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PLASMA AND ERYTHROCYTE CHOLINESTERASE VALUES FOR THE COMMON LONG-NOSED ARMADILLO, *DASYPUS NOVEMCINCTUS*

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ABSTRACT: Plasma and erythrocyte cholinesterase activities were determined for 40 free-living and 12 captive common long-nosed armadillos (*Dasypus novemcinctus*) in order to establish normal values for monitoring pesticide exposure. Plasma cholinesterase activity ranged from 105 to 549 U/liter with no sexual or seasonal differences. Plasma values from captive animals were significantly lower than those from wild armadillos. Erythrocyte cholinesterase activity ranged from 2,915 to 15,126 U/liter with no differences detected between captive and wild animals or between sexes. However, erythrocyte cholinesterase values varied seasonally. Erythrocyte and plasma cholinesterase activities were not significantly correlated. Packed cell volume ranged from 24 to 51% and did not vary significantly between captive and wild samples, between sexes or among seasons. However, both whole blood and erythrocyte cholinesterase activities showed significant negative correlations with packed cell volume. Controlled experiments are needed to find the factors responsible for the statistically significant difference between plasma cholinesterase activities of captive and wild armadillos. The seasonal variation in erythrocyte cholinesterase activity and the negative correlation between erythrocyte cholinesterase activity and packed cell volume can be explained by an hypothesis that relates the variation in erythrocyte cholinesterase activity to variation in erythrocyte turnover rate. Future work should involve experiments to test this hypothesis.

Key words: *Dasypus novemcinctus*, common long-nosed armadillo, blood cholinesterase levels, normal values, toxicology, clinical study.

INTRODUCTION

The common long-nosed armadillo, *Dasypus novemcinctus* is a generalist insectivore whose diet includes nearly every type of invertebrate, small vertebrates, and even some fruits (Redford, 1985; Wirtz et al., 1985) present in surface soil and litter. As a top predator of the soil/leaf litter community, the armadillo is likely to be exposed to toxins and pesticide residues reaching the soil surface. Thus, this species is useful for sampling and measuring these residues throughout its geographic range which extends from Argentina to the southeastern United States (Wetzel, 1985). The usefulness of armadillos for surveying organochlorine residues has been demonstrated (Wheeler et al., 1975).

Presently, organophosphate and carbamate compounds are the most widely used pesticides in agricultural, and municipal and domestic pest control programs (Marquis, 1986). The primary mode of action of these compounds is to inhibit cholinesterases and thereby cause death by cholinergic hyperstimulation (Marquis, 1986). Measurements of blood and tissue cholinesterase activities are useful for assaying organophosphate and carbamate exposure. Blood cholinesterase activity is relatively easy to measure and is correlated with cholinesterase levels in nervous tissues. Organophosphate and carbamate compounds are thought to degrade in the environment more rapidly than organochlorine compounds; however, several reports of primary and secondary toxicity to wildlife have followed organophosphate applications (White et al., 1979; Zinkl et al., 1979; Henny et al., 1985). The purpose of this study was to investigate the armadillo as a bioassay for carbamate and organophosphate impacts on the environment. This report provides preliminary findings on the normal values of plasma and erythrocyte cholinesterase activities of wild and captive armadillos.

TABLE 1. Packed cell volume (PCV) and blood cholinesterase activities in wild and captive armadillos.

				Cholinesterase activity (U/liter)[a]			
		PCV (%)		Plasma		Erythrocyte	
Sex	*n*	$\bar{x}$ ± SE	Range	$\bar{x}$ ± SE	Range	$\bar{x}$ ± SE	Range
Wild male	19	37.5 ± 1.5	24–50	380 ± 14.2	282–492	6,684 ± 761.3	2,915–15,126
Wild female	21	39.5 ± 0.9	28–48	371 ± 10.2	286–516	6,329 ± 687.8	3,753–13,329
Pooled	40	38.6 ± 0.9		376[b] ± 8.7		6,498 ± 505.2	
Captive male	7	42.9 ± 1.6	39–51	329 ± 5.7	307–339	5,107 ± 251.3	4,248–5,795
Captive female	5	37.8 ± 1.2	35–42	329 ± 70.4	105–549	5,800 ± 386.1	5,107–7,058
Pooled	12	40.8 ± 1.3		329[b] ± 27.6		5,429 ± 236.5	

[a] Units/liter = micromoles of acetylthiocholine hydrolyzed per liter per minute at 37 C.
[b] Significantly different; $P < 0.05$, two-tailed Student's *t*-test.

MATERIALS AND METHODS

Blood samples (2 to 3 ml) were collected from 21 female and 19 male armadillos from a wild population in the San Felasco Hammock State Preserve (Alachua County, Florida, USA; 29°42′ to 29°45′N, 82°26′ to 82°28′W) and from seven males and five females maintained in captivity at the Florida Institute of Technology (Melbourne, Florida 32904, USA). San Felasco Hammock State Preserve is a 2,400 ha site that is not treated with pesticides. Armadillos from this site were assumed to have minimal exposure to organophosphate and carbamate residues. Captive animals were collected from several different locations in Florida and were maintained under controlled conditions for at least 22 mo ($\bar{x}$ = 36 mo). The housing facilities, maintenance care and diet of the captive animals are described by Storrs (1987). All armadillos in the captive colony are periodically dipped for mites using an organophosphate miticide (Para Mite, Vet-Kem, Dallas, Texas 75234, USA) but the animals sampled in this study had not been treated for several months.

Captive animals were sedated with a combination of fentanyl citrate and droperidol 0.11 ml/kg (Innovar-Vet, Pitman-Moore, Washington Crossing, New Jersey 08560, USA) and bled from the saphenous vein. Wild animals were bled via the median caudal vein while conscious or sedated with a combination of ketamine hydrochloride 25 mg/kg (Ketaset, Bristol Laboratories, Syracuse, New York 13201, USA) and acepromazine maleate 0.3 mg/kg (Promace, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA). Packed cell volume was determined using a microhematocrit centrifuge. A 200 µl aliquot of whole blood was added to 1,800 µl of sterile water to lyse the erythrocytes for whole blood cholinesterase determination. The remaining blood sample was centrifuged at 2,000 rpm for 3 min to separate plasma. The plasma and hemolyzed whole blood were stored at −20 C until analyzed.

Plasma and whole blood cholinesterase activities were determined by the method of Ellman et al. (1961) using a commercial diagnostic kit and following the manufacturer's recommendations (Reagenset, Cholinesterase, Boehringer Mannheim Diagnostics, Indianapolis, Indiana 46250, USA). Enzyme activities were measured at 37 C and are reported in international enzyme units per liter (U/liter). Erythrocyte cholinesterase values were calculated from the packed volume and plasma and hemolyzed whole blood values.

Packed cell volumes and plasma and erythrocyte cholinesterase values were compared between sexes, and between captive and wild samples using Student's *t*-tests. Seasonal differences in the wild population were tested using Kruskal-Wallis analysis of variance by ranks (December to February, winter; March to May, spring; June to August, summer; September to November, fall). Pearson product moment correlation coefficients were calculated for the following combinations of variables: plasma cholinesterase activity versus erythrocyte cholinesterase activity, packed cell volume versus erythrocyte cholinesterase activity, and packed cell volume versus whole blood cholinesterase activity.

RESULTS

Mean values, standard errors and ranges of packed cell volume, and plasma and erythrocyte cholinesterase activities are presented in Table 1. Overall, plasma cholinesterase activity ranged from 105 to 549 U/liter and erythrocyte cholinesterase activity ranged from 2,915 to 15,126 U/liter. Erythrocyte cholinesterase activity com-

prised between 85 and 96% of whole blood cholinesterase activity. There were no statistically significant differences between sexes for packed cell volume ($t = 0.2$, $P > 0.05$), plasma cholinesterase activity ($t = 0.3$, $P > 0.05$), or erythrocyte activity ($t = 0.2$, $P > 0.05$). Mean values of packed cell volume and erythrocyte cholinesterase activity did not differ significantly between wild and captive samples ($t = 1.3$ and $t = 1.1$, respectively; $P > 0.05$). Conversely, plasma cholinesterase activity differed significantly between samples from wild and captive animals ($t = 2.2$, $P < 0.05$).

Figure 1 (A, B and C) illustrates the seasonal variation in packed cell volume, plasma cholinesterase, and erythrocyte cholinesterase activities, respectively, of the wild population. Erythrocyte cholinesterase activity showed significant variation among seasons ($H = 39.6$, $P < 0.001$). The samples collected in spring (March to May) had the lowest mean and variance in erythrocyte cholinesterase values. The samples collected in fall and winter had the highest mean erythrocyte cholinesterase values. The hematocrit and plasma cholinesterase activities did not show significant seasonal variation ($H = 3.0$ and $H = 3.0$, respectively; $P > 0.05$).

Plasma and erythrocyte cholinesterase activities were not significantly correlated ($r = -0.09$, $P > 0.05$). Conversely, both whole blood cholinesterase and erythrocyte cholinesterase activities showed significant negative correlations with packed cell volume ($r = -0.33$ and $r = -0.64$, respectively; $P < 0.005$).

DISCUSSION

Several factors, including differences in diet, method of restraint and pesticide exposure, may explain why captive animals tended to have lower plasma cholinesterase activities than the wild population. These factors are among those known to affect human plasma cholinesterase levels (Whittaker, 1986). Therefore, further experiments are needed to explain this variation in plasma cholinesterase activity of the armadillo.

The statistically significant seasonal variation in erythrocyte cholinesterase in the wild population is intriguing. In order to show that the low spring and summer cholinesterase activities resulted from exposure to cholinesterase inhibitors, pesticide residues would have to be demonstrated in the tissues or ingesta. Also, a similar pattern of depressed cholinesterase activity in other tissues would be expected. There is not enough information to attribute this seasonal variation to a particular cause; however, the findings that plasma cholinesterase activities were not similarly depressed in spring and summer and plasma and erythrocyte cholinesterase activities were not significantly correlated indicate that pesticide exposure is a less probable explanation for the above. In addition, these armadillos lived in a 2,400 ha nature preserve that was not treated with pesticides. Because of the armadillos' small home ranges (Layne and Glover, 1977; L. H. Herbst, unpubl. data) it is unlikely that these animals wandered out of the preserve into residential or agricultural areas that might be sprayed seasonally. Therefore, some other factors may be responsible for the observed seasonal variation in erythrocyte cholinesterase activity.

Erythrocyte cholinesterase is synthesized in the bone marrow and activity is known to decrease with the age of the cell (Harris and Kellermeyer, 1970; Whittaker, 1986). Thus, it is possible that the seasonal pattern in erythrocyte cholinesterase activity may be related to seasonal changes in erythrocyte age or turnover rate. For example, in winter months a higher frequency of armadillos with regenerative anemia could result in raised reticulocyte counts and increased erythrocyte cholinesterase activity. Presently, the only evidence to support this hypothesis is that erythrocyte cholinesterase activity tends to be higher in animals with low packed cell volume. The highly significant negative correlation between erythrocyte cholin-

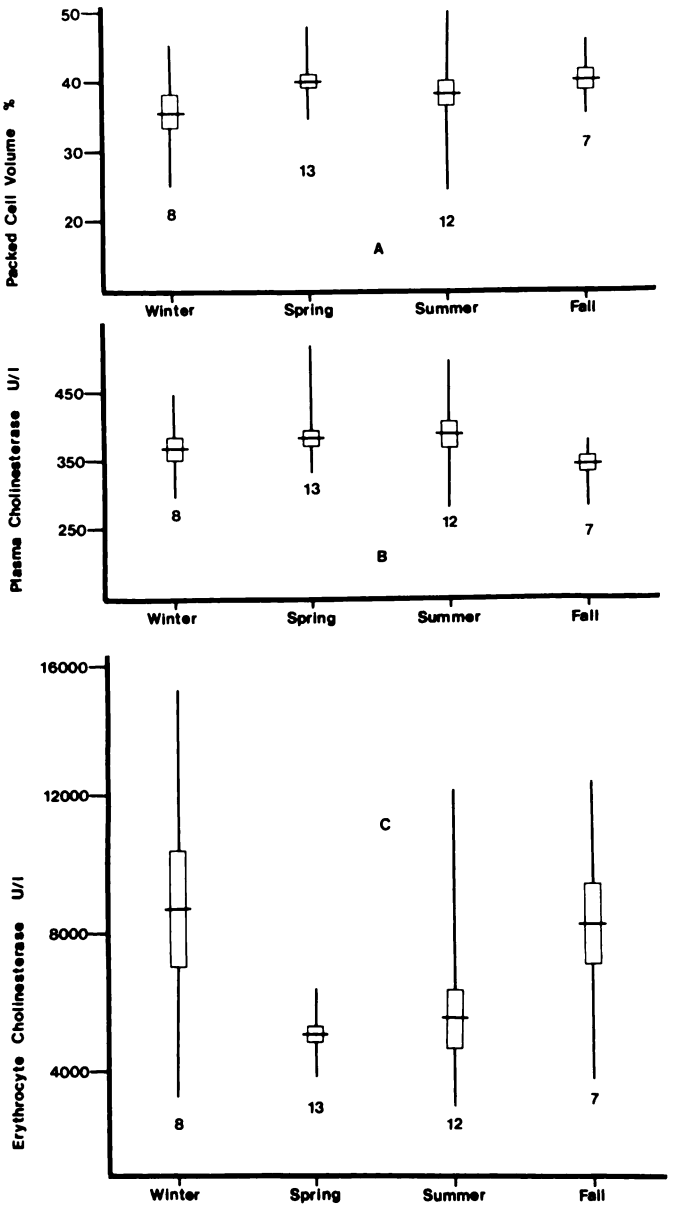

FIGURE 1. Seasonal variation in packed cell volume (A), plasma cholinesterase activity (B), and erythrocyte cholinesterase activity (C) of wild armadillos. Horizontal bars and boxes represent means and standard errors. Vertical bars represent the ranges. Numbers below the vertical bars are sample sizes.

esterase activity and packed cell volume and the fact that erythrocytes are responsible for most of the cholinesterase activity of whole blood explains why whole blood cholinesterase activity had a negative correlation with packed cell volume. If erythrocyte cholinesterase activity was constant or independent of packed cell volume whole blood activity would be expected to have a positive correlation with packed cell volume. Further investigation of the potential factors involved in producing seasonal changes in erythrocyte enzyme activities is recommended.

A limitation of our data is that erythrocyte cholinesterase activities are calculated values that accumulate errors in measuring plasma and whole blood activities. Therefore, a more direct assay method for erythrocyte cholinesterase is desirable to confirm the findings reported herein.

The cholinesterase activity of armadillo plasma is low compared to plasma from many domestic animals (Ecobichon and Comeau, 1973; Sundlof et al., 1984; A. I. Webb, unpubl. data). Armadillo plasma values are similar to values found in goats and rats (Ecobichon and Comeau, 1973). These low normal values make plasma measurements less sensitive for detecting cholinesterase depression caused by pesticide exposure. Hassan et al. (1981) used the same assay method as our study and found a residual cholinesterase activity of 105 U/liter following maximum inhibition of human plasma cholinesterase with organophosphate and heat. Because armadillo plasma cholinesterase activities averaged only three to four times this minimal value, a search for tissues with higher normal activities to monitor pesticide exposure is recommended.

Low normal activities of plasma cholinesterase have been related to organophosphate sensitivity in some species (DiGiacomo et al., 1987). Thus, there is a possibility that armadillos are also sensitive to cholinesterase inhibitors. Although signs of toxicity have not been observed in captive armadillos periodically dipped with organophosphate, we recommend caution when using cholinesterase inhibitors for parasite control in armadillos (also see Divers, 1978) until toxicity testing is conducted to assess the sensitivity or resistance of these animals to these compounds.

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