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Brain Cholinesterase Activity in Fledgling Starlings: Implications for Monitoring Exposure of Songbirds to ChE Inhibitors

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Exposure of songbirds to organophosphate and carbamate pesticides is often determined by comparing the brain cholinesterase (ChE) activity of individuals that have been exposed with that of unexposed birds of the same species (e.g., see Busby et al. 1981, 1982; Hamilton et al. 1981). Knowledge of the normal pattern of ChE development in the brains of nestling and fledgling passerines is a necessary prerequisite for monitoring exposure to ChE inhibitors, and assessing the impacts of these inhibitors on the reproductive success and survival of songbirds. In a previous study, Grue et al. (1981) reported that ChE activity in the brains of wild nestling European starlings (Sturnus vulgaris), 3 to 18 days old, varied significantly with age and appeared to increase toward adult levels at a constant rate. Grue et al. (1981) hypothesized that if brain ChE activity continued to develop at a constant rate, levels in the brains of fledgling starlings about 28 days old would be comparable to those in the brains of adults. The present study was conducted to test this hypothesis.

MATERIALS AND METHODS

As part of a study to compare the sensitivity of nestling and adult European starlings to an organophosphate pesticide, we monitored the nesting activity of free-living starlings within 100 wooden nest boxes on the Patuxent Wildlife Research Center during April, May, and June 1980. Nestlings were collected at 6 (n = 27), 16 (n = 19), and 18 (n = 10) days of age, sacrificed by CO_2 asphyxiation, and frozen (about -15° C). Additional 18-day old nestlings (1-3/nest, n = 42) were sealed within their nest boxes (n = 33) so that they could receive food from the adults, but could not escape. (Nestling starlings fledge when 19-23 days of age, Kessel 1957). We prevented nestlings from leaving their nest box by placing a piece of wire (2.5 x 5.0 cm mesh) over the outside of the entrance hole (diameter = 5.0 cm). We also raised the nests within the nest boxes by supporting each nest with a piece of 2.5 x 2.5 cm mesh wire at a height which permitted the nestlings to easily protrude their heads through the entrance hole and receive food from the adults.

When nestlings within the sealed boxes were 23 days old, they

were removed, banded, and placed in a large outdoor pen (1.8 x 3 x 3.6 m) equipped with several perches. Commercial turkey starter (Turkey Starter AP [medicated] Crumpels, Beacon Milling Co., Inc., Cayuga, NY[1]), live mealworms (Tenebrio molitor, Rainbow Mealworms, Compton, CA), live and frozen crickets (Acheta domestica, Ghann's Cricket Farm, Inc., Augusta, GA), Nebraska Brand bird of prey diet (Central Nebraska Packing Co., North Platte, NE), and running water were available at all times. Two captive adult starlings accustomed to the diets were placed in the pen as models for the fledglings. Fledglings were weighed and sacrificed at 30 (n = 10), 45 (n = 8), or 60 (n = 4) days of age, and frozen. Twenty-four captive adult (≥1 year old) male and female starlings (12/sex) were also weighed, sacrificed, and frozen.

We determined brain ChE levels colorimetrically using the methods of Ellman et al. (1961) as described by Hill and Fleming (1982). We used a Bausch and Lomb Spectronic 70 spectrophotometer connected to a strip-chart recorder to measure the rate of the enzyme reaction. To facilitate extraction, we removed brains while the tissue was frozen. Acetylthiocholine iodide (Sigma Chemical Co., St. Louis, MO) was used as the substrate. All samples were randomized and analyzed in duplicate at room temperature (21-24 °C) on a single day; the average ChE activity for each specimen was used in all calculations. Brain ChE activity was expressed as μmoles acetylthiocholine iodide hydrolyzed per minute per gram of tissue (wet weight).

We used linear regression (Snedecor and Cochran 1967:135) to determine the relationship between age and brain ChE activity in nestling and fledgling starlings. To find a descriptive curve which fit the data, we investigated setting Y (ChE activity) equal to the following functions of X (age): [1] AX, $A + BX$, AB^{BX}, AX^{B}, $A + B\log(X)$, $1/A + BX$, $A + B/X$, and $X/A + BX$. For each function, we transformed the data so that a line could be fit to the transformed values using linear regression. To compare fits, however, the data and the fitted lines were transformed back to the original units. The sums of the squared residuals of the original data about the curves in [1] were used in calculating the r values. In addition, we used one-way analyses of variance (ANOVA, Snedecor and Cochran 1967:258) to test for significant ($P<0.05$) differences between (1) brain ChE levels in captive adult males and females and (2) body weights of 30-, 45-, and 60-day old fledglings and weights of captive adult males and females. Duncan's multiple range tests (Duncan 1955; Kramer 1956) were used to separate means. Data for nestlings and fledglings from different nests were used to calculate all means.

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[1] Use of trade names or names of suppliers is for identification purposes only and does not constitute endorsement by the Federal government.

RESULTS AND DISCUSSION

Only 22 of the 42 18-day old nestlings held in captivity (52%) survived to 30, 45, or 60 days of age. Survivors, however, appeared to be in good condition at sacrifice; body weights were comparable to those of captive adults (Table 1).

Table 1. Body weights (g) of fledgling and adult (≥1 year old) starlings

	18[a]	Fledglings			Adults	
		30	45	60	Male	Female
n	24	10	8	4	12	12
Mean[b]	70.1^{X}	69.6^{X}	71.0^{X}	78.5^{Y}	70.2^{X}	76.0^{Y}
SD	6.9	5.2	3.6	5.4	6.3	4.9
Range	52-87	58-77	66-78	71-80	58-79	69-78

[a] Age in days
[b] Means with different superscripts differ significantly, $P<0.05$, one-way ANOVA with Duncan's multiple range test.

Brain ChE activity in 30-, 45-, and 60-day old fledglings did not vary significantly (Table 2). However, ChE levels in the brains of 60-day old fledglings were significantly greater (28.4%) than those of 18-day olds, and were only about 90 percent of brain ChE

Table 2. Brain ChE activity in captive fledgling and adult starlings

Age	ChE Activity[a]			
	n	Mean[b]	SD	Range
18 days	10	14.1^{X}	1.3	12.1-15.6
30 days	10	17.5^{Y}	1.8	15.2-20.5
45 days	8	17.3^{Y}	2.1	12.8-19.3
60 days	4	$18.1^{Y,Z}$	1.3	16.3-19.3
Adult male[c]	12	20.7^{Z}	2.8	18.5-25.9
Adult female[c]	12	$20.0^{Y,Z}$	2.9	17.8-24.2

[a] µmoles acetylthiocholine iodide hydrolyzed per minute per gram of tissue (wet weight).
[b] Means with different superscripts differ significantly, $P<0.05$, one-way ANOVA with Duncan's multiple range test.
[c] Age ≥ 1 year.

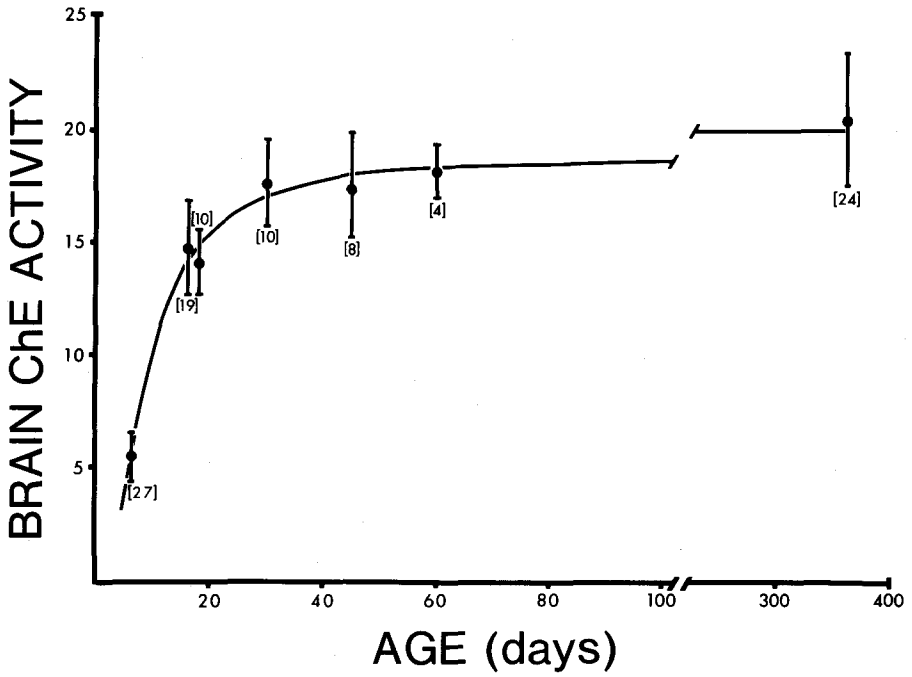

Figure 1. Brain cholinesterase (ChE) activity in free-living nestling, and captive fledgling and adult (≥365 days old) starlings. ChE activity is expressed as μmoles acetylthiocholine iodide hydrolyzed per minute per gram of tissue (wet weight). Data for adult male and female starlings were combined because differences in brain ChE activity were not significant (Table 2). Of the equations evaluated (see text), $Y = A + B/X$ (line shown above), where A = 20.1 and B = -89.3, provided the best fit of the data ($r = 0.95$). Numbers in brackets are sample sizes.

levels in captive adult males and females (Table 2). Differences between ChE levels in the brains of 30- and 45-day old starlings and ChE levels in the brains of captive adult males were statistically significant. Brain ChE levels in adult males and females did not differ significantly.

Graphical representation of our data for 6-, 16-, 18-, 30-, 45-, 60-day old, and captive adult (age ≥ 365 days) starlings suggests that brain ChE activity in nestlings (3-18 days old) increases linearly toward adult levels until fledging, after which brain ChE increases more slowly with age (Fig. 1 and Grue et al. 1981). Of the descriptive equations we evaluated, $Y = A + B/X$ provided the best fit of the data ($r = 0.95$). Results suggest that development of brain ChE in nestling starlings follows a S-shaped curve, a pattern similar to that observed in developing mallard (Anas platyrhynchos) embryos (Hoffman and Eastin 1981). However, additional data for 1-day old nestling starlings and adult starlings of known ages will be necessary to test this hypothesis.

For this reason, the descriptive equation given should not be used to predict brain ChE levels in starlings outside the range of the data on which the equation was based. Nor should brain ChE levels given be used as controls in subsequent studies without qualification because calibration standards do not exist for the assay.

The pattern of development of brain ChE activity in altricial birds appears to differ temporally from that observed in precocial and semi-precocial (Pettingill 1970:371) species. In precocial and semi-precocial species, brain ChE activity appears to increase to adult levels during embryonic development (Hoffman and Eastin 1981); young and adults have similar activity levels (Ludke et al. 1975, Hudson et al. 1972, and Table 3).

Table 3. Brain ChE activity in precocial bird species of different ages.

Species	Age	ChE Activity[a] n	Mean	SD	Reference
Mallard (*Anas platyrhynchos*)	1 day	15	9.6	0.8	Hoffman and Eastin (1981)
	16 days	6	9.1	1.4	W. J. Fleming
	31 days	4	8.9	0.4	(unpublished data)
	61 days	6	9.5	1.3	
	6 months	48	11.9	1.0	Fleming and Grue (1981)
Japanese Quail (*Coturnix c. japonica*)	2 weeks	20	10.1	1.0	E. F. Hill
	3 weeks	20	10.3	1.4	(unpublished data)
	8 weeks	34	12.6	1.0	
Laughing Gull (*Larus atricilla*)	1 day[b]	1	16.1[c]	-	White et al. (1979)
	1 week	1	15.6	-	
	2 weeks	2	22.0	4.0	
	4 weeks	2	20.7	4.5	
	5 weeks	1	21.7	-	
	5 weeks	1	12.3	-	
	adult	9	18.5	3.7	

[a] μmoles acetylthiocholine iodide hydrolyzed per minute per gram of tissue (wet weight). Data not separated by sex because brain ChE activity in adult male and female birds appears not to differ significantly (e.g., see Ludke et al. 1975, Yawetz et al. 1979, Grue 1982, Grue et al. 1982, and the present study).

[b] Age estimated to nearest day or week based on body weight data presented by Schreiber and Schreiber (1980).

[c] Brain ChE assays were conducted on a single day.

If the pattern of brain ChE development we observed in starlings is representative of altricial species, age must be considered when diagnosing exposure of nestling and fledgling songbirds to ChE inhibitors. Although age-related reductions in brain ChE in fledgling starlings (9-14%) were less than the degree of brain ChE inhibition generally accepted as indicative of exposure to a ChE inhibitor (≥20%, Ludke et al. 1975), age-dependent differences may be an important consideration in diagnosing exposure to organophosphates and carbamates. Noncontaminant related environmental factors (e.g., temperature stress and food restriction) cause reductions (10-17%) in the brain ChE activity of birds (Rattner 1982) similar to those related to age in our starlings. These reductions are well below the degree of brain ChE inhibition diagnostic of organophosphate- or carbamate-related mortality (≥ 50%, Ludke et al. 1975). However, if the effects are compounded, noncontaminant-related reductions in brain ChE could equal or exceed the 20 percent inhibition criterion indicative of exposure to anti-cholinesterases. Noncontaminant-related factors (including age) probably account for the normal variability in the brain ChE activity of free-living birds. However, unlike most of these factors, age can often be controlled for in the field.

Age-related reductions in brain ChE may also result in greater sensitivity of young altricial birds to ChE inhibitors than adults. Since nestlings and fledglings may have less ChE per unit of brain tissue than adults, less exposure to ChE inhibitors may be required to induce behavioral effects or mortality in young of altricial species. This appears to be true for starlings, where 5-day old nestlings were nearly twice as sensitive as adults to an oral dose of an organophosphate pesticide (C. E. Grue and B. K. Shipley, unpublished manuscript). The median lethal dose for 15-day old starlings was, however, similar to that for adults.

Additional data are necessary to determine the validity of extrapolating our results to other altricial bird species. Greater knowledge of the patterns of development of brain ChE activity in precocial and altricial species will be of value in monitoring exposure of birds to organophosphate and carbamate pesticides. Comparisons between the patterns of brain ChE development in several altricial and precocial species may indicate that a general relationship between age and brain ChE activity exists within each group. Predictive models could then be derived which may facilitate diagnosis of exposure to ChE inhibitors when suitable controls are not available. If brain weight is highly correlated with ChE activity and age, it may also be possible to use brain weight as an indicator of ChE activity when age is not known.

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REFERENCES

Busby DG, Pearce PA, Garrity NR (1981) Brain cholinesterase response in songbirds exposed to experimental fenitrothion spraying in New Brunswick, Canada. Bull Environm Contam Toxicol 26: 401-406

Busby DG, Pearce PA, Garrity NR (1982) Brain cholinesterase inhibition in forest passerines exposed to experimental aminocarb spraying. Bull Environm Contam Toxicol 28:225-229

Duncan DB (1955) Multiple range and multiple F tests. Biometrics 11:1-42

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88-95

Fleming WJ, Grue CE (1981) Recovery of cholinesterase activity in five avian species exposed to dicrotophos, an organophosphate pesticide. Pestic Biochem Physiol 16:129-135

Grue CE (1982) Response of common grackles to dietary concentrations of four organophosphate pesticides. Arch Environm Contam Toxicol 11:617-626

Grue CE, Powell GVN, Gladson NL (1981) Brain cholinesterase (ChE) activity in nestling starlings: implications for monitoring exposure of nestling songbirds to ChE inhibitors. Bull Environm Contam Toxicol 26:544-547

Grue CE, Powell GVN, Gorsuch CH (1982) Assessing effects of organophosphates on songbirds: comparison of a captive and a free-living population. J Wildl Manage 46:766-768

Hamilton GA, Hunter K, Ruthven AD (1981) Inhibition of brain acetylcholinesterase activity in songbirds exposed to fenitrothion during aerial spraying of forests. Bull Environm Contam Toxicol 27:856-863

Hill EF, Fleming WJ (1982) Anticholinesterase poisoning of birds: monitoring and diagnosis of acute poisoning. Environm Toxicol Chem 1:27-38

Hoffman DJ, Eastin WC Jr (1981) Effects of malathion, diazinon, and parathion on mallard embryo development and cholinesterase activity. Environm Res 26:472-485

Hudson RH, Tucker RK, Haegele MA (1972) Effect of age on sensitivity: acute oral toxicity of 14 pesticides to mallard ducks of several ages. Toxicol Appl Pharmacol 22:556-561

Kessel B (1957) A study of the breeding biology of the European starling (Sturnus vulgaris L.) in North America. Am Midl Nat 58:257-331

Kramer CY (1956) Extension of multiple range tests to group means with unequal numbers of replication. Biometrics 12:307-310

Ludke JL, Hill EF, Dieter MP (1975) Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitors. Arch Environm Contam Toxicol 3:1-21

Pettingill OS Jr (1970) Ornithology in laboratory and field, 4th edn. Burgess Publishing Company, Minneapolis, 524 pp

Rattner BA (1982) Diagnosis of anticholinesterase poisoning in birds: effects of environmental temperature and underfeeding on cholinesterase activity. Environm Toxicol Chem 1:329-335

Schreiber EA, Schreiber RW (1980) Breeding biology of laughing gulls in Florida. Part II: nesting parameters. J Field Ornithol 51:340-355

Snedecor GW, Cochran WG (1967) Statistical methods, 6th edn. Iowa State University Press, Ames, 593 pp

White DH, King KA, Mitchell CA, Hill EF, Lamont TG (1979) Parathion causes secondary poisoning in a laughing gull breeding colony. Bull Environm Contam Toxicol 23:281-284

Yawetz A, Agosin M, Perry AS (1979) Metabolism of parathion and brain cholinesterase inhibition in four species of wild birds. Pestic Biochem Physiol 11:294-300

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