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ENZYME ACTIVITIES IN PLASMA, KIDNEY, LIVER, AND MUSCLE OF FIVE AVIAN SPECIES

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ABSTRACT: Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) were determined in plasma, kidney, liver, and muscle from five species of captive birds. Few differences occurred in plasma activities between sexes but considerable differences occurred between species. All five enzymes were detected in each of the tissues sampled. Relative enzyme activities in liver, kidney, and muscle were similar for each species. CPK activitiy was much higher in muscle than in liver or kidney and, of the five enzymes studied, may be the best indicator of muscle damage. Most of the other enzymes were more evenly distributed among the three tissues, and no organ-specific enzyme could be identified for liver or kidney. Because of interspecific variations in plasma enzyme activities, it is important to establish baseline values for each species to ensure accurate interpretation of results.

INTRODUCTION

In human and veterinary medicine, serum or plasma activities of organ-specific enzymes are often used in clinical diagnosis. Even when enzymes specific to certain organs have not been identified, the source of cellular enzymes in plasma can sometimes be predicted by studying activities of several enzymes if their relative distribution in major organs is known (Freedland and Kramer, 1970; Schmidt and Schmidt, 1974). The development of microtechniques has made clinical plasma enzyme analyses possible in birds, but little is known about the relationships between tissue and plasma enzyme activities. As a result, the significance of elevated plasma enzyme activities in birds is often unclear. Normal plasma or serum enzyme activities are known for a variety of avian species (McDaniel and Chute, 1961; Mulley, 1979; Driver, 1981; Gee et al., 1981; Franson, 1982; Westlake et al., 1983), but less information is available for the activities of these enzymes in tissues (Cornelius et al., 1959; Fowler, 1970; Franson, 1982). We report here normal activities of five enzymes in plasma, liver, kidney, and muscle from captive barn owls (*Tyto alba* Scopoli), screech owls (*Otus asio* L.), black-crowned night herons (*Nycticorax nycticorax* L.), canvasbacks (*Aythya valisineria* Wilson), and redheads (*Aythya americana* Eyton).

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MATERIALS AND METHODS

Twenty apparently healthy adults of each species (10 male and 10 female, except for 11 male and 9 female black-crowned night herons) were selected from colonies maintained at the Patuxent Wildlife Research Center, Laurel, Maryland. Samples were collected from nonbreeding barn owls, screech owls, and black-crowned night herons in May 1982, and from nonbreeding canvasbacks and redheads in August 1982. Owls were fed whole animal diets of chicks, mice, and turkey poults, while ducks were maintained on commercial developer pellets (Beacon Feeds, Cayuga, New York 13034, USA). Herons received a combination of commercial bird-of-prey diet (Nebraska®, Animal Spectrum, Inc., Lincoln, Nebraska 68516, USA) and smelt.

Birds were bled via jugular venipuncture with ammonium heparinized syringes between 0830 and 1100 hr, and plasma was harvested after centrifugation at 985 *g* for 15 min and refrigerated immediately at 4 C. All plasma enzyme activities were assayed within 4 hr of collection. Following blood collection, birds were killed by CO_2 asphyxiation and about 1 g each of kidney, liver, and pectoralis muscle was removed im-

mediately after death, weighed to the nearest 0.01 g, and placed in screw-top polyethylene vials. Cold phosphate buffer (0.1 M, pH 7.4) equivalent to 10× the sample weight was added to each vial, and samples were frozen at −80 C. On the day of analysis, samples were thawed and tissue was homogenized for 20 sec with a teflon pestle at 1,725 rpm. Homogenates were centrifuged for 20 min at 985 *g*, supernatants were recovered and refrigerated at 4 C, and enzyme analyses were completed within 4 hr of sample preparation.

Activities of alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.3.1), creatine phosphokinase (CPK, EC 2.7.3.2), and lactate dehydrogenase (LDH, EC 1.1.1.27) were determined in plasma and tissue homogenate supernatants with a centrifugal analyzer (CentrifiChem®, Union Carbide Corp., Rye, New York 10580, USA), according to manufacturer's recommendations. Duplicate samples were used in all analyses.

Two-way analysis of variance was used to compare enzyme activities among species and between sexes for each tissue and enzyme. Comparisons of enzyme activities among and within tissues were conducted using a multivariate profile analysis for each species (Morrison, 1976). All enzyme activities were log transformed before statistical analysis to obtain homogeneous variances among groups. Tukey's multiple comparison procedure (Neter and Wasserman, 1974) was used to quantify species and sex differences. The Bonferroni multiple comparison procedure (Morrison, 1976) was used to quantify the differences identified in the profile analysis.

RESULTS AND DISCUSSION

Significant species differences occurred in plasma enzyme activities, even between the closely related canvasbacks and redheads (Table 1). Activity of CPK was much higher in barn owls and black-crowned night herons than in the other three species, LDH was similar among all species except screech owls, and plasma ALP activity was up to 10 times greater in black-crowned night herons than in most other species. Plasma AST activity was much lower in waterfowl than in owls or herons, and ALT activity was relatively low in all species. Although samples were collected from nonbreeding birds, the potential effect of seasonal variation on plasma enzyme activities should be considered when comparing values for owls and herons with canvasbacks and redheads. Sex differences were observed in plasma enzyme activities only for ALT and LDH in black-crowned night herons and ALP in barn owls.

Although there were considerable species differences, plasma activities of LDH or CPK were usually highest, and ALT or AST lowest. Results for several other avian species indicated similar relative levels of enzyme activity. Captive black ducks (*Anas rubripes* Brewster) had a similar plasma enzyme profile (Franson, 1982), and Mulley (1979) reported that LDH had the highest serum activity of four enzymes studied in wild Australian black ducks (*Anas superciliosa* Gmelin). Similarly, Gee et al. (1981) observed high serum LDH activity in Anseriformes and Falconiformes. Driver (1981) reported high plasma CPK activity in wild mallards (*Anas platyrhynchos* L.), but in chickens CPK exhibited the lowest activity of seven plasma enzymes studied (Mitruka and Rawnsley, 1977). In bald eagles (*Haliaeetus leucocephalus* L.), plasma LDH activity was greater than AST, CPK, or ALT (Dieter and Wiemeyer, 1978).

All five enzymes were detected in each of the three tissues sampled, and a similar overall pattern in relative enzyme activity occurred among species for each tissue (Fig. 1, Table 2). This pattern was most obvious in muscle, where CPK activity was highest in all species, followed by LDH, AST, ALT; only minimal ALP activity was evident. In kidney, ALT and ALP activities were lowest in all species, activities of LDH and CPK were highest in three species, and AST was usually intermediate. Liver LDH and AST activities were greatest in all species, ALP was lowest in three species, and CPK and ALT were often intermediate.

Elevated serum or plasma activities of CPK, LDH, and AST are used in clinical

TABLE 1. Plasma activities (IU/liter) of five enzymes in barn owls (BAOW), screech owls (SCOW), black-crowned night herons (BCNH), canvasbacks (CANV), and redheads (REDH). Mean = geometric mean, CV = coefficient of variation.

Sex		BAOW	SCOW	BCNH	CANV	REDH
			Alanine aminotransferase			
M	Mean	10A[a]	28B	7A	19B	21B
	CV	30.8	6.1	20.2	6.3	8.6
	Range	3–20	21–39	3–11	14–28	14–31
	n	10	10	11	11	9
F	Mean	18A	41B	16A[b]	15A	20A
	CV	27.3	18.2	17.2	6.7	4.9
	Range	5–38	10–96	7–32	11–20	14–23
	n	7	10	7	10	10
			Aspartate aminotransferase			
M	Mean	131AB	148B	127A	15C	23D
	CV	4.5	4.2	5.7	8.1	12.0
	Range	98–208	102–200	87–186	11–20	13–34
	n	10	10	10	11	9
F	Mean	116AB	166B	94A	11C	22D
	CV	4.6	8.3	7.9	9.5	11.0
	Range	92–164	100–293	65–212	8–20	12–34
	n	10	10	9	10	10
			Alkaline phosphatase			
M	Mean	22A	24A	273B	40A	26A
	CV	9.7	14.6	7.7	22.5	14.8
	Range	14–36	15–54	157–543	20–232	13–72
	n	10	10	11	11	9
F	Mean	52A[b]	32AC	179	36AB	19AB
	CV	16.7	19.8	8.0	18.0	10.0
	Range	15–114	17–152	111–405	14–88	10–26
	n	9	10	9	10	10
			Creatine phosphokinase			
M	Mean	723A	96D	376B	70C	97CD
	CV	4.7	8.7	11.2	8.3	12.9
	Range	644–1,310	53–191	164–944	46–122	48–280
	n	10	10	8	11	9
F	Mean	584A	144D	412B	74C	108CD
	CV	4.8	14.0	8.2	11.7	15.4
	Range	404–962	53–477	246–892	42–146	44–366
	n	10	10	9	10	10
			Lactate dehydrogenase			
M	Mean	154A	93B	187A	140AB	199A
	CV	8.6	9.5	8.3	5.6	4.4
	Range	76–358	59–171	95–342	72–184	148–286
	n	10	10	11	10	8
F	Mean	111A	117A	83A[b]	120A	219B
	CV	5.5	7.5	10.8	4.8	6.4
	Range	63–168	79–263	42–207	79–184	144–382
	n	10	10	9	10	10

[a] Means in the same row not sharing letters in common are significantly different ($P < 0.05$).

[b] Significantly different from males ($P < 0.05$).

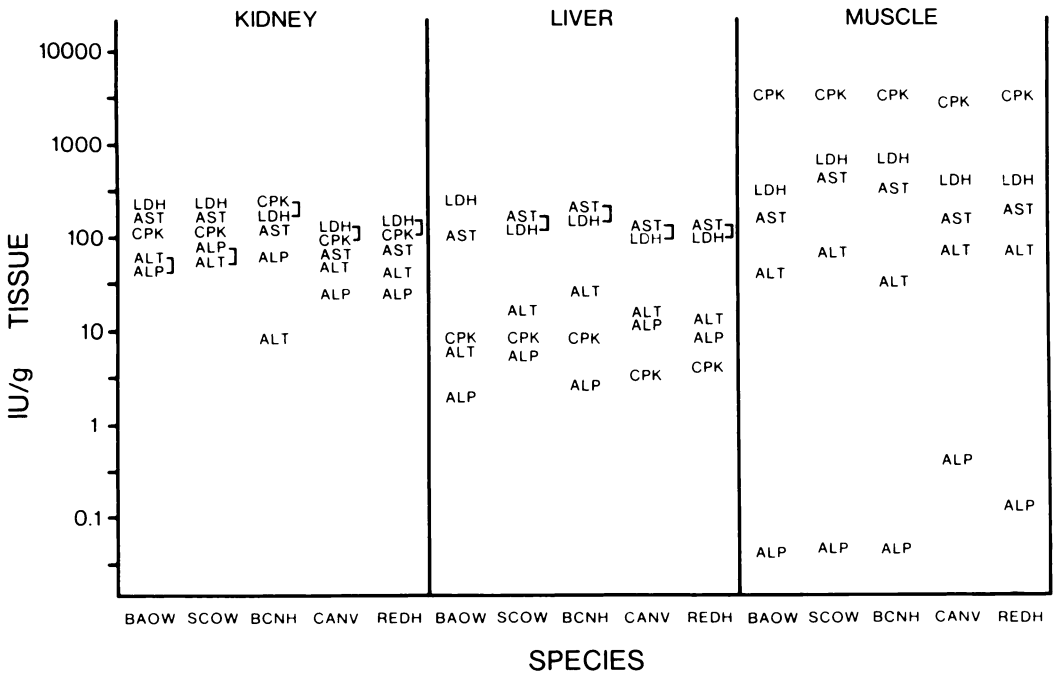

FIGURE 1. Tissue activities (geometric means, IU/g) of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) in barn owls (BAOW), screech owls (SCOW), black-crowned night herons (BCNH), canvasbacks (CANV), and redheads (REDH). Activities within a column are significantly different ($P < 0.05$), except those connected by brackets.

veterinary medicine for detection of muscle disease in mammals (Duncan and Prasse, 1977). A panel of these three enzymes may also be useful in birds because these enzymes exhibited the highest muscle activity in all species (Fig. 1). Creatine phosphokinase may be the best enzyme of those studied to use for predicting muscle damage because its activity is much lower in liver and kidney than in muscle; further support for this use is given by Hollands et al. (1980) who used plasma CPK activity to identify turkeys with degenerative myopathy. Activities of LDH and AST are more similar among the three tissues, so their use alone may reflect disease of other organs, especially liver.

Because of the wide distribution in other tissues, little evidence exists that any of the five enzymes studied can be used as a specific indicator of liver damage. Fowler (1970) observed increased plasma AST activity after carbon tetrachloride administration in White Leghorn cockerels and Khaki Campbell ducklings, but suggested this increased activity may have been due to damage to some tissue other than liver, because several tissues contained significant AST activity. Ibrahim et al. (1980) reported a slight increase in plasma AST activity in domestic chickens fed a high concentration of rapeseed meal, which was considered hepatotoxic, but activities of isocitrate dehydrogenase and malate dehydrogenase showed a greater response. Plasma ALT activity has been suggested as a specific indicator of liver damage in waterfowl (Szaro et al., 1978), but data from canvasbacks and redheads indicate higher ALT activity in both kidney and muscle, and earlier work with mallards, black ducks, and chickens showed greater

TABLE 2. Tissue activities (IU/g) of five enzymes in barn owls (BAOW), screech owls (SCOW), black-crowned night herons (BCNH), canvasbacks (CANV), and redheads (REDH). Mean = geometric mean, CV = coefficient of variation for log-transformed data. n = 20.

Tissue	BAOW	SCOW	BCNH	CANV	REDH
			Alanine aminotransferase		
Kidney					
Mean	48	54	8	52	41
CV	10.5	6.6	9.9	4.7	6.6
Range	14–78	34–79	6–12	28–66	31–65
Liver					
Mean	6	17	23	16	12
CV	21.0	8.0	9.4	8.5	8.4
Range	3–12	10–22	12–42	9–22	9–18
Muscle					
Mean	40	68	29	66	64
CV	5.3	4.1	9.9	7.6	6.9
Range	28–52	41–85	14–47	33–116	32–102
			Aspartate aminotransferase		
Kidney					
Mean	170	168	134	70	57
CV	3.1	2.3	2.4	1.9	2.2
Range	114–209	140–213	106–161	58–85	49–70
Liver					
Mean	113	163	182	131	116
CV	4.4	3.3	3.2	4.0	4.9
Range	78–158	112–229	119–249	96–180	75–161
Muscle					
Mean	180	371	298	152	186
CV	2.8	2.3	3.6	4.0	5.0
Range	144–260	285–443	186–407	114–209	128–330
			Alkaline phosphatase		
Kidney					
Mean	36	57	52	26	25
CV	19.7	4.7	6.7	7.8	5.4
Range	10–84	39–78	36–92	14–39	17–36
Liver					
Mean	2	5	3	12	8
CV	155.3	28.6	26.7	9.8	8.9
Range	ND*–4	2–10	2–6	6–19	6–11
Muscle					
Mean	0.04	0.05	0.05	0.26	0.11
CV	30.1	26.2	32.0	40.4	53.2
Range	ND–0.2	ND–0.18	ND–0.24	0.09–0.68	ND–0.65
			Creatine phosphokinase		
Kidney					
Mean	132	116	193	112	128
CV	4.1	3.2	2.6	2.2	1.7
Range	91–175	83–156	152–266	86–138	113–150

TABLE 2. Continued.

Tissue	BAOW	SCOW	BCNH	CANV	REDH
Liver					
Mean	9	9	9	4	4
CV	12.8	11.5	13.8	31.8	23.0
Range	5–14	6–15	6–16	2–12	3–7
Muscle					
Mean	3,275	3,058	3,105	2,536	2,940
CV	1.7	1.7	2.1	1.5	2.7
Range	2,598–4,400	2,268–3,640	1,966–3,894	1,901–3,137	1,928–3,896
			Lactate dehydrogenase		
Kidney					
Mean	192	188	178	121	134
CV	2.7	1.8	2.6	1.6	1.5
Range	138–265	156–225	140–253	110–145	115–160
Liver					
Mean	261	160	164	113	108
CV	4.7	3.6	3.5	6.7	4.4
Range	164–414	112–242	125–261	47–176	68–149
Muscle					
Mean	342	628	580	434	375
CV	2.2	1.9	2.6	3.3	2.6
Range	278–441	528–783	445–815	253–589	273–505

* ND = Not detected.

ALT activity in kidney than in liver (Cornelius et al., 1959; Franson, 1982). An inverse relationship exists between mammalian body weight and liver ALT activity, so plasma ALT activity is a good indicator of liver necrosis in small mammals, but birds generally have low hepatic ALT activity which is not related to body weight (Cornelius, 1963).

Kidney damage is usually measured in terms of glomerular function, but if cell necrosis occurred, one might expect the greatest increase in plasma activities of those enzymes which exhibited the highest tissue activities (Fig. 1). It may be possible to differentiate kidney from liver and muscle as sources of these three enzymes, because muscle CPK activity is much greater, and liver CPK activity is much less than that in kidney.

With the possible exception of high plasma CPK activities resulting from muscle necrosis, it seems that diagnosis of organ damage in birds using ALT, AST, LDH, CPK, and ALP may be difficult. However, by studying the relative activities of several enzymes in an organ, prediction of organ involvement on the basis of plasma activities of the same enzymes may be possible (Schmidt and Schmidt, 1974). Also, it is important to determine baseline plasma enzyme activities for each species studied, because wide interspecific variation exists. Comparison of plasma activities in one species to established norms of another even closely related species could result in misinterpretation.

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