Comp. Biochem. Physiol. Vol. 82A, No. 4, pp. 837–846, 1985
Printed in Great Britain

0300-9629/85 $3.00 + 0.00

SEASONAL, SEXUAL AND AGE-RELATED VARIATION IN THE BLOOD COMPOSITION OF THE BROWN PELICAN (*PELECANUS OCCIDENTALIS*)

STEVEN H. WOLF*, RALPH W. SCHREIBER†, LAWRENCE KAHANA‡ and J. J. TORRES§
*606 Ontario Ave., Tampa, Florida 33606, †Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, California 90007, ‡2919 Swan Ave., Tampa, Florida 33606 and §Department of Marine Science, University of South Florida, 140 Seventh Ave. South, St. Petersburg, Florida 33701, USA. Telephone: (813) 893-9131

(*Received* 13 *March* 1985)

Abstract—1. Serum chemistries of a group of captive/crippled brown pelicans (*Pelecanus occidentalis*) were measured at monthly intervals for 14 months using a sequential multiple analyzer.

2. Serum components related to bone and fat metabolism decreased with age until about 24 months old.

3. Protein- and fat-related serum components were elevated between December and May, approximately coinciding with the breeding season of the captive birds and the winter–spring season of the geographic region.

4. Wild brown pelicans showed reduced serum cholesteral and glucose, relative to captives. Wild birds and captives maintained on sea-water had higher serum electrolytes than captives maintained on fresh water.

5. Captive, egg-laying females had elevated levels of serum calcium, inorganic phosphorus and triglycerides relative to non-breeding females.

INTRODUCTION

A strict piscivore, the brown pelican (*Pelecanus occidentalis*) exists at the top of the marine food chain. As such, much interest has been directed toward the interaction of this species with pesticides (Thompson *et al.*, 1977; King *et al.*, 1978; Blus *et al.*, 1979; Schreiber, 1980b). Despite these interests the blood chemistry of *P. occidentalis* has received little attention (Balasch *et al.*, 1974).

Sub-lethal effects of pesticides and other toxic pollutants must be interpreted within the framework of normal physiological changes accompanying development and maturation, reproduction and molt cycles. Blood chemical parameters provide a sensitive indicator of an individual's physiological status, but because many factors, both external (food availability, day length and temperature), and internal (parasites and age), influence the levels of blood components (Siegel, 1980), meaningful interpretation of blood samples requires a large data base for comparison. Serial sampling of captive specimens can establish the baseline data required to assess individual blood chemical variation that is not feasible using capture–recapture techniques in the field.

The brown pelican is well suited for a study of blood chemistry. They are large, allowing sufficient sample volume for analysis of many blood components and for repetitive sampling without altering the bird's physiological state. They breed successfully in captivity (Nesbitt *et al.*, 1980). Their behavior and ecology (Schreiber, 1976b, 1977, 1980a), and physiology (Bartholomew and Dawson, 1954; Schmidt-Nielsen and Fange, 1958; Schreiber, 1976a; Blus and Keahey, 1978) are well documented.

This study examined selected blood chemical parameters of captive adult and immature brown pelicans of both sexes over a full year to gain insight into seasonal and individual variations. Samples from healthy wild brown pelicans are compared to gauge the relevance of the generated baseline to birds in the natural environment.

MATERIALS AND METHODS

Individual *Pelecanus occidentalis* were selected from the population of permanently crippled pelicans maintained in captivity at the Suncoast Sea-bird Sanctuary, a rehabilitation center located along the Gulf beach of Pinellas County, Florida, USA (27°55′N, 82°51′W). Birds with minimum impairment were selected in order to provide a group as close to normal as possible; all were captives sufficiently long to have recovered from the injuries that brought them to the sanctuary.

Twenty-three birds comprising four age categories were selected based on plumage characteristics (Palmer, 1962; Schreiber *et al.*, in preparation) when the study began in June, 1982. Sex was determined based on culmen length, a length of greater than 300 mm was considered a male, less than 300 mm a female (Schreiber, unpublished data). The study group consisted of: four pairs of adults from five to over 13 years old; four female and three male sub-adults from 24–36 months old; four female and two male immatures that fledged in the 1981 breeding season; one female and one male hatch-year that fledged in the 1982 breeding season. Blood was drawn from sixteen birds (three pairs of adults, three female and two sub-adults, two female and one male immature and two hatch-years), the remaining seven birds comprised a control group. Blood samples were also taken from 14 wild adult brown pelicans captured by hand near the sanctuary, and from three birds hatched from the study group starting from an age of 11–12 weeks.

All birds were housed together in a 7 × 15 m outdoor pen at the sanctuary. A small fiber-glass pool was provided and filled with fresh water daily except for the four days preceding the last sampling date. During that period the pool was filled daily with sea-water from the Gulf of Mexico. Birds were fed a variety of local fish purchased frozen from a distributor. All fish were species eaten by local pelicans (Schreiber, unpublished data). Birds consumed approx. 0.5 kg/bird/day, similar to the intake of a captive adult reported by Schreiber (1976a). The amount varied with the ambient temperature and weather conditions, ranging from 0.3 kg/bird/day in the summer to over 0.8 kg/bird on some winter days.

Between June 1982 and July 1983, blood samples were drawn once a month between 10:30–13:30 hr from the experimental group. Approximately 4 ml of blood were drawn from the ulnaris profundus vein or the brachial vein; sampling was usually completed within 2–4 min and no longer than 10 min after capture. Birds were photographed (head, side and back profile) and weighed to the nearest 50 g at each sampling period. Birds in the control group were handled in an identical manner except that no blood was drawn. Birds were starved for 17–20 hr.

Whole blood samples were allowed to clot (30 min to 2 hr) and then centrifuged at 1500 *g* for 10 min to separate serum. Levels of the following serum components were measured using a computer controlled sequential multiple analyzer (SMAC, Technicon Corporation) located at the Damon Medical Laboratory in Tampa, Florida (detailed procedures of analysis outlined in Wolf, 1984): glucose, creatinine, uric acid, cholesterol, triglycerides, total protein, albumin fraction, alkaline phosphatase, calcium, phosphorus, lactate dehydrogenase, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, sodium, potassium, chloride and total carbon dioxide. The globulin fraction was determined by subtracting the albumin fraction from the total protein value. Actual amounts of hemoglobin in the serum were determined to gauge hemolysis in approx. 10% of the samples (Crosby and Furth, 1956). Hemolysis in the remaining samples was qualitatively gauged by comparing their color with known standards.

Means and correlations were calculated using SAS (Helwig and Council, 1979). All values in the text are reported as mean ± standard error of the mean ($\bar{x} \pm SE$) unless otherwise noted.

RESULTS

Birds fell into four age classes based on blood chemical similarities: (1) The three fledglings (FL), 11–12 weeks old; (2) the hatch-year (HY) group of one male and female that became part of the study at ages five and seven months, respectively and continued through their tenth month; (3) immature (I) birds, from 10 to 24 months of age, or when they attained sub-adult plumage, and (4) adults (A), approximately two years or older. Wild birds (W) were all adults sampled between December and May.

Significant seasonal variations were found in several blood components. Seasonal variations of highest significance were found by dividing the year into two periods: December to May and June to November, herein referred to as winter–spring and summer–fall, respectively.

Glucose

Glucose level varied from 192 to 477 mg/dl ($\bar{x} = 315 \pm 4$ mg/dl, $N = 181$). Levels generally increased from the fledgling to the immature stage (Table 1), but a significant decrease was found in adults, and wild birds were significantly lower than all except fledglings.

Non-protein nitrogen

Creatinine levels were relatively stable (range = 0.3–3.0 mg/dl, $\bar{x} = 1.0 \pm 0.03$ mg/dl, $N = 167$), with no significant variation related to age, sex, season or captive status.

Uric acid levels ranged from 1.8 to 18.6 mg/dl ($\bar{x} = 5.6 \pm 0.2$ mg/dl, $N = 182$) and increased significantly with age after the high values of fledglings (Table 1). Wild birds exhibited significantly elevated values over captive adults. In addition, uric acid levels of captives were significantly higher in the winter–spring than in summer–fall (Table 1).

Table 1. Serum glucose and non-protein nitrogen levels of captive and wild brown pelicans

Group*	Glucose (mg/dl)†	Uric acid (mg/dl)		
		Annual†	June–Nov†	Dec–May†
FL	223 ± 12 (3)‡	7.6 ± 0.3 (3)‡		
HY	299 ± 18 (9)b	4.8 ± 0.5 (9)b		
IM	334 ± 7 (53)c	5.1 ± 0.2 (53)b	4.8 ± 0.3 (27)‡	5.4 ± 0.2 (26)‡
A	308 ± 5 (119)b	5.9 ± 0.2 (120)‡	5.4 ± 0.3 (72)‡§	6.7 ± 0.4 (48)b
W	208 ± 7 (14)‡			9.4 ± 0.8 (12)c

*FL = fledgling; HY = hatch-year; IM = immature; A = adult; W = wild in this and all following tables.
†$\bar{x} \pm SE(N)$.
‡Means in each column not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).
§Designates a significant seasonal variation of a parameter within a group at the $P < 0.05$ level (t-test).

Table 2. Protein levels and hematocrits of captive brown pelicans

Group	Total protein (g/dl)*	Albumin (g/dl)*	Globulin (g/dl)*	Albumin/globulin*	Hematocrit (% packed cells)*
FL	5.3 ± 0.4 (3)†	2.8 ± 0.4 (3)†	2.5 ± 0.1 (3)†b	1.1 ± 0.1 (3)†	41.9 ± 1.0 (3)†b
HY	4.1 ± 0.2 (9)bc	1.7 ± 0.1 (9)b	2.4 ± 0.1 (9)†b	0.7 ± 0.04 (9)bc	44.4 ± 1.1 (8)†b
IM	4.0 ± 0.1 (53)b	1.8 ± 0.04 (53)b	2.3 ± 0.04 (53)†	0.8 ± 0.02 (53)b	43.1 ± 0.3 (39)†
A	4.4 ± 0.1 (119)c	1.8 ± 0.04 (119)b	2.7 ± 0.1 (119)b	0.7 ± 0.02 (119)c	45.0 ± 0.4 (83)b

*$\bar{x} \pm SE$ (N).
†Means in each column not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).

Protein

Fledglings had significantly higher total protein levels than either hatch-year or immature birds, due to an elevated albumin fraction (Table 2). Total protein levels were significantly higher in adults than in immatures because of an elevated globulin fraction. Protein levels of wild birds did not differ significantly from those of captive adults (Table 3).

Total protein levels of captive birds were elevated in winter–spring. In adults this increase was due mainly to an increase in the albumin fraction, as reflected by an increase in the albumin/globulin (A/G) ratio (Table 3). In immature birds the A/G ratio did not change and elevated total protein values resulted from equal increases of both albumin and globulin fractions. Total protein levels of wild birds were not significantly different from captive adults. However, wild birds did have significantly higher levels of globulins.

Total protein levels were moderately correlated with hematocrits in this study (Table 4). Adults had significantly elevated hematocrit values in the winter–spring, while the hematocrit of immatures followed a similar, but non-significant, seasonal trend (Table 3).

Lipids

Cholesterol levels of the immature birds were significantly higher than those of adults (Table 5). Cholesterol levels were also significantly elevated in winter–spring in both immatures and adults (Fig. 1). Adult males had significantly higher levels than females throughout the year, while immature birds showed no such sexual difference (Table 5). Wild birds had significantly lower cholesterol levels when compared with adult captives at the same time of year.

Serum triglycerides varied from 6 to 238 mg/dl ($\bar{x} = 55 \pm 3$ mg/dl, $N = 178$) in the non-breeding captive birds and no significant age or seasonal variations were found. The only major fluctuations of this component were found in laying females (see below).

Table 3. Seasonal variations in protein levels and hematocrits of brown pelicans with comparisons to wild birds

	Total protein (g/dl)		Albumin/globulin		Hematocrit (% packed cells)	
Groups	June–Nov*	Dec–May*	June–Nov*	Dec–May*	June–Nov*	Dec–May*
IM	3.7 ± 0.1§ (27)	4.4 ± 0.1† (26)	0.8 ± 0.03† (27)	0.8 ± 0.2† (26)	42.3 ± 0.6† (13)	43.5 ± 0.4† (26)
A	4.1 ± 0.1§ (71)	4.9 ± 0.1b (48)	0.7 ± 0.02b‡ (71)	0.8 ± 0.03† (48)	43.9 ± 0.5†‡ (39)	46.0 ± 0.5b (44)
W		4.5 ± 0.3†b (14)		0.6 ± 0.04b (14)		44.2 ± 1.0†b (11)

*$\bar{x} \pm$ SE(N).
†Means in each column not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).
‡Designates a significant seasonal variation of a parameter within a group at the $P < 0.05$ level (t-test).
§Designates a significant seasonal variation of a parameter within a group at the $P < 0.001$ level (t-test).

Table 4. Correlations of protein and lipid related serum components of captive brown pelicans

	Correlation coefficients (r)†		
Correlations*	IM + A	IM	A
TP vs PCV	0.425** (134)	0.591** (44)	0.356** (90)
TP vs CHL	0.418** (172)	0.732** (53)	0.490** (119)
CHL vs WT	0.262** (170)	NS‡	0.274* (117)

*TP = total protein; PCV = packed cell volume (hematocrit) CHL = cholesterol; WT = weight.
†'*' = $P < 0.05$; '**' = $P < 0.001$.
‡No significant correlation.

Table 5. Seasonal and sexual variations of cholesterol levels of captive brown pelicans with comparisons to wild birds

	Cholesterol (mg/dl)			
	Annual		Both sexes	
Group	Male*	Female*	June–Nov*	Dec–May*
IM	224 ± 7 (19)†	223 ± 5 (34)†	205 ± 5 (27)†§	242 ± 5 (26)†
A	206 ± 5 (62)†b‡	191 ± 4 (58)b	182 ± 3 (72)b§	224 ± 5 (48)b
W	185 ± 9 (7)b	190 ± 15 (17)b		188 ± 8 (14)c
FL			304 ± 33 (3)c	
HY			185 ± 10 (9)†b	

*$\bar{x} \pm$ SE(N).
†Means in each column not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).
‡Designates a significant sexual or seasonal variation of a parameter at the $P < 0.05$ level (t-test).
§Designates a significant sexual or seasonal variation of a parameter at the $P < 0.001$ level (t-test).

Table 6. Seasonal and sexual variation of captive brown pelican weights with comparisons to wild birds

Group	Weight (g)* June–Nov	Weight (g)* Dec–May
Male IM	3488 ± 80 (10)†	3648 ± 88 (10)†
A	3283 ± 44 (37)b‡	3512 ± 52 (28)†b
W		3314 ± 99 (7)b
Female IM	2801 ± 48 (18)c	2881 ± 62 (16)c
A	2606 ± 36 (42)†d	2791 ± 54 (20)c
W		2760 ± 87 (5)c

*$\bar{x} \pm SE(N)$.

†Means in each column not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).

‡Designates a significant seasonal variation of a parameter within a group at the $P < 0.05$ level (t-test).

Captive male pelicans for both the adult and immature groups averaged 600–800 g heavier than females. This sexual difference, although somewhat less pronounced (~550 g), was also found in wild birds and reflects actual size differences between sexes. Both sexes of captive adult birds averaged ~200 g heavier in the winter–spring (Table 6). Immatures showed the same general trend in weight as adults (Fig. 2), but the seasonal difference was not significant. However, the immatures were heavier than adults by 100–200 g throughout the year. Weights of captive birds correlated weakly with cholesterol levels (Table 4).

Weights of control and wild birds did not differ significantly from weights of experimental birds.

Enzymes

Sample numbers for lactate dehydrogenase (LDH) and serum glutamic oxaloacetic transaminase (SGOT) were reduced due to hemolysis, limiting possible comparisons and no significant variations of these enzymes were found. LDH levels varied from 321 to 636 units/l ($\bar{x} = 474 \pm 11$ units/l, $N = 50$) for samples with little or no hemolysis (<70 mg hemoglobin/dl serum). SGOT levels ranged from 90 to 251 units/l ($\bar{x} = 151 \pm 3$ units/l, $N = 111$) for samples with less than 125 mg hemoglobin/dl serum. Serum glutamic pyruvic transaminase (SGPT) activities were relatively unaffected by hemolysis, but no age, sex or seasonal variations could be detected ($\bar{x} = 27 \pm 1$ units/dl, $N = 176$, range = 1–130 units/l). However, the wild birds examined had significantly higher ($P < 0.001$, t-test) SGPT levels ($\bar{x} = 53 \pm 9$ units/l, $N = 14$) when compared to captive birds.

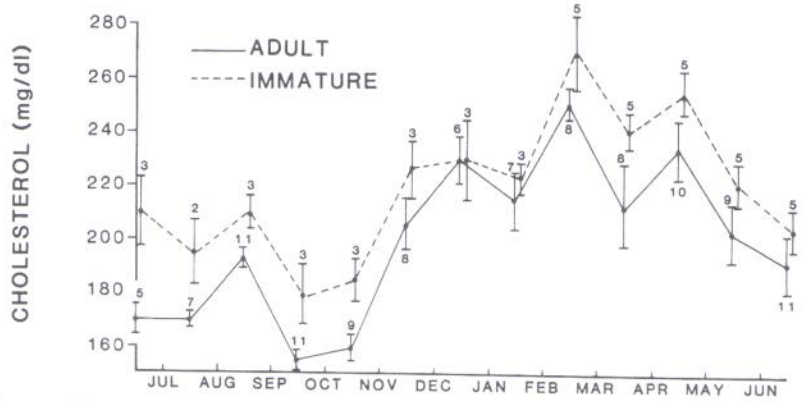

Fig. 1. Seasonal changes in serum cholesterol of captive immature and adult brown pelicans. Error bars represent standard error (SE); number of observations (N) are shown at each point.

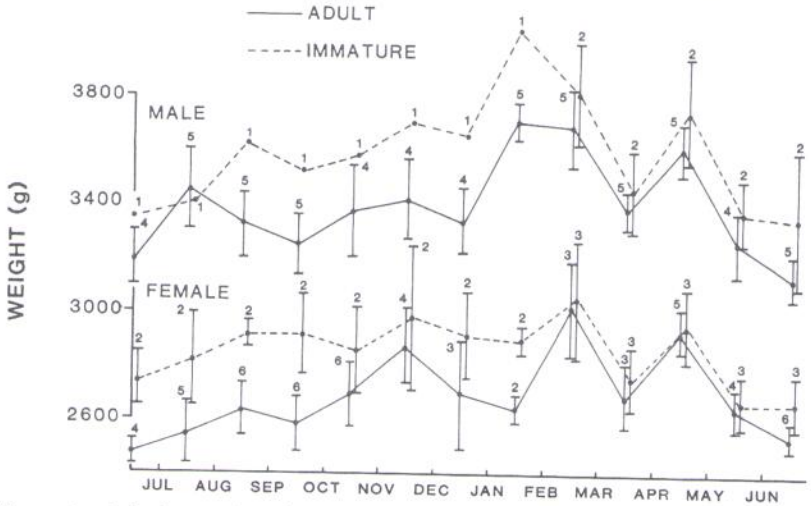

Fig. 2. Seasonal weight changes in male (upper) and female (lower) captive brown pelicans. Error bars represent standard error (SE); number of observations (N) are shown at each point.

Calcium metabolism

Calcium (Ca), inorganic phosphorus (inorgP) and alkaline phosphatase (alkPh) levels generally decreased with age (Table 7). Although inorgP levels had decreased by the fifth month of age, all three components remained at significantly higher levels than those of the immatures during January when the birds were approx. 10 months old. Ca, inorgP and alkPh levels then decreased sharply to the same levels of the immature birds.

InorgP and Ca levels were significantly higher in the winter–spring than in summer–fall in immature birds. Only inorgP levels were increased in winter–spring in adult birds. InorgP levels were also higher in males than in females for both immatures and adults (Table 8). AlkPh levels were higher in males of both groups, but showed no significant seasonal variations (Table 8).

Wild birds showed no significant differences from the values of Ca, inorgP or alkPh reported for the captive adults (Table 8).

Electrolytes

Levels of sodium (Na), potassium (K), chloride (Cl) and total carbon dioxide (CO_2) were not found to vary with sex or age. CO_2 values may have varied due to atmospheric exchange before analysis, and K values included some hemolyzed samples in the means. However, these data were included in the calculation of anion gap:

$$\text{anion gap} = [\text{Na}] + [\text{K}] - [\text{CO}_2] - [\text{Cl}].$$

Wild birds exhibited significantly higher levels of Na, Cl and CO_2 and lower K levels than the captive birds maintained on fresh water (Table 9). Their mean anion gap was one half that of the captive birds. For four days prior to the last sampling date, the captive birds had only sea-water ($\sim$35‰ salinity) in their pool. The only significant difference between captives maintained on sea-water and wild birds was the elevated K levels of the captives (Table 9).

Plumage and molt

Captive adults molted head, neck, back and breast feathers between June and September. They molted head and neck feathers again between November and January. This molt occurred several months before the corresponding molt in the local wild population (Schreiber *et al.*, in preparation). The molt cycle was more variable in birds with sub-adult plumage. Colors were not as intense, or were speckled with remnants of the preceding color phase. Sub-adult molt of head and neck did not occur until November, which was followed by another molt in several months. Birds younger than two years showed the same general molt pattern as sub-adults. The molt of the hatch-year birds at 10 months included the back feathers and resulted in the same plumage as the other immature birds. Remige and rectrix molt was difficult to discern in the captive birds because of prior wing injuries and the poor condition of feathers damaged by life in captivity.

Laying females

Captive birds began courtship and nest building in mid November, 2–3 months earlier than typical of the local wild pelicans (Schreiber, 1980a). Egg-laying commenced in early December and continued until late April.

Blood components of two laying females were compared with those of five non-breeding adult females for the same time period and those components that differed significantly are presented in

Table 7. Serum calcium, inorganic phosphorus and alkaline phosphatase levels of captive and wild brown pelicans

Group	Calcium* (mg/dl)	Inorganic phosphorus* (mg/dl)	Alkaline phosphatase* (Units/l)
FL	11.5 ± 0.3 (3)†	12.2 ± 2.2 (3)†	1794 ± 268 (3)†
HY	10.7 ± 0.4 (9)†	4.6 ± 0.3 (9)b	2528 ± 244 (9)†
IM	9.4 ± 0.1 (53)b	3.7 ± 0.1 (53)c	1138 ± 71 (47)b
A	9.0 ± 0.1 (119)c	4.0 ± 0.1 (120)bc	1060 ± 58 (110)b
W	9.4 ± 0.1 (10)bc	4.9 ± 0.4 (10)b	1383 ± 233 (9)b

*$\bar{x} \pm SE(N)$.
†Means in each column not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).

Table 8. Seasonal and sexual variations of serum alkaline phosphatase and inorganic phosphorus in captive brown pelicans with comparisons to wild birds

	Alkaline phosphatase (units/l)		Inorganic phosphorus (mg/dl)			
	Annual		Annual		Seasonal	
Group	Male*	Female*	Male*	Female*	June–Nov*	Dec–May*
IM	1490 ± 124†§ (17)	938 ± 63† (30)	4.1 ± 0.2†‡ (19)	3.5 ± 0.1† (34)	3.5 ± 0.1†‡ (27)	4.0 ± 0.2† (26)
A	1264 ± 86†§ (57)	840 ± 64† (53)	4.3 ± 0.2†‡ (62)	3.7 ± 0.1† (58)	3.6 ± 0.1†§ (72)	4.5 ± 0.2†b (48)
W	1612 ± 285† (7)	855 ± 110† (7)	4.7 ± 0.5† (7)	5.4 ± 0.5b (3)		4.9 ± 0.4b (10)

*$\bar{x} \pm SE(N)$.
†Means in each column not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).
‡Designates a significant seasonal or sexual variation of a parameter within a group at the $P < 0.05$ level (t-test).
§Designates a significant seasonal or sexual variation of a parameter within a group at the $P < 0.001$ level (t-test).

Table 9. Serum electrolyte levels of captive brown pelicans maintained on fresh water and sea-water with comparisons to wild birds

	Captive		Wild	
	Freshwater†	Sea-water‡	Non-layer§	Layer**
Na*	143 ± 1 (167)‡‡ [120–162]	155 ± 3 (15)b [117–165]	154 ± 1 (10)b [147–159]	132 ± 0 (2)‡‡ [132]
K	4.4 ± 0.1 (167)‡‡ [1.5–9.9]	5.2 ± 0.2 (15)b [4.0–6.3]	3.8 ± 0.5 (9)‡‡ [2.1–6.2]	4.2 ± 0.9 (2)‡‡ [3.3–5.1]
Cl	87 ± 2 (159)‡‡ [54–122]	121 ± 2 (15)b [90–128]	119 ± 1 (10)b [113–125]	74 ± 8 (2)‡‡ [66–81]
CO_2	11 ± 0.4 (150)‡‡ [1–23]	13 ± 1 (15)‡‡b [7–20]	14 ± 1 (10)b [10–19]	9 ± 3 (2)‡‡b [6–12]
GAP††	50 ± 1 (150)‡‡ [18–86]	27 ± 4 (15)b [−7–65]	25 ± 2 (9)b [19–36]	54 ± 4 (2)‡‡ [50–57]

*All values are $\bar{x} \pm SE(N)$, [range] in mEq/l.
†Pool filled daily with fresh water preceding sampling.
‡Pool filled with sea-water (~35‰ salinity) four days preceding sampling.
§Assumed non-layer due to sex or "normal" Ca, inorgP and triglycerides.
**Assumed in some stage of laying due to highly elevated levels of Ca, inorgP and triglycerides.
††GAP = anion gap = [Na] + [K] − [Cl] − [total CO_2].
‡‡Means across each line not followed by a common letter are significantly different at the $P < 0.05$ level (t-test).

Table 10. Ca and inorgP levels of laying females increased 67 and 47%, respectively, relative to non-breeding females and were highly correlated ($r = 0.785$ at $P < 0.001$, $N = 15$). The level of triglycerides increased almost 15 times in laying females. Body weights of the laying females were somewhat elevated, but the difference was not significant.

Studies of domestic fowl have reported elevated Ca and inorgP values preceding first egg-laying by 1–2 weeks and continuing for several days after incubation begins (Sturkie, 1976). Most observations reported for captive laying females in this study were within 2–3 weeks of egg-laying or loss of clutch, but values remained elevated for 4–8 weeks in some cases.

Two wild adult females captured in April 1983 showed levels of Ca, inorgP and triglycerides similar to those of captive laying females (Table 10). Although these two birds were captured in a marine environment, they had reduced levels of Cl and elevated anion gaps relative to other wild birds and these levels were similar to those of captive birds maintained on fresh water (Table 9).

DISCUSSION

Age related variation

Significant differences in levels of several serum components determine age divisions of brown pelicans (Table 11), the most noticeable being those related to calcium metabolism. Increased levels of alkaline phosphatase are associated with osteoblastic activity in growing mammals and birds (Bell, 1971). Increased levels of this enzyme in fledgling pelicans, along with elevated calcium and inorganic phosphorus, suggest very active bone formation until they reach approximately 10 months of age (Table 7). These levels remained moderately elevated for birds 10–24 months old, suggesting a continuation of bone formation through the second year.

The high serum uric acid levels and albumin/globulin ratios of fledgling pelicans (Tables 1 and 2) are a reflection of their large daily food intake, a relationship which has been established in the domestic fowl (Leveille and Sauberlich, 1961; Featherston, 1969). Decreases in serum albumin levels in older birds suggest that the high levels were associated with rapid growth and/or maintenance of a large protein reserve at the time young pelicans leave the nest.

As in wild brown pelicans, fledglings weighed the same or more than adults (Schreiber, 1976a). Hatch-year birds were heavier than fledglings, but the decreased cholesterol levels and strenuous exercise associated with flight suggest an increase in muscle mass rather than fat reserves. Weight remained relatively constant through the hatch-year period and dropped as the birds entered immature plumage,

Table 10. Serum components of captive brown pelicans significantly altered during laying with comparisons to wild birds

Group*	Triglycerides† (mg/dl)	Calcium† (mg/dl)	Inorganic P† (mg/dl)
AF	42 ± 5 (17)‡ [21–81]	9.3 ± 0.2 (20)‡ [7.4–10.5]	4.3 ± 0.2 (20)‡ [2.1–6.3]
CBF	614 ± 130 (15)b [99–1735]	15.5 ± 1.0 (15)b [10.8–22.5]	6.3 ± 0.4 (15)b [4.2–9.3]
WBF	912 ± 90 (2)b [822–1002]	18.2 ± 0.5 (2)b [17.7–18.6]	8.0 ± 0.2 (2)b [7.8–8.1]

*AF = captive adult and sub-adult non-breeding females for the Dec–May period; CBF = captive breeding females No. 107 and No. 122, sampled from Nov–April; WBF = wild females assumed at some stage of laying.
†$\bar{x} \pm SE(N)$, [range].
‡Means in each column not followed by a common letter are significantly different at the $P < 0.001$ level (t-test).

Table 11. Summary of serum chemical and weight comparisons of captive brown pelicans

Comparison	Parameters that increase	Parameters that decrease
Fledgling to immature	Glucose	Albumin, calcium, inorganic phosphorus, alkaline phosphatase, uric acid, cholesterol
Immature to adult	Globulin	Calcium, inorganic phosphorus, alkaline phosphatase, cholesterol, weight
Male to female		Alkaline phosphatase, cholesterol (adults only), weight
Summer–fall to winter–spring	Albumin, globulin (immatures only) hematocrit, uric acid, cholesterol, weight (adults only)	
Captive to wild		Glucose, albumin, cholesterol
Maintenance on sea–water to freshwater	Anion gap	Sodium, potassium, chloride
Non-breeding female to breeding female	Triglycerides, calcium, inorganic phosphorus	

possibly due to utilization of fat reserves during the associated molt and/or a general metabolic shift, as reflected by the changes in serum components related to bone metabolism.

Reduced globulin levels in immature birds accounted for the difference in total protein levels between immatures and adults (Table 2). Stimulation of the immune system as the birds age may result in the increased globulin fraction. α and β globulins have been shown to make up part of the lipoproteins associated with yolk formation (Sturkie, 1976). The globulin difference between immature and adult birds was more pronounced in females in this study: estrogens associated with the maturation of the females may act to increase globulin levels.

Elevated weight and cholesterol levels suggest that immature birds maintain a higher fat reserve than adults. This reserve is probably important for survival until the immature birds become as adept as adults at catching fish (Orians, 1969).

No significant differences were found in the blood composition of non-laying brown pelicans in sub-adult and adult plumage. This suggests that sub-adults and adults are physiologically quite similar, a supposition supported by the fact that pelicans in sub-adult plumage have bred successfully at three years of age (Williams and Joanen, 1974; Blus and Keahey, 1978).

Seasonal variations

Seasonal variations in blood composition have been described in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*, de Graw *et al.*, 1979), and the Canada goose (*Branta canadensis interior*, Mori and George, 1978). Both those species are intracontinental migrants with distinct annual cycles divisible into periods such as premigratory fattening, wintering, breeding and molting, which have definite behavioral and physiological correlates.

Seasonality for birds living in tropical or sub-tropical environments often is less distinct. However, the captive brown pelicans in this study exhibited significantly elevated values of serum components related to protein and fat metabolism from December to May, as compared to June to November (Table 11). The transition between seasons is not marked by an obvious behavioral change such as migration. Some fledglings do move south along the Florida Gulf coast during their first fall (Schreiber, 1976b), but a large number of pelicans remain in the area year round. Although the boundaries between seasons are not physically or physiologically distinct, the December–May and June–November divisions resulted in highly significant differences in seasonal means of blood components. Although no "pre-migratory fattening" occurred in this study, weights and serum cholesterol were elevated from December to May (Tables 5 and 6), resulting in a correlation similar to that in the white-crowned sparrow (deGraw *et al.*, 1979). The actual composition of the pelican's diet (type of fish eaten) did not vary markedly throughout the study, but the amount consumed did increase 2–3 times on cold or rainy days. This increased food intake is the probable cause of the same protein- and lipid-related increases reported in domestic fowl (Sim *et al.*, 1980).

Increased protein and fat reserves may offer the local brown pelicans a buffer against the related stresses of the winter season. Summer rainfall is quite heavy, but of short duration and is followed by sunshine, giving the birds a chance to dry wet plumage. Winter rains associated with the passage of weather systems may extend for longer periods of time. The rainfall and lower temperatures probably result in a greater expenditure of energy just to maintain body temperature. In addition, the high winds that accompany the passage of weather systems and associated reduced water clarity may make both locating and capturing fish difficult. Thus, at a time when birds require more energy, food intake may be reduced, indicating the necessity of an energy reserve.

Almost certainly, the overlap of the captive birds' breeding season (November–June) and the seasonality of protein- and fat-related blood components is correlated. The stable food supply of our captive birds apparently resulted in a nesting chronology similar to that of brown pelicans living farther south

in the Caribbean (Schreiber, 1980a). Reproduction in wild birds has been shown to be governed more by adequate food supply than by environmental temperature (Wingfield *et al.*, 1983). Once a level of energy reserves sufficient for egg production and incubation is attained, nesting commences. Ashmole (1971) suggests that these energy reserves are the controlling factors in initiation of nesting in tropical regions where seasonal fluctuations of climate and food supply are minimal.

Most avian species undergo a complete molt following breeding (Pettingill, 1970) which can result in reduced hematocrits and serum protein levels (Mori and George, 1978; deGraw *et al.*, 1979; Driver, 1981). In the present study a molt of head, neck, back and breast feathers for most birds began approximately in July. Schreiber (1976a) reported a complete molt of a captive adult brown pelican between July and November. If the captive pelicans of this study molted flight feathers during this period, the added requirement for protein may have depleted reserves and enhanced the seasonality of blood components.

Captive vs wild birds

The brown pelican, being piscivorous, must rely almost entirely on gluconeogenesis to maintain its serum glucose level and glycogen supply. As a result, serum glucose levels are probably not affected by immediate feeding state, as has been shown in the carnivorous black vulture (*Coragypus atratus*, Migliorini *et al.*, 1973). However, glucose levels of wild pelicans were quite low relative to captive birds (Table 1), most likely related to a difference in exercise. A decrease in exercise can result in decreased glucose utilization and a subsequent rise of serum glucose levels. Differences in individual activity levels coincide with the observed glucose variations between groups. Flyers (wild birds and fledglings) had the lowest glucose levels. One captive adult was a capable flyer and its mean glucose level (239 mg/dl) was similar to that of wild pelicans (208 mg/dl) and contributed to the lower value for adults.

Captive brown pelicans had higher albumin and cholesterol levels and slightly higher body weights than the wild birds, a result similar to that found in captive and wild white-crowned sparrows (deGraw *et al.*, 1979). The stable diet and limited exercise both contribute to serum cholesterol and albumin elevations in captives.

Marine vs freshwater

A change from maintenance on fresh water to sea-water led to significant alteration of the captive pelicans' serum electrolyte levels (Tables 9 and 11). The anion gap is a convenient means of assessing electrolyte relationships (Murphy and Henry, 1979). In its simplest form the anion gap is calculated by subtracting the routinely measured anions (Cl and HCO_3, measured as total CO_2) from the cations (Na and K). The possible erroneous K and CO_2 values noted in these results are not enough to explain the two-fold difference in anion gap values between pelicans in a freshwater and marine environment. Anion gap fluctuations were highly correlated with chloride levels ($r = 0.927$ at $P < 0.001$, $N = 185$) which suggests that serum chloride levels drop in response to changes of unmeasured cations (ionic calcium and magnesium) and anions (phosphate and proteins).

Female reproductive condition

Deposition of most egg components during the 24–30 hr preceding oviposition is higher than the rate they can be absorbed in the gut. Thus, adequate body reserves must exist prior to the onset of laying and these reserves are reflected by changes in blood composition (Sturkie, 1976). The laying pelicans of this study showed the same general trends in blood composition reported for other species (Mori and George, 1978; Bacon *et al.*, 1980; McRae and Dimmick, 1982). Neutral lipids make up a large percent of the total egg lipid and egg formation is noted by an elevation of serum triglycerides. Individual levels of triglycerides for laying pelicans ranged from 99 to 1735 mg/dl with higher levels found just before egg formation. Serum calcium and inorganic phosphorus levels fluctuate in response to construction or mobilization of specialized medullary bone related to eggshell formation (Simkiss and Taylor, 1971). Serum calcium and inorganic phosphorus levels of laying females of this study averaged almost two times the levels of non-breeding birds (Table 10).

An interesting difference noted in this study is the length of time that serum components related to egg production remained elevated in the pelicans. Burkholder (1974) reported increased calcium levels in the red-winged blackbird (*Agelaius phoeniceus*) approximately 10 days before laying commenced and levels dropped to near normal 6–7 days after incubation began. In the present study most samples were taken within several weeks of oviposition. However, one bird still had increased level of calcium, inorganic phosphorus and triglycerides when brooding a four-week-old chick. With an adequate food supply, females may remain in a reproductive state even when brooding chicks in anticipation of rapid renesting after nest failure or fledging of the chicks.

Two wild females pelicans sampled during the breeding season of the local population had increased levels of serum components associated with egg formation (Table 10). In addition, they had serum electrolyte levels and anion gaps similar to those of birds maintained on fresh water (Table 9). It is probable that the blood components elevated at the time of egg formation (proteins and lipids) have a net negative charge which is balanced by a drop in chloride levels (causing the anion gap to increase) to maintain serum electroneutrality.

SUMMARY

Blood components of captive/crippled brown pelicans revealed significant variations related to season, age, sex and captive state. Elevated levels of serum components related to bone metabolism in younger birds suggest very active bone formation until approximately 10 months of age. These levels remained moderately elevated for birds 10–24 months old suggesting a continuation of bone formation through the second year. Increased levels of protein- and/or lipid-related components in younger birds are

most likely related to the energy reserves these birds require for growth and maturation until they are as adept as adults at catching fish.

Both adult and immature birds displayed seasonal variations in protein- and lipid-related serum components. Highest levels of protein and lipid reserves were found between December and May. This period corresponds with the captive birds' breeding season as well as local winter, suggesting that these reserves are important in coping with the energy demands related to winter survival and reproduction.

Differences in serum components between wild and captive birds suggest that exercise and time after feeding are important factors in determining the levels of serum components such as glucose, cholesterol and uric acid. In addition, electrolyte levels varied significantly between captives maintained on freshwater and wild birds or captives maintained on sea-water. Captive breeding females had high levels of serum calcium, inorganic phosphorus and triglycerides relative to non-breeding birds. These levels were highest near the time of egg formation. However, levels remained increased even while adults were brooding young chicks, suggesting that with an adequate food supply, ovaries remained in an active state, perhaps to allow renesting.

Acknowledgements—The staff and facilities of the Suncoast Seabird Sanctuary were indispensable in this study. In particular, we would like to thank Carl Almquist, Jennie Grimes, Dianna King and Barbara Suto. Damon Medical Laboratories of Tampa donated their services and thanks are due to Elaine Stevens in particular. Dr H. Albers and Dr C. Marcum contributed both valuable advice and equipment. Dr S. Grossman and Dr G. Woolfenden provided advice and a critical review of the manuscript. We sincerely thank all these individuals. Special thanks are due to the Wolfs who provided both encouragement and help throughout this study.

REFERENCES

Ashmole N. P. (1971) Seabird ecology and the marine environment. In *Avian Biology* (Edited by Farner D. S. and King J. R.), Vol. I, pp. 223–286. Academic Press, New York.

Bacon W. L., Brown K. I. and Musser M. A. (1980) Changes in plasma calcium, phosphorus, lipids and estrogens in turkey hens with reproductive state. *Poultry Sci.* **59,** 444–452.

Balasch J. S., Palomeque J., Palacios L., Musquera S. and Jimenez M. (1974) Hematological values of some great flying and aquatic-diving birds. *Comp. Biochem. Physiol.* **49,** 137–145.

Bartholomew G. A., Jr. and Dawson W. R. (1954) Temperature regulation in young pelicans, herons and gulls. *Ecology* **35,** 466–472.

Bell D. J. (1971) Plasma enzymes. In *Physiology and Biochemistry of the Domestic Fowl* (Edited by Bell D. J. and Freeman B. M.), Vol. II, pp. 963–971. Academic Press, New York.

Blus L. J., Cromartie E., Mcnease L. and Joanen T. (1979) Brown pelican: Population status, reproductive success and organochlorine residues in Louisiana, 1971–1976. *Bull. Envir. Contam. Toxicol.* **22,** 128–135.

Blus L. J. and Keahey J. A. (1978) Variation in reproductivity with age in the brown pelican. *Auk* **95,** 128–134.

Burkholder T. J. (1974) The relation of serum calcium levels to the reproductive cycle and egg production of the female red-winged blackbird, *Agelaius phoeniceus*. *Physiol. Zool.* **47,** 242–251.

Crosby W. H. and Furth F. W. (1956) A modification of the benzidine method for measurement of hemoglobin in plasma and urine. *Blood* **11,** 380–383.

deGraw W. A., Kern M. D. and King J. R. (1979) Seasonal changes in the blood composition of captive and free-living white-crowned sparrows. *J. comp. Physiol.* **129,** 151–162.

Driver E. A. (1981) Hematological and blood chemical values of mallard (*Anas p. platyrhynchos*), drakes before, during and after remige moult. *J. Wildl. Dis.* **17,** 413–421.

Featherston W. R. (1969) Nitrogenous metabolites in the plasma of chicks adapted to high protein diets. *Poultry Sci.* **48,** 646–652.

Helwig J. T. and Council K. A. (1979) *SAS User's Guide*. SAS Institute, Inc., Raleigh, North Carolina.

King K. A., Flickinger E. L. and Hildebrand H. H. (1978) Shell thinning and pesticide residues in Texas aquatic bird eggs, 1970. *Pestic. Monit. J.* **12,** 16–21.

Leveille G. A. and Sauberlich H. E. (1961) Influence of dietary protein levels on serum protein components and cholesterol in the growing chick. *J. Nutrition* **74,** 500–504.

McRae W. A. and Dimmick R. W. (1982) Body fat and blood-serum values of breeding wild bobwhites. *J. Wildl. Dis.* **46,** 268–271.

Migliorini R. H., Linder C., Moura J. L. and Viega J. A. S. (1973) Gluconeogenesis in a carnivorous bird (black vulture). *Am. J. Physiol.* **225,** 1389–1392.

Mori J. G. and George J. C. (1978) Seasonal changes in serum levels of certain metabolites, uric acid and calcium in the migrating Canada goose (*Branta canadensis interior*). *Comp. Biochem. Physiol.* **59B,** 263–269.

Murphy J. E. and Henry J. B. (1979) Evaluation of renal function, and water, electrolyte and acid–base balance. In *Clinical Diagnosis and Management by Laboratory Methods* (Edited by Henry J. B.), pp. 135–152. W. B. Saunders, Philadelphia.

Nesbitt S. A., Harris B. A., Fenelon S. A. and King D. M. (1980) Reproduction of brown pelicans in captivity. *Wildl. Soc. Bull.* **8,** 259–262.

Orians G. H. (1969) Age and hunting success in the brown pelican (*Pelecanus occidentalis*). *Anim. Behav.* **17,** 316–319.

Palmer R. S. (1962) *Handbook of North American Birds*, pp. 271–280. Yale University Press, New Haven.

Pettingill O. S., Jr. (1970) *Ornithology in Laboratory and Field*, pp. 188–199. Burgess, Minneapolis.

Schmidt-Nielsen K. and Fange R. (1958) The function of the salt gland in the brown pelican. *Auk* **75,** 282–289.

Schreiber R. W. (1976a) Growth and development of nestling brown pelicans. *Bird Banding* **47,** 19–39.

Schreiber R. W. (1976b) Movements of color marked brown pelicans. *Bird Banding* **47,** 101–111.

Schreiber R. W. (1977) Maintenance behavior and communication in the brown pelican. *Ornithological Monographs*, No. 22, pp. 1–78. The American Ornithologist's Union, Washington D.C.

Schreiber R. W. (1980a) Nesting chronology of the eastern brown pelican *Auk* **97,** 491–508.

Schreiber R. W. (1980b) The brown pelican: An endangered species? *BioSci.* **30,** 742–747.

Schreiber R. W., Schreiber E. A. and Anderson D. W. (in preparation). Plumage and molt cycles of the brown pelican (*Pelecanus occidentalis*).

Siegel H. S. (1980) Physiological stress in birds. *BioSci* **30,** 529–534.

Sim J. S., Kitts W. D. and Bragg D. B. (1980) Effect of dietary egg yolk on serum cholesterol levels of white leghorn chickens. *Poultry Sci.* **59,** 1812–1817.

Simkiss K. and Taylor T. G. (1971) Shell formation. In

Physiology and Biochemistry of the Domestic Fowl (Edited by Bell D. J. and Freeman B. M.), Vol. 3, pp. 1331–1343. Academic Press, New York.

Sturkie P. D. (1976) *Avian Physiology*, 3rd edn. Springer, New York.

Thompson N. P., Courtney C. H., Forrester D. J. and White F. H. (1977) Starvation–pesticide interactions in juvenile brown pelicans. *Bull. Envir. Contam. Toxicol.* **17,** 485–490.

Williams L. E. and Joanen T. (1974) Age of first nesting in the brown pelican. *Wilson Bull.* **86,** 279–280.

Wingfield J. C., Moore M. C. and Farner D. S. (1983) Endocrine responses to inclement weather in naturally breeding populations of white-crowned sparrows (*Zonotrichia leucophrys pugetensis*). *Auk* **100,** 56–62.

Wolf S. H. (1984) Seasonal, age and sex variations in the blood composition of the brown pelican, *Pelecanus occidentalis*. M.S. Thesis, University of South Florida.