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Seasonal Brain Acetylcholinesterase Activity in Three Species of Shorebirds Overwintering in Texas

Christine A. Mitchell and Donald H. White

U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Gulf Coast Field Station, P.O. Box 2506, Victoria, TX 77902-2506

Depression of brain acetylcholinesterase (AChE) activity has been used as an indicator of exposure to, or death due to organophosphate compounds in bird populations (ZINKL et al. 1978; DEWEESE et al. 1979; WHITE et al. 1979; FELTON et al. 1981; ZINKL et al. 1981; HILL & FLEMING 1982). Many of the factors that affect AChE activity have been examined. Effect of the animal's sex (LUDKE et al. 1975), age (SHELLHAMMER 1961; GRUE et al. 1981), and nutritional state (BRUST et al. 1971) have been studied, as well as, the inhibition and recovery patterns of AChE (WEISS 1958; BENKE & MURPHY 1974; STENERSEN 1979; FLEMING 1981), its regional distribution in the brain (APRISON et al. 1964; GIBSON et al. 1969), and its circadian periodicity (RUSSELL 1968; HANIN et al. 1970; OWASOYO et al. 1980). Additionally, other factors like postmortem conditions (LUDKE et al. 1975), storage regimes (WEISS 1958; LUDKE et al. 1975), and assay conditions (GIBSON et al. 1969; HILL & FLEMING 1982) have all been delineated.

For diagnostic interpretation, AChE activity of affected birds is compared to that of normal birds (controls), so the collection of controls is very important. There are many advantages to using controls collected at times other than when an organophosphate-poisoning is suspected. In Texas, for example, large areas of agricultural land are sprayed with organophosphate insecticides making it difficult to find birds that have not potentially been exposed to these AChE inhibitors. For rare or migratory birds, adequate numbers of control birds might not immediately be available when needed. Finally, if brains salvaged at other times of the year could be used, additional healthy wild birds need not be sacrificed to serve as controls. Seasonal variation must be understood before this can be a viable alternative. RUSSELL (1968) examined AChE activity in the median eminence, adenohypophysis, and neurohypophysis of experimentally manipulated white-crowned sparrows *(Zonotrichia leucophyrys)* but found no effect of different light-dark regimes (8L 16D or 20L 4D). She did find a difference in AChE activity in the adenohypophysis between photosensitive and photorefractory birds, but the biological significance of the photorefractory birds is not clear nor is it known how these specific AChE levels related to whole brain levels. SHELLHAMMER (1961) did show some seasonal variation in whole brains of 2 species of wild mice, so we wanted to examine whether seasonal variation exists in wild birds, and additionally to see if exposure to organophosphate chemicals is taking place.

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MATERIALS AND METHODS

Western sandpipers *(Calidris mauri)*, long-billed dowitchers *(Limnodromus scolopaceus)*, and American avocets *(Recurvirostra americana)* were collected between July 1979 and February 1980 at the mouth of an agricultural drainage system that enters the Laguna Madre 8 km south of Port Mansfield, Texas (26°14'N, 97°26'W) The collection dates and sample size (Table 1) varied somewhat mainly due to the different migration patterns of the 3 species. The collections were all made during late morning (1000 - 1200 h), and the total collection time on any given day was <1 h. Birds were shot, placed on wet ice usually within 1 h postmortem, and then frozen later in the day. They remained frozen until the intact brains were removed from partially thawed birds, put into glass jars, and immediately refrozen. In November and December 1980, the brains were assayed to determine AChE activity. The colorimetric method of ELLMAN et al. (1961) as modified by HILL & FLEMING (1982) was used. The whole brain was homogenized in the w. sandpipers and dowitchers, but because of the large size only one-half (divided longitudinally) of each avocet brain was used. All samples, from initial collection to final assay, were handled as identically as possible to minimize variation.

RESULTS AND DISCUSSION

Since organophosphate pesticides were being used on agricultural croplands in Texas in July and August, the data from that period were excluded from the following statistical analyses. There was no significant difference ($P>0.01$, analysis of variance) in average AChE activity among dates for the 3 species (Table 1). Since no differences existed, an average AChE activity for each species was calculated combining the 3 dates. The averages $\pm$ 2 standard deviations were 22.8 $\pm$ 5.60, 16.2 $\pm$ 3.40, and 19.4 $\pm$ 4.50 for the w. sandpipers, dowitchers, and avocets, respectively.

As other investigators (LUDKE et al. 1975) have noted, there was no significant difference ($P>0.05$, analysis of variance) in the AChE levels we found between males and females of a species. The average AChE activity $\pm$ 1 standard deviation was 22.0 $\pm$ 1.94 and 23.4 $\pm$ 3.14 for male and female w. sandpipers, 16.4 $\pm$ 1.69 and 16.1 $\pm$ 1.76 for dowitchers, and 19.4 $\pm$ 2.29 and 19.4 $\pm$ 2.28 for avocets. The sex ratio was 41:59, 44:56, and 50:50, respectively.

WEISS (1958) showed a curvilinear relationship between brain weight and AChE activity on a unit weight basis, although no statistics were given. Our data for w. sandpipers and dowitchers showed a significant non-linear relationship ($P<0.05$, F-test) that was best fitted by a parabolic line. Although a relationship existed, the r^2 value for both species was <0.25, therefore, the regression line would not be helpful in predicting or adjusting AChE levels based on brain weight.

TABLE 1. Brain AChE activity in 3 species of shorebirds

Species Date Collected	N	AChE activity[a] $\bar{x} \pm 2$ SD
Western sandpiper		
25 July 1979	20	20.3 ± 5.56
11 Oct. 1979	20	23.6 ± 7.76
1 Dec. 1979	20	23.1 ± 3.18
15 Feb. 1980	20	21.6 ± 4.06
Long-billed dowitcher		
11 Oct. 1979	20	17.0 ± 2.34
1 Dec. 1979	20	15.7 ± 3.98
15 Feb. 1980	15	15.9 ± 3.36
American avocet		
25 Aug. 1979	3	15.7 ± 7.98
11 Oct. 1979	10	19.5 ± 5.66
1 Dec. 1979	10	18.8 ± 3.68
15 Feb. 1980	10	19.9 ± 4.08

[a] AChE activity expressed as µmoles AtChI hydrolyzed per min per gram of brain tissue.

Because the birds were feeding at the mouth of an agricultural drainage system that serviced tens of thousands of acres of cropland, the potential existed for exposure to agricultural chemicals both during and after periods of application. The closest fields to the collection site were about 15 km away. Although organophosphate chemicals are considered to be relatively non-persistent in the environment, they can persist in water and soil in appreciable quantities for weeks and even months after application (MILES & HARRIS 1978; SHAROM et al. 1980). WILLIAMS & SOVA (1966) and HOLLAND et al. (1967) found that fish at the mouths of rivers polluted with organophosphate materials, either from organophosphate chemical plants or agriculture, had reduced AChE activity. The frequency distribution of AChE activity for w. sandpipers (Fig. 1) shows a shift to the left in the July sample. This shift is also apparent in the avocet data (Fig. 1), but the sample size for the August collection was small. The brain AChE activity of 2 w. sandpipers and 1 avocet fall below the $\bar{x} \pm 2$ SD range established above, with inhibition of 25, 36, and 43%. This would strongly indicate exposure to an AChE inhibitor. There is no significant difference ($P>0.40$, t-test) in AChE activity between male and female w. sandpipers in the July collection, which might suggest that males and females are not differentially affected by AChE inhibitors.

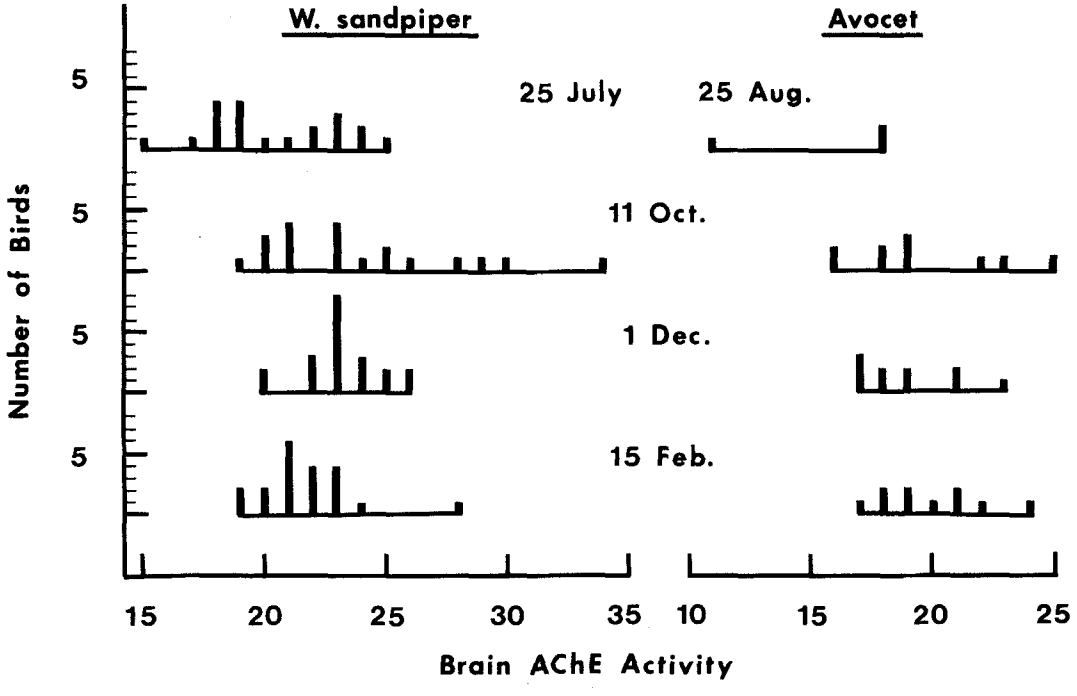

FIGURE 1. Frequency distribution of brain AChE activity expressed as μmoles of AtChI hydrolyzed per min per gram of brain tissue.

CONCLUSIONS

There was no seasonal variation in average brain AChE activity for the 3 species of wild birds collected between October and February. Further work needs to be done, however, covering an even broader time frame which includes the reproductive cycle. It appears that some birds feeding at the mouth of an agricultural drain, at some distance from the nearest pesticide applications, were affected by AChE inhibitors.

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