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ENZYME ACTIVITIES IN PLASMA, LIVER AND KIDNEY OF BLACK DUCKS AND MALLARDS

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Abstract: Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured in plasma, liver, and kidney, and gamma-glutamyl transferase (GGT) was measured in liver and kidney of black ducks *(Anas rubripes)*. Activities of ALT, AST, GGT, and ornithine carbamyl transferase (OCT) were assayed in plasma, liver, and kidney of game-farm mallards *(Anas platyrhynchos)*. Appreciable OCT and AST activity occurred in both liver and kidney. Activities of ALT, CPK, ALP and GGT were higher in kidney, while LDH was higher in liver. GGT was detected in plasma from one of four mallards.

INTRODUCTION

Clinical enzymology is routinely used for evaluation of organ pathology in domestic mammals, but little information is available for birds concerning the significance of elevated plasma or serum enzyme levels. Before diagnostic criteria can be established for birds it is necessary to determine normal enzyme levels in plasma or serum, and relative activities in various tissues. Some plasma and serum survey work has been done. Gee et al. (1981) reported serum values for several enzymes in 12 species of captive birds, including the whooping crane *(Grus americana)*, Aleutian Canada goose *(Branta canadensis leucopareia)*, arctic peregrine falcon *(Falco peregrinus tundrius)*, and masked bobwhite quail *(Colinus virginianus ridgwayi)*. Driver (1981) analyzed mallard *(Anas platyrhynchos)* plasma for alkaline phosphatase (ALP), aspartate aminotransferase (AST), and creatine phosphokinase (CPK). McDaniel and Chute (1961) measured alanine aminotransferase (ALT), AST, and lactate dehydrogenase (LDH) in chicken plasma. Less data are available for tissue enzyme activities, although the distribution of ALT and AST has been measured in various tissues of chickens and domestic ducks (Fowler, 1970; Cornelius et al., 1959). Literature on general and comparative enzyme biochemistry of birds has been surveyed by Pan (1971). The objective of the present study was to determine the activities of several enzymes in plasma, liver, and kidney of black ducks *(Anas rubripes)* and game-farm mallards *(Anas platyrhynchos)*.

MATERIALS AND METHODS

Four (two males, two females) 3-yr-old game-farm mallards were obtained from Frost Game Farms.[1] Ten (three males, seven females) 12- to 20-wk-old black ducks were obtained from a colony at Patuxent Wildlife Research Center.[2] Mallards had been maintained for 5 mo in indoor pens with a photoperiod of 12 hr light per 24 hr, and were reproductively inactive. Black ducks were hatched and raised in outdoor pens. Both species received commercial duck rations and

[1] Frost Game Farms, Coloma, Wisconsin 54930, USA.

[2] Patuxent Wildlife Research Center, Laurel, Maryland 20708, USA.

water ad libitum. Blood and tissue samples were collected between 0900 and 1100 hr in August and September of 1981.

Birds were bled by jugular venipuncture with partially evacuated heparinized glass tubes.[1] Tubes were centrifuged at 985g for 15 min and plasma was harvested. Following blood collection, ducks were killed by halothane inhalation and approximately 1 gm each of liver and kidney was removed immediately after death and transferred to glass homogenization tubes. Nine ml of cold phosphate buffer (0.1 M, pH 7.4) was added to each tube, and tissue was homogenized 20 sec with a teflon pestle at 1725 rpm. Homogenates were centrifuged 20 min at 985g and supernatants were recovered. Both tissue homogenate supernatants and plasma were refrigerated at 4 C immediately after preparation, and enzyme activities were assayed within 4 hr of sample collection.

Ornithine carbamyl transferase (OCT, EC 2.1.3.3) was measured colorimetrically (Bagrel et al., 1975). ALT (EC 2.6.1.2), AST (EC 2.6.1.1), LDH (EC 1.1.1.27), CPK (EC 2.7.3.2), ALP (EC 3.1.3.1) and gamma-glutamyl transferase (GGT, EC 2.3.2.2) were assayed with a centrifugal analyzer[2] using manufacturer's recommended procedures. Duplicate samples were used in all analyses. Data are reported as arithmetic means ± one standard deviation (SD).

RESULTS

Detectable plasma GGT activity was found in only one of four mallards (Table 1). Because of the low frequency of detection, GGT was not assayed in black duck plasma. In mallards, GGT was found primarily in kidney, ALT activity was over four times higher in kidney than liver, and mean AST and OCT activities were similar in these tissues (Table 1). Black duck plasma and tissue activities are listed in Table 2. Mean GGT, ALT, CPK, and ALP activities were three to 142 times higher in kidney than liver, LDH activity was nearly three times higher in liver, and AST activity was similar in liver and kidney (Table 2).

DISCUSSION

Use of different methods and species prevents comparison of absolute plasma enzyme activities in mallards and black ducks with earlier work. However, considering relative mean values, Gee et al. (1981) reported LDH to be highest in serum of four species of geese followed by AST, ALP, and ALT. Chicken plasma is

TABLE 1. Enzyme activities in mallard plasma, liver, and kidney (means ± SD).[a]

Enzyme	Tissue		
	Plasma (n=4)	Liver (n=4)	Kidney (n=4)
ALT	12.0 ± 3.4	12.5 ± 3.4	57.2 ± 18.8[b]
AST	13.5 ± 4.6	48.8 ± 8.4	53.0 ± 9.3
GGT	5.0[c]	ND[d]	10.2 ± 1.3
OCT	15.4 ± 5.8	18.8 ± 1.4	23.4 ± 3.2

[a]Units are IU/l for plasma and IU/g for liver and kidney.
[b]Significantly different from liver value (Students t-test, $P < 0.005$).
[c]Value represents one sample; below detectable limits in three others.
[d]Not detected in two birds, < 0.02 IU/g in two others.

[1] Vacutainer®, Becton-Dickinson, Rutherford, New Jersey 07070, USA.

[2] CentrifiChem®, Union Carbide Corp., Rye, New York 10580, USA.

TABLE 2. Enzyme activities in black duck plasma, liver, and kidney (means ± SD) [a]

Enzyme	Tissue		
	Plasma (n=9)	Liver (n=10)	Kidney (n=10)
ALT	20.1 ± 4.3	12.4 ± 4.7	36.6 ± 11.7 [b]
AST	18.6 ± 8.2	46.8 ± 7.1	40.0 ± 4.4
CPK	265.1 ± 144.5	3.8 ± 0.9	91.0 ± 11.9 [b]
ALP	131.8 ± 38.7	1.9 ± 0.6	19.8 ± 3.1 [b]
LDH	244.7 ± 81.8	162.6 ± 28.5	58.8 ± 7.6 [b]
GGT	ND [c]	0.08± 0.03	11.4 ± 3.4 [b]

[a] Units are IU/l for plasma and IU/g for liver and kidney.
[b] Significantly different from liver value (Students t-test, $P<0.005$).
[c] Not determined.

also reported to contain higher activity of AST than ALT (McDaniel and Chute, 1961). The pattern of these four enzymes in black duck plasma was somewhat different with LDH highest, followed by ALP, and nearly equal values for ALT and AST. Activity of CPK in chicken serum has been reported to range from 2.4 to 8.1 IU/l (Mitruka and Rawnsley, 1977) much less than the mean of 265 IU/l found in black duck plasma. High CPK activity has also been found in plasma of wild mallards (Driver, 1981). Mean serum GGT activity ranged from 1 to 4 IU/l in 12 diverse avian species (Gee et al., 1981). Values in ducks may be even lower, since GGT was not detected in three of four mallard plasma samples. Mallard plasma OCT activity in the present study was somewhat higher than that reported for control mallards in earlier studies (Szaro et al., 1978; 1981).

In a variety of mammals, elevated serum or plasma OCT activity is considered highly specific for liver damage (Coles, 1980; Drotman and Lawhorn, 1978; St. Aubin and Geraci, 1977), but the literature on OCT in birds is contradictory. Cohen and Brown (1960) considered OCT to be absent from avian liver, and Fowler (1970) reported no OCT activity in plasma, liver, or kidney from cockerels or ducks. According to Tamir and Ratner (1963), OCT is present in chick kidney but absent in liver, and elevated plasma OCT has been used as an indicator of kidney damage in mallards (Szaro et al., 1978; 1981). Since similar OCT activity occurred in mallard liver and kidney in the present study, it is possible that OCT could be released as a result of damage to either tissue.

Although mammalian kidney has much higher GGT activity than liver, increased serum GGT is associated with cholestasis and bile duct damage and is used an an indicator of liver pathology (Rico et al., 1977; Shull and Hornbuckle, 1979; Ford, 1974). Data from mallards and black ducks also indicate much higher GGT levels in kidney than liver but it is unknown if increased serum or plasma activity would result from the same mechanism as in mammals.

Cornelius et al. (1959) reported higher ALT activity in chicken kidney than in liver and, although the difference was not as great in the present study, mallard and black duck kidney ALT also exceeded liver ALT. In mammals, liver ALT activity is inversely related to body weight (Cornelius, 1963), and high serum ALT is considered specific for liver damage in the smaller species (Coles, 1980). Since avian ALT is not liver-specific, further work needs to be done to determine relative ALT release following liver and kidney damage before its diagnostic use can be evaluated. The activity of AST was similar in liver and

kidney from mallards and black ducks, which agrees with the results of Fowler (1970) in cockerels and ducks. Fowler (1970) found this enzyme relatively evenly distributed between several duck tissues so AST used alone may have little diagnostic significance.

Since LDH is reported to occur in most avian tissues (Clarkson and Richards, 1971), its usefulness for diagnosing liver or kidney pathology is probably limited. However, in black ducks the relative distribution between liver and kidney suggests appreciable plasma activity would be more likely a result of liver damage than kidney damage. Avian kidney tubule epithelium has an alkaline phosphatase-positive brush border (Siller, 1971) which may contribute to the higher ALP activity in kidney. The significance of higher CPK activity in black duck kidney than liver is unknown.

None of the enzymes studied were organ-specific for liver or kidney. Because of its larger relative size, the liver could be expected to contribute more to plasma enzyme activity following tissue damage than would the kidney. However, further work needs to be done to relate histopathologic damage with plasma or serum enzyme activities. Data concerning patterns of enzyme release from damaged tissue would be of significant value in avian clinical pathology.

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