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CHANGES IN MALLARD (*ANAS PLATYRHYNCHOS*) SERUM CHEMISTRY DUE TO AGE, SEX, AND REPRODUCTIVE CONDITION

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ABSTRACT: Selected serum constituents were analyzed from 50 adult mallards (*Anas platyrhynchos*) of both sexes during several stages of reproduction: pre-egg laying, egg laying, incubating, molting, and postreproductive. Similar assays were conducted on sera from ducklings aged 5 to 58 days. Values for total protein (TPR), albumin (ALB), glucose (GLU), gamma-glutamyl transferase (GGT), calcium (CA), phosphorus (PHOS) and magnesium (MG) differed by sex. When all data were combined and analyzed for sex-related differences within each reproductive condition separately, all assays except lactate dehydrogenase (LD-L), cholinesterase (CHE), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRN) and direct bilirubin (BIDI) differed between sexes during one or more reproductive periods. Each assay showed differences among the various reproductive conditions regardless of gender. The pattern of change differed between sexes. All assays except ALB, GLU, CA and MG showed age-related changes. Lipemia in the sample interfered with all chemistries except TPR, LD-L and CA. Results indicate that when using clinical chemistry as a diagnostic tool in the mallard, age and reproductive condition should be determined in order to compare the data to appropriate control values.

Key words: Mallard, *Anas platyrhynchos*, serum chemistry, clinical pathology, reproductive biology, experimental study.

INTRODUCTION

Serum chemistry and enzyme concentrations are used routinely in clinical medicine to monitor organ system functions. Physiological factors such as age, sex, or species will affect normal concentrations of most serum constituents (Cornish, 1971). A growing body of literature documents these effects and examines appropriate assay techniques for both domestic chickens (e.g., McDaniel and Chute, 1961; Freeman, 1984) and captive psittacines (Altman, 1978; Campbell and Coles, 1986; Lewandowski et al., 1986). Although less information is available about serum chemistry values of other avian species, studies of several species of wild birds (including waterfowl) have shown differences due to species (Gee et al., 1981; Westlake et al., 1983), sex (Gee et al., 1981; Kocan and Pitts, 1976), age (Kocan and Pitts, 1976), molt (Driver, 1981) and captive versus wild-caught (Driver, 1981; Perry et al., 1986). Time of year also has been associated with differences in activity of some serum enzymes in the canvasback (*Aythya valisineria*) (Kocan and Pitts, 1976; Perry et al., 1986), with the implication that differing reproductive conditions contributed to these changes. The present study was designed primarily to examine possible effects of the reproductive cycle (including egg laying, incubation and the reproductive molt) on the clinical chemistry of mallards (*Anas platyrhynchos*) of both sexes. Young from the mated pairs were examined to determine age effects.

Clinical chemistry data are useful when studying sublethal effects of pathogens or toxicants in a laboratory setting or assessing organ system malfunction in wild-caught animals. The mallard is becoming a commonly used laboratory species, particularly in preregistration safety testing of pesticides regulated by the United States Environmental Protection Agency (1982). Historically, the mallard has been used as a sentinel species by the United States Fish and Wildlife Service as part of the National Contaminant Biomonitoring Program to monitor environmental contamination (Jacknow et al., 1986). The present study provided information about sources of innate variability for the selected serum

constituents and determined normal values for ducks of various ages, sex, and reproductive conditions. The absolute values of the data presented should be used cautiously by other laboratories prior to establishing that techniques, reagents and equipment used produce similar results.

MATERIALS AND METHODS

Fifty pairs of 7-mo-old mallard ducks were purchased from Whistling Wings, Inc. (Hanover, Illinois 61041, USA) and housed in outdoor pens (1.3 × 5.3 m) from April to August 1986. Birds were provided Purina® (St. Louis, Missouri 63160, USA) Game Bird Layer Feed and water ad libitum. Each hen was allowed to progress to egg production and establish and incubate a clutch on her own schedule. Ducklings used for determination of age effects were either from clutches hatched and reared by the experimental birds or from ducklings purchased at 1 day of age and housed indoors under 12:12 L:D, 24 C for the duration of the study. Ducklings were provided Purina® Game Bird Starter Chow and water ad libitum. Reproductive condition was designated as pre-egg laying (if the birds were in breeding plumage but had not yet begun laying eggs), egg laying, incubating, molting and postreproductive (when the molt was complete). Reproductive condition of the male was designated the same as that of the female with whom he was paired until he began the postreproductive molt.

Two blood samples were drawn from each bird within each reproductive period and from young birds at selected intervals. Birds were denied access to food for 18 hr prior to drawing blood samples to minimize the occurrence of lipemic samples. Blood samples were collected via jugular venipuncture, transferred to glass evacuated tubes (Vacutainer®, Becton-Dickinson, Rutherford, New Jersey 07070, USA) and allowed to clot at 4 C. Clots were centrifuged at 3,000 *g* for 10 min and the serum was stored at −70 C until assayed.

Clinical measurements and assay methods included total protein (TPR; Gornall et al., 1949), albumin (ALB; Doumas et al., 1971), glucose (GLU; Neese et al., 1976), lactate dehydrogenase (LD-L; EC 1.1.1.27; Wacker et al., 1956), alanine aminotransferase (ALT; EC 2.6.1.2; Bergmeyer et al., 1978), aspartate aminotransferase (AST; EC 2.6.1.1; Bowers et al., 1975), amylase (AMY; Pierre, et al., 1976), gamma-glutamyl transferase (GGT; EC 2.3.2.2; Szasz, 1969), alkaline phosphatase (ALP; Bowers and McComb, 1966; Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clincial Physiology, 1974), cholinesterase (CHE; EC 3.1.1.7 and EC 3.1.1.8; Ellman et al., 1961), calcium (CA; Gitelman, 1967; Kessler and Wolfman, 1964), phosphorus (PHOS; Daly and Ertingshausen, 1972), magnesium (MG; Mann and Yoe, 1956), uric acid (UA; Trinder, 1969), creatinine (CRN; Jaffe, 1896; Henry et al., 1974) total bilirubin (BITO; Winsten and Cehelyk, 1969) and direct bilirubin (BIDI; Van den Bergh and Snapper, 1913). All assays were conducted with a Gilford Impact 400 Autoanalyzer (Ciba Corning Diagnostics, Oberlin, Ohio 44074, USA) using reagents and procedures supplied by the manufacturer except where noted below. Linearity of kinetic assays was verified by determining absorbance over a 3 min time interval and selecting a read time where the change in absorbance was constant. Samples were serially diluted and assayed to confirm linearity within the concentration range observed. Samples with high concentrations producing nonlinear kinetics were diluted appropriately with buffer and reanalyzed. Reference normal and abnormal sera purchased from Ciba Corning Diagnostics were included within each run for quality control. Amount of hemolysis and lipemia of each sample was noted.

Five of the assays were modified from the techniques recommended by Ciba Corning Diagnostics. Three of the assays (LD-L, AMY, and UA) used Ciba Corning Diagnostics reagent kits and were altered to reduce the sample and reagent volumes. For the LD-L assay, the sample volume was reduced from 25 µl to 10 µl. For the AMY assay, sample volume was reduced from 20 µl to 5 µl and reagent volume increased from 0.5 ml to 0.7 ml. For the UA assay, sample volume was decreased from 50 µl to 40 µl. For the LD-L and AMY enzymatic assays, lag times were adjusted to assure that the reactions were read during the linear portion of the kinetic curve and conversion factors used to calculate amount of substrate hydrolyzed/liter/min were corrected to account for differences in volumes used. Appropriate standard curves were developed for the UA assay. GGT assays were conducted using reagents and procedures from Boehinger Mannheim Diagnostics, Inc. (Indianapolis, Indiana 46250, USA) due to the increased ease of solubility of their reagents as compared to reagents supplied by Ciba Corning Diagnostics. The CHE assay followed an adaptation of the technique of Ellman et al. (1961) and used acetylthiocholine (ACTI) as a substrate rather than pseudothiocholine as available in commercial kits. This method was selected because mallard serum consists of about 70% butyrylcholinesterase and 30% acetylcholinesterase (ACHE) (A. Fairbrother, unpubl. data). Since ACHE has a low binding affinity to pseudo- or

TABLE 1. Serum chemistry and enzyme values (mean, $\bar{x}$; standard deviation, SD) for nonreproductive adult mallards.

	Sex					
	Male			Female		
Assay[a]	$\bar{x}$	SD	n[b]	$\bar{x}$	SD	n[b]
TPR (g/dl)	3.8	0.7	13	4.2	0.5	35
ALB (g/dl)	1.5	0.4	13	1.7	0.2	33
GLU (mg/dl)[c]	185	47	13	215	34	34
AMY (U/liter)	2,631	630	13	2,766	684	35
CHE (U/liter)	794	249	13	812	197	32
ALT (U/liter)	26.3	8.0	13	29.9	9.9	33
AST (U/liter)	16.2	4.3	13	15.8	4.7	33
GGT (U/liter)	7.7	4.2	13	8.0	4.8	35
ALP (U/liter)	26.3	8.0	13	44.2	22.7	35
LD-L (U/liter)	199	83	13	147	80	33
CA (mg/dl)	9.4	1.9	13	9.8	1.1	35
MG (mEq/liter)	1.8	0.4	13	1.8	0.3	35
PHOS (mg/dl)	2.9	1.0	13	3.0	1.0	35
UA (mg/dl)	4.0	1.3	13	4.5	1.8	35
CRN (mg/dl)	0.25	0.08	13	0.28	0.07	33
BITO (mg/dl)	0.16	0.05	10	0.16	0.04	33
BIDI (mg/dl)	0.07	0.01	13	0.07	0.01	31

[a] See text for definition of assay abbreviations.
[b] Sample size.
[c] Significantly different between sexes ($P \leq 0.05$).

butyrylthiocholine, it was necessary to change substrates to measure total CHE activity. ACTI and buffers used in the reaction were purchased from Sigma Chemical Co. (St. Louis, Missouri 63178, USA). Final volume of reagents used in the test were 2.9 µl of 0.075 M acetylthiocholine as substrate and 14.7 µl 0.1 M 5,5-dithio-bis-(2-nitrobenzoic acid) in 382 µl of pH 8.0 phosphate buffer. Sample volume was 60 µl serum. Assays were conducted at 35 C and 405 nm wavelength. Samples were read for 15 sec following a 60 sec lag time.

All samples were assayed in duplicate. If replicates differed by more than 10%, the sample was reanalyzed. Results of the duplicates were averaged prior to statistical analysis. All statistical analyses were conducted using the SAS statistical package for personal computers (SAS Institute Inc., 1985). First, all data were combined and a two-way analysis of variance (ANOVA) was conducted to determine which assays were affected by slight to severe hemolysis or lipemia. Samples that were sufficiently hemolyzed and/or lipemic to significantly interfere with an assay were not included in subsequent statistical analyses for that test. Prior to further analysis, data from samples collected from each bird within the same reproductive period were averaged to give one value per bird per reproductive period. The data were then examined for normality, kurtosis and skewness. A $\log_2$ transformation of the data for all the assays was necessary to normalize the distribution and reduce heterogeneity of variance prior to further comparative analyses. Means and standard deviations reported in the Tables were computed from nontransformed data. To determine which assays produced results that differed by sex and/or reproductive condition, a two-way ANOVA was conducted on data from adult birds. Because both main effects and interactions were significant, the model was simplified and a one-way ANOVA was conducted within each sex to determine which assays varied significantly by reproductive state. Significant ANOVA tests were followed by Student-Newman-Keuls pair-wise comparisons to determine which means differed from each other. Student's t-tests were conducted for each assay to examine sex-related differences within each reproductive period. Because some of the values from birds <60 days of age were repeated samples from the same birds, autocorrelation difficulties prohibited statistical comparisons among age groups of these birds. Therefore, data are presented graphically to illustrate time trends. All data from young birds in each age class were combined, regardless of housing conditions, since values did not differ between these two groups. Significance was set at $P \leq 0.05$.

TABLE 2. Serum chemistry and enzyme values (mean, $\bar{x}$; standard deviation, SD) for adult female mallards of differing reproductive states.

	Reproductive condition[a]											
	PE			EL			INC			MOLT		
Assay[b]	$\bar{x}$	SD	n[c]	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
TPR (g/dl)	5.6	2.9[d]	39	6.3	1.2[d]	18	4.4	0.6	20	4.5	1.2	12
ALB (g/dl)	2.0	0.3	28	2.3	0.2[d]	6	1.6	0.2	20	1.7	0.2	6
GLU (mg/dl)	238	21	36	258	51	10	211	53	20	199	30	11
AMY (U/liter)	3,058	527	34	3,821	741[d]	9	2,700	626	20	2,346	1,012	10
CHE (U/liter)	1,337	280[d]	34	1,563	592[d]	9	1,002	266	19	894	219	8
ALT (U/liter)	31.0	10.3	31	34.2	19.4	11	30.6	13.1	19	41.1	17.1	6
AST (U/liter)	18.0	3.4	34	23.7	6.7[d]	16	22.1	7.4[d]	20	22.6	12.6[d]	7
GGT (U/liter)	19.8	19.8	35	199.6	283[d]	11	7.5	4.7	20	20.8	36.9	9
ALP (U/liter)	63.6	56.8[d]	37	124.9	56.7[d]	11	34.3	15.8	20	36.0	18.1	10
LD-L (U/liter)	165	50	34	177	57	16	215	107	20	268	2.2[d]	7
CA (mg/dl)	14.0	4.1[d]	39	21.9	5.6[d]	19	10.3	2.0	20	10.6	4.2	12
MG (mEq/liter)	2.3	0.5[d]	35	3.6	0.8[d]	11	1.6	0.3	20	1.6	0.5	10
PHOS (mg/dl)	4.6	1.7[d]	37	8.1	2.4[d]	12	3.7	1.0	20	4.1	2.2	11
UA (mg/dl)	5.2	1.1	37	9.1	5.1[d]	12	5.5	1.7[d]	20	4.9	1.7	11
CRN (mg/dl)	0.34	0.06	31	0.33	0.15	8	0.42	0.15	20	0.33	0.08	6
BITO (mg/dl)	0.23	0.08	39	0.43	0.28[d]	17	0.20	0.11	20	0.21	0.05	11
BIDI (mg/dl)	0.07	0.04	17	0.15	0.22	4	0.06	0.04	16	0.06	0.01	4

[a] PE, Pre-egg laying; EL, Egg laying; INC, Incubating; MOLT, Molting.
[b] See text for definition of assay abbreviations.
[c] Sample size.
[d] Significantly different from nonreproductive value ($P \leq 0.05$).

TABLE 3. Serum chemistry and enzyme values (mean, $\bar{x}$; standard deviation, SD) for adult male mallards of differing reproductive states.

	Reproductive condition[a]											
	PE			EL			INC			MOLT		
Assay[b]	$\bar{x}$	SD	n[c]	$\bar{x}$	SD	n[c]	$\bar{x}$	SD	n[c]	$\bar{x}$	SD	n[c]
TPR (g/dl)	4.6	0.6[d]	40	4.5	0.8[d]	18	4.2	0.5[d]	18	3.9	0.8	24
ALB (g/dl)	1.8	0.2[d]	35	1.6	0.2	14	1.7	0.3	17	1.5	0.3	19
GLU (mg/dl)	234	33[d]	38	233	32[d]	18	199	26	18	185	29	24
AMY (U/liter)	3,123	583	37	2,869	614	17	3,203	785[d]	18	2,991	748	23
CHE (U/liter)	1,326	344[d]	36	1,380	399[d]	17	984	470	17	983	452	20
ALT (U/liter)	34.6	9.4	36	35.8	13.1	18	27.6	12.1[d]	16	28.4	19.2	20
AST (U/liter)	17.3	4.0	39	20.5	8.0	18	20.8	15.7	17	18.1	8.1	20
GGT (U/liter)	8.5	7.6	38	10.6	12.6	19	9.3	6.0	18	16.5	36.0[d]	23
ALP (U/liter)	40.2	25.3[d]	39	44.1	44.8	19	38.4	48.0	18	35.3	44.2	24
LD-L (U/liter)	168	66	39	219	107	18	263	203	17	202	152	20
CA (mg/dl)	10.9	1.0[d]	40	11.0	1.9[d]	19	9.9	1.0	18	9.3	2.2	24
MG (mEq/liter)	2.0	0.2[d]	38	2.0	0.4[d]	18	1.8	0.4	18	1.8	0.9	23
PHOS (mg/dl)	3.7	0.9[d]	40	3.6	0.9[d]	19	2.8	0.5	18	3.1	1.4	24
UA (mg/dl)	5.2	1.2[d]	40	5.2	1.5[d]	19	5.7	1.9[d]	18	4.7	2.3[d]	24
CRN (mg/dl)	0.35	0.08[d]	38	0.36	0.10[d]	18	0.34	0.12	17	0.30	0.12	20
BITO (mg/dl)	0.22	0.09	40	0.20	0.09	17	0.18	0.04	15	0.20	0.08	22
BIDI (mg/dl)	0.07	0.02	28	0.06	0.01	8	0.07	0.02	9	0.08	0.05	15

[a] PE, Pre-egg laying; EL, Egg laying; INC, Incubating; MOLT, Molting.
[b] See text for definition of assay abbreviations.
[c] Sample size.
[d] Significantly different from nonreproductive values ($P \leq 0.05$).

TABLE 4. Serum chemistry and enzyme values (mean, $\bar{x}$; standard deviation, SD) for juvenile mallards.

	Age (days)								
	5			10			18		
Assay[a]	$\bar{x}$	SD	n[b]	$\bar{x}$	SD	n[b]	$\bar{x}$	SD	n[b]
TPR (g/dl)	3.4	0.6	15	4.7	1.4	19	4.3	1.3	36
ALB (g/dl)	1.4	0.2	20	1.7	0.2	8	1.5	0.3	17
GLU (mg/dl)	239	54	20	224	56	12	215	93	8
AMY (U/liter)	3,230	760	34	3,788	869	21	3,984	1,297	44
CHE (U/liter)	1,423	696	17	1,259	274	8	984	559	5
ALT (U/liter)	21.3	9.1	34	36.8	15.1	20	30.5	10.5	45
AST (U/liter)	22.3	7.4	26	54.9	26.4	22	88.5	54.1	44
GGT (U/liter)	1.2	2.8	38	3.7	2.2	15	4.6	3.6	32
ALP (U/liter)	411	89	23	560	110	12	386	194	9
LD-L (U/liter)	425	153	22	650	291	12	629	251	18
CA (mg/dl)	13.0	10.3	26	12.0	2.4	12	9.6	1.7	18
MG (mEq/liter)	2.8	0.8	26	2.4	0.4	12	1.8	0.7	9
PHOS (mg/dl)	7.9	2.8	26	10.5	1.9	12	7.6	1.3	17
UA (mg/dl)	12.2	5.4	39	11.1	3.4	24	10.9	3.8	44
CRN (mg/dl)	0.47	0.42	3	0.27	0.10	10	0.55	0.65	8
BITO (mg/dl)	0.40	0.11	8	0.30	0.15	6	0.43	0.31	7
BIDI (mg/dl)	0.08	0.02	3	0.06	0.02	4	0.10	0.04	3

[a] See text for definition of assay abbreviations.
[b] Sample size.

RESULTS

When the data from adult mallards of all reproductive conditions were combined, TPR, ALB, GLU, GGT, CA, PHOS, and MG values differed significantly by sex. When data were analyzed for sex-related differences within each reproductive condition separately, all assays except LD-L, CHE, AST, ALT, CRN, and BIDI differed between sexes during one or more reproductive periods. For nonreproductive birds, GLU was the only assay whose values differed between males and females (Table 1). Hens and drakes in "pre-egg laying" condition had significantly different concentrations of ALB, GGT, CA, PHOS, and MG. Laying hens differed from drakes in the ALB, AMY, GGT, CA, PHOS, MG, UA, and BITO assays. Incubating birds had sex-related differences in ALB, AMY, and PHOS concentrations. In molting birds, only AMY differed between the sexes. Within each sex, values from all tests differed among reproductive conditions for all assays, although the pattern of change was not the same for both sexes. This is consistent with statistical analyses indicating both a significant main effect for reproductive condition and a significant interaction between sex and reproductive condition.

In the hen, egg laying significantly influenced serum enzyme activity and chemistry concentrations, increasing values in 12 of the 17 assays (Table 2). The greatest increase occurred in GGT where activities in egg laying hens increased tenfold over the other reproductive periods. AMY doubled during egg laying while other constituents increased a statistically significant, although lesser, amount. Values of 12 of the assays in samples from the drakes also differed by reproductive condition (Table 3). GGT activity in molting males was twice their nonreproductive values. Although 11 other constitutents showed statistically significant reproductive-related differences, they differed from their nonreproductive values by <60%.

Means and standard deviations for each assay from young mallards are presented in Table 4. AMY, CHE, AST, LD-L, UA,

TABLE 4. Continued.

Age (days)											
26			42			51			58		
$\bar{x}$	SD	n^b	$\bar{x}$	SD	n^b	$\bar{x}$	SD	n^b	$\bar{x}$	SD	n^b
5.4	1.6	25	4.0	0.8	4	3.3	0.7	23	3.2	1.0	23
—	—	—	1.6	0.4	4	1.4	0.3	23	1.4	0.4	23
—	—	—	189	27	3	191	46	24	186	45	9
3,751	576	26	3,005	302	4	2,782	691	24	2,395	699	23
—	—	—	827	253	2	559	304	9	818	248	19
32.3	12.9	27	26.1	7.0	4	35.3	13.9	23	23.9	7.1	21
41.5	21.2	25	9.4	5.1	4	20.3	8.2	23	17.4	5.7	23
5.3	3.7	25	5.3	5.7	4	4.5	3.4	23	6.1	3.6	19
—	—	—	217	32	4	272	70	24	185	47	21
—	—	—	169	70	4	263	162	24	233	83	23
—	—	—	10.9	1.6	4	9.8	1.9	24	8.4	1.8	23
—	—	—	2.0	0.2	4	1.6	0.5	24	1.6	0.5	21
—	—	—	6.2	1.3	4	6.2	1.6	24	5.0	1.7	23
7.5	2.2	23	4.0	0.7	4	4.1	1.6	24	4.0	1.8	23
—	—	—	0.28	0.10	4	0.27	0.10	24	0.21	0.11	21
—	—	—	0.20	0.0	2	0.20	0.08	20	0.17	0.05	12
—	—	—	0.06	0.0	3	0.07	0.04	16	0.06	0.02	23

and PHOS were all elevated in young birds and decreased with increasing age until reaching adult, postreproductive values (Fig. 1). The other serum constituents appeared to be unaffected by age.

Several of the assays were affected by the presence of lipemia or hemolysis in the sample. GLU, GGT, CHE, MG, and ALP were sensitive to even small amounts of lipid. ALT, AST, BIDI, and CRN assays were unaffected by lipemia but were affected if the samples were severely hemolyzed. ALB was affected by both factors and TPR, LD-L, and CA were unaffected by either parameter. Preliminary tests showed that nonfasted ducks had large amounts of lipid in their serum, especially hens in laying condition. The 18 hr fast reduced the degree of lipemia and the number of affected samples.

DISCUSSION

The results indicate that age, sex, and reproductive condition of mallards can significantly affect interpretation of serum enzyme and chemistry values.

CRN and blood urea nitrogen (BUN) measurements probably are of little value in diagnosing renal pathology in the mallard. Birds do not convert much of their creatine to creatinine and what little is produced is resorbed in the renal tubule (Skadhauge, 1983). Therefore, normal serum CRN values are very low (<0.5 IU/L) and increased values are not necessarily indicative of pathologic changes of the renal glomeruli as in mammals (Skadhauge, 1983). BUN also is present in very low concentrations (1.4 mg/100 ml in chickens; Sykes, 1971) in avian plasma. About 30% of the filtered urea is reabsorbed in the renal tubules. The end result is that only 1% to 10% of total urinary nitrogen is urea; 80% to 85% is uric acid and 10% is ammonia (Skadhauge, 1983). A decrease in renal tubular absorption following renal insult would be difficult to detect since normal values of CRN and BUN are so low. Therefore, UA measurements are the most sensitive indicators of renal function in mallards.

Birds produce very little bilirubin since they almost totally lack the enzyme biliverdin reductase that converts biliverdin to

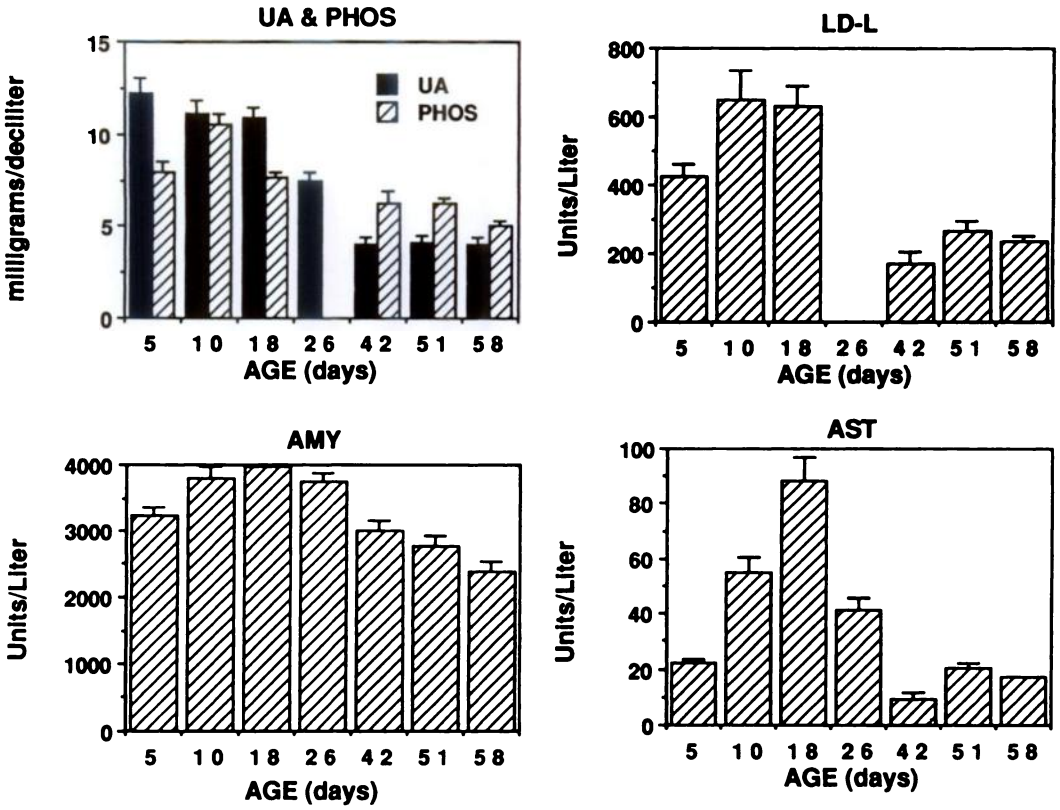

FIGURE 1. Mean (±SE) values of selected serum chemistries and enzymes of juvenile mallards of differing ages demonstrating age-related changes. UA, uric acid; PHOS, phosphorus; LD-L, lactate dehydrogenase; AMY, amylase; AST, aspartate aminotransferase.

bilirubin (Hill, 1983). Therefore, measurements of total and direct bilirubin (BITO and BITI, respectively) within mallard serum are not sensitive indicators of bile formation or bile duct occlusion. Although not commercially available, a better assay would be a measurement of serum biliverdin concentrations.

The AMY values measured in the study were higher than any values previously published for mallards or other birds (e.g., maximum 904 U/liter reported in a sandhill crane from Florida by Gee et al., 1981). The results were repeatable using sera from mallards purchased from multiple sources and by using two comparable autoanalyzers with the same reagents. We hypothesized that the high values were due to the interference of endogenous sugars with the AMY test reaction. The Ciba Corning Diagnostics AMY assay is based on a series of coupled reactons that convert maltotetraose to 6-phosphogluconate + NADH, catalyzed initially by AMY. Recently, Diagnostic Chemicals Ltd. (Bethany, Connecticut 06525, USA) marketed a new AMY test based on the AMY-catalyzed degradation of a synthetic substrate, *p*-Nitrophenyl-α-D-maltoheptaoside-blocked, in place of starch. Preliminary tests in our laboratory indicate that the new reaction also produces the same high results for mallard serum AMY, suggesting that the assays both measure AMY without interference from other endogenous substrates.

Egg laying increased the mean values of 12 of the 17 chemistries measured in the hens (Table 2). ALB, CA, PHOS, and MG were all increased, as would be expected due to their involvement in egg

production (Lewandowski et al., 1986). GLU also has been shown to be elevated during the breeding season in psittacines (Lewandowski et al., 1986). However, the most notable increase was in GGT which increased from a non-egg laying value of 8 IU/liter to a mean of 198 IU/liter during the egg laying condition (Table 2). Why the increase should occur is unclear since GGT is produced by the liver in most animals including the chicken (Ibrahim et al., 1980). AMY and AST were slightly but significantly elevated during the egg laying period. Generally, AMY is produced by the pancreas (Wallach, 1978) while distribution of AST in avian tissues varies among species. In ducks, the distribution of AST is, in descending order, skeletal muscle, heart, kidney, brain, and liver. Moderate increases are often associated with soft tissue injury (Lewandowski et al., 1986). In drakes, slight but significant changes in TPR, GLU, CHE, CA, PHOS, and MG occurred during the time when they were reproductively active and their hens were either in the pre-egg laying or egg laying reproductive periods, possibly as a result of increased concentrations of testosterone and/or changes in food consumption.

LD-L increased in hens during molt and in young birds. This enzyme generally is associated with muscle and integument (Lewandowski et al., 1986; Wallach, 1978), both of which are affected during the molt and growth stages, although Franson (1982) found significant amounts in the livers of black ducks. Values of this enzyme from wild-caught mallards may be difficult to interpret, since the birds often are subject to considerable trauma during trapping and handling prior to obtaining the blood sample which may result in elevated LD-L concentrations.

Analyses of the effects of hemolyzed or lipemic samples indicated that these factors will interfere with some of the assays performed. For those assays that are affected only by large amounts of hemolysis or lipemia, a suitable dilution often can be found to overcome these effects. Commercially available methods of removing lipids from serum (e.g., Liposol®, Ann Arbor, Michigan 48104, USA) are expensive and did not prove very efficacious in avian serum in our laboratory. Birds are prone to having lipemic blood samples, particularly within 1 to 2 hr after a feeding bout or during egg laying. In controlled laboratory studies, lipemia can be minimized by restricting access to food for 12 hr prior to drawing the blood sample. However, a recently published study by Wilson and Miles (1988) shows that plasma uric acid in chickens decreases with time postfeeding from 10.5 mg/dl at 2 hr postprandial to 7.4 mg/dl at 24 hr. This suggests that values obtained for uric acid in our study may be lower than would have been measured if feed were not restricted. A normal circadian pattern to UA values is likely since the mallard generally does not feed during the night. Therefore, a sample taken immediately prior to the first morning feeding bout would have lower UA value than one taken shortly after feeding.

To verify the applicability of our data to mallards in general, blood samples were obtained from 85 12-wk-old mallards that had been purchased from Wild Wings Game Farm (Hugo, Minnesota 55038, USA) as part of a field study being conducted by the U.S. Fish and Wildlife Service. The birds had been kept in a 0.8 ha enclosure at a wildlife refuge for 3 mo

TABLE 5. Selected serum chemistry values (mean, $\bar{x}$; standard deviation, SD) from 12-wk-old mallards maintained in outdoor enclosures on a national wildlife refuge.

Assay[a]	$\bar{x}$	SD	n
AMY (U/liter)	3,380	1,364	84
ALT (U/liter)	28.3	8.3	82
AST (U/liter)	33.4[b]	10.4	82
GGT (U/liter)	7.4	6.0	86
LD-L (U/liter)	729[b]	183	87
CA (mg/dl)	12.3	2.5	90
UA (mg/dl)	3.9	1.2	86

[a] See text for definition of assay abbreviations.
[b] Significantly different from reference data set.

prior to obtaining the blood samples. All of their enzyme and chemistry values fell within our established normal range (within two standard deviations of the mean) except for AST and LD-L (Table 5). Elevation of these two enzymes may have been due to soft-tissue injuries sustained during capture and handling of the birds prior to obtaining the samples.

Measurement of serum enzyme and chemistry concentrations can be an important tool for use in monitoring the health of mallards in laboratory experiments, on game farms, and in the natural, free-flying environment. However, correct interpretation of increased values depends upon knowledge of innate and environmental factors contributing to normal, nonpathologic changes in enzyme concentrations. This study has shown that mallards in reproductive condition, particularly egg laying hens and molting hens and drakes, can have significant elevations in many of their serum constituents. Young birds also have significantly higher values for certain serum enzymes than do adult birds. Therefore, when using clinical chemistry as a diagnostic tool in the mallard, age and reproductive condition should be determined in order to compare the data to appropriate control values.

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LITERATURE CITED

ALTMAN, R. 1978. Clinical pathology. *In* Zoo and wild animal medicine, M. E. Fowler (ed.). W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 392–394.

BERGMEYER, H. U., P. SCHEIBE, AND A. W. WAHLEFELD. 1978. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clinical Chemistry 24: 58–73.

BOWERS, G. N., H. U. BERGMEYER, AND D. W. MOSS. 1975. International Federation of Clincial Chemistry methods for the measurement of catalytic concentration of enzymes. Clinica Chemica Acta 61: F11–F42.

———, AND R. B. MCCOMB. 1966. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. Clinical Chemistry 12: 70.

CAMPBELL, T. W., AND E. H. COLES. 1986. Avian clinical pathology. *In* Veterinary clinincal pathology, E. H. Coles (ed.). W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 279–301.

COMMITTEE ON ENZYMES OF THE SCANDINAVIAN SOCIETY FOR CLINICAL CHEMISTRY AND CLINICAL PHYSIOLOGY. 1974. Recommended methods for the determination of four enzymes in blood. Scandinavian Journal of Clinical Laboratory Investigations 32: 291.

CORNISH, H. H. 1971. Problems posed by observations of serum enzyme changes in toxicology. CRC Critical Reviews in Toxicology 1: 1–32.

DALY, J. A., AND G. ERTINGSHAUSEN. 1972. Direct method for determining inorganic phosphorus in serum with the "Centrifichem". Clinical Chemistry 18: 263.

DOUMAS, B. T., W. WATSON, AND H. G. BIGGS. 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chemica Acta 31: 87–96.

DRIVER, E. A. 1981. Hematological and blood chemical values of mallard, *Anas platyrhynchos platyrhynchos*, drakes before, during and after remige moult. Journal of Wildlife Diseases 17: 413–421.

ELLMAN, G. L., D. K. COURTNEY, V. ANDRES, AND R. M. FEATHERSTRON. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemistry and Pharmacology 7: 88–95.

FRANSON, J. C. 1982. Enzyme activities in plasma, liver and kidney of black ducks and mallards. Journal of Wildlife Diseases 18: 481–488.

FREEMAN, B. M. 1984. Appendix: Biochemical and physiological data. *In* Physiology and biochemistry of the domestic fowl, Vol. 5, B. M. Freeman (ed.). Academic Press, New York, New York, pp. 412.

GEE, G. F., J. W. CARPENTER, AND G. L. HENSLER. 1981. Species differences in hematological values of captive cranes, geese, raptors, and quail. The Journal of Wildlife Management 45: 463–483.

GITELMAN, H. J. 1967. An improved automated procedure for the determination of calcium in biological specimens. Analytical Biochemistry 18: 521.

GORNALL, A. G., C. J. BARDAWILL, AND M. M. DAVID. 1949. Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry 177: 751–766.

HENRY, R. J., D. C. CANNON, AND J. W. WINKELMAN.

1974. Clinical chemistry: Principles and technics, 2nd ed. Harper and Row, Hagerstown, Maryland, 1106 pp.

HILL, K. J. 1983. Physiology of the digestive tract. *In* Physiology and biochemistry of the domestic fowl, Vol. 4, B. M. Freeman (ed.). Academic Press, London, England, pp. 31–49.

IBRAHIM, I., D. J. HUMPHREYS, J. B. J. STODULSKI, AND R. HILL. 1980. Plasma enzyme activities indicative of liver cell damage in laying fowl given a diet containing 20 per cent of rapeseed meal. Research in Veterinary Science 28: 330–335.

JACKNOW, J., J. L. LUDKE, AND N. C. COON. 1986. Monitoring fish and wildlife for environmental contaminants: The National Contaminant Biomonitoring Program. U.S. Department of the Interior, Fish and Wildlife Service, Fish and Wildlife Leaflet 4, Washington, D.C., 15 pp.

JAFFE, M. 1896. Uber den niederschlag, welchen pikrinsaure in normalen harn erzeugt und uber eine neue reaction des kreatinines. Hoppe Seylers Z. Physiological Chemistry 10: 391–400.

KESSLER, G., AND M. WOLFMAN. 1964. An automated procedure for the simultaneous determination of calcium and phosphorus. Clinical Chemistry 10: 686–703.

KOCAN, R. M., AND S. M. PITTS. 1976. Blood values of the canvasback duck by age, sex and season. Journal of Wildlife Diseases 12: 341–346.

LEWANDOWSKI, A. H., T. W. CAMPBELL, AND G. J. HARRISON. 1986. Clinical chemistries. *In* Clinical avian medicine and surgery, G. J. Harrison and L. R. Harrison (eds.). W. B. Saunders Co., Philadelphia, Pennsylvania, pp. 192–200.

MANN, C. K., AND J. H. YOE. 1956. Spectrophotometric determination of magnesium with sodium 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-napthalene-1-(2-hydroxybenzene-5-sulfonate). Analytical Chemistry 28: 202–208.

MCDANIEL, L. S., AND H. L. CHUTE. 1961. Enzyme activity levels in chicken plasma. American Journal of Veterinary Research 22: 99–103.

NEESE, J. W., P. DUNCAN, D. BAYSE, M. ROBINSON, T. COOPER, AND C. STEWART. 1976. Development and evaluation of a hexokinase/glucose-6-phosphate dehydrogenase procedure for use as a national glucose reference method. United States Department of Health, Education and Welfare, Washington, D.C., Number (CDC)77-8330, 7 pp.

PERRY, M. C., H. H. OBRECHT, B. K. WILLIAMS, AND W. J. KUENZEL. 1986. Blood chemistry and hematocrit of captive and wild canvasbacks. The Journal of Wildlife Management 50: 435–441.

PIERRE, K. J., K. K. TUNG, AND H. NADG. 1976. A new enzymatic kinetic method for determination of α-amylase. Clinical Chemistry 22: 1219.

SAS INSTITUTE INC. 1985. SAS/STAT® Guide for Personal Computers, Version 6 Edition. SAS Institute Inc., Cary, North Carolina, 378 pp.

SKADHAUGE, E. 1983. Formation and composition of urine. *In* Physiology and biochemistry of the domestic fowl, Vol. 4, B. M. Freeman (ed.). Academic Press, London, England, pp. 108–135.

SYKES, A. H. 1971. Formation and composition of urine. *In* Physiology and biochemistry of the domestic fowl, Vol. 1, D. J. Bell and B. M. Freeman (eds.). Academic Press, London, England, pp. 233–278.

SZASZ, G. 1969. Kinetic photometric method for serum gamma-glutamyl transpeptidase. Clinical Chemistry 15: 124–136.

TRINDER, P. 1969. Determination of blood glucose using 4-amino-phenazone. Journal of Clinical Pathology 22: 246.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1982. Pesticide assessment guidelines, subdivision E, hazard evaluation: Wildlife and aquatic organisms. EPA-540/9-82-024. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C., 86 pp.

VAN DEN BERGH, A. A. H., AND J. SNAPPER. 1913. Die farbstoffe des blutserums eine quantitative bestimmung des bilirubin in blukserum. Deutsch Archives Klinical Medicine 110: 540.

WALLACH, J. 1978. Interpretation of diagnostic tests, 3rd ed. Little, Brown and Co., Boston, Massachusetts, pp. 56–59.

WACKER, W. E. C., D. D. ULMER, AND B. L. VALLEE. 1956. Metalloenzymes and myocardial infarction. II. Malic and lactic dehydrogenase activities and zinc concentrations in serum. New England Journal of Medicine 255: 449–456.

WESTLAKE, G. E., A. D. MARTIN, P. I. STANLEY, AND C. H. WALKER. 1983. Control enzyme levels in the plasma, brain and liver from wild birds and mammals in Britain. Comparative Biochemistry and Physiology 76C: 15–24.

WILSON, H. R., AND R. D. MILES. 1988. Research notes: Plasma uric acid of broiler breeder and leghorn male chickens: Effect of feeding time. Poultry Science 67: 345–347.

WINSTEN, S., AND B. CEHELYK. 1969. A rapid micro diazo technique for measuring total bilirubin. Clinical Chemistry Acta 25: 441.

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