only nestlings). Lack of fit tests were not significant for house wrens (P=0.24, n=53, r^2=0.87) or starlings (P=0.10, n=55, r^2=0.93) indicating a linear relationship between total ChE activity and age. The increase in total ChE activity with age was significantly more rapid (P<0.001) in brain of house wrens than starlings. For adults, mean (SD) total ChE activity in brain was 35.9 (4.1) and 26.6 (3.0) μmoles AThCh hydrolyzed/min/g brain (FAM=8×10^{-4} M for substrate) for house wrens and starlings, respectively. For both species, total ChE activity in brain was significantly less in nestlings at fledging age than adults (P<0.001 and <0.01 for house wrens and starlings, respectively); total ChE activity in brain was 42 and 24% lower (house wrens and starlings, respectively) in nestlings at fledging age than adults. For adults, total ChE activity was significantly greater (P<0.01) in brain of house wrens than starlings; however, total ChE activity in brain of nestlings at fledging age was not significantly different (P=0.65) between species. For nestlings of both species, total ChE activity in brain increased directly with brain weight (Figs. 3–4). The increase in activity with brain weight was significantly greater (P<0.001) for house wrens

than starlings. No relationship between total ChE activity in brain and brain weight was evident in adults. For each species, total ChE activity in brain was greater in adults than that predicted from the relationship between total ChE activity and brain weight for nestlings.

Acetylcholinesterase activity comprised all of the ChE activity in brain of adults and nearly all of the ChE activity in brain of nestlings of both species. Minor but significant BChE activity was detected in brain of young nestlings of both species. For house wrens, significant BChE activity ($P<0.04$ and <0.07 for zero-day and 2 day old nestlings, respectively) comprised 3% of the ChE activity in brain of young nestlings. For starlings, significant BChE activity ($P<0.01$, <0.02, and <0.05 for zero-day, 2 day old, and 4 day old nestlings, respectively) comprised 7% of the ChE activity in brain of zero day and 2 day old nestlings and 3% of the ChE activity in brain of 4 day old nestlings.

Substrate inhibition of AChE (butyrylcholinesterase is not subject to substrate inhibition [Fairbrother et al., 1991]) was evident in assays for total ChE in brain conducted with a substrate concentration 6.25 times greater (FAM of 5×10^{-3} M) than optimal. For adults, mean total ChE activity in brain decreased 15 and 12% for house wrens and starlings, respectively, in assays conducted with high-concentration substrate. Regression equations of total ChE activity (Y) in brain with age (X) for assays conducted with high-concentration substrate ($Y=4.84+0.83X$, $r^2=0.88$, $n=97$, $P<0.001$ and $Y=4.67+0.61X$, $r^2=0.90$, $n=88$, $P<0.001$ for house wrens and starlings, respectively) indicated the rate of change for total ChE activity in brain with age was reduced by 11 and 12% for house wrens and starlings, respectively. The degree of substrate inhibition appears to vary little with species or developmental stage.

DISCUSSION

House wrens and starlings had similar age-dependent patterns of AChE and BChE activities in plasma. Butyrylcholinesterase activity in plasma increased throughout the nestling period and after fledging. In contrast, AChE activity in plasma declined rapidly immediately after hatching and remained constant throughout the remainder of the nestling stage; the decline in AChE post fledging was more pronounced in house wrens than starlings. Increasing BChE activity with size or age was also observed in plasma of the nestlings of Eurasian tree sparrows (*Passer montanus*), bluebirds, and blackbirds suggesting a general age-dependent trend in BChE activity in plasma among passerines (Thompson, 1991; Gard and Hooper, 1993; Wolfe and Kendall, 1998). The pattern of decline in AChE activity in plasma of house wrens was similar to that observed by Gard and Hooper (1993) in bluebirds and by Wolfe and Kendall (1998) in blackbirds.

In contrast with starlings, blackbirds, and bluebirds, AChE activity comprised a considerably larger component of total ChE activity in plasma of house wrens. At fledging age, AChE activity in plasma comprised 42% of total ChE activity in house wrens as compared with 9% in starlings. Gard and Hooper (1993) found AChE activity constituted only 12–15% of total ChE activity in bluebirds and starlings at fledging age. For 8–10 day old blackbirds, AChE activity comprised 16% of the total ChE activity in plasma (Wolfe and Kendall, 1998). These trends were further reflected post fledging in sexually mature adults. Acetylcholinesterase activity in house wrens was 20% compared with 5% in starlings. For adults, AChE comprised only 4.7% and 10% of total ChE activity in plasma of bluebirds and starlings, respectively (Gard and Hooper, 1993) and 6% of total ChE activity in plasma of blackbirds (Wolfe and Kendall, 1998). Butyrylcholinesterase activity in plasma is similar for house wrens and starlings. Consequently, greater AChE activity results in a higher total ChE activity in house wrens (2.26 μmoles AThCh hydro-

lyzed/min/ml plasma in adults) than starlings (1.86 and 1.0 μmoles AThCh hydrolyzed/min/ml plasma in adults from this study and Gard and Hooper [1993], respectively). Acetylcholinesterase activity may also account for the greater total ChE activity in plasma of house wrens than bluebirds or blackbirds (1.5 and 1.85 μmoles AThCh hydrolyzed/min/ml plasma in adults of bluebirds [Gard and Hooper, 1993] and blackbirds [Wolfe and Kendall, 1998], respectively).

In altricial species, the onset of mobility seems to be related to the rate of change in ChE activity in brain with age. In colonial waterbirds, Custer and Ohlendorf (1989) noted greater mobility in 15 day old snowy egrets than similarly aged black-crowned night-herons and great egrets. Snowy egrets are able to leave the nest temporarily as early as 10 days post hatch (St. Clair et al., 1979 cited by Parsons and Master, 2000). In contrast, great egrets and black-crowned night-herons are not able to leave the nest for short periods until 21–25 and 28 days, respectively, after hatching (Davis, 1993; Dwyer, 1988 cited by McCrimmon et al., 2001). Rates for ChE activity (Y) in brain with age (X) are correspondingly greater for snowy egrets ($Y=11.43+0.68X$) than either black-crowned night-herons ($Y=8.69+0.39X$) or great egrets ($Y=8.39+0.38X$) (Custer and Ohlendorf, 1989). The rate of change for ChE activity in brain with age apparently is related inversely to the period of development required for mobility in waterbirds. A similar relationship is apparent in passerines. House wrens and bluebirds fledge in 25 to 30% less time than starlings. Rates for ChE activity (Y) in brain with age (X) were greater for house wrens ($Y=5.83+0.93X$) and bluebirds ($Y=3.03+0.94X$ [Gard and Hooper, 1993]) than starlings ($Y=5.07+0.69X$ and $Y=5.30+0.64X$ from this study and Gard and Hooper [1993], respectively). However, similar ChE activity in brain is predicted at fledging for these passerine species. Based on regressions, predicted ChE activity at fledging was 19.8 and 18.9 μmoles AThCh hydrolyzed/min/g brain for house wren at 15 days and starling at 20 days, respectively. Relationships for bluebird and starling predicted ChE activity of 18.1 and 18.7 μmoles AThCh hydrolyzed/min/g brain for bluebird at 16 days and starling at 21 days, respectively (Gard and Hooper, 1993). Similarities in predicted ChE activity in brain at fledging suggest that, although the rate of change for ChE activity in brain with chronologic age may be different among species, passerines may hold in common a specific level of ChE activity associated with the mobility required at fledging. This relationship suggests that a consistent relationship, although not necessarily causal, between ChE activity in brain and ontogeny independent of chronologic age is likely present in nestlings of passerines.

We observed patterns of age-dependent change in ChE activities in starling nestlings of known-age that were similar to those observed by Gard and Hooper (1993). Patterns of change in AChE and BChE activities in plasma and relationships for ChE activity (Y) in brain with age (X) were similar ($Y=5.07+0.69X$ and $Y=5.30+0.64X$ from this study and Gard and Hooper [1993], respectively; <5% difference in predicted ChE activity for nestlings of the same age). However, ChE activity was 17% greater in brain of our adult starlings (26.6 vs. 22.7 from this study and Gard and Hooper [1993], respectively). Butyrylcholinesterase activity in plasma in our adult starlings was 90% greater than starlings assayed by Gard and Hooper (1993) due to twice the level of BChE activity. Development of ChE activity in brain follows a sigmoidal pattern of increase to at least 1 yr in age (Grue and Hunter, 1984). Furthermore, trends of increasing BChE activity with age, including an increase post fledging, suggest that BChE activity in plasma continues to increase over the post-fledging period. The comparison of results suggest that the activities of ChE in brain and BChE in plasma of adult starlings of unspecified age as-

sayed by Gard and Hooper (1993) are consistent with activities that may be expected for adult birds that are younger than those we assayed. Cholinesterase activity was considerably greater in adults of species with shorter nesting periods (35.2 and 35.9 μmoles AThCh hydrolyzed/min/g tissue in bluebirds [Gard and Hooper, 1993] and house wrens, respectively) than adult starlings. However, a lack of accurate models of ChE activity with age and data from known-age birds post fledging to sexual maturity limit the value of an interspecies comparison of ChE activity in adults.

Trends in ChE activity in the embryo determine ChE activity in plasma of chicks immediately after hatching (Lyles et al., 1980; Smucker and Wilson, 1990). However, maturation of the blood-brain barrier, which reduces the passage of large, polar molecules, may not be complete for a significant period after hatching (Benzo, 1986). The origin of BChE that produced low levels of BChE activity in brain of young house wren and starling nestlings is not known but may be the result of an initial mobilization of BChE from the liver and an incomplete blood-brain barrier. If the source of AChE activity in plasma is the brain, maturation of the blood-brain barrier would reduce the transfer of AChE, resulting in decreased activity in plasma with age. Alternatively, the decline in AChE activity in plasma may be the result of a decline in AChE in muscle as muscle fiber matures (Smucker and Wilson, 1990; Farage-Elawar, 1991). Further research is necessary to fully understand the origins of ChE activity in plasma of avian species.

Age-dependent relationships for toxicologic sensitivity to ChE inhibitors are more complex in passerines than studies of starlings indicate. Newly hatched starling nestlings were more sensitive than adults when exposed to dicrotophos (Grue and Shipley, 1984). Using mortality as a toxicologic endpoint for exposure to terbufos and diazinon, Wolfe and Kendall (1998) found that, although both starling and blackbird adults were less sensitive than nestlings as expected, starling adults were much less so than nestlings or blackbird adults. In contrast, blackbird adults were only slightly less sensitive than nestlings. Although A-esterase and hepatic monooxygenase activities among other metabolic factors cannot be discounted, evidence favors B-esterases as prominent mediators of toxicologic sensitivity to ChE inhibitors (Chambers, 1992). The complex relationships in deferential sensitivity to ChE inhibitors in passerines may be due B-esterase composition.

Gard and Hooper (1993) hypothesized that the decrease in sensitivity to ChE inhibitors with age in passerines may be due to greater ChE activity per gram of brain, increasing levels of BChE in plasma, or possibly maturation of the blood-brain barrier. Butyrylcholinesterase activity in plasma is inhibited more rapidly and to a larger degree than ChE activity in brain (Hill and Fleming, 1982). Butyrylcholinesterase may be reacting with the active oxon form of organophosphorus (OP) compounds that might have inhibited ChE activity in brain and, consequently, increased BChE activity in plasma would decrease the effect of OP compounds, thereby partially accounting for decreasing sensitivity in older birds (Gard and Hooper, 1993). In vivo removal of BChE activity from adult starling plasma and tissues with an inhibitor (iso-OMPA) largely selective for BChE substantially increased sensitivity to diazinon (a reduction in the dose lethal to 50% of exposed birds [LD_{50}] from approximately 1,300 mg/kg to less than 200 mg/kg) supporting the contention that BChE in plasma reduces sensitivity to OP compounds (Leopold, 1996). However, in vivo removal of BChE activity also revealed that sensitive CaE forms in plasma have the potential to contribute to a reduction in sensitivity to OP compounds (Leopold, 1996).

Differences in the quantity and nature of forms of CaE in plasma relative to species, size, and trophic status supports the

contention that CaE plays a role in the detoxication of lipophilic xenobiotic esters in the diet of birds (Thompson and Walker, 1994). Although not inconsistent with the notion that increased BChE activity as birds age is protective, the contention that CaE plays a role in detoxification implies that variation in CaE may be partially responsible for interspecies differences in toxicologic sensitivity to ChE-inhibitors. However, the toxicologic buffering capacities of BChE and sensitive forms of CaE in plasma do not seem to explain the differential toxicity of ChE-inhibitors (diazinon, methyl parathion, aldicarb, oxamyl) in 2–3 day old starlings (Parker and Goldstein, 2000). Insensitive forms of CaE in plasma and brain may play a role in differential toxicologic response in young nestlings and, moreover, these CaE forms, apparently found in nestlings but not adults, may contribute to increased toxicologic sensitivity of nestlings (Parker and Goldstein, 2000). In addition to toxicologic buffering properties of B-esterases, differences in modes of toxicity (direct or requiring metabolic activation) and mechanisms of enzyme reactivation and detoxification and pharmacodynamics for specific ChE-inhibitors must be considered in assessing relationships in sensitivity (Wallace, 1992). Cholinesterase activity in brain or BChE activity in plasma do not seem to explain the greater toxicologic sensitivity of blackbirds to ChE inhibitors since ChE activity in brain is similar in starlings and blackbirds and BChE activity in plasma is actually greater in blackbirds than starlings (Wolfe and Kendall, 1998). Furthermore, the differential tolerance for loss of AChE activity in plasma of black birds and starlings exposed to ChE inhibitors (retention of 16.3 and 50% of control activity in blackbirds exposed to terbufos and diazinon, respectively, vs. <1% in starlings exposed to either compound) at doses near the LD_{50} suggests that AChE in plasma may play a role in or relate to factors affecting the relative difference in toxicologic response between species (Wolfe and Kendall, 1998). Potential differences in the CaE components as well as differences in the response of AChE in plasma to ChE inhibitors may contribute to the complex interspecies relationship in toxicologic response to ChE inhibitors in passerines.

House wrens and starlings generally have similar patterns of age-dependent changes in ChE activity in plasma and brain; however, AChE activity in plasma was four to six times greater in older nestlings and adults of house wrens than starlings. If the role of AChE in plasma in mediating toxicologic sensitivity to ChE inhibitors is to be investigated, the high level of AChE activity in plasma suggests the house wren should be a candidate for comparative studies. Given that ChE activity in brain is more directly related to ontogeny than chronologic age, comparison of toxicologic response in nestlings of passerines based on ChE activity in brain would be better facilitated by comparing effects related to periods proportional to the time to fledging than specific ages. Considering AChE activity in plasma and developmental rate as a factors in species comparisons may further the understanding of the differential sensitivity of passerines to ChE inhibitors. Because field studies generally rely on a limited number of representative passerine species, understanding toxicologic relationships among species is important to fully evaluate the impact of ChE inhibitors on multiple-species assemblages of passerines found in most habitats.

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