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Age-Dependent Changes in Activity of Mallard Plasma Cholinesterases

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ABSTRACT: Plasma acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity was measured repeatedly in 27 mallard (*Anas platyrhynchos*) ducklings between 7 and 85 days of age to determine age-dependent changes in enzyme activity. Plasma AChE, BChE, and total cholinesterase (ChE) activity decreased significantly with age. The relative proportion of AChE in total ChE activity also decreased slightly with age. Since some anti-ChE chemicals can selectively inhibit AChE or BChE activity, characterization of age-dependent changes in the activity of each enzyme may be necessary to accurately identify the occurrence of pesticide exposure.

Key words: mallard, *Anas platyrhynchos*, cholinesterase, age-dependent changes, repeated measures.

Inhibition of brain and plasma cholinesterase (ChE) activity is commonly used for diagnosing wildlife exposure to organophosphorus and carbamate insecticides. The ChE activity of animals with suspected exposure to these insecticides can be compared to the activity of normal control animals of the same species to determine relative inhibition. Animal age also can be a critical factor for making valid comparisons. Brain ChE activity of European starlings (*Sturnus vulgaris*) increases several-fold between hatching and attainment of adult size (Grue and Hunter, 1984; Robinson et al., 1988), making it necessary to compare juveniles of the same age to determine appropriately the occurrence and degree of ChE inhibition. Age-dependent increases in brain ChE activity have also been observed in nestling great egrets (*Casmerodius albus*), snowy egrets (*Egretta thula*), and black-crowned night-herons (*Nycticorax nycticorax*) (Custer and Ohlendorf, 1989). Precocial and semi-precocial species that have been studied differ from altricial species in the developmental pattern of brain ChE activity. Hoffman and Eastin (1981) found that brain ChE activity in mallards (*Anas platyrhynchos*) increases to adult levels during embryonic development. In other precocial and semi-precocial species, young and adults also have similar brain ChE activity levels (Hudson et al., 1972; Ludke et al., 1975; White et al., 1979; Grue and Hunter, 1984).

Plasma ChE activity, on the other hand, does vary with age in some precocial birds. Fairbrother et al. (1990) reported that plasma total ChE activity in normal mallards decreased by 43% between 5 and 58 days of age. Lyles et al. (1980) reported that plasma ChE activity decreased in developing chickens (*Gallus domesticus*), with a rapid decrease in acetylcholinesterase (AChE) activity after hatch, while butyrylcholinesterase (BChE) activity was relatively constant. These findings have been attributed to differences in the source of each enzyme. High levels of AChE at hatching appear to be associated with release of AChE from maturing muscle fibers, while BChE is synthesized and secreted by the liver (Lyles et al., 1980).

The present study was conducted to document age-dependent changes in AChE and BChE in growing mallards. Since some anti-ChE chemicals can selectively inhibit AChE or BChE activity (Aldridge, 1953; Silver, 1974), characterization of age-dependent changes in the activity of each enzyme and their relative contribution to total ChE activity may be necessary to accurately identify the occurrence of pesticide exposure.

Day-old mallard ducklings, purchased from Whistling Wings, Inc. (Hanover, Illinois 61041, USA), were housed in brooders maintained at 39 C and provided with Purina® (St. Louis, Missouri 63166, USA) gamebird starter and water *ad libitum*.

TABLE 1. Mean (±SE) and range for plasma acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and total cholinesterase (ChE) activity[a] in 27 mallard ducklings at five ages.

Age (days)	Enzyme activity			Percent AChE[b]
	AChE	BChE	Total ChE	
7	302 ± 14	1,045 ± 55[c]	1,343 ± 64[c]	22.4[c]
	161, 440	645, 1,524	913, 1,885	15, 37
14	276 ± 16	1,014 ± 51	1,290 ± 61	21.8
	120, 456	495, 1,407	616, 1,711	15, 35
29	286 ± 17	938 ± 24	1,224 ± 28	21.4
	172, 538	706, 1,154	878, 1,445	15, 39
50	208 ± 13	784 ± 35	992 ± 40	20.3
	90, 361	465, 1,276	556, 1,547	14, 36
85	184 ± 12[c]	744 ± 32[c]	923 ± 33	18.4[c]
	109, 338	489, 1,071	680, 1,244	12, 37

[a] Enzyme activity is expressed as micromoles of acetylthiocholine hydrolyzed/min/l plasma.
[b] Median value and range for percent AChE activity of total ChE.
[c] Means based on sample size of 26 ducklings.

Brooder temperatures were reduced to 35 C at 7 days of age. Ducklings were moved to outdoor ground pens (2.5 × 5.0 m) at 21 days of age. Blood samples (2 ml) were collected from each of 27 ducklings at 7, 14, 29, 50, and 85 days of age by jugular venipuncture. The blood was collected in blood collection tubes (Vacutainer®, Bectin Dickson, Rutherford, New Jersey 07070, USA) containing sodium heparin and kept on wet ice until centrifugation. Each sample was centrifuged for 10 min at 2 to 5 C at 3,000 × g. Plasma was collected in plastic vials (Sardstedt®, Princeton, New Jersey 08540, USA) and stored at −75 C until analysis. After the last collection period, all samples were analyzed at the same time in random order. Methods for conducting ChE assays are described in Fairbrother et al. (1990). AChE activity was determined by incubating samples in the presence of 1×10^{-5} M iso-OMPA (tetraisopropylpyrophosphoramide, Sigma Chemical Co., St. Louis, Missouri 63178, USA) (Aldridge, 1953). BChE was calculated as the remainder of total ChE minus AChE.

The mean activity for AChE, BChE, and total ChE decreased with increasing age in mallard ducklings (Table 1). The temporal changes in ChE activity were examined by a repeated measures analysis of variance. Significant ($P \leq 0.0001$) linear trends over time were observed for each measurement. There was no evidence ($P > 0.08$) of curvilinear trends. A test for serial correlation using the Durbin-Watson statistic (Durbin and Watson, 1971) did not detect serial correlation in total ChE and BChE data, but did for AChE. Linear regression of enzyme activity on age was used to describe the linear trends as follows: 1) AChE = (308.8 ± 11.0, SE) − (1.6 ± 0.2) Days, $R^2 = 0.24$; 2) BChE = (1056.8 ± 30.6) − (4.1 ± 0.7) Days, $R^2 = 0.23$, and 3) total ChE = (1364.3 ± 35.3) − (5.7 ± 0.8) Days, $R^2 = 0.30$. The percent of AChE in total ChE activity decreased slightly with age (Table 1). However, percent of AChE varied much more among individuals than within individuals, as observed in the ranges for percent AChE (Table 1). The mean percent AChE for each bird ranged from 15 to 37%. Variation between individuals in the relative proportions of AChE and BChE activity may help explain variability in individual sensitivity to pesticide effects and illustrates the advantage of using repeated measurements of ChE activity as a means of separating possible pesticide effects from individual variabilitiy (Fairbrother et al., 1989).

Because age-dependent changes in plasma AChE and BChE activity exist, similar

age birds are necessary when evaluating exposure of young mallards to ChE inhibitors. It is not known if the relationships observed in mallards and chickens apply to other precocial or altricial young, but given the amount of change observed in plasma ChE activity during growth, age should be a critical factor when evaluating exposure to ChE inhibitors. More research is needed to determine how changes in brain and plasma ChE activity during growth relate to age-related differences in pesticide sensitivity.

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