Bull. Environm. Contam. Toxicol. 26, 544-547 (1981)

Brain Cholinesterase (ChE) Activity in Nestling Starlings: Implications for Monitoring Exposure of Nestling Songbirds to ChE Inhibitors

Christian E. Grue, George V. N. Powell, and Nancy L. Gladson

U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD 20811

The effects of organophosphate and carbamate pesticides on the reproductive success of wild passerines are poorly known. Since these pesticides are often applied during the breeding season, there is concern that they may have detrimental effects on the survival of nestling songbirds. Exposure to these chemicals is usually determined by monitoring brain cholinesterase (ChE) activity relative to unexposed birds (e.g., see DEWEESE et al. 1979, WHITE et al. 1979, ZINKL et al. 1979). Therefore, knowledge of normal brain ChE activity in nestling passerines is a necessary prerequisite for monitoring exposure to and assessing the impacts of ChE inhibitors on the reproductive success of songbirds. Our objective was to determine the relation between age and brain ChE activity in wild nestling starlings (*Sturnus vulgaris*).

MATERIALS AND METHODS

While conducting a study to determine the effects of an organophosphate pesticide on parental care in wild female starlings, we monitored nesting activity in 75 nest boxes located on the Patuxent Wildlife Research Center between March and July 1979. Nestlings, usually entire broods, were collected at 3, 8, 11-14, and 18 days of age. Six adult starlings (3 of each sex) were collected from their nest boxes. All birds were sacrificed by CO_2 asphyxiation and frozen (about -10°C) before brain ChE assays.

We determined brain ChE levels colorimetrically using methods described by ELLMAN et al. (1961), as modified by DIETER and LUDKE (1975) and HILL (1979). 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 frozen. Each whole brain was weighed and homogenized in 10X its weight of cold 0.05 M Tris buffer (*p*H=8.0). Acetylthiocholine iodide (Sigma Chemical Co., St. Louis, Missouri) was used as the substrate. All samples were analyzed concurrently and in duplicate; the average activity was used in all calculations. Activities were expressed as µmoles acetylthiocholine iodide hydrolyzed per min per g of tissue. We determined the rela-

0007-4861/81/0026-0544 $01.00

tion between nestling age and brain ChE levels using linear regression (SNEDECOR and COCHRAN 1967:135). We tested for significant ($P<0.05$) differences in ChE activity between broods of the same age using one-way analysis of variance (SNEDECOR and COCHRAN 1967:258). Ten broods, each containing four 13-day old young, were randomly selected for use in this analysis.

RESULTS AND DISCUSSION

Brain ChE activity in nestling starlings was age dependent and increased linearly toward adult levels (Fig. 1). ChE levels

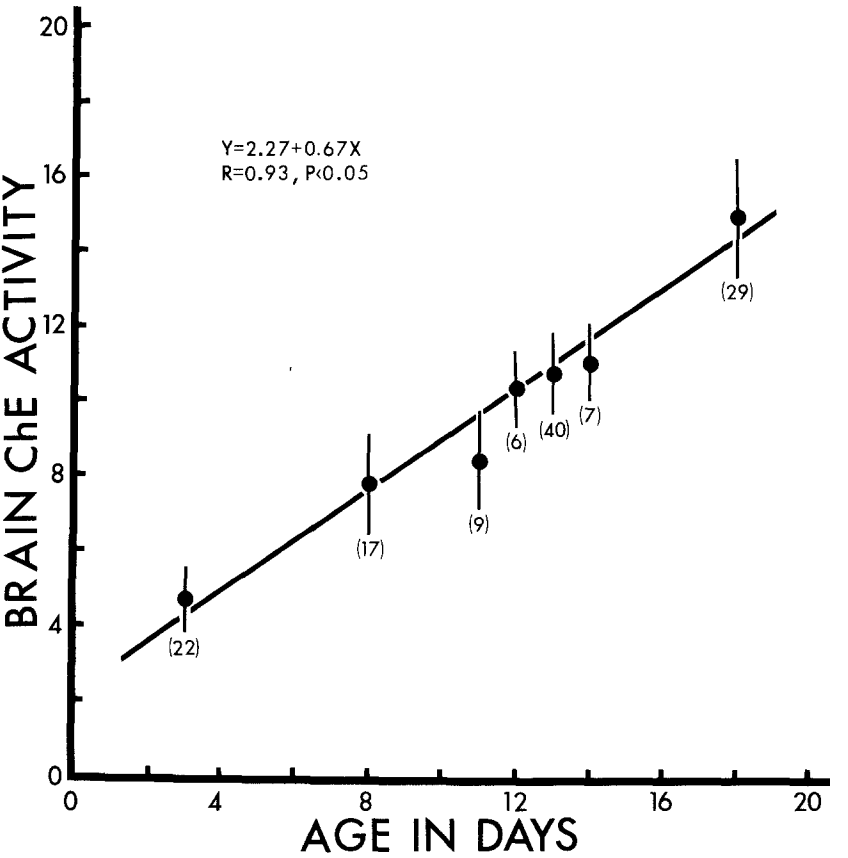

FIGURE 1. Levels of brain cholinesterase (ChE) activity in nestling starlings. ChE activity is expressed as µmoles acetylthiocholine iodide hydrolyzed per min per g of tissue. Points represent means; sample sizes are given in parentheses. Vertical lines about means denote one standard deviation. Brain ChE activities given should not be used as controls in subsequent studies because calibration standards do not exist for the assay.

in 18-day old nestlings ($\bar{\chi}$=14.9, *SD*=1.6) were 70.0 percent of those found in brains of adults ($\bar{\chi}$=21.3, *SD*=1.4), though young were within 1 to 3 days of fledging (KESSEL 1957). If brain ChE continues to develop at a constant rate (Fig. 1), we would expect fledglings to reach adult levels at 28 days of age. Differences in brain ChE activity between broods of 13-day old starlings were not significant (F=1.8, df=9, P>0.05).

The pattern of development of brain ChE activity appears to differ temporally between altricial and precocial bird species. In precocial species, brain ChE activity appears to increase to adult levels during embryonic development (D. J. HOFFMAN, unpubl. data); young and adults have similar activity levels (LUDKE et al. 1975; WHITE et al. 1979; W. J. FLEMING, pers. comm.).

If the pattern of brain ChE development we observed in starlings occurs in other altricial species, age must be considered when monitoring the degree of exposure altricial nestlings sustain from pesticides that are ChE inhibitors. Nestlings selected for comparison of treated and control areas should be the same age; otherwise, an age-related correction factor, possibly species specific, must be derived.

We do not know the survival implications of age-dependent brain ChE activity in nestling songbirds with respect to exposure to organophosphate and carbamate pesticides. Age-related sensitivity of altricial songbirds to ChE inhibitors has not been investigated. However, since nestling starlings have less ChE per g of brain tissue than adults, it is possible that less exposure to ChE inhibitors would be required to induce behavioral effects or mortality in young of altricial species.

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 young birds to organophosphate and carbamate pesticides.

ACKNOWLEDGMENTS

We thank C. Christian, C. Gorsuch, B. Keyes, and M. McChesney for monitoring nests, collecting nestlings, and conducting the cholinesterase assays. B. Wieland and L. Thomas provided the necessary secretarial assistance. E. H. Dustman, W. J. Fleming, and D. J. Hoffman kindly reviewed the manuscript.

REFERENCES

DEWEESE, L. R., C. J. HENNY, R. L. FLOYD, K. A. KOBAL, and A. W. SCHULTZ: U. S. Fish Wildl. Ser. Spec. Sci. Rep. - Wildl. No. 224 (1979).

DIETER, M. P., and J. L. LUDKE: Bull. Environm. Contam. Toxicol. 13, 257 (1975).
ELLMAN, G. L., K. D. COURTNEY, V. ANDRES, and R. M. FEATHERSTONE: Biochem. Pharmacol. 7, 88 (1961).
HILL, E. F.: Lab. Anim. Sci. 29, 349 (1979).
KESSEL, B.: Am. Midl. Nat. 58, 257 (1957).
LUDKE, J. L., E. F. HILL, and M. P. DIETER: Archiv. Environm. Contam. Toxicol. 3, 1 (1975).
SNEDECOR, G. W., and W. G. COCHRAN: Statistical methods. 6 ed. Ames, Iowa: Iowa State Univ. Press 1967.
WHITE, D. H., K. A. KING, C. A. MITCHELL, E. F. HILL, and T. G. LAMONT: Bull. Environm. Contam. Toxicol. 23, 281 (1979).
ZINKL, J. G., C. J. HENNY, and P. J. SHEA: In Animals as monitors of environmental pollutants. Washington, D.C.: Nat. Acad. Sci. 1979.