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BLOOD PROFILES FOR A WILD POPULATION OF GREEN TURTLES (*CHELONIA MYDAS*) IN THE SOUTHERN BAHAMAS: SIZE-SPECIFIC AND SEX-SPECIFIC RELATIONSHIPS

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ABSTRACT: Blood biochemical profiles and packed cell volumes were determined for 100 juvenile green turtles, *Chelonia mydas*, from a wild population in the southern Bahamas. There was a significant correlation of body size to 13 of the 26 blood parameters measured. Only plasma uric acid and cholesterol were significantly different between male and female turtles. The relationship between total plasma proteins and plasma refractive index was significant. The equation for converting refractive index (Y) to total plasma proteins (X) is $Y = 1.34 + 0.00217(X)$.

Key words: Green turtle, *Chelonia mydas*, sea turtle, blood chemistry, packed cell volume, size and sex variation.

INTRODUCTION

Blood profiles have been used successfully to diagnose chelonian diseases and can be used to assess the physiological status of populations (Rosskopf and Woerpel, 1982; Jacobson et al., 1991). Establishing baseline blood chemical profiles for healthy, wild populations of endangered sea turtle species has been cited as a high priority (Lutcavage, 1990; Balazs and Pooley, 1991). Within the past decade, there has been an alarming worldwide increase in the incidence of cutaneous fibropapilloma in green turtles (*Chelonia mydas*) (Jacobson et al., 1989; Balazs and Pooley, 1991). To help assess the causes of this disease and to evaluate populations that are potentially at risk, baseline studies are needed for natural populations of marine turtles that are not negatively impacted by marine debris, pollution, or habitat degradation. In addition, assessing the physiological status of populations of endangered species is critical to developing appropriate management and conservation plans.

There is little information on blood values of wild sea turtles. Most studies present only a few chemical parameters based on small sample sizes of captive-reared animals or animals of unknown histories; these are reviewed by Dessauer (1970), Dozy et al. (1964), Frair (1977a), and Bonnet (1979). Turtles maintained in captivity may have significantly different blood chemistries as a result of artificial diets or stressful conditions. Comparisons among studies also are hindered by use of different analytical techniques (Bolten, Jacobson and Bjorndal, unpubl. data).

Our objective was to determine the blood chemistry profiles for a wild population of juvenile green turtles on their feeding grounds in waters around the island of Inagua in the southern Bahamas. This population, which is in a remote area protected by the Bahamas National Trust as a wildlife sanctuary, has been the subject of long-term research. The nutritional ecology (Bjorndal, 1980, 1990), individual growth rates (Bjorndal and Bolten, 1988, 1989) and sex ratios (Bolten et al., 1992) of this population have been studied. All turtles in this population appear to be clinically healthy; no turtles have been observed with cutaneous fibropapillomas. We are aware of no other complete blood chemistry profiles for a natural population of green turtles.

METHODS

The study area was described by Bjorndal and Bolten (1988). To minimize the effects of location and season, all turtles were caught at one

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location (21°07'N, 73°34'W), and blood samples were taken during a 10-day field season in April 1988. Turtles were caught by diving from a motor boat onto the turtle. The turtle was brought into the boat and taken to shore where blood samples were obtained within 30 min of capture.

Blood samples were collected from the dorsal, cervical sinus by a non-injurious technique (Owens and Ruiz, 1980) using 22-gauge needles and 7-ml heparinized vacutainer tubes. To determine packed cell volumes (PCV), two 75-mm capillary tubes were filled from the vacutainer tube and centrifuged 5 min in a micro-capillary centrifuge Model MB (Damon/IEC, Needham Heights, Massachusetts, USA) for 5 min. Packed cell volume was recorded as the mean percent packed cell volume of the two capillary tubes. Whole blood in the vacutainer tubes was centrifuged for 5 min. Percent concentration of solids by weight was measured on a small subsample of plasma with a hand-held Model 10431 refractometer (American Optical, Buffalo, New York, USA) and converted to refractive index (Anonymous, 1986). The remaining plasma was pipetted into cryogenic vials and stored in liquid nitrogen. There was no evidence of hemolysis. No more than 15 min elapsed from the time that the blood was drawn until storage of the plasma in liquid nitrogen.

Plasma samples were transported in liquid nitrogen to the University of Florida and stored at −70 C until analyzed. Plasma samples were evaluated using an Olympus AU5061 autoanalyzer (Olympus Corporation, Lake Success, New York, USA) (Hodgin et al., 1988). In a study to establish a protocol for analysis of sea turtle blood chemistry, the Olympus AU5061 was found to have excellent precision (Bolten, Jacobson and Bjorndal, unpubl. data).

The sex of immature green turtles cannot be determined from external characteristics. Circulating testosterone titers have been used successfully to determine the sex of immature green turtles (Owens et al., 1978; Wibbels, 1988). Testosterone concentrations were measured according to a radioimmunoassay method developed for sea turtles (Wibbels, 1988; Wibbels et al., 1990). Turtles with testosterone levels <10 picograms/ml (pg/ml) were classifed as females; turtles with titers >20 pg/ml were classified as males; turtles with values between 10 and 20 pg/ml were classified as undetermined. We used this conservative classification to ensure accurate assignment of sex.

Following blood sampling, straight-line carapace length (SCLm) from anterior to posterior point of midline (nuchal notch to posterior notch) was measured to the nearest 1 mm with anthropometer calipers (GPM model 101, Swiss Precision Instruments, Carlstadt, New Jersey, USA). Turtles were tagged on their flippers with plastic tags and released in the vicinity of capture.

The correlation of SCLm with the blood chemistries and differences between values for male and female turtles were evaluated using Spearman rank correlation, ANOVA and chi-square (SAS Institute Inc., 1982). The relationship between plasma refractive index and plasma total protein was evaluated by linear regression with total protein as the independent variable. The regression met the assumptions of homogeneous variance about the regression line and normal distribution of the residuals about the line (Zar, 1984). Unless otherwise stated, alpha = 0.05 for all analyses.

RESULTS

Packed cell volumes were determined for 38 males, 60 females, and 8 turtles of undetermined sex. The mean (SD) of PCV was 35.2% (3.2) (Table 1). Packed cell volume was not significantly related to difference in sex, or to body size (Table 2). The SCLm ranged from 248 to 669 mm for these animals.

Blood chemistry profiles for 100 green turtles, with a range of SCLm from 248 to 679 mm, are presented in Table 1. There were 41 males, 53 females, and six turtles of undetermined sex. Body size distributions of males and females were not significantly different (chi-square = 1.74, df = 1, $P = 0.188$). Therefore, the effect of size was independent of the effect of sex on the blood chemistry values.

Only two blood chemistry parameters were significantly different between males and females (Table 2). The mean (SD) uric acid value for males was 1.8 (0.6) mg/dl compared with 1.4 (0.5) mg/dl for females ($P = 0.0026$). The mean (SD) cholesterol value for males was 196 (51) mg/dl compared with 235 (51) mg/dl for females ($P = 0.0004$).

Thirteen blood parameters were significantly correlated with turtle size; of those, seven were inversely correlated (Table 2). The variability (R^2) accounted for by body size for these thirteen blood parameters ranged from 4% to 25%. The parameter

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TABLE 1. Packed cell volume (n = 106) and blood chemistry values (n = 100) for juvenile green turtles from a wild population on foraging grounds in the southern Bahamas, April 1988.

Parameters	Mean	SD	CV[a]	Range
Packed cell volume (%)	35.2	3.2	9	26.4–42.0
Glucose (mg/dl)	114	15	13	87–167
Sodium (meq/l)	172	5	3	157–183
Potassium (meq/l)	5.3	0.6	11	4.1–6.9
Chloride (meq/l)	113	5	4	100–130
Carbon dioxide (meq/l)	14	5	36	4–26
Ion balance[b,c] (meq/l)	44	7	16	23–59
Urea nitrogen (BUN) (mg/dl)	7	5	71	2–37
Creatinine (mg/dl)	0.5	0.1	20	0.3–0.9
BUN/creatinine ratio[b]	14	16	114	3–107
Uric acid (mg/dl)	1.5	0.6	40	0.5–3.5
Calcium (mg/dl)	9.1	2.1	23	1.6–12.2
Phosphorus (mg/dl)	6.7	1.2	18	3.8–10.9
Total protein (g/dl)	5.1	0.8	16	2.6–6.9
Albumin (g/dl)	1.5	0.2	13	0.6–2.1
Globulin (g/dl)[b]	3.6	0.7	19	1.9–5.2
Albumin/globulin ratio[b]	0.4	0.1	25	0.3–0.7
Ionized calcium (mg/dl)[b]	4.8	1.2	25	0.8–7.7
Total bilirubin (mg/dl)	0.1	0.04	40	0–0.3
Alkaline phosphatase (U/l)	43	16	37	13–95
Lactic dehydrogenase (U/l)	135	61	45	48–342
SGOT (AST)[d] (U/l)	178	50	28	31–389
SGPT (ALT)[e] (U/l)	6	3	50	1–17
Cholesterol (mg/dl)	217	53	24	73–365
Triglycerides (mg/dl)	172	85	49	43–413
Iron (mcg/dl)	55	15	27	19–88

[a] Coefficient of variation.
[b] Calculated values.
[c] Ion balance = Na − (Cl + CO_2).
[d] Aspartate aminotransferase.
[e] Alanine aminotransferase.

with the largest correlation coefficient was total protein.

Plasma refractive index was measured for 57 green turtles, with SCLm's ranging from 248 to 669 mm. The relationship between plasma refractive index and total plasma proteins was significant ($P < 0.001$, df = 56, R^2 = 92.9%; Fig. 1).

DISCUSSION

Packed cell volume had no significant relationship with sex or body size in this study. Grumbles et al. (1990) also found no significant difference in PCV between sexes in wild adults of the closely related species *Chelonia agassizi*. Wood and Ebanks (1984) and Frair (1977b) reported a significant correlation of body size with PCV. However, these studies used captive green turtles and incorporated a greater body size range than that in our study. As in our study, Frair and Shah (1982) reported a significant correlation between total protein and carapace length for green turtles.

Mean blood chemistry values reported by Norton et al. (1990) for three green turtles from Florida fall within the range of our blood chemistry values, except for sodium, which was lower in the Florida turtles. Mean (SD) total bilirubin values reported as 4.2 (0.9) mg/dl for the Florida turtles by Norton et al. (1990) should have been reported as 0.1 (0.1) mg/dl (E. R. Jacobson, pers. comm.).

The 100 green turtles evaluated for blood chemistry included five individuals, with a range of SCLm from 248 to 274 mm,

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TABLE 2. Relationships of body size (straight carapace length) and sex with packed cell volume (PCV) and blood chemistries for green turtles in the southern Bahamas, April 1988. Relation with body size was analyzed using Spearman rank correlation; differences between male and female turtles were analyzed with ANOVA.

Parameters	Straight-carapace length[a]		Male/female differences[b]	
	r	*P*	*F*	*P*
Packed cell volume (%)	0.012	0.9044	0.31	0.5792
Glucose (mg/dl)	−0.098	0.3331	0.57	0.4529
Sodium (meq/l)	−0.046	0.6485	0.76	0.3844
Potassium (meq/l)	−0.035	0.7319	0.13	0.7145
Chloride (meq/l)	−0.334	0.0007	0.17	0.6776
Carbon dioxide (meq/l)	0.173	0.0858	0.90	0.3449
Ion balance[c,d] (meq/l)	0.074	0.4658	1.05	0.3083
Urea nitrogen (BUN) (mg/dl)	−0.198	0.0479	0.32	0.5702
Creatinine (mg/dl)	0.379	0.0001	0.46	0.4986
BUN/creatinine ratio[c]	−0.370	0.0002	1.68	0.1977
Uric acid (mg/dl)	−0.378	0.0001	9.59	0.0026
Calcium (mg/dl)	−0.207	0.0387	0.72	0.3975
Phosphorus (mg/dl)	−0.043	0.6713	2.64	0.1077
Total protein (g/dl)	0.503	0.0001	1.58	0.2126
Albumin (g/dl)	0.443	0.0001	1.91	0.1698
Globulin (g/dl)[c]	0.452	0.0001	1.06	0.3060
Albumin/globulin ratio[c]	−0.021	0.8391	0.47	0.4964
Ionized calcium (mg/dl)[c]	−0.475	0.0001	0.07	0.7941
Total bilirubin (mg/dl)	0.108	0.2839	0.76	0.3856
Alkaline phosphatase (U/l)	−0.307	0.0019	1.16	0.2846
Lactic dehydrogenase (U/l)	0.174	0.0826	1.14	0.2886
SGOT (AST)[e] (U/l)	−0.004	0.9677	0.01	0.9264
SGPT (ALT)[f] (U/l)	−0.085	0.4027	0.86	0.3564
Cholesterol (mg/dl)	0.196	0.0513	13.65	0.0004
Triglycerides (mg/dl)	0.228	0.0225	0.00	0.9620
Iron (mcg/dl)	0.292	0.0032	0.75	0.3902

[a] For PCV, $n = 106$; for blood chemistries, $n = 100$.
[b] For PCV, $n = 38$ males and 60 females; for blood chemistries, $n = 41$ males and 53 females.
[c] Calculated values.
[d] Ion balance = $Na - (Cl + CO_2)$.
[e] Aspartate aminotransferase.
[f] Alanine aminotransferase.

which had unpigmented plasma; this is in contrast to the yellow pigmented plasma observed in the larger turtles. Because the yellow pigmentation is probably a result of plant pigments (Nakamura, 1980), we believe that these turtles recently had entered the benthic foraging habitat from the open ocean, pelagic habitat and had not yet completed the switch from an omnivorous to the herbivorous diet of the seagrass *Thalassia testudinum* (Bjorndal, 1985). We re-analyzed the data for the differences between males and females and for the effect of body size with these five individuals removed to avoid the potential confounding effect of diet on blood chemistries. The only change in significance between males and females from those shown in Table 2 was a significant difference for phosphorus; the male mean (SD) was 7.0 (1.2) mg/dl whereas the female mean (SD) was 6.5 (1.2) mg/dl ($P = 0.0400$). The only changes in significance of relationships for body size from those shown in Table 2 were that BUN and triglycerides no longer were significant ($P = 0.5325$, $P = 0.2024$, respectively).

In mammals, total plasma protein values often are determined by refractometry, using conversion equations developed from regression of plasma refractive index values and values for total plasma proteins

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measured by the biuret method (Coles, 1986). Refractometry also has been used to determine plasma proteins in reptiles, but the use of mammalian conversion equations may not be accurate because the appropriate conversions need to be established for each species. For the green turtles in our study, the significant relationship between refractive index and total plasma proteins (Fig. 1) yielded the conversion equation

$$Y = 1.34 + 0.00217(X)$$

where Y is the plasma refractive index, and X is the plasma protein concentration (g/dl). Substances other than proteins can affect the concentration of total solids in plasma, and thus change the relationship between refractive index and total plasma proteins. Regression equations should be generated and compared for green turtles in different seasons, on different diets, and at different locations.

One must be cautious when comparing our results with those in the literature based on animals that are captive-reared, fed unnatural diets, or fall outside of the body size range that we studied. Captive-reared green turtles usually are fed a diet high in protein compared with their natural, herbivorous diet. For example, Dessauer (1970) noted that the plasma potassium values reported for green turtles by Holmes and McBean (1964) were low compared with other reptiles. However, Holmes and McBean's (1964) results were based on captive turtles fed a diet of shrimp. In contrast, our values for potassium were within the range for reptiles reported by Dessauer (1970). Similarly, low values for plasma sodium compared with our results have been reported for smaller, captive green turtles (Holmes and McBean, 1964; Kooistra and Evans, 1976).

The mean PCV values reported here are higher than those for captive green turtles reported by Berkson (1966), Lapennas and Lutz (1982), and Frair (1977a) but lower than those reported by Wood and Ebanks (1984). Diet, health, exercise and stress could affect the PCV of turtles maintained in captivity (Frair, 1977a).

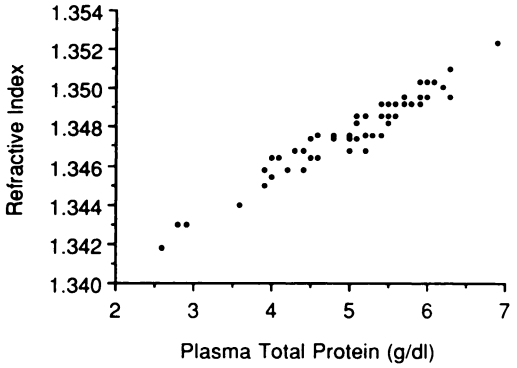

FIGURE 1. Relationship between plasma refractive index and plasma total protein for 57 green turtles in the southern Bahamas, April 1988. The regression equation is $Y = 1.34 + 0.00217(X)$ ($P < 0.001$), where Y is the plasma refractive index and X is the plasma protein concentration (g/dl).

Because of differences in blood chemistries between turtles from natural habitats and captive facilities, it may be inappropriate to use captive animals fed unnatural diets to assess many physiological parameters and extrapolate the results to wild populations. In addition, clinical diagnoses using blood chemistries must take into account the diet of the turtle.

Many of the parameters in Table 1 have a large variance. Although some of the variation may have resulted from sampling non-fasting turtles or from stress of capture, the high degree of variation underscores the importance of large sample sizes for characterizing a population.

Our future investigations on the blood chemistries of green turtles will evaluate the effect of seasonal and geographic factors and natural diet differences (e.g., algae versus seagrasses) on blood parameters. Because of the low variation in both monthly water temperatures and monthly nutrient content of the primary diet plant *T. testudinum* in Inagua (Bjorndal, 1980), we would not expect great seasonal variation in blood chemistries for green turtles there. However, seasonal changes in blood chemistries should be evaluated, particularly in temperate habitats. To more rig-

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orously test the relationship of body size to blood parameters, we will expand the range of body sizes evaluated.

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