Adrenal and Hematological Responses to Stress in Juvenile Green Turtles (*Chelonia mydas*) with and without Fibropapillomas

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Abstract

This study reports baseline adrenocortical, hematological, and plasma biochemical values for clinically healthy juvenile green turtles from a discrete population at Kaneohe Bay, island of Oahu, Hawaii. Using a general linear modeling program, we compared mean values for these parameters with mean values of a group afflicted with green turtle fibropapillomas (GTFP). Turtles of similar size classes from both groups were collected under the same conditions in the same study area and season at the same time of the day. Corticosterone, hematological, and enzymatic responses to acute and chronic stress were characterized for each group at four different sampling periods: 0 h (within 2 min of capture), 1 h, 3–4 h, and 24 h postcapture. On the basis of the differences identified between groups and times within a group, we conclude that turtles with GTFP are chronically stressed and immunosuppressed.

Introduction

Green turtle fibropapillomas (GTFP) afflict several green turtle (*Chelonia mydas*) populations in epidemic proportions throughout the world (Wil-

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liams et al. 1994). The primary etiology is unknown, although a virus has been suggested as the causative agent. Chronic stress coupled with environmental factors (e.g., water temperature, excessive solar radiation, and pollutants) that impair the immune system are among other possible etiologic factors (Balazs 1991; Aguirre et al. 1994*a*, 1994*b*).

The effects of chronic stress in captive reptiles have been addressed previously (Hoff, Frye, and Jacobson 1984; Lance 1990); however, stress in wild populations remains largely uninvestigated. Detection of a stress response in an animal population is difficult because of the diverse physiological and psychological responses to different stressors. This is complicated by the high variability of behavioral, autonomic, and neuroendocrine responses in individuals. In addition, there has been a failure to correlate measures of stress with meaningful changes in the well-being of animals (Fiennes 1982; Breazile 1987; Spraker 1993). Nevertheless, several physiological responses to a stressor, including adrenocortical, hematologic, and biochemical parameters, are measurable (Moberg 1987). An increase in hematocrit, an elevation of circulating corticosterone, and changes in blood cell counts, enzymes, and heterophil/lymphocyte (H/L) ratios have been used to determine stress levels in several reptile species (Duggan 1981; Moberg 1985; Mahapatra, Mahata, and Maiti 1991; Kreger and Mench 1993). These parameters may provide basic information on the response of clinically healthy and tumored turtles to environmental stressors and the correlation of those stressors with disease and immunity (Aguirre 1991).

The objective of this study was to determine baseline adrenal, hematological, and biochemical values of clinically healthy green turtles and turtles with GTFP. In addition, the responses to acute (capture) stress and chronic (disease) stress responses were determined in both groups.

Material and Methods

Field Sampling

Serial blood samples were collected from five clinically healthy turtles and five turtles with GTFP, at Kaneohe Bay (21°30′ N, 15⁻°50′ W), island of Oahu, Hawaii, September 20–22, 1993. We captured green turtles by hand alive and unharmed while snorkeling from a boat. Each turtle was brought into the boat, and a blood specimen was taken by venipuncture from the dorsal postoccipital sinuses (Owens and Ruiz 1980) immediately (within 2 min) after capture. Additional blood samples were taken at 1 h, 3–4 h, and 24 h postcapture prior to the turtles' release into the wild. Blood (3–5 mL) was collected with a 21-gauge needle and 5-mL syringes. Blood collection

time did not exceed 30 s and only occasionally exceeded 15 s. After the first bleeding, turtles were transported to the Hawaii Institute of Marine Biology on Coconut Island in Kaneohe Bay, where they were kept cool on their plastron in a shady place and covered with wet towels until the next bleeding took place. Between the 3–4-h and 24-h sampling periods, turtles were kept overnight in a saltwater (26°C) tank.

Each blood sample was split into two heparinized Vacutainer tubes. One tube was sent overnight in Blue Ice packs (Rubbermaid, Wooster, Ohio) to Veterinary Diagnostics (West Sacramento, Calif.) for a complete hemogram and differential. The plasma of the second tube was separated by centrifugation and split in two vials. One vial was immediately stored in an ultra-freezer at −70°C for hormonal analysis. A second vial was sent to the SmithKline Beecham Laboratory (Honolulu, Hawaii) for a 25-element blood chemistry analysis. Hemolyzed samples were discarded.

After the 24-h bleeding period, turtles were measured, tagged, and weighed following techniques previously described (Balazs, Forsyth, and Kam 1987). All turtles were thoroughly examined for the presence of fibropapillomas. A description of the size, number, and location of tumors was made in turtles with GTFP. Turtles were coded by their degree of tumor severity on a scale of 1–4, with a tumor score of 4 being the most severe case (Balazs 1991).

Corticosterone Radioimmunoassay

Plasma samples were analyzed for corticosterone with standard radioimmunoassay (RIA) procedures. Specimens were extracted with 5 mL diethyl ether prior to RIA analysis. Each sample was analyzed in duplicate, and results were corrected for the extraction efficiency of 83% ± 6.1%. Standard curves were prepared in phosphate-buffered saline (PBS) with known amounts of radioinert corticosterone (10, 25, 50, 100, 250, 500, 1,000, and 2,000 pg/mL) purchased from Amersham Corporation (Arlington Heights, Ill.). The minimum concentration per tube, which was distinguishable from zero, was 18.3 pg/mL. Cross-reactivities of the corticosterone antiserum (no. 07-189016, ICN Biomedicals, Costa Mesa, Calif.) with other steroids were 6.1% for desoxycorticosterone, 0.29% for progesterone, 0.19% for cortisol, and <0.1% for all other steroids examined.

Pooled plasma samples (approximately 740 pg/mL) were assayed serially in volumes of 10, 25, 50, 75, and 100 µL (final volume of 100 µL with charcoal-stripped plasma). The inhibition curve for corticosterone was parallel to the standard curve, with the test of homogeneity of regression indicating that the curves did not differ. Further characterization of the assay involved

measurement of known amounts (5, 10, 25, 50, 100, 250, and 500 pg) of the radioinert hormone in 100 μL charcoal-stripped plasma. For corticosterone ($Y = 10.1 + 0.947X$, where Y is the amount of corticosterone measured [pg] and X is the amount of corticosterone added [pg]; $R^2 = 0.8793$), interassay and intrassay coefficients of variation were 7.9% and 10.3%, respectively. Aliquots from each sample (100 μL) were extracted with 5 mL diethyl ether. After vortexing for 1 min, the aqueous phase was frozen in a dry-ice methanol bath and the ether phase poured off into 12 × 75-mm glass tubes and evaporated. Then, PBS buffer was added to standards (250 μL) and dried samples (300 μL). The tritiated hormone (100 μL) and each antiserum (100 μL) were added to all standard and sample tubes. All tubes were incubated overnight at 4°C. After incubation, 250 μL of dextran-coated charcoal (0.5% charcoal and 0.05% dextran) was added to each tube to separate the unbound hormone from the antibody-bound fraction. All tubes were centrifuged (1,200 rpm) for 10 min. A sample (0.5 mL) of the supernatant was drawn from each tube and mixed with 3.5 mL of scintillation cocktail (Scintiverse BD, Fisher Scientific) in plastic scintillation vials. All vials were counted for 1 min, and hormone titers in ng/mL were calculated with the standard curve generated in the assays (Gross et al. 1993).

Plasma Biochemistry

In the SmithKline Beecham Laboratory, plasma samples were analyzed with an Olympus AU5061 autoanalyzer (Olympus, Lake Success, N.Y.) as suggested by Bolten, Jacobson, and Bjorndal (1992). Among the 25 parameters analyzed, plasma chemistry profiles including total protein, selected enzyme activity, urea nitrogen, cholesterol, glucose, and creatinine were compared and correlated with hematologic and hormonal values in both healthy turtles and turtles with tumors.

Hemogram and Differential

Hematologic erythrocyte (RBC) count, leukocyte (WBC) count, hemoglobin (HGB), packed cell volume (PCV, or hemotocrit), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured on a Coulter Counter, model S-Plus IV (Coulter Electronics, Hialeah, Fla.), in accordance with standard laboratory procedures. The WBC differential counts (heterophils, neutrophils, lymphocytes, eosinophils, and basophils) were performed manually as described by Hawkey and Dennett (1989). The blood characteristics, in addition to H/L ratios, were used to evaluate and compare values

of turtles of this study with similar data from other green turtle studies and those of other marine turtle species. The blood data were also used to evaluate responses to capture stress, parasites, and GTFP.

Statistical Analysis

Statistical analysis was conducted on raw and log-transformed data by means of the univariate approach for repeated-measures (split-plot) ANOVA design (SAS Institute 1990). This general linear model was used to determine differences between hormone, blood cell count, and plasma chemistry values for both turtle groups. This analysis assessed between-group effects, within-time effects (sampling periods within a group), and interactions between group and time effects. Least significant difference (0.05) values for comparing means in a split-plot design were based on formulas from standard errors for a split-plot design (Steel and Torrie 1980). Differences were considered significant at $\alpha \leq 0.05$. Data are expressed as mean, SD, and range of values for each blood parameter.

Results

Five clinically healthy, juvenile green turtles with a mean weight of 16.9 ± 3.1 kg (13–22 kg) and a mean straight carapace length (SCL) of 48.7 ± 3.2 cm (44.7–52.9 cm) served as the control group. Five green turtles with a mean weight of 21 ± 7.7 kg (15–36 kg) and a mean SCL of 53.5 ± 6.0 cm (48.4–64.9 cm) presented multiple cutaneous and conjunctival fibropapillomas (fig. 1). Their score for overall degree of tumor severity for the turtles with GTFP averaged 2.8 ± 0.4 (2–3).

Corticosterone Values

The repeated-measures ANOVA revealed significant interactions between time and group for corticosterone levels. Mean corticosterone concentrations in plasma increased from 0 h to 1 h postcapture in both groups and were significantly higher in turtles with GTFP. For both groups, mean concentration levels peaked 3–4 h postcapture. During the 24-h period, corticosterone concentrations remained at peak levels for the turtles with GTFP but significantly declined in the healthy group (fig. 2), although not to the concentration of the capture sample (0 h).

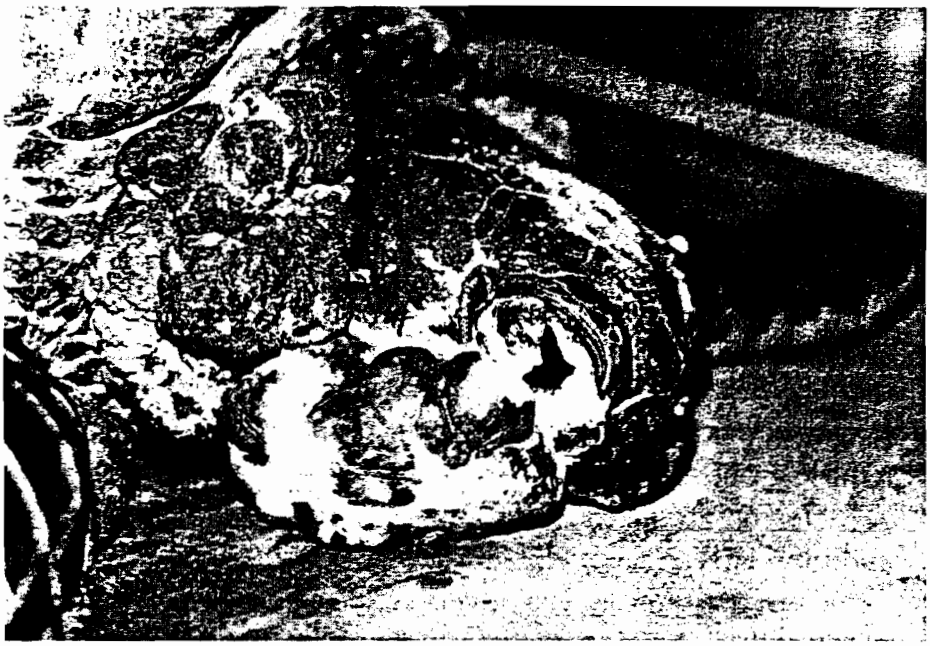


*Fig. 1. Juvenile green turtle (*Chelonia mydas*) demonstrating severe ocular and neck fibropapillomas.*

Plasma Biochemistry Values

Mean, SD, and range for blood biochemistry profiles for clinically healthy green turtles (table 1) and turtles with fibropapillomas (table 2) are reported

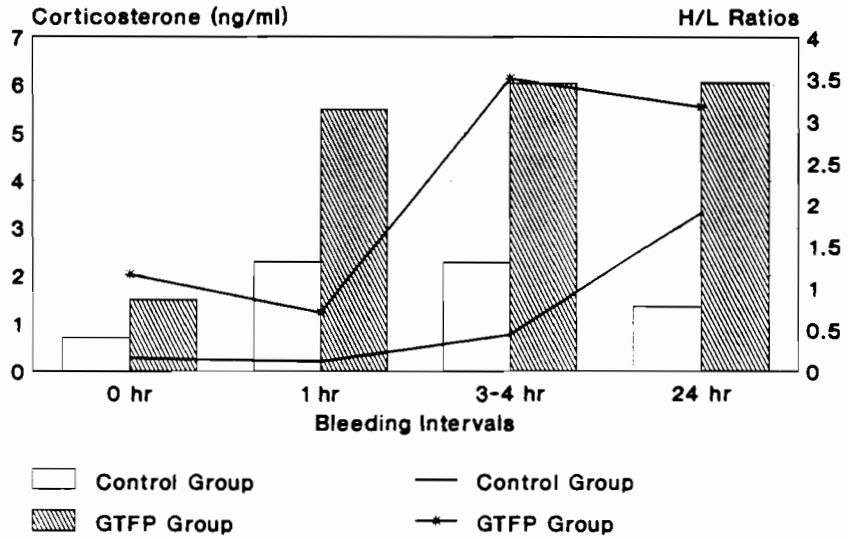


*Fig. 2. Mean corticosterone concentrations and H/L ratios at four bleeding intervals for juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas, captured at Kaneohe Bay, island of Oahu, Hawaii, 1993.*

for each bleeding interval. Total protein values for both groups remained relatively constant, but a slight hypoproteinemia was evident in the GTFP group (3.44 ± 0.62 g/dL) 24 h postcapture when compared with the healthy turtles (4.3 ± 0.22 g/dL). Albumin levels for the control group increased from 0.92 (± 0.38) g/dL at initial bleeding to 1.25 (± 0.44) g/dL 3–4 h postcapture. Significantly higher globulin values were identified in the initial and final sampling periods for the healthy turtles. Except for the bleeding period immediately after capture (0 h), total bilirubin values were higher for the control group. Triglyceride levels were higher for the control group at all times, significantly declining over time. Glucose levels differed between groups 3–4 h postcapture, with the control group manifesting a slight hyperglycemia (fig. 3).

Blood enzymes, including alanine amino transferase (ALAT), asparmate amino transferase (ASAT), and lactate dehydrogenase (LDH), presented similar values in both groups and remained unchanged from the 0-h to the 3–4-h period. Levels of ASAT and LDH significantly increased in the GTFP group in the 24-h period. Alkaline phosphatase values were consistently higher at all times for healthy turtles. Gamma glutamyl transferase (GGT) levels were higher for the GTFP group at 0 h and significantly decreased between 1 h and 3–4 h postcapture (fig. 4).

Higher urea nitrogen values and blood urea notrogen (BUN)/creatinine ratios were detected in the GTFP group at all times. These ratios significantly increased between the 3–4-h and 24-h sampling periods in this group. Iron and phosphorus levels were significantly higher for the control group, but no group or time differences were detected for sodium, potassium, and chloride (fig. 5).

Hematological Values

Mean, SD, and range of hematological values for clinically healthy green turtles (table 3) and turtles with GTFP (table 4) were summarized for each bleeding period. The turtles with GTFP had significantly lower HGB and PCV values (fig. 6). The RBC counts declined in the control group over the 24-h sampling period. No significant differences were detected between groups for MCV, MCH, or MCHC. The MCV and MCH values significantly increased in turtles with fibropapillomas between the 3–4-h and 24-h sampling periods.

Higher WBC counts were identified in healthy turtles when compared with turtles with GTFP, except 24 h postcapture when WBC counts increased significantly for sick turtles and declined for healthy turtles. Heterophils, neutrophils, lymphocytes, eosinophils, and basophils were analyzed as both absolute numbers and percentages, with WBC count as a covariate (fig. 7). Heterophils

TABLE 1

*Plasma corticosterone and biochemistry values for each sampling period for clinically healthy green turtles (*Chelonia mydas*), Kaneohe Bay, island of Oahu, Hawaii, 1993*

Plasma Biochemistry Variable	0 h	
	Mean ± SD	Range
Corticosterone (ng/mL)	.70 ± .24	.46–.98
Protein (g/dL)	4.32 ± .58	3.8–5.3
Albumin (g/dL)	.93 ± .38	.6–1.3
Globulin (g/dL)	2.98 ± .66	2.4–3.6
Albumin/globulin ratio	.35 ± .17	.2–.5
Total bilirubin (mg/dL)	.14 ± .11	.0–.3
Direct bilirubin (mg/dL)	.10 ± .11	.0–.3
Indirect bilirubin (mg/dL)	.08 ± .08	.0–.2
Alanine amino transferase (U/L)	5.80 ± 4.71	2.0–14
Asparmate amino transferase (U/L)	141.00 ± 51.20	81–223
Alkaline phosphatase (U/L)	42.40 ± 10.69	27–52
Gamma glutamyl transferase (U/L)	.40 ± .55	.0–1.0
Lactate dehydrogenase (U/L)	109.40 ± 49.53	55–171
Blood urea nitrogen (BUN) (mg/dL)	1.00 ± 1.00	.0–2.0
Creatinine (mg/dL)	.26 ± .09	.2–.4
BUN/creatinine ratio	3.66 ± 4.15	.0–10
Uric acid (mg/dL)	.84 ± .23	.6–1.2
Calcium (mg/dL)	8.42 ± 1.02	7.2–9.7
Phosphorus (mg/dL)	7.88 ± .93	6.9–9.3
Cholesterol (mg/dL)	117.80 ± 32.77	92–173
Triglycerides (mg/dL)	181.80 ± 104.64	62–351
Glucose (mg/dL)	86.60 ± 13.90	76–111
Iron (µg/dL)	46.00 ± 31.13	25–99
Sodium (meq/L)	152.20 ± 2.39	149–155
Potassium (meq/L)	4.98 ± .84	4.0–6.1
Chloride (meq/L)	109.00 ± 6.20	102–117

1 h		3–4 h		24 h	
Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
2.29 ± 1.33	1.20–4.57	2.70 ± .88	2.02–3.83	1.36 ± .91	.36–2.52
4.46 ± .46	3.8–5.1	4.06 ± .48	3.5–4.8	4.30 ± .22	4.0–4.6
1.20 ± .41	.6–1.5	1.25 ± .43	.6–1.5	1.00 ± .46	.6–1.4
2.73 ± .52	2.4–3.5	2.73 ± .52	2.4–3.5	2.98 ± .66	2.4–3.6
.48 ± .19	.2–.6	.48 ± .19	.2–.6	.40 ± .23	.2–.6
.16 ± .11	.0–.3	.16 ± .11	.0–.3	.14 ± .09	.0–.2
.06 ± .05	.0–.1	.08 ± .04	.0–.1	.08 ± .04	.0–.1
.08 ± .08	.0–.2	.10 ± .07	.0–.2	.06 ± .05	.0–.1
3.60 ± 1.95	1.0–5.0	3.20 ± 2.28	.0–6.0	4.60 ± 1.82	3.0–7.0
127.20 ± 28.46	85–155	123.20 ± 19.80	97–147	140.20 ± 41.13	91–202
42.00 ± 10.34	26–55	38.40 ± 8.70	24–45	38.80 ± 6.53	30–46
.80 ± .84	.0–2.0	1.00 ± 1.00	.0–2.0	1.00 ± .71	.0–2.0
135.20 ± 56.13	54–189	99.00 ± 28.59	75–146	210.60 ± 52.55	148–266
.70 ± .84	.0–2.0	1.60 ± .89	.0–2.0	2.20 ± .84	1.0–3.0
.28 ± .16	.0–.4	.28 ± .04	.2–.3	.26 ± .05	.2–.3
3.63 ± 4.07	.0–9.8	6.02 ± 3.66	.0–10	8.68 ± 3.97	5.0–15.0
1.10 ± .30	.6–1.4	1.36 ± .50	.9–2.2	1.04 ± .15	.8–1.2
9.16 ± 1.44	7.2–11.1	9.58 ± 2.12	6.5–12.1	7.22 ± .49	6.6–7.7
7.94 ± .77	7.1–9.0	7.36 ± .72	6.4–8.3	7.56 ± .86	6.8–9.0
121.60 ± 27.24	99–167	107.80 ± 19.57	89–136	121.00 ± 27.34	98–165
166.60 ± 27.24	62–307	109.00 ± 49.07	57–167	97.00 ± 48.09	41–153
101.00 ± 11.89	87–118	143.40 ± 40.63	88–195	106.00 ± 13.58	84–121
55.20 ± 32.20	27–110	38.80 ± 10.18	28–49	45.20 ± 14.91	29–69
155.40 ± 1.67	154–158	154.80 ± 3.11	151–158	153.80 ± 3.11	150–157
4.92 ± .31	4.4–5.2	4.54 ± .43	4.0–5.0	4.02 ± .44	3.5–4.7
112.40 ± 4.72	106–117	114.80 ± 5.07	110–122	107.40 ± 3.51	103–112

TABLE 2

*Plasma croticosterone and biochemistry values for each sampling period for green turtles (*Chelonia mydas*) with fibropapillomas, Kaneohe Bay, island of Oahu, Hawaii, 1993*

Plasma Biochemistry Variable	0 h	
	Mean ± SD	Range
Corticosterone (ng/mL)	1.49 ± 1.71	.3–4.46
Protein (g/dL)	3.96 ± .31	3.6–4.3
Albumin (g/dL)	1.12 ± .36	.5–1.4
Globulin (g/dL)	2.46 ± .31	2.0–2.8
Albumin/globulin ratio	.48 ± .08	.4–.6
Total bilirubin (mg/dL)	.20 ± .10	.1–.3
Direct bilirubin (mg/dL)	.08 ± .05	.0–.1
Indirect bilirubin (mg/dL)	.18 ± .05	.1–.2
Alanine amino transferase (U/L)	4.00 ± 1.00	3.0–5.0
Asparmate amino transferase (U/L)	128.00 ± 40.03	91–178
Alkaline phosphatase (U/L)	23.00 ± 9.46	12–37
Gamma glutamyl transferase (U/L)	.80 ± .84	.0–2.0
Lactate dehydrogenase (U/L)	145.80 ± 46.25	102–223
Blood urea nitrogen (BUN) (mg/dL)	8.10 ± 8.11	2.0–22.0
Creatinine (mg/dL)	.26 ± .09	.2–.4
BUN/creatinine ratio	39.00 ± 42.19	5.0–110
Uric acid (mg/dL)	1.00 ± .29	.5–1.2
Calcium (mg/dL)	7.88 ± 2.46	4.8–11.4
Phosphorus (mg/dL)	5.88 ± .96	4.5–6.8
Cholesterol (mg/dL)	116.80 ± 13.29	97–130
Triglycerides (mg/dL)	86.00 ± 34.84	57–144
Glucose (mg/dL)	78.80 ± 6.91	68–86
Iron (µg/dL)	26.60 ± 9.84	10–35
Sodium (meq/L)	154.60 ± 7.23	150–167
Potassium (meq/L)	4.76 ± .61	4.1–5.5
Chloride (meq/L)	116.00 ± 4.36	112–123

1 h		3–4 h		24 h	
Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
5.50 ± 2.50	2.57–8.61	6.03 ± 2.20	2.14–7.38	6.04 ± 2.31	2.52–8.83
4.04 ± .61	3.2–4.6	3.76 ± .62	3.0–4.5	3.44 ± .59	3.1–4.5
1.16 ± .28	.7–1.4	1.14 ± .27	.7–1.4	1.14 ± .27	.7–1.4
2.46 ± .36	1.9–2.8	2.44 ± .34	1.9–2.7	2.36 ± .29	2.0–2.7
.48 ± .08	.4–.6	.46 ± .09	.4–.6	.46 ± .09	.4–.6
.06 ± .13	.0–.3	.02 ± .05	.0–.1	.06 ± .09	.0–.2
.04 ± .05	.0–.1	.02 ± .05	.0–.1	.02 ± .05	.0–.1
.12 ± .11	.0–.2	.06 ± .09	.0–.2	.12 ± .08	.0–.2
4.20 ± 2.17	1.0–6.0	4.20 ± 3.11	.0–7.0	4.00 ± 1.41	2.0–5.0
147.0 ± 2.17	110–190	140.80 ± 37.44	87–185	202.60 ± 44.18	146–260
25.60 ± 13.01	17–48	23.40 ± 12.82	14–45	26.80 ± 9.09	16–41
1.20 ± 1.30	.0–3.0	.40 ± .55	.0–1.0	.60 ± .55	.0–1.0
173.40 ± 65.90	120–275	133.60 ± 38.16	81–187	371.20 ± 254.18	87–747
7.80 ± 9.09	.0–23.0	8.00 ± 8.52	.0–22	12.20 ± 10.83	27.0–24.0
.30 ± .07	.2–.4	.28 ± .08	.2–.4	.24 ± .09	.1–.3
33.66 ± 46.73	.0–115	36.00 ± 44.64	.0–110	74.68 ± 92.20	6.7–230
1.16 ± .30	.9–1.6	1.26 ± .38	.7–1.7	1.26 ± .21	1.1–1.6
8.14 ± 1.20	6.8–9.8	8.70 ± 1.48	6.4–10.4	7.18 ± .58	6.6–8.0
6.38 ± 1.40	4.4–7.9	6.56 ± 1.12	4.9–7.6	6.74 ± 1.03	6.0–8.4
106.20 ± 25.70	66–130	97.60 ± 21.87	62–120	104.00 ± 24.09	63–125
62.60 ± 23.97	30–87	54.00 ± 17.76	26–72	57.60 ± 14.74	37–78
94.20 ± 9.50	82–104	116.20 ± 23.98	95–155	100.60 ± 20.43	76–127
31.60 ± 11.70	13–43	31.40 ± 12.95	10–45	22.00 ± 11.11	9.0–34.0
153.60 ± 3.13	151–159	152.00 ± 1.87	150–154	152.80 ± 2.17	151–156
4.64 ± .27	4.2–4.9	4.18 ± .37	3.8–4.8	4.10 ± .24	3.8–4.3
116.40 ± 2.97	113–121	115.60 ± 7.50	109–126	108.80 ± 4.76	103–116

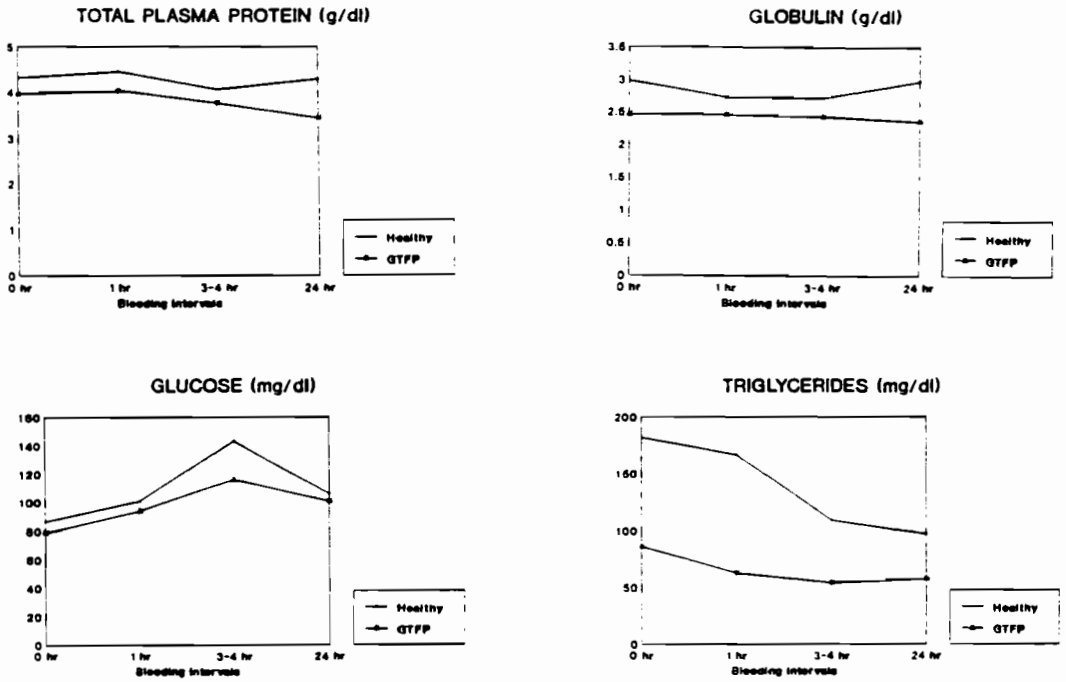


*Fig. 3. Mean total plasma protein, globulin, triglyceride, and glucose values at four bleeding intervals for juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas, captured at Kaneohe Bay, island of Oahu, Hawaii, 1993.*

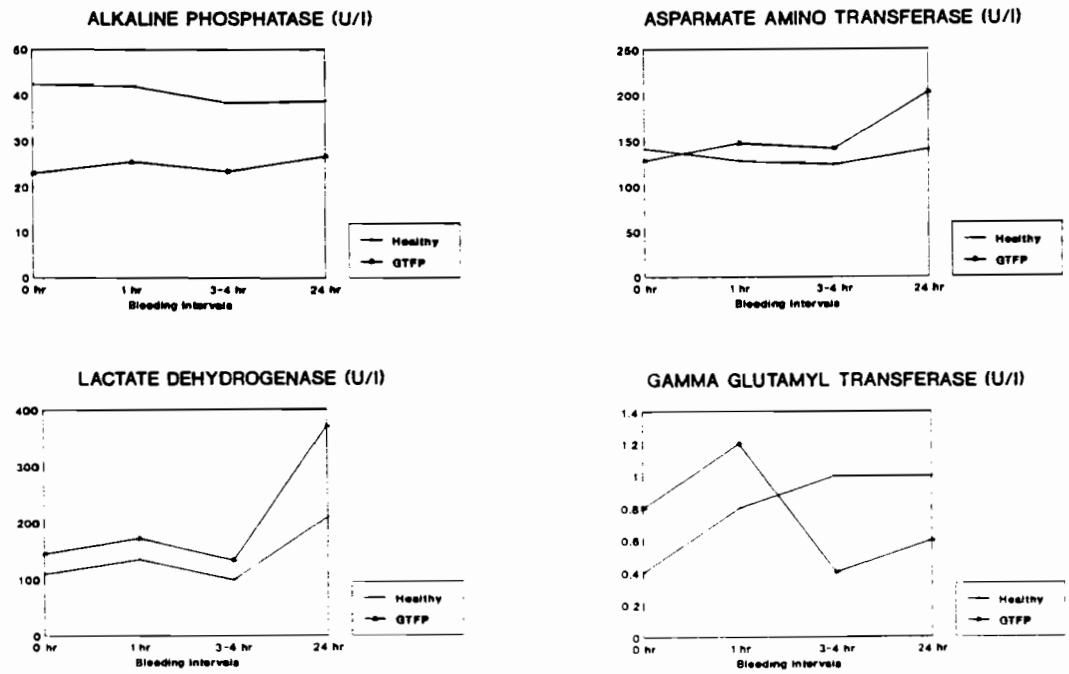


*Fig. 4. Mean ASAT, LDH, ALAT, and GGT values at four bleeding intervals for juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas, captured at Kaneohe Bay, island of Oahu, Hawaii, 1993.*

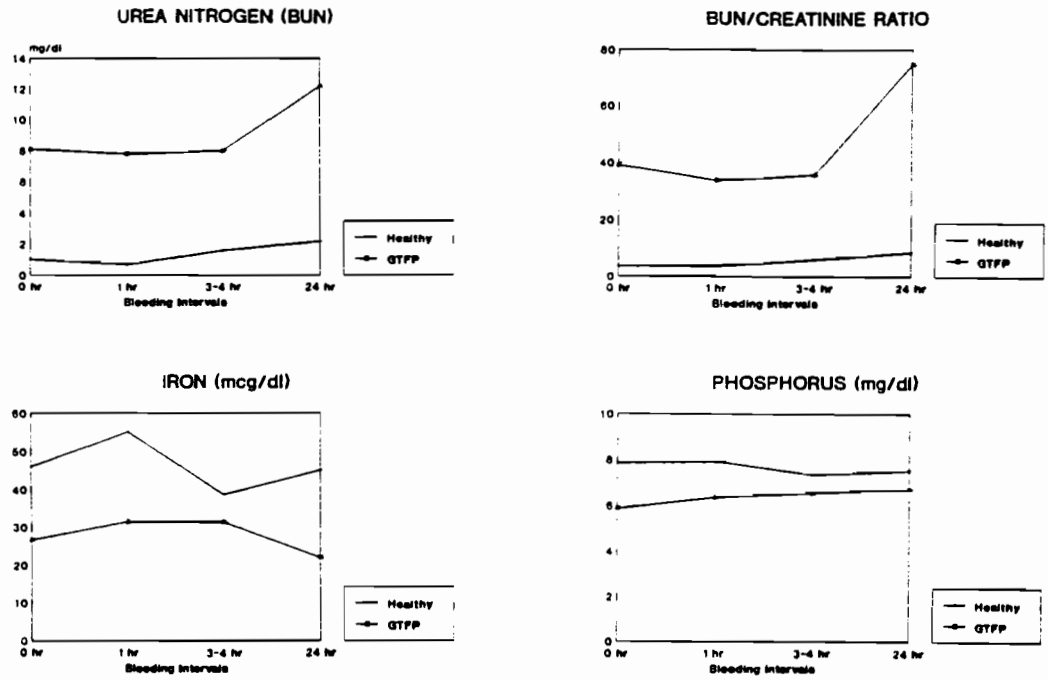


*Fig. 5. Mean BUN, BUN/creatinine ratio, and values for iron and phosphorus at four bleeding intervals for juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas, captured at Kaneohe Bay, island of Oahu, Hawaii, 1993.*

increased significantly for both groups over the 24-h sampling period. Differences were significant between groups at 0-h, 1-h, and 3–4-h periods, with higher absolute numbers in the GTFP group. Significant neutrophilia was detected for the group with GTFP. Consistently higher number and percentages of lymphocytes were detected for the healthy turtles, except during the 24-h period, when numbers were similar for both groups. A significant lymphocytopenia occurred for both groups 24 h postcapture. Number and percentages of eosinophils and basophils were similar between groups at all times. Ratios of H/L were similar for both groups at the initial sampling but increased significantly for the GTFP group 1 h, 3–4 h, and 24 h postcapture. This difference was evident when a significant increase in ratios occurred between the time of the initial sampling and 1 h postcapture for the same group (fig. 2).

Discussion

Corticosterone Values

This study documents baseline (0 h) levels of plasma corticosterone concentrations for clinically healthy juvenile Hawaiian green turtles. These lev-

TABLE 3

*Hematological values for each sampling period for clinically healthy green turtles (*Chelonia mydas*), Kaneohe Bay, island of Oahu, Hawaii, 1993*

	0 h			1 h	
Hematological Variable	Mean ± SD		Range	Mean ± SD	
PCV (%)	31.20	± 4.97	28–40	31.00 ±	4.00
RBC (10^6/mL)	.48	± .16	.23–.63	.47 ±	.13
HGB (g/dL)	8.54	± 1.44	7.3–10.9	8.52 ±	1.14
MCV (fL)	725.00	± 312.35	444–1261	681.60 ±	150.81
MCH (pg)	198.18	± 83.52	115.9–339.1	188.76 ±	51.52
MCHC (g/dL)	27.50	± 1.25	26.7–29.7	27.50 ±	1.30
WBC (10^3/mL)	9.34	± 4.44	3.3–15.5	7.82 ±	3.22
Heterophils (10^3/mL)	876.20	± 441.52	429–1581	530.40 ±	264.38
Heterophils (%)	10.400	± 4.67	5–17	7.80 ±	3.96
Neutrophils (10^3/mL)	711.60	± 915.87	156–2325	251.40 ±	193.21
Neutrophils (%)	5.60	± 5.41	2–15	3.20 ±	1.64
Lymphocytes (10^3/mL)	6118.20	± 2864.54	2739–9180	5478.60 ±	2670.34
Lymphocytes (%)	67.80	± 15.40	51–85	68.80 ±	10.08
Eosinophils (10^3/mL)	1197.80	± 1269.34	99–2635	1031.20 ±	811.29
Eosinophils (%)	12.00	± 12.77	1–32	13.60 ±	10.26
Basophils (10^3/mL)	495.60	± 407.04	0–930	528.40 ±	443.12
Basophils (%)	6.75	± 1.71	5–9.0	6.60 ±	4.34
H/L ratio	.16	± .07	.09–.27	.12 ±	.07

els were compared with those of a group of turtles with GTFP that were captured under similar conditions. Plasma corticosterone concentrations of green turtles increased in response to acute stress in both control and GTFP groups. Our study demonstrated that the response of juvenile Hawaiian green turtles to acute stress was similar to that reported for other reptiles (Duggan 1981; Mahapatra et al. 1991; Gregory 1994).

Plasma glucocorticoid concentrations have been used as a quantitative index of stress levels in a population. The positive correlation of increased plasma corticosterone concentrations with acute stress (capture, restraint, and repeated bleeding) in Hawaiian *Chelonia mydas* in our study was similar to that in findings for loggerhead turtles (*Caretta caretta*) (Wibbels et al. 1990; Gregory 1994). The highest corticosterone levels were identified 3–4 h postcapture. Corticosterone levels for the control group did not correlate with levels reported for *C. caretta* caught at Port Canaveral (Gregory 1994).

	3–4 hr			24 h		
Range	Mean ± SD		Range	Mean ± SD		Range
28–38	28.00 ±	4.53	25–36	26.80 ±	4.66	24–35
.31–.64	.40 ±	.12	.28–.59	.34 ±	.09	.19–.44
7.4–10.4	7.48 ±	1.35	5.7–9.4	7.46 ±	1.31	6.8–9.8
545–935	735.80 ±	207.00	441–929	851.20 ±	296.47	545–1316
145.5–277.4	199.76 ±	70.47	116.9–275.0	235.96 ±	78.44	156.8–357.9
26.4–29.7	26.82 ±	3.77	21.1–30.8	27.88 ±	1.21	26.2–29.2
3.8–10.8	9.38 ±	3.77	6.8–16.0	7.08 ±	1.58	5.0–8.8
324–990	1812.80 ±	1221.62	255–3200	4029.60 ±	1375.94	2520–5229
3–11	20.20 ±	14.31	3–41	55.80 ±	9.42	42–65
100–540	683.40 ±	797.74	136–2080	493.60 ±	424.66	120–1162
1–5	6.00 ±	4.36	2–13	7.00 ±	5.66	2–14
2774–8715	5645.40 ±	2727.40	2652–8800	2255.60 ±	570.74	1660–2904
56–83	59.80 ±	21.56	39–90	33.00 ±	10.07	20–47
228–2268	809.80 ±	714.73	170–1898	274.00 ±	190.61	88–540
6–28	9.40 ±	9.71	2–26	4.00 ±	3.46	1–9
200–1260	428.60 ±	282.18	146–800	205.00 ±	83.44	146–264
3–14	4.60 ±	2.88	2–9	2.50 ±	.71	2–3
.04–.18	.44 ±	.40	.03–1.05	1.90 ±	.87	.89–3.15

Glucocorticoids are known to reduce immunity and thus increase susceptibility to infectious agents in birds and mammals (Spraker 1993). Few studies, however, are available related to chronic stress in reptiles (Lance 1990). Corticosterone monitoring and selected blood parameters may provide an index of interrenal function and quantification of chronic stress and immunosuppression in a sea turtle population. An increase in PCV, a shift in corticosterone, and lymphocytopenia have been described among the physiologic changes due to chronic stress in reptiles. Lance (1990) reported that juvenile alligators (*Alligator mississippiensis*) responded to chronic stress with chronically elevated corticosterone secretion and changes in the leukogram. Significantly higher levels of corticosterone, larger H/L ratios, and increased neutrophilia in turtles with GTFP indicated that this group was immunosuppressed and chronically stressed. Corticosterone-related immunosuppression is manifested by neutrophilia, lysis and margination of T

TABLE 4

Hematological values for each sampling period for green turtles (Chelonia mydas) *with fibropapillomas, Kaneohe Bay, island of Oahu, Hawaii, 1993*

	0 h			1 h	
Hematological Variable	Mean ± SD		Range	Mean ± SD	
PCV (%)	21.80 ±	5.40	13–27	20.40 ±	8.79
RBC (10^6/mL)	.32 ±	.10	.24–.49	.29 ±	.12
HGB (g/dL)	5.78 ±	1.82	2.9–7.6	5.42 ±	2.63
MCV (fL)	705.00 ±	170.14	510–871	701.80 ±	211.51
MCH (pg)	186.16 ±	58.73	120.8–245.2	183.84 ±	64.85
MCHC (g/dL)	26.06 ±	2.60	22.3–28.7	25.86 ±	2.73
WBC (10^3/mL)	6.98 ±	3.49	3–11	6.30 ±	5.60
Heterophils (10^3/mL)	1716.20 ±	283.59	1320–2060	1190.40 ±	464.48
Heterophils (%)	30.80 ±	16.45	12–55	25.80 ±	10.55
Neutrophils (10^3/mL)	627.20 ±	795.96	53–2014	444.40 ±	183.00
Neutrophils (%)	13.25 ±	16.58	3–38	11.80 ±	8.41
Lymphocytes (10^3/mL)	3564.00 ±	2929.82	720–6798	3285.40 ±	3403.31
Lymphocytes (%)	44.40 ±	23.16	15–66	44.80 ±	14.20
Eosinophils (10^3/mL)	574.40 ±	185.81	420–880	1193.00 ±	1493.54
Eosinophils (%)	9.20 ±	2.95	6–14	15.00 ±	6.25
Basophils (10^3/mL)	603.50 ±	799.65	33–1760	300.33 ±	424.66
Basophils (%)	6.00 ±	6.88	1–16	4.00 ±	2.65
H/L ratio	1.15 ±	1.10	.21–2.40	.70 ±	.52

cells, monocytes, and eosinophils, and decrease of lymphoid cell proliferation (Breazile 1987, 1988).

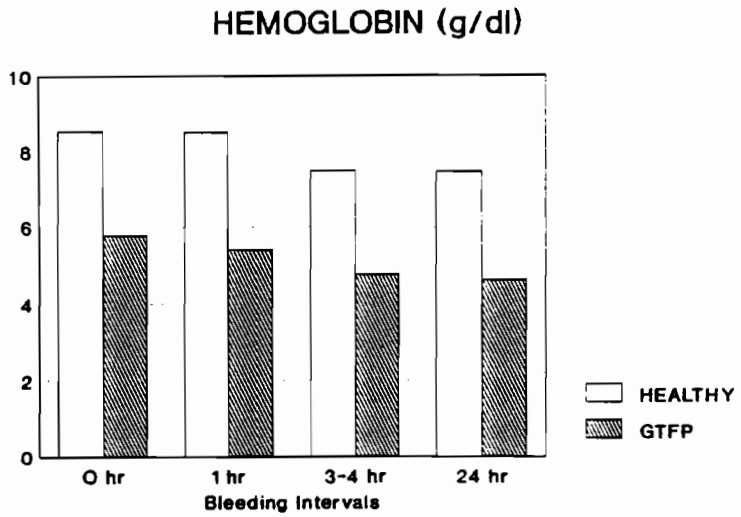
Plasma Biochemistry Values

Changes in blood biochemistry values in sea turtles may be related to their physiological state or may be indicators of chronic or pathologic conditions. Alterations in the blood chemistry of these reptiles, however, may be affected by many intrinsic and extrinsic factors (Lutz and Dunbar-Cooper 1987). Most plasma biochemical values in this study were not comparable with those of other studies that were based on values obtained from maricultured or captive-reared animals (Dessauer 1970; Bonnet 1979). Mean blood biochemistry values for the control group, however, were similar to values reported for green turtles

	3–4 hr			24 h		
Range	Mean ± SD		Range	Mean ± SD		Range
6–27	20.20 ±	5.54	12–25	18.40 ±	5.22	10–24
.15–1.46	.34 ±	.09	.21–.45	.22 ±	.10	.07–.35
1.4–7.9	4.78 ±	1.70	2.3–6.8	4.64 ±	1.24	2.8–5.9
400–893	619.80 ±	191.82	375–820	938.20 ±	301.84	600–1429
93.3–250.0	151.30 ±	71.82	71.9–228.6	241.58 ±	94.07	154–400
23.3–29.3	23.40 ±	4.50	18.3–28.2	25.46 ±	2.22	22.2–28.0
1.3–15.8	6.98 ±	3.12	3.2–11.3	9.48 ±	2.47	7.3–13.3
468–1738	2388.60 ±	1343.79	1170–4290	5989.20 ±	1848.99	3869–8113
11–36	35.20 ±	21.45	15–60	62.60 ±	8.14	53–74
232–689	699.00 ±	630.89	288–1638	1047.60 ±	406.47	400–1425
3–23	10.00 ±	7.44	5–21	12.00 ±	6.12	4–18
312–9006	3277.60 ±	2791.26	312–7119	2259.60 ±	1069.44	1045–3857
24–58	43.00 ±	26.09	4–65	23.60 ±	7.70	11–30
143–3792	1041.20 ±	777.56	210–2147	248.33 ±	133.25	146–399
8–24	13.00 ±	5.61	6–19	2.33 ±	0.58	2–3
33–790	159.50 ±	51.60	100–226	86.50 ±	19.09	73–100
1.6	1.75 ±	.50	1–2	1.00 ±	.0	1.0–1.0
.19–1.5	3.46 ±	5.83	.23–13.75	3.16 ±	2.03	1.76–6.73

in Florida (Norton, Jacobson, and Sundberg 1990) and juvenile green turtles from the Bahamas (Bolten and Bjorndal 1992). Significantly smaller values of BUN (1.5 ± 1.14 mg/dL, 0–4) were identified in the Hawaiian green turtles when compared with those of juvenile turtles from the Bahamas (7 ± 5 mg/dL, 2–37) and Florida (26 ± 25 mg/dL). Similarly, the Hawaiian turtles presented higher LDH concentrations (172 ± 134 U/L, 54–681) than the Bahamas sample (135 ± 61 U/L, 48–342).

Hypoalbuminemia, hypoglobulinemia, hypophosphatemia, and lower triglyceride blood levels observed in the turtles with GTFP were indicative of a chronic, debilitating condition. The hypoferremia observed in turtles with GTFP can be considered a host defense mechanism in response to infection. Serum iron levels fall in several reptile and mammal species during an infectious process (Kluger 1979). Increase in plasma enzyme concentrations is due to leakage of enzymes from damaged cells and increased secretion by affected



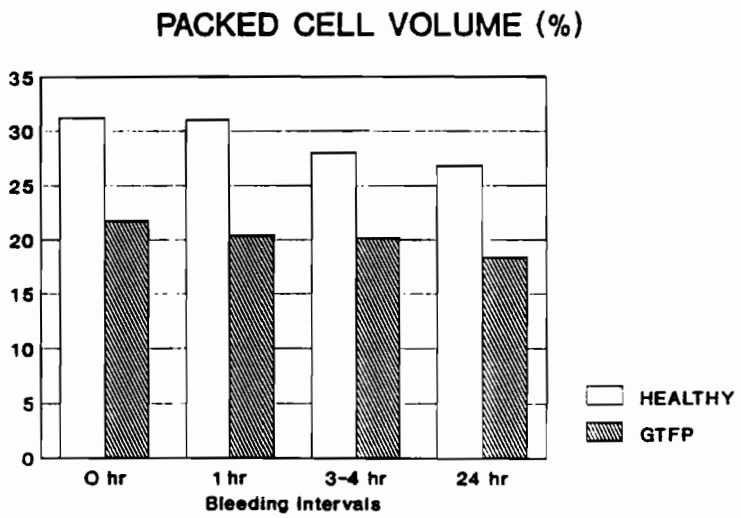
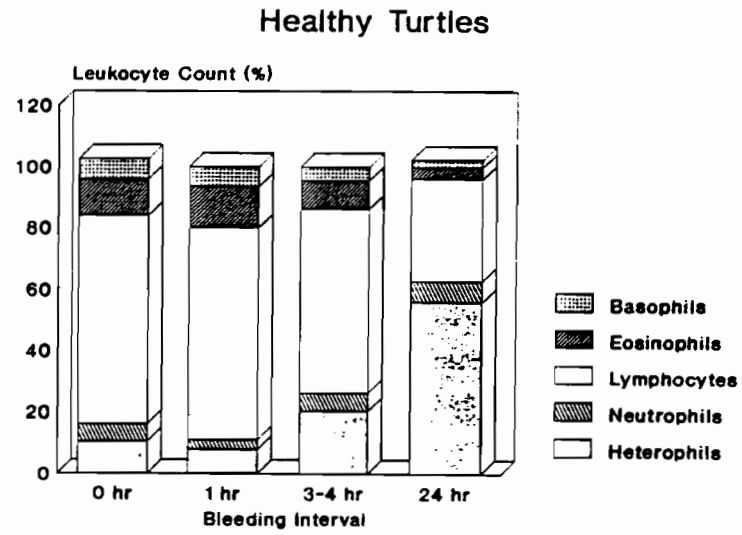


*Fig. 6. Mean HGB and PCV values at four bleeding intervals for juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas, captured at Kaneohe Bay, island of Oahu, Hawaii, 1993.*

tissues. For example, in mammals, elevated ASAT, ALAT, and LDH activities can be detected during muscle damage or exertion due to capture stress (Spraker 1993). Enzymatic levels identified for both turtle groups in this study did not provide sufficient evidence to indicate capture-related stress. In a clinical sense, the higher levels of ASAT and LDH in the turtles afflicted with fibropapillomas were another indication of chronic stress. Higher levels of alkaline phosphatase in the GTFP group indicated an increase in metabolic rate as a coping mechanism for disease or a chronic condition.



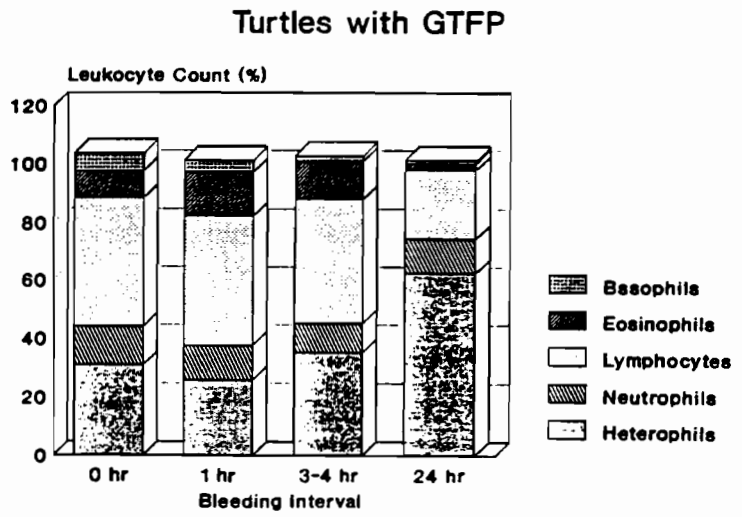


*Fig. 7. Mean percentages for different types of leukocytes at four bleeding intervals for juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas, captured at Kaneohe Bay, island of Oahu, Hawaii, 1993.*

Hematological Values

Hematological values are useful parameters that indicate the health status or state of disease in green turtles. Many factors, however, such as age, sex, season, stress, diet, circulating hormones, temperature, oxygen pressure, and body hydration affect these blood values (Duguy 1970). Although ex-

tensive work on blood parameters and their correlation with turtle physiological profiles is needed, this study provided baseline data for juvenile green turtles during early autumn in Kaneohe Bay, island of Oahu, Hawaii. This information was compared with data for a group of turtles of similar age that were afflicted with GTFP and were collected under similar conditions in the same study site and season.

Limited data on hematological values of sea turtles have been previously reported (Frair 1977*a*, 1977*b*; Wood and Ebanks 1984; Grumbles et al. 1990; Norton et al. 1990). The mean PCV reported for our control group, 29.5 ± 4.55% (24%–40%), is comparable to those of other studies reporting PCV values of 25%–31.6% for *C. mydas* (Thorson 1968; Frair 1977*b*), 10%–40% for nesting *Chelonia agassizii* from Mexico (Grumbles et al. 1990), 36% (± 7%) for green turtles from Florida (Norton et al. 1990), and 26.4%–42% for juvenile green turtles from the Bahamas (Bolten and Bjorndal 1992). In this study, hematocrit values for the GTFP group were significantly lower (20.2 ± 6.0, 6%–27%). In addition, HGB and RBC counts were significantly lower in tumored turtles, which indicates that these animals presented a nonregenerative anemia and a chronic condition (papillomas).

The absence of monocytes or azurophilic granulocytes was considered normal, as they have not been identified in sea turtles (Wood and Ebanks 1984; Cannon 1992). During this study, monocytes were identified in two turtles with GTFP, at 33 and 113 $10^3/\mu L$. Monocytosis suggests a chronic infectious process or other immunogenic stimulation. Monocyte proliferation is associated with granulomata (Frye 1991).

Eosinophils and basophils were identified in both groups as well-characterized and distinct cell types for juvenile Hawaiian green turtles. Absolute eosinophil counts were similar for both groups. We expected a severe eosinophilia in the group with GTFP, caused by the increased numbers of spirorchid trematode eggs and marine leeches (*Ozobranchus branchiatus*) in their tumors. Eosinophilia has been reported in alligators infested with leeches (Glassman, Holbrook, and Bennett 1979).

Heterophilia, lymphocytopenia, eosinopenia, and neutrophilia occurred in both turtle groups within 24 h postcapture. These changes in the leukogram have been reported in other species as due, in part, to the stress caused by capture (Lance 1990; Hajduk, Copland, and Schultz 1992). Nevertheless, statistical differences in absolute numbers and percentages of cells were significant between the two groups. The severe lymphocytopenia and neutrophilia observed in turtles with GTFP indicate a suppression or inhibition of the immune system in these animals.

Ratios of H/L represent a reliable measure of chronic stress in other species (Gross and Siegel 1983). These are less variable than the absolute cell num-

bers and have a positive correlation with corticosterone levels. In this study, green turtles with fibropapillomas showed a significant increase in H/L ratios and a positive correlation with corticosterone increase, which provides evidence of chronic stress. The only reports on H/L ratios in reptiles were found in alligators, ball pythons (*Python regius*), and blue-tongued skinks (*Tiliqua scincoides*) (Kreger and Mench 1993). Ratios of H/L did not provide evidence of the degree of susceptibility of turtles to fibropapillomas or trematode eggs.

Conclusion

Green turtles afflicted with fibropapillomas were immunosuppressed and chronically stressed prior to being subjected to capture stress. The higher corticosterone concentrations and the positive correlation with H/L ratios provided this evidence. In addition, several biochemical values indicated that GTFP turtles were suffering from a chronic, debilitating condition. The hematologic values suggested a nonregenerative anemia. The marked lymphocytopenia and neutrophilia observed in the GTFP group indicated a suppression of the immune system. Clinically healthy turtles responded similarly to acute stress in comparison with other reptile species. The results of this study provide evidence that inhibition of the interrenal response may be associated with a chronic condition (possibly GTFP). The immunosuppression resulting from GTFP could easily make the afflicted animals more susceptible to other infectious agents, such as spirorchid tremadodes, that may exacerbate the condition and cause severe mortality (Aguirre et al. 1994). More information is needed on hormonal and hematologic values in turtles of different age class, sex, and reproductive states during various seasons.

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