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# CELLULAR AND HORMONAL RESPONSES TO STRESS AND SPIRORCHID TREMATODE EGGS OF HAWAIIAN GREEN TURTLES (*CHELONIA MYDAS*) WITH AND WITHOUT FIBROPAPILLOMAS

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# PREFACE

This report was prepared under contract as part of the Southwest Fisheries Science Center Honolulu Laboratory's research program on threatened and endangered species of marine turtles. The study relates to fibropapillomatosis, a tumor-forming disease of green turtle (*Chelonia mydas*) populations in Hawaii, Florida, and selected locations elsewhere worldwide. The incidence of this life-threatening disease has reached epidemic proportions and represents a serious threat to the survival of the species. The nature of this disease and its cause must be determined in order to develop an effective long-term management and containment program. The present report by Dr. Alonso Aguirre constitutes progress in that direction which must be followed by additional research.

Because this report was prepared by an independent investigator, the findings, conclusions, recommendations and other statements do not necessarily reflect the views of the National Marine Fisheries Service, NOAA.

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## EXECUTIVE SUMMARY

This study reports baseline hormonal, hematological, and plasma biochemistry values for clinically healthy juvenile green turtles from a discrete population at Kaneohe Bay, Island of Oahu, Hawaii. Mean values for these parameters were compared with the mean values of a group of turtles afflicted with green turtle fibropapillomas (GTFP) using a general linear modeling program. Turtles from both groups of similar size classes were collected under the same conditions, study area, time of the day, and season. The hormonal, humoral, and enzymatic responses to acute and chronic stress were characterized for each group at four different sampling periods: 2 minutes within capture (0 hr), 1 hr, 3-4 hr, and 24 h post-capture. The differences identified between groups and times within a group provided a basis to conclude that sick turtles had been chronically stressed and immunosuppressed prior to being subjected to acute or capture stress. In addition, histopathologic and electron microscopic examinations were performed of biopsies collected from tumors of the GTFP group. Cellular reaction and tumor progression suggested a viral etiology with spirorchid trematode eggs and their associated granulomata being secondary to tumor development. It is hypothesized that a primary infectious agent causes immunosuppression and predisposition to secondary bacterial and parasitic infections. Further research should emphasize the transmission and identification of this agent to provide possible insights and solutions for the control, treatment, and prevention of this disfiguring, debilitating, and lethal disease.

## INTRODUCTION

Green turtle fibropapillomatosis (GTFP) is a condition afflicting several green turtle (*Chelonia mydas*) populations in epidemic proportions throughout the world. Although a virus has been suggested as the causative agent, the primary etiology is unknown. Chronic stress coupled with environmental factors (e.g., water temperature, excessive solar radiation) impairing the immune system and spirorchid trematode eggs have been listed among many other possible etiologic factors (Balazs and Pooley, 1991).

The effects of chronic stress in captive reptiles have been previously addressed (Hoff et al., 1984; Lance, 1990); however, stress monitoring in wild populations remains largely uninvestigated since measuring the stress levels in an animal population is difficult due to the diversity of physiologic and psychologic responses to different stressors. This is complicated by the high variability of behavioral, autonomic, and neuroendocrine responses within individuals. In addition, there has been a failure to correlate measures of stress and meaningful changes in the well-being of animals (Fiennes, 1982; Breazile, 1987; Spraker, 1993). Nevertheless, several physiologic responses to a stressor, including biochemical, cellular, and hormonal parameters are measurable (Moberg, 1987). For example, an increase in hematocrit, high concentrations of corticosterone, changes in blood cell counts and enzymes, and determination of heterophil/lymphocyte ratios have provided an index to determine stress levels in several reptile species (Duggan, 1981; Moberg, 1985; Mahapatra et al., 1991; Kreger and Mench, 1993). These parameters may provide basic information on the response differences of clinically healthy and GTFP turtles to environmental stressors and their correlation to disease and immunity (Aguirre, 1991).

More than 50 species of digenean trematodes have been reported in green turtles worldwide. Ova from three trematode species of the family Spirorchidae have been described in tumors of Hawaiian green turtles including *Learedius learedi* (Price, 1934), *Carettacola hawaiiensis* (Dailey et al., 1991), and *Hapalotrema dorsopora* (Dailey et al., 1992). The cellular response to the presence of these blood fluke eggs has been reported as a capsule of epithelioid macrophages surrounded by fibrotic tissue (J. Harshbarger, pers. comm.). If trematode ova are the causative agent of GTFP, the lesions observed in green turtles represent a host response characterized by a cutaneous, foreign body fibrosis associated with papillary epidermal hyperplasia. No progressive characterization of this cellular response, however, from early to advanced growths has been described.

The objectives of this study were to determine baseline blood hormonal, biochemical, and cellular values of a group of clinically healthy green turtles. These values were compared to

parameters collected from a group of turtles with GTFP. The acute (capture) stress and chronic (disease) stress responses were measured and evaluated using hormonal and hematological values. In addition, normal skin and tumors from early to advanced growths were evaluated by histopathologic examination to characterize the progression of tumor development and the possible cellular response to spirorchid trematode eggs.

## MATERIALS 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, 157°50'W), Island of Oahu, Hawaii, during 20-22 September 1993. Green turtles were captured 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 post-occipital sinuses (Owens and Ruiz, 1980) immediately after capture. This sample was taken within 2 minutes from touching the animal in the water. Subsequent bleedings were performed at 1 h, 3-4 h, and 24 h post-capture, prior to their release back into the wild. Blood (3-5 ml) was collected using a 21 gauge needle and 5-ml syringes. Collection time did not exceed 30 sec and only occasionally exceeded 15 sec. After the first bleeding, turtles were transported to the facilities of the University of Hawaii (Hawaii Institute of Marine Biology) on Coconut Island in Kaneohe Bay where they were kept cool in a shady place and covered with wet towels until the next bleeding occurred. For the 24-h sampling, turtles were kept overnight in a salt water (26°C) tank.

Each blood sample was split in 2 heparinized Vacutainer® tubes. One tube was sent overnight in blue ice to California Veterinary Diagnostics, Inc. (West Sacramento, CA), for a complete hemogram and differential. The plasma of a second sample was separated by centrifugation, split in two vials, and immediately stored in an ultrafreezer at -70° until analyzed. Hemolyzed samples were discarded. One sample was sent to the SmithKline Beecham Laboratory (Honolulu, HI) for a 25-element blood chemistry analysis, and another sample was sent to the BEECS Reproductive Analysis Core, University of Florida (Gainesville, FL) for hormonal analysis.

After the 24-h bleeding, turtles were measured, tagged, and weighed following techniques previously described (Balazs et al., 1987). 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). Multiple tissue biopsies from the prickle cell layer of the epidermis of tumors at several growth stages were collected from each of the five

green turtles manifesting fibropapillomas. In addition, every tumor from each turtle was individually coded from 1 to 4 based on gross appearance according to size, pigmentation, surface, attachment, and location. Normal skin biopsies from five clinically healthy turtles served as controls. Biopsy specimens were fixed in 10% neutral buffered formalin for histopathologic evaluation. A biopsy of the earliest growth for each individual with GTFP was fixed in Karnovsky's solution and held at 4 C until processed for both light and transmission electron microscopy.

## Laboratory Techniques

### Hormone Radioimmunoassays

*Validation Summary*--Plasma samples were analyzed for corticosterone, estradiol 17-β, and testosterone using standard radioimmunoassay (RIA) procedures. Specimens were extracted with 5 ml diethyl ether prior to RIA analysis. Each sample was analyzed in duplicate and corrected for the extraction efficiency of 83 ± 6.1% for corticosterone, 92 ± 2.4% for estradiol, and 87 ± 3.3% for testosterone.

Standard curves were prepared in PBS buffer with known amounts of radioinert corticosterone (10, 25, 50, 100, 250, 500, 1000, and 2000 pg/ml), and estradiol and testosterone (1, 5, 10, 25, 50, 100, 250, 500, and 1000 pg/ml) purchased from Amersham Corporation (Arlington Heights, IL). The minimum concentration per tube which was distinguishable from zero was 18.3 pg/ml for corticosterone, 5.2 pg/ml for estradiol, and 12.2 pg/ml for testosterone. Cross-reactivities of the corticosterone antiserum (#07-189016, ICN Biomedicals Inc., Costa Mesa, CA) with other steroids were 6.1% for desoxycorticosterone, 0.29% for progesterone, 0.19% for cortisol, and <0.1% for all other steroids examined. Cross-reactivities of the estradiol antiserum with other steroids were 11.2% for estrone, 1.7% for estriol, <1% for estradiol-17α, and <0.1% for all other steroids examined. Cross reactivities of the testosterone antiserum (#07-189016, ICN Biomedicals Inc., Costa Mesa, CA) with other steroids were 18.75% for 5α-dihydrotestosterone, 3.0% for 5α-androstenediol, <1.0% for androstenedione, and 0.1% for all other steroids examined.

Pooled samples (approximately 740 pg/ml of corticosterone, 950 pg/ml of estradiol, and 880 pg/ml for testosterone) were assayed serially in 10, 25, 50, 75, and 100 µl volumes (final volume of 100µl with charcoal-stripped plasma). The inhibition curve for each hormone 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 100µl charcoal-stripped plasma. For corticosterone [$Y = 10.1 + 0.947X$; Y = amount of corticosterone measured (pg); X = amount of corticosterone added (pg); $R^2 = 0.8793$]; interassay and intrassay coefficients of

variation were 7.9 and 10.3% respectively. For estradiol [Y = 1.13 + 0.93X; Y = amount of estradiol measured (pg); X = amount of estradiol added (pg); $R^2$ = 0.9211]; interassay and intrassay coefficients of variation were 8.7 and 10.3% respectively. For testosterone [Y = 4.25 + 0.932X; Y = amount of testosterone measured (pg); X = amount of testosterone added (pg); $R^2$ = 0.8969]; interassay and intrassay coefficients of variation were 9.2 and 11.1%, respectively.

*Sample Analysis*--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 x 75 mm glass tubes and evaporated. 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 (1200 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) in plastic scintillation vials (Fisher Scientific, Pittsburgh, PA). All vials were counted for 1 minute and hormone titers in ng/ml were calculated using the standard curve generated in the assays (Gross et al., 1993).

Sexing of turtles was attempted by measuring testosterone (T) concentrations based on reported values for female loggerhead turtles (*Caretta caretta*) of T≤ 31 pg/ml and for males T≥ 80 pg/ml (Wibbels et al. 1990). In addition, estradiol/testosterone ratios were compared (Gross et al. 1993).

**Plasma Biochemistry**

In the SmithKline Beecham Laboratory, plasma samples were analyzed using an Olympus AU5061 autoanalyzer (Olympus Corporation, Lake Success, NY) as suggested by Bolten et al. (1992). Among the 25 parameters, plasma chemistry profiles including total protein, selected enzyme activity, uric acid, cholesterol, glucose, and creatinine were compared and correlated to hematologic and hormonal values in both, healthy turtles and turtles with tumors.

**Hemogram and Differential**

Hematologic erythrocyte count (RBC), leukocyte count (WBC), hemoglobin (HGB), hematocrit or packed cell volume (PCV), 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, FL) in accordance to standard laboratory procedures. The WBC differential counts (heterophils, neutrophils, lymphocytes,

eosinophils, and basophils) were performed manually as previously described (Hawkey and Dennett, 1989). These blood characteristics, in addition to heterophil/lymphocyte ratios, were used to evaluate and compare values in sea turtles sampled and to determine responses to capture stress, parasites, or unknown conditions related to GTFP.

**Histopathology and Electron Microscopy**

For histopathology, fixed normal skin and fibropapilloma lesions were embedded in paraffin, sectioned 6-$\mu$m thick and stained with hematoxylin and eosin. The skin lesions for electron microscopy were washed with 0.2 M Sorenson's phosphate buffer pH 7.3 and were postfixed in 1.0% osmium tetroxide for 1 hour. The tissues were washed through two changes of deionized $H_2O$, dehydrated through a graded acetone series, infiltrated with and embedded in Medcast-Araldite 502 Resin® (Ted Pella Inc., Redding, CA). Semi-thin, 1-2$\mu$m survey sections were cut from the blocks, stained with Methylene Blue-Azure II-Basic Fuchsin, and examined with a light microscope (Hayat 1986). Ultrathin sections from the tumor biopsies of two turtles were placed on copper grids, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope by Dr. John Chandler (Colorado State University, Department of Anatomy and Neurobiology, Fort Collins, CO 80523).

**Statistical Analysis**

Statistical analysis was conducted on raw and log-transformed data using the univariate approach for repeated measures (split-plot) analysis of variance (ANOVA) design (SAS Institute Inc., 1990). This general linear model was used to determine differences between hormone, blood cell count, and plasma chemistry values for both turtle groups. The procedure assessed differences between group effects, within time effects (sampling periods within a group), and interactions between group and time effects. Differences were considered significant at $\alpha = 0.05$. Data were expressed as mean, standard deviation (SD), and range of values for each blood parameter.

## RESULTS

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

## Hormonal Values

The repeated measures ANOVA revealed significant interactions between time and group for corticosterone levels. Mean corticosterone concentrations in plasma increased from 0 to 1 h post-capture in both groups, but were significantly higher ($p \leq 0.05$) in turtles with GTFP. For both groups, mean concentration levels peaked 3-4 h post-capture. During the 24-h period, corticosterone concentrations remained at peak levels for the turtles with GTFP, but significantly declined in the healthy group (Fig. 1). Estradiol (Fig. 2) and testosterone (Fig. 3) concentrations, and estradiol/testosterone ratios (Fig. 4) for individual turtles are summarized for both groups.

## Plasma Biochemistry Values

Mean, standard deviation, and range for blood biochemistry profiles for clinically healthy green turtles (Table 1) and turtles with fibropapillomas (Table 2) are reported for each bleeding interval. A significant decline of total protein values for both groups occurred between 3-4 h and 24 h post-capture, and hypoproteinemia was evident in the GTFP group (3.44 ± 0.62 g/dl) at the 24-h sampling period when compared with the group free of GTFP (4.3 ± 0.22 g/dl). Albumin levels were higher for the control group. Albumin values remained constant for the GTFP group; however, these significantly increased from 0.92 (SD ± 0.38) g/dl at initial bleeding to 1.25 (±0.44) g/dl 3-4 h post-capture. Higher globulin values were identified at all sampling periods for the control group. Except for the bleeding immediately after capture, total bilirubin values were higher for the control group. Total bilirubin declined over 3-4 h in turtles with GTFP from 0.2 ± 0.1 mg/dl to 0.06 ± 0.09 mg/dl (Tables 1 and 2).

Blood alanine amino transferase (ALAT), asparmate amino transferase (ASAT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) values were similar in both groups and remained unchanged over the 24-h period, excepting for ASAT and LDH which significantly increased in the GTFP group from 141 ± 37 U/l to 203 ± 44 U/l and from 134 ± 38 U/l to 371 ± 254 U/l, respectively. Alkaline phosphatase values were consistently higher at all times for the GTFP group and no sampling period differences were identified within groups. Significantly higher urea nitrogen values and BUN/creatinine ratios were detected in the GTFP group at all times. These ratios significantly increased between the 3 to 4-h and 24-h sampling periods in this group. Uric acid and creatinine levels did not differ between groups; however, these values significantly increased in the control group over the 3-4 h period (Tables 1 and 2).

Calcium values were similar between groups but levels significantly declined between the 3-4 h period and the 24-h period. Higher phosphorus levels were identified in the group

free of GTFP for all sampling periods. Significantly higher ($p \leq 0.05$) cholesterol values were detected between groups during two sampling periods (3-4 h and 24 hr). Triglyceride levels were also higher for the control group at all times, and they significantly declined over time for both groups. Glucose levels differ between groups 3-4 h post-capture, with the control group manifesting significant hyperglycemia. Iron levels were higher for the control group, but no group or time differences where detected for sodium, potassium, and chloride (Tables 1 and 2).

**Hematological Values**

Mean, standard deviation, 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 tumors had significantly lower HGB and PCV values. The RBC, HGB, and PCV values significantly declined over the 24-h period for both groups. No significant differences were detected for MCV, MCH, and MCHC between groups excepting for MCHC that had higher values at the 3-4 h period for the control group. The MCV and MCH values significantly increased in turtles with fibropapillomas between the 3-4 h and 24-h sampling periods (Tables 3 and 4).

Higher WBC values were identified in healthy turtles when compared to turtles with GTFP, except 24 h post-capture when WBC values significantly increased for sick turtles and declined for healthy turtles. Heterophils, neutrophils, lymphocytes, eosinophils, and basophils were analyzed both as absolute numbers and percentages using WBC as a covariate. Heterophils significantly increased over the 24-h sampling period for both groups. Differences were significant between groups at 0-hr, 1-hr, and 3-4 h periods, with higher absolute numbers in the control group. Significant neutrophilia was detected for the group with GTFP. Consistently higher number and percentages of lymphocytes were detected for the healthy turtles, excepting for the 24-h period when numbers were similar for both groups. A significant lymphocytopenia occurred for both groups 24 h post-capture. Numbers and percentages of eosinophils and basophils were similar between groups at all times (Fig. 5).

Heterophil/lymphocyte (H/L) ratios were similar for both groups at the initial sampling, but ratios significantly increased for the GTFP group at 1-hr, 3-4 hr, and 24-h post-capture. This difference was evident when a significant increase in ratios occurred between the initial sampling and 1-h post-capture for the same group (Fig. 1).

**Histopathology**

Multiple sections of 23 tumor biopsies from early growths to well-developed tumors from five turtles with GTFP were analyzed under light microscopy. Five skin biopsies from five healthy

turtles were also analyzed. The histopathologic findings correlated with the gross tumor ranking of 1 to 4; therefore, the description was sequenced based on these scores.

**Skin Biopsies**

The skin contained a few vessels within the dermis that were cuffed with lymphocytes, plasma cells, and an excessive amount of pigment. In most cases, the thickness of the epidermis was relatively uniform composed by four to eight cell layers and within normal limits. The keratin layer was thin and relatively uniform. The dermis presented a papillary layer and a well-defined reticular layer composed of large bundles of collagen. Dermal papillary projections into the epidermis were also observed (Fig. 6). There was one small focus in which the epidermis and keratin layer were moderately infiltrated with lymphocytes and a few plasma cells. Epidermal cells in this specific focus had undergone mild hyperplasia and one vessel underneath was characterized by lymphocytic and heterophilic cuffing. Focal dermatitis within a lymphocytic epidermis was observed in three biopsies. These foci were characterized by mild acanthosis and early necrosis within the dermis (Fig. 7). There were marine leeches attached to the skin at several of these sites. The ulcers were relatively shallow, but surrounded by inflammatory cells.

**Tumor Score 1**

Six tumor biopsies ranging from 2 by 3 mm to 5 by 10 mm were characterized as small, papillary, early growths within the epidermis and dermis with extremely early pseudoepitheliomatous hyperplasia, acanthosis of the epidermis, and a reaction of the superficial dermal mesoderm. Tumors were plaque-like (flat sarcoid) in shape and tightly adherent to the underlying epidermis (Fig. 8). This epithelium covered a layer of fibroblastic cell proliferation tissue and had a slightly excessive amount of keratin over the epidermis. The tumors initially proliferated at the basal layer of epidermis and then progressed downward. Small blister formations were observed in three tumors. Few microorganisms were present on the surface of these tumors, including bacteria, protozoa, and fungi within the keratin clefts. Occasionally, cells of the epidermis presented vacuole degeneration, suggesting early degeneration. Spirorchid trematode eggs were not identified in these tumors.

**Tumor Score 2**

Nine tumor biopsies ranging from 8 by 15 mm to 25 by 25 mm were characterized as papillary tumors with or without black pigmentation. The epithelium covering these tumors had undergone acanthosis and early pseudoepitheliomatous hyperplasia covering a proliferation layer of fibroblastic cells (Fig. 9). Tumors were structurally forming folds and papillary projections. The core

of the papillary projections was composed of fusiform cells surrounded by finely fibrillar cytoplasm. Focal areas of the epithelium had undergone ballooning degeneration and nuclear necrosis (Fig. 10). Underneath these areas of ballooning degeneration, a mild hyperplasia of the basal cell layer was observed with a reaction within the upper dermis characterized by proliferation of fusiform or fibroblastic-type cells. Areas of ulceration on the surface of two tumors extended into the superficial dermal tissue. The ulcers in the epidermis of these biopsies were characterized by swollen and hypertrophic degeneration of the epithelium with structures similar to intranuclear inclusion bodies (Fig. 11). The inclusions were characterized by margination of the chromatin large basophilic structures within the nucleus, some of which occupied the entire nucleus. Vessels surrounding these areas presented lymphocytic infiltration and were cuffed with plasma cells. A moderate number of macrophages containing black pigment were present throughout one tumor. A mild granulomatous reaction was associated with or surrounding spirorchid trematode ova in five tumor biopsies. These granulomas were observed within the tumor and within the connective tissue adjacent to the tumor (Fig. 12). An excessive amount of keratin was present on the surface of some areas of the epidermis. Bacterial colonies were found within those areas. Several areas and vessels underneath the tumors were surrounded by lymphocytes, macrophages, and plasma cells.

**Tumor Score 3**

Four tumors ranging from 14 by 22 mm to 55 by 57 mm ranked with a score of 3. These cauliflower-like tumors were larger, more solid, and pedunculated or nodular in shape. Their base was formed primarily by fusiform cells with elongated nuclei surrounded by fibrillar cytoplasm. The surface of this mass covered by epithelium had undergone acanthosis and severe pseudoepitheliomatous hyperplasia. The superficial dermal cellular component proliferated forming the fibrous component of these tumors. This portion was composed of fusiform cells with elongated nuclei. In all the tumors, mature collagen was evident. The neoplastic cells formed interweaving bundles in some areas and sheet-like layers in others. The dermal proliferation occurred under the epithelial layer. The epithelium presented ulcerations and was covered with extensive amounts of fibrin and keratin. There was a moderate degree of spongiosis within the epidermis in several areas of one tumor. The bed of the tumors was highly infiltrated with neutrophils, lymphocytes, plasma cells, and macrophages. Bacteria were found on the surface of the ulcerated areas. Trematode ova surrounded by granulomas were present in three tumor biopsies, and mites were identified in the crevices of two tumors. Marine leeches on the surface of four tumors were also observed.

**Tumor Score 4**

Four biopsies ranging from 47 by 68 mm to 66 by 115 mm were described as growths with extensive papillary, sessile or pedunculated masses. Their cauliflower-like surface presented several areas of ulceration that progressed through the epidermis and into the superficial dermal tissues. The inflammatory response around these ulcers was primarily formed by lymphocytes, plasma cells, and heterophils. Both the keratin and epidermal layers were heavily infiltrated with these inflammatory cells. These ulcers were multifocal and possibly associated with parasitic bite wounds caused by marine leeches. Granulomas and spirorchid trematode eggs were evident in three of the tumors with severe perivascular lymphocytic infiltration (Fig. 13).

## Electron Microscopy

Light microscopy examination of five tumor biopsies stained with Methylene Blue-Azure II-Basic Fuchsin revealed the same lesions described for the histopathologic examination. The electron microscopic findings of one turtle specimen included multiple epidermal folds associated with dermal proliferation and epidermis thicker than normal. Dermal papillae were projected towards the epidermis, and intercellular spaces were enlarged. Increased numbers of subcellular organelles, endoplasmic reticulum, mitochondria, hypertrophy, and hyperplasia of the stratum spinosum with pleomorphic cells were also identified. Granules with electron-dense bodies about 150 nm in diameter similar to viral particles were observed in the more superficial cells of the epidermis. Ultrastructurally, in deeper areas of the dermis cellular clusters were identified around the blood vessels including lymphocytes, heterophils, plasma cells, and macrophages. Trematode eggs were observed in the more superficial cells of the epidermis.

# DISCUSSION

## Hormonal Values

This study documents baseline levels of corticosterone concentrations for clinically healthy juvenile Hawaiian green turtles 2 minutes post-capture. These levels were compared to a group of turtles afflicted with GTFP captured under similar conditions. Plasma corticosterone concentrations of green turtles increased in response to acute stress in both control and GTFP groups. Our study demonstrates that juvenile Hawaiian green turtle response to acute stress was similar to that demonstrated in 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 to acute stress (capture and repeated bleeding) in Hawaiian green turtles in our study were similar to findings in loggerheads (Wibbels et al., 1990; Gregory, 1994). The highest corticosterone levels were identified 3-4 h post-capture. The results of our study for the control group corticosterone levels did not correlate to findings in *C. caretta* caught at Port Canaveral, Florida (Gregory, 1994). Since smaller levels of corticosterone were detected in the Hawaii sample (2.3 ± 1.3 ng/ml, 60 minutes post-capture) compared to the Florida sample (5.0 ng/ml, 60 minutes post-capture). Caution should be taken when comparing data between two species from different physiographic regions. In addition, differences may be related to season, age, diurnal rhythm, reproductive condition, and restraint method.

Corticosterone monitoring and selected blood parameters may provide an index of interrenal function and quantification of chronic stress and immunosuppression in a population. Glucocorticosteroids are known to reduce immunity predisposing an animal to be more susceptible to infectious agents. Few studies, however, are available related to chronic stress in reptiles (Lance, 1990). An increase in hematocrit, a shift in glucocorticosteroids, and lymphocytopenia have been described among the physiologic changes due to chronic stress observed in reptiles. Lance (1990) reported that juvenile alligators (*Alligator mississippiensis*) responded to chronic stress by chronically elevated corticosterone secretion and changes in the leukogram. Significantly higher levels of corticosterone, larger heterophil/lymphocyte ratios, and higher 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 cells, monocytes, and eosinophils; and decrease of lymphoid cell proliferation (Breazile 1987, 1988).

Results from our study in sexing juvenile Hawaiian green turtles based on testosterone levels were unreliable due to variation in methods when compared with work done by Wibbels et al. (1990, 1993) and Bolten and Bjorndal (1992). In our study, a different testosterone-labeled antibody was used, and sex was not confirmed by laparoscopy. Further research testing of the RIA technique developed by Gross et al. (1993) with controls coupled with estradiol/testosterone ratios is necessary to establish hormonal levels in Hawaiian green turtles from different size classes. No correlation was observed between individual corticosterone and testosterone levels. Testosterone suppression has been documented in stress response in tree lizards (*Urosaurus ornatus*) (Moore et al., 1991), alligators (Lance, 1990), and other reptile species (Gregory, 1994).

## 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 biochemistry values in this study were not comparable to other studies which 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 when compared to values reported for green turtles in Florida (Norton et al., 1990), and juvenile green turtles from the Bahamas (Bolten and Bjorndal, 1992). Significantly smaller values of BUN (1.5 ± 1.14 mg/dl, range 0-4) were identified in the Hawaiian greens when compared to juvenile turtles from Bahamas (7 ± 5 mg/dl, range 2-37) and Florida (26 ± 25 mg/dl). Similarly, the Hawaiian turtles presented higher LDH concentrations (172 ± 134 U/l, range 54-681) than the Bahamas sample (135 ± 61 U/l, range 48-342).

Hypoproteinemia, hypoalbuminemia, hypoglobulinemia, hypophosphatemia, and lower cholesterol and triglyceride blood levels observed in the turtles with GTFP were indicative of a chronic, debilitating condition. Increase in plasma enzyme concentrations is due to leakage of enzymes from damaged cells and increased secretion by affected tissues. For example, elevated ASAT, ALAD, 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 myopathy. Clinically, 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.

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

Limited information on some hematological values of sea turtles has been previously reported (Frair, 1977a,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 other studies reporting PCV values of 25-31.6% for *C. mydas* (Thorson, 1968; Frair, 1977b); 10-40% in nesting *C. agassizi* from Mexico (Grumbles et al., 1990); 36%(±7) in Florida green turtles (Norton et al., 1990); and 26.4-42% in juvenile green turtles from Bahamas (Bolten and Bjorndal, 1992). In this study, hematocrit values for the GTFP group were considerably lower (20.2 ± 6.0, range 6-27%). In addition, HGB and RBC were also significantly lower in tumored turtles indicating that these animals presented a nonregenerative anemia, a chronic condition (GTFP), and inanition.

The absence of monocytes or azurophilic granulocytes was considered normal as they had 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 $10^3/\mu l$ respectively. Monocytosis suggests a chronic infectious process or other immunogenic stimulation. Monocyte proliferation is associated with granulomata (Frye, 1991).

Abundant numbers of eosinophils and basophils were identified in both groups. These cells were common and well characterized as distinct cell types for juvenile Hawaiian green turtles. We expected a severe eosinophilia in the group with GTFP, caused by the increased numbers of spirorchid trematode eggs and marine leeches (*O. branchiatus*) in their tumors. This cellular response has been reported for alligators infested with leeches (Glassman et al., 1979). Absolute eosinophil counts were similar for both groups.

Heterophilia, lymphocytopenia, eosinopenia, and neutrophilia occurred in both turtle groups within 24 h post-capture. These changes in the leukogram have been reported as part of the stress caused by capture in other species (Lance, 1990; Hajduk et al., 1992). Nevertheless, statistically significant differences in absolute numbers and percentages of cells were remarkable between the two groups (Fig. 5). The severe lymphocytopenia and neutrophilia observed in turtles with GTFP indicate a suppression or inhibition of the immune system in these animals.

Heterophil/lymphocyte ratios represent a reliable measure of chronic stress in other species (Gross and Siegel, 1983). These are less variable than the absolute cell numbers and have a positive correlation with corticosterone levels. In this study, green turtles afflicted with fibropapillomas showed a significant increase in H/L ratio and a positive correlation with corticosterone increase, providing evidence of chronic stress (Fig. 1). 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). This parameter, however, did not provide evidence of the degree of susceptibility of turtles to fibropapillomas or trematode eggs.

The humoral and hormonal responses to stress by both turtle groups indicated that the GTFP animals were immunosuppressed and chronically stressed before being subjected to an acute stress situation (i.e., capture and repeated bleeding). Turtles with GTFP in this study, although affected by a tumor score 3, were vigorous and in good condition. Possibly, during the more advanced stages of the disease, trematode parasites proliferate and easily invade the tissues of immunosuppressed animals.

**Histopathology and Electron Microscopy**

The histopathology of green turtle fibropapillomas has been described for Florida green turtles (Jacobson et al., 1989) and Hawaiian green turtles (Aguirre et al., 1994). Our study did not provide evidence that spirorchid trematode eggs were the initial cause of fibropapilloma formation. The lesions observed did not represent a host response characterized by a cutaneous, foreign body fibrosis associated with papillary epidermal hyperplasia.

All the biopsies analyzed suggested that these fibropapillomas had a viral etiology - most likely a papilloma virus as hypothesized earlier (Jacobson, 1991). The early ballooning degeneration, the intranuclear structures, and the nuclear necrosis of the epithelium were lesions highly suggestive of a viral infection of the epidermis, extending to the underlying dermis. As the lesions progressed, secondary agents were captured within the neoplastic tissue. These agents included trematode ova-resulting in granulomata, mites, and bacteria. Secondary bacterial infections caused the ulcerated areas of the epidermis, whereas in other areas the primary infection produced epithelionecrosis and ulceration followed by bacterial proliferation. Vessels surrounding the tumor were cuffed by lymphocytes suggesting that turtles were responding to the neoplastic tissue in addition to granulomas induced by trematode ova.

Two hypothetical scenarios are suggested: (a) heavy spirorchid trematode infections coupled with other intrinsic or extrinsic stressors lead to immunosuppression predisposing turtles to an infectious agent, or (b) a primary infectious agent (i.e., virus) causes immunosuppression and the tumors, leading to severe trematode infections and death. Hypothesis b is supported based on the findings of this study. Tumor formation and enhancement by a virus-induced stress has been documented in other species (Riley, 1981). Acute viral infections increase corticosterone concentrations modulating host immunocompetence.

Three of the five skin biopsies presented a mild to moderate dermatitis apparently caused by marine leeches. These areas of

ulceration and irritation may increase the susceptibility of green turtles to skin penetration by an infectious agent.

## CONCLUSIONS

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 heterophil/lymphocyte ratios provided this evidence. In addition, several biochemistry values indicated that GTFP turtles were hypoproteinemic and suffering from a chronic, debilitating condition. The hematologic values demonstrated a nonregenerative anemia. The severe lymphocytopenia and neutrophilia observed in this group indicated a suppression or inhibition of the immune system in these animals. Clinically healthy turtles responded similarly to acute stress when compared to other reptile species. The results of this study support that inhibition of the interrenal response may be associated with a chronic condition (GTFP). More information is needed in hormonal and hematologic values in turtles of different age class, sex, and reproductive state, during various seasons. Based on the histopathologic findings, spirorchid trematode eggs were not the initial cause of tumor formation in the tissues analyzed. Our study supported evidence that the cellular reaction and tumor formation may be associated with an infectious agent, most likely a fibropapilloma virus. Further research in the identification of this agent by immunohistochemical localization of the antigen or detection via gene probes will provide possible insights for the control, treatment, and prevention of this disfiguring and debilitating disease.

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Table 1. Mean, standard deviation, and range of 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 | 0 hr | | | 1 hr | | | 3-4 hr | | | 24 hr | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VARIABLE | Mean | ±SD[a] | Range | Mean | ±SD | Range | Mean | ±SD | Range | Mean | ±SD | Range |
| Corticosterone (ng/ml) | 0.70 | 0.24 | 0.46-0.98 | 2.29 | 1.33 | 1.20-4.57 | 2.70 | 0.88 | 2.02-3.83 | 1.36 | 0.91 | 0.36-2.52 |
| Protein (g/dl) | 4.32 | 0.58 | 3.8-5.3 | 4.46 | 0.46 | 3.8-5.1 | 4.06 | 0.48 | 3.5-4.8 | 4.30 | 0.22 | 4.0-4.6 |
| Albumin (g/dl) | 0.93 | 0.38 | 0.6-1.3 | 1.20 | 0.41 | 0.6-1.5 | 1.25 | 0.43 | 0.6-1.5 | 1.00 | 0.46 | 0.6-1.4 |
| Globulin (g/dl) | 2.98 | 0.66 | 2.4-3.6 | 2.73 | 0.52 | 2.4-3.5 | 2.73 | 0.52 | 2.4-3.5 | 2.98 | 0.66 | 2.4-3.6 |
| Albumin/Globulin Ratio | 0.35 | 0.17 | 0.2-0.5 | 0.48 | 0.19 | 0.2-0.6 | 0.48 | 0.19 | 0.2-0.6 | 0.40 | 0.23 | 0.2-0.6 |
| Total Bilirubin (mg/dl) | 0.14 | 0.11 | 0.0-0.3 | 0.16 | 0.11 | 0.0-0.3 | 0.16 | 0.11 | 0.0-0.3 | 0.14 | 0.09 | 0.0-0.2 |
| Direct Bilirubin (mg/dl) | 0.10 | 0.11 | 0.0-0.3 | 0.06 | 0.05 | 0.0-0.1 | 0.08 | 0.04 | 0.0-0.1 | 0.08 | 0.04 | 0.0-0.1 |
| Indirect Bilirubin (mg/dl) | 0.08 | 0.08 | 0.0-0.2 | 0.08 | 0.08 | 0.0-0.2 | 0.10 | 0.07 | 0.0-0.2 | 0.06 | 0.05 | 0.0-0.1 |
| ALAT (SGPT)[b] (U/l) | 5.80 | 4.71 | 2.0-14 | 3.60 | 1.95 | 1.0-5.0 | 3.20 | 2.28 | 0.0-6.0 | 4.60 | 1.82 | 3.0-7.0 |
| ASAT (SGOT)[c] (U/l) | 141.00 | 51.20 | 81-223 | 127.20 | 28.46 | 85-155 | 123.20 | 19.80 | 97-147 | 140.20 | 41.13 | 91-202 |
| Alkaline Phosphatase (U/l) | 42.40 | 10.69 | 27-52 | 42.00 | 10.34 | 26-55 | 38.40 | 8.70 | 24-45 | 38.80 | 6.53 | 30-46 |
| GGT[d] (U/l) | 0.40 | 0.55 | 0.0-1.0 | 0.80 | 0.84 | 0.0-2.0 | 1.00 | 1.00 | 0.0-2.0 | 1.00 | 0.71 | 0.0-2.0 |
| LDH[e] (U/l) | 109.40 | 49.53 | 55-171 | 135.20 | 56.13 | 54-189 | 99.00 | 28.59 | 75-146 | 210.60 | 52.55 | 148-266 |
| Urea Nitrogen (BUN) (mg/dl) | 1.00 | 1.00 | 0.0-2.0 | 0.70 | 0.84 | 0.0-2.0 | 1.60 | 0.89 | 0.0-2.0 | 2.20 | 0.84 | 1.0-3.0 |
| Creatinine (mg/dl) | 0.26 | 0.09 | 0.2-0.4 | 0.28 | 0.16 | 0.0-0.4 | 0.28 | 0.04 | 0.2-0.3 | 0.26 | 0.05 | 0.2-0.3 |
| BUN/Creatinine Ratio | 3.66 | 4.15 | 0.0-10 | 3.62 | 4.07 | 0.0-9.8 | 6.02 | 3.66 | 0.0-10 | 8.68 | 3.97 | 5.0-15.0 |
| Uric Acid (mg/dl) | 0.84 | 0.23 | 0.6-1.2 | 1.10 | 0.30 | 0.6-1.4 | 1.36 | 0.50 | 09-2.2 | 1.04 | 0.15 | 0.8-1.2 |
| Calcium (mg/dl) | 8.42 | 1.02 | 7.2-9.7 | 9.16 | 1.44 | 7.2-11.1 | 9.58 | 2.12 | 6.5-12.1 | 7.22 | 0.49 | 6.6-7.7 |
| Phosphorus (mg/dl) | 7.88 | 0.93 | 6.9-9.3 | 7.94 | 0.77 | 7.1-9.0 | 7.36 | 0.72 | 6.4-8.3 | 7.56 | 0.86 | 6.8-9.0 |
| Cholesterol (mg/dl) | 117.80 | 32.77 | 92-173 | 121.60 | 27.24 | 99-167 | 107.80 | 19.57 | 89-136 | 121.00 | 27.34 | 98-165 |
| Triglycerides (mg/dl) | 181.80 | 104.64 | 62-351 | 166.60 | 88.97 | 62-307 | 109.00 | 49.07 | 57-167 | 97.00 | 48.09 | 41-153 |
| Glucose (mg/dl) | 86.60 | 13.90 | 76-111 | 101.00 | 11.89 | 87-118 | 143.40 | 40.63 | 88-195 | 106.00 | 13.58 | 84-121 |
| Iron (mcg/dl) | 46.00 | 31.13 | 25-99 | 55.20 | 32.20 | 27-110 | 38.80 | 10.18 | 28-49 | 45.20 | 14.91 | 29-69 |
| Sodium (meq/l) | 152.20 | 2.39 | 149-155 | 155.40 | 1.67 | 154-158 | 154.80 | 3.11 | 151-158 | 153.80 | 3.11 | 150-157 |
| Potassium (meq/l) | 4.98 | 0.84 | 4.0-6.1 | 4.92 | 0.31 | 4.4-5.2 | 4.54 | 0.43 | 4.0-5.0 | 4.02 | 0.44 | 3.5-4.7 |
| Chloride (meq/l) | 109.00 | 6.20 | 102-117 | 112.40 | 4.72 | 106-117 | 114.80 | 5.07 | 110-122 | 107.40 | 3.51 | 103-112 |

[a]Standard Deviation
[b]Alanine Amino Transferase
[c]Aspermate Amino Transferase
[d]Gamma Glutamyl Transferase
[e]Lactate Dehydrogenase

Table 2. Mean, standard deviation, and range of plasma corticosterone and biochemistry values for each sampling period for green turtles (*Chelonia mydas*) afflicted with fibropapillomas, Kaneohe Bay, Island of Oahu, Hawaii, 1993.

| PLASMA BIOCHEMISTRY | 0 hr | | | 1 hr | | | 3-4 hr | | | 24 hr | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VARIABLE | Mean | ±SD[a] | Range | Mean | ±SD | Range | Mean | ±SD | Range | Mean | ±SD | Range |
| Corticosterone (ng/ml) | 1.49 | 1.71 | 0.3-4.46 | 5.50 | 2.50 | 2.57-8.61 | 6.03 | 2.20 | 2.14-7.38 | 6.04 | 2.31 | 2.52-8.83 |
| Protein (g/dl) | 3.96 | 0.31 | 3.6-4.3 | 4.04 | 0.61 | 3.2-4.6 | 3.76 | 0.62 | 3.0-4.5 | 3.94 | 0.59 | 3.1-4.5 |
| Albumin (g/dl) | 1.12 | 0.36 | 0.5-1.4 | 1.16 | 0.28 | 0.7-1.4 | 1.14 | 0.27 | 0.7-1.4 | 1.14 | 0.27 | 0.7-1.4 |
| Globulin (g/dl) | 2.46 | 0.31 | 2.0-2.8 | 2.46 | 0.36 | 1.9-2.8 | 2.44 | 0.34 | 1.9-2.7 | 2.36 | 0.29 | 2.0-2.7 |
| Albumin/Globulin Ratio | 0.48 | 0.08 | 0.4-0.6 | 0.48 | 0.08 | 0.4-0.6 | 0.46 | 0.09 | 0.4-0.6 | 0.46 | 0.09 | 0.4-0.6 |
| Total Bilirubin (mg/dl) | 0.20 | 0.10 | 0.1-0.3 | 0.06 | 0.13 | 0.0-0.3 | 0.02 | 0.05 | 0.0-0.1 | 0.06 | 0.09 | 0.0-0.2 |
| Direct Bilirubin (mg/dl) | 0.08 | 0.05 | 0.0-0.1 | 0.04 | 0.05 | 0.0-0.1 | 0.02 | 0.05 | 0.0-0.1 | 0.02 | 0.05 | 0.0-0.1 |
| Indirect Bilirubin (mg/dl) | 0.18 | 0.05 | 0.1-0.2 | 0.12 | 0.11 | 0.0-0.2 | 0.06 | 0.09 | 0.0-0.2 | 0.12 | 0.08 | 0.0-0.2 |
| ALAT (SGPT)[b] (U/l) | 4.00 | 1.00 | 3.0-5.0 | 4.20 | 2.17 | 1.0-6.0 | 4.20 | 3.11 | 0.0-7.0 | 4.00 | 1.41 | 2.0-5.0 |
| ASAT (SGOT)[c] (U/l) | 128.00 | 40.03 | 91-178 | 147.0 | 32.71 | 110-190 | 140.80 | 37.44 | 87-185 | 202.60 | 44.18 | 146-260 |
| Alkaline Phosphatase (U/l) | 23.00 | 9.460 | 12-37 | 25.60 | 13.01 | 17-48 | 23.40 | 12.82 | 14-45 | 26.80 | 9.09 | 16-41 |
| GGT[d] (U/l) | 0.80 | 0.84 | 0.0-2.0 | 1.20 | 1.30 | 0.0-3.0 | 0.40 | 0.55 | 0.0-1.0 | 0.60 | 0.55 | 0.0-1.0 |
| LDH[e] (U/l) | 145.80 | 46.25 | 102-223 | 173.40 | 65.90 | 120-275 | 133.60 | 38.16 | 81-187 | 371.20 | 254.18 | 87-747 |
| Urea Nitrogen (BUN) (mg/dl) | 8.10 | 8.11 | 2.0-22.0 | 7.80 | 9.09 | 0.0-23.0 | 8.00 | 8.52 | 0.0-22 | 12.20 | 10.83 | 2.0-24.0 |
| Creatinine (mg/dl) | 0.26 | 0.09 | 0.2-0.4 | 0.30 | 0.07 | 0.2-0.4 | 0.26 | 0.08 | 0.2-0.4 | 0.24 | 0.09 | 0.1-0.3 |
| BUN/Creatinine Ratio | 39.00 | 42.19 | 5.0-110 | 33.66 | 46.73 | 0.0-115 | 36.00 | 44.64 | 0.0-110 | 74.68 | 92.20 | 6.7-230 |
| Uric Acid (mg/dl) | 1.00 | 0.29 | 0.5-1.2 | 1.16 | 0.30 | 0.9-1.6 | 1.26 | 0.38 | 0.7-1.7 | 1.26 | 0.21 | 1.1-1.6 |
| Calcium (mg/dl) | 7.88 | 2.46 | 4.8-11.4 | 8.14 | 1.20 | 6.8-9.8 | 8.70 | 1.48 | 6.4-10.4 | 7.18 | 0.58 | 6.6-8.0 |
| Phosphorus (mg/dl) | 5.88 | 0.96 | 4.5-6.8 | 6.38 | 1.40 | 4.4-7.9 | 6.56 | 1.12 | 4.9-7.6 | 6.74 | 1.03 | 6.0-8.4 |
| Cholesterol (mg/dl) | 116.80 | 13.29 | 97-130 | 106.20 | 25.70 | 66-130 | 97.60 | 21.87 | 62-120 | 104.00 | 24.09 | 63-125 |
| Triglycerides (mg/dl) | 86.00 | 34.84 | 57-144 | 62.60 | 23.97 | 30-87 | 54.00 | 17.76 | 26-72 | 57.60 | 14.74 | 37-78 |
| Glucose (mg/dl) | 78.80 | 6.91 | 68-86 | 94.20 | 9.50 | 82-104 | 116.20 | 23.98 | 95-155 | 100.60 | 20.43 | 76-127 |
| Iron (mcg/dl) | 26.60 | 9.84 | 10-35 | 31.60 | 11.70 | 13-43 | 31.40 | 12.95 | 10-45 | 22.00 | 11.11 | 9.0-34.0 |
| Sodium (meq/l) | 154.60 | 7.23 | 150-167 | 153.60 | 3.13 | 151-159 | 152.00 | 1.87 | 150-154 | 152.80 | 2.17 | 151-156 |
| Potassium (meq/l) | 4.76 | 0.61 | 4.1-5.5 | 4.64 | 0.27 | 4.2-4.9 | 4.18 | 0.37 | 3.8-4.8 | 4.10 | 0.24 | 3.8-4.3 |
| Chloride (meq/l) | 116.00 | 4.36 | 112-123 | 116.40 | 2.97 | 113-121 | 115.60 | 7.50 | 109-126 | 108.80 | 4.76 | 103-116 |

[a]Standard Deviation
[b]Alanine Amino Transferase
[c]Aspartate Amino Transferase
[d]Gamma Glutamyl Transferase
[e]Lactate Dehydrogenase

Table 3. Mean, standard deviation, and range of hematological values for each sampling period for clinically healthy green turtles (*Chelonia mydas*), Kaneohe Bay, Island of Oahu, Hawaii, 1993.

| HEMATOLOGICAL VARIABLE | 0 hr | | | 1 hr | | | 3-4 hr | | | 24 hr | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | ±SD[a] | Range | Mean | ±SD | Range | Mean | ±SD | Range | Mean | ±SD | Range |
| Packed Cell Volume (%) | 31.20 | 4.97 | 28-40 | 31.00 | 4.00 | 28-38 | 28.00 | 4.53 | 25-36 | 26.80 | 4.66 | 24-35 |
| Erythrocytes ($10^6$/µl) | 0.48 | 0.16 | 0.23-0.63 | 0.47 | 0.13 | 0.31-0.64 | .40 | 0.12 | 0.28-0.59 | 0.34 | 0.09 | 0.19-0.44 |
| Hemoglobin (g/dl) | 8.54 | 1.44 | 7.3-10.9 | 8.52 | 1.14 | 7.4-10.4 | 7.48 | 1.35 | 5.7-9.4 | 7.46 | 1.31 | 6.8-9.8 |
| MCV[b] (fl) | 725.00 | 312.35 | 444-1261 | 681.60 | 150.81 | 545-935 | 735.80 | 207.00 | 441-929 | 851.20 | 296.47 | 545-1316 |
| MCH[c] (pg) | 198.18 | 83.52 | 115.9-339.1 | 188.76 | 51.52 | 145.5-277.4 | 199.76 | 70.47 | 116.9-275.0 | 235.96 | 78.44 | 156.8-357.9 |
| MCHC[d] (g/dl) | 27.50 | 1.25 | 26.7-29.7 | 27.50 | 1.30 | 26.4-29.7 | 26.82 | 3.77 | 21.1-30.8 | 27.88 | 1.21 | 26.2-29.2 |
| Leukocytes ($10^3$/µl) | 9.34 | 4.44 | 3.3-15.5 | 7.82 | 3.22 | 3.8-10.8 | 9.38 | 3.77 | 6.8-16.0 | 7.08 | 1.58 | 5.0-8.8 |
| Heterophils ($10^3$/µl) | 876.20 | 441.52 | 429-1581 | 530.40 | 264.38 | 324-990 | 1812.80 | 1221.62 | 255-3200 | 4029.60 | 1375.94 | 2520-5229 |
| Heterophils (%) | 10.400 | 4.67 | 5-17 | 7.80 | 3.96 | 3-11 | 20.20 | 14.31 | 3-41 | 55.80 | 9.42 | 42-65 |
| Neutrophils ($10^3$/µl) | 711.60 | 915.87 | 156-2325 | 251.40 | 193.21 | 100-540 | 683.40 | 797.74 | 136-2080 | 493.60 | 424.66 | 120-1162 |
| Neutrophils (%) | 5.60 | 5.41 | 2-15 | 3.20 | 1.64 | 1-5 | 6.00 | 4.36 | 2-13 | 7.00 | 5.66 | 2-14 |
| Lymphocytes ($10^3$/µl) | 6118.20 | 2864.54 | 2739-9180 | 5478.60 | 2670.34 | 2774-8715 | 5645.40 | 2727.40 | 2652-8800 | 2255.60 | 570.74 | 1660-2904 |
| Lymphocytes (%) | 67.80 | 15.40 | 51-85 | 68.80 | 10.08 | 56-83 | 59.80 | 21.56 | 39-90 | 33.00 | 10.07 | 20-47 |
| Eosinophils ($10^3$/µl) | 1197.80 | 1269.34 | 99-2635 | 1031.20 | 811.29 | 228-2268 | 809.80 | 714.73 | 170-1898 | 274.00 | 190.61 | 88-540 |
| Eosinophils (%) | 12.00 | 12.77 | 1-32 | 13.60 | 10.26 | 6-28 | 9.40 | 9.71 | 2-26 | 4.00 | 3.46 | 1-9 |
| Basophils ($10^3$/µl) | 495.60 | 407.04 | 0-930 | 528.40 | 443.12 | 200-1260 | 428.60 | 282.18 | 146-800 | 205.00 | 83.44 | 146-264 |
| Basophils (%) | 6.75 | 1.71 | 0.0-9.0 | 6.60 | 4.34 | 3-14 | 4.60 | 2.88 | 2-9 | 2.50 | 0.71 | 2-3 |
| Heterophil/Lymphocyte Ratio | 0.16 | 0.07 | 0.09-0.27 | 0.12 | 0.07 | 0.04-0.18 | 0.44 | 0.40 | 0.03-1.05 | 1.90 | 0.87 | 0.89-3.15 |

[a]Standard Deviation
[b]Mean Corpuscular Volume
[c]Mean Corpuscular Hemoglobin
[d]Mean Corpuscular Hemoglobin Concentration

Table 4. Mean, standard deviation, and range of hematological values for each sampling period for green turtles (*Chelonia mydas*) afflicted with fibropapillomas, Kaneohe Bay, Island of Oahu, Hawaii, 1993.

| HEMATOLOGICAL VARIABLE | 0 hr | | | 1 hr | | | 3-4 hr | | | 24 hr | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | ±SD[a] | Range | Mean | ±SD | Range | Mean | ±SD | Range | Mean | ±SD | Range |
| Packed Cell Volume (%) | 21.80 | 5.40 | 13-27 | 20.40 | 8.79 | 6-27 | 20.20 | 5.54 | 12-25 | 18.40 | 5.22 | 10-24 |
| Erythrocytes ($10^6$/µl) | 0.32 | 0.10 | 0.24-0.49 | 0.29 | 0.12 | 0.15-1.46 | 0.34 | 0.09 | 0.21-0.45 | 0.22 | 0.10 | 0.07-0.35 |
| Hemoglobin (g/dl) | 5.78 | 1.82 | 2.9-7.6 | 5.42 | 2.63 | 1.4-7.9 | 4.78 | 1.70 | 2.3-6.8 | 4.64 | 1.24 | 2.8-5.9 |
| MCV[b] (fl) | 705.00 | 170.14 | 510-871 | 701.80 | 211.51 | 400-893 | 619.80 | 191.82 | 375-820 | 938.20 | 301.84 | 600-1429 |
| MCH[c] (pg) | 186.16 | 58.73 | 120.8-245.2 | 183.84 | 64.85 | 93.3-250.0 | 151.30 | 71.82 | 71.9-228.6 | 241.58 | 94.07 | 154-400 |
| MCHC[d] (g/dl) | 26.06 | 2.60 | 22.3-28.7 | 25.86 | 2.73 | 23.3-29.3 | 23.40 | 4.50 | 18.3-28.2 | 25.46 | 2.22 | 22.2-28.0 |
| Leukocytes ($10^3$/µl) | 6.98 | 3.49 | 3-11 | 6.30 | 5.60 | 1.3-15.8 | 6.98 | 3.12 | 3.2-11.3 | 9.48 | 2.47 | 7.3-13.3 |
| Heterophils ($10^3$/µl) | 1716.20 | 283.59 | 1320-2060 | 1190.40 | 464.48 | 468-1738 | 2388.60 | 1343.79 | 1170-4290 | 5989.20 | 1848.99 | 3869-8113 |
| Heterophils (%) | 30.800 | 16.45 | 12-55 | 25.80 | 10.55 | 11-36 | 35.20 | 21.45 | 15-60 | 62.600 | 8.14 | 53-74 |
| Neutrophils ($10^3$/µl) | 627.20 | 795.96 | 53-2014 | 444.40 | 183.00 | 232-689 | 699.00 | 630.89 | 288-1638 | 1047.60 | 406.47 | 400-1425 |
| Neutrophils % | 13.25 | 16.58 | 3-38 | 11.80 | 8.41 | 3-23 | 10.00 | 7.44 | 5-21 | 12.00 | 6.12 | 4-18 |
| Lymphocytes ($10^3$/µl) | 3564.00 | 2929.82 | 720-6798 | 3285.40 | 3403.31 | 312-9006 | 3277.60 | 2791.26 | 312-7119 | 2259.60 | 1069.44 | 1045-3857 |
| Lymphocytes (%) | 44.40 | 23.16 | 15-66 | 44.80 | 14.20 | 24-58 | 43.00 | 26.09 | 4-65 | 23.60 | 7.70 | 11-30 |
| Eosinophils ($10^3$/µl) | 574.40 | 185.81 | 420-880 | 1193.00 | 1493.54 | 143-3792 | 1041.20 | 777.56 | 210-2147 | 248.33 | 133.25 | 146-399 |
| Eosinophils (%) | 9.20 | 2.95 | 6-14 | 15.00 | 6.25 | 8-24 | 13.00 | 5.61 | 6-19 | 2.33 | 0.58 | 2-3 |
| Basophils ($10^3$/µl) | 603.50 | 799.65 | 33-1760 | 300.33 | 424.66 | 33-790 | 159.50 | 51.60 | 100-226 | 86.50 | 19.09 | 73-100 |
| Basophils (%) | 6.00 | 6.88 | 1-16 | 4.00 | 2.65 | 1-6 | 1.75 | 0.50 | 1-2 | 1.00 | 0.0 | 1.0-1.0 |
| Heterophil/Lymphocyte Ratio | 1.15 | 1.10 | 0.21-2.40 | 0.70 | 0.52 | 0.19-1.5 | 3.46 | 5.83 | 0.23-13.75 | 3.16 | 2.03 | 1.76-6.73 |

[a]Standard Deviation
[b]Mean Corpuscular Volume
[c]Mean Corpuscular Hemoglobin
[d]Mean Corpuscular Hemoglobin Concentration

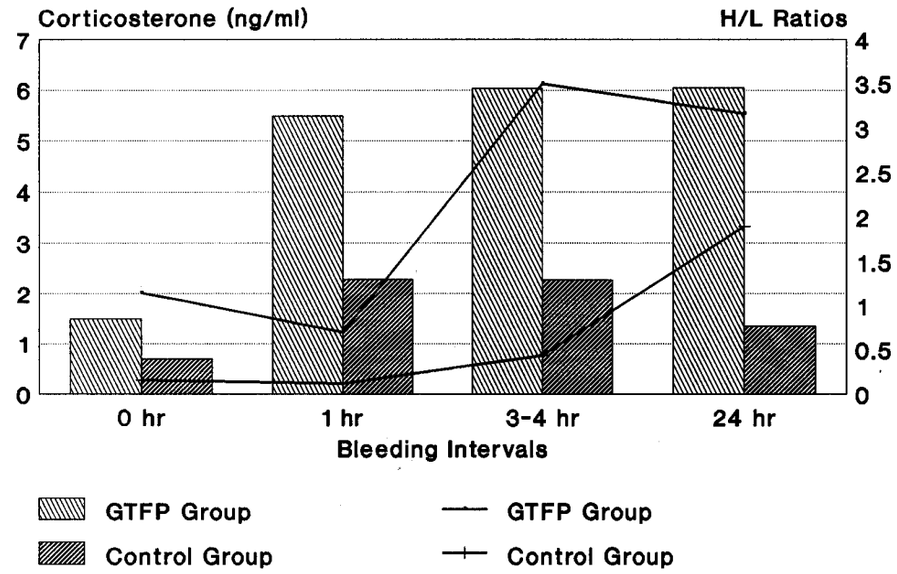



Figure 1. Mean corticosterone concentrations and heterophil/ lymphocyte ratios at four bleeding intervals for juvenile green turtles (Chelonia mydas) with and without fibropapillomas captured at Kaneohe Bay, Island of Oahu, Hawaii, September 1993.

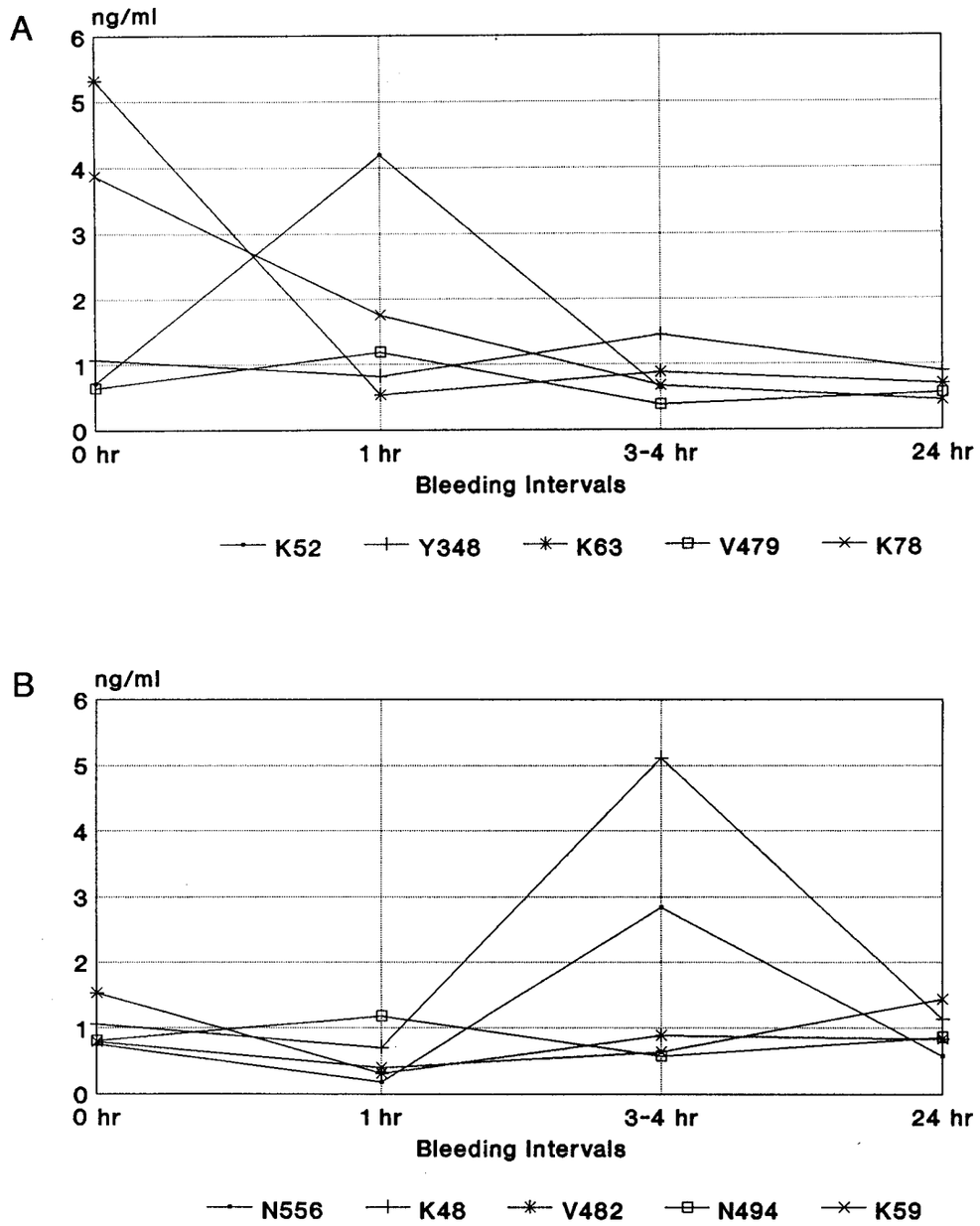



Figure 2. Estradiol 17-ß concentrations at four bleeding intervals for individual juvenile green turtles (Chelonia mydas) with (A) and without (B) fibropapillomas captured at Kaneohe Bay, Island of Oahu, Hawaii, September 1993.

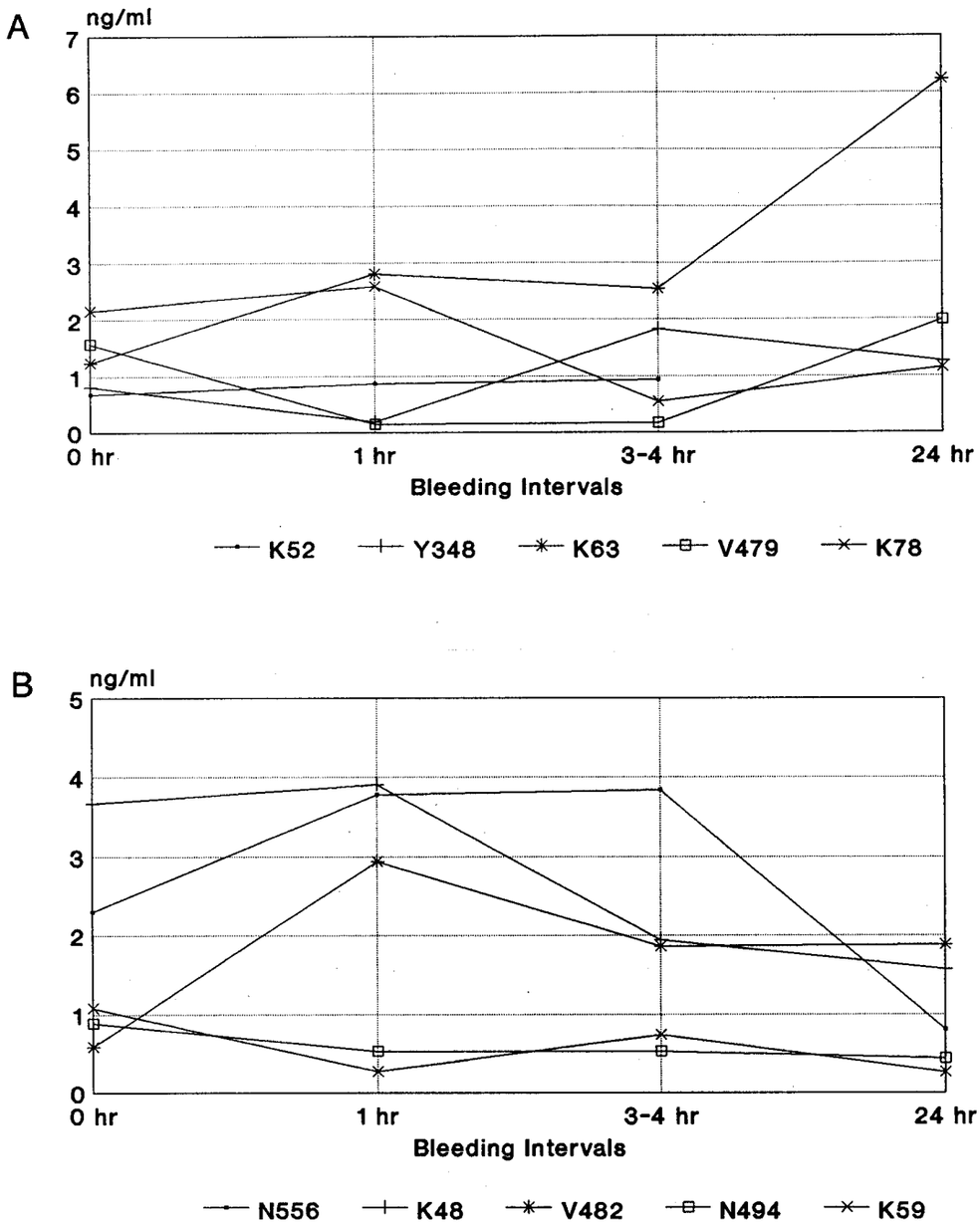



Figure 3. Testosterone concentrations at four bleeding intervals for individual juvenile green turtles (Chelonia mydas) with (A) and without (B) fibropapillomas captured at Kaneohe Bay, Island of Oahu, Hawaii, September 1993.

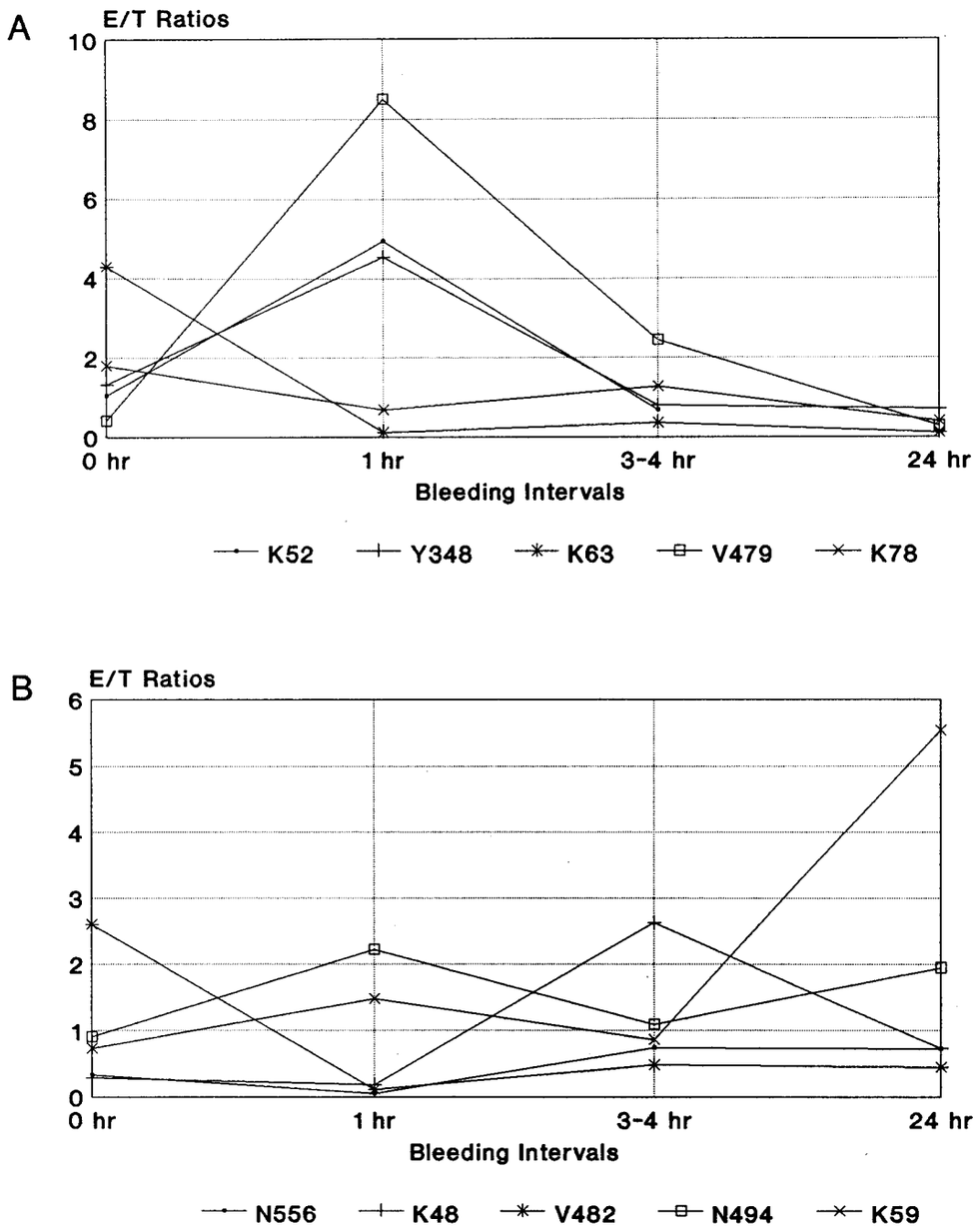



Figure 4. Estradiol 17-ß/testosterone ratios at four bleeding intervals for individual juvenile green turtles (Chelonia mydas) with (A) and without (B) fibropapillomas captured at Kaneohe Bay, Island of Oahu, Hawaii, September 1993.

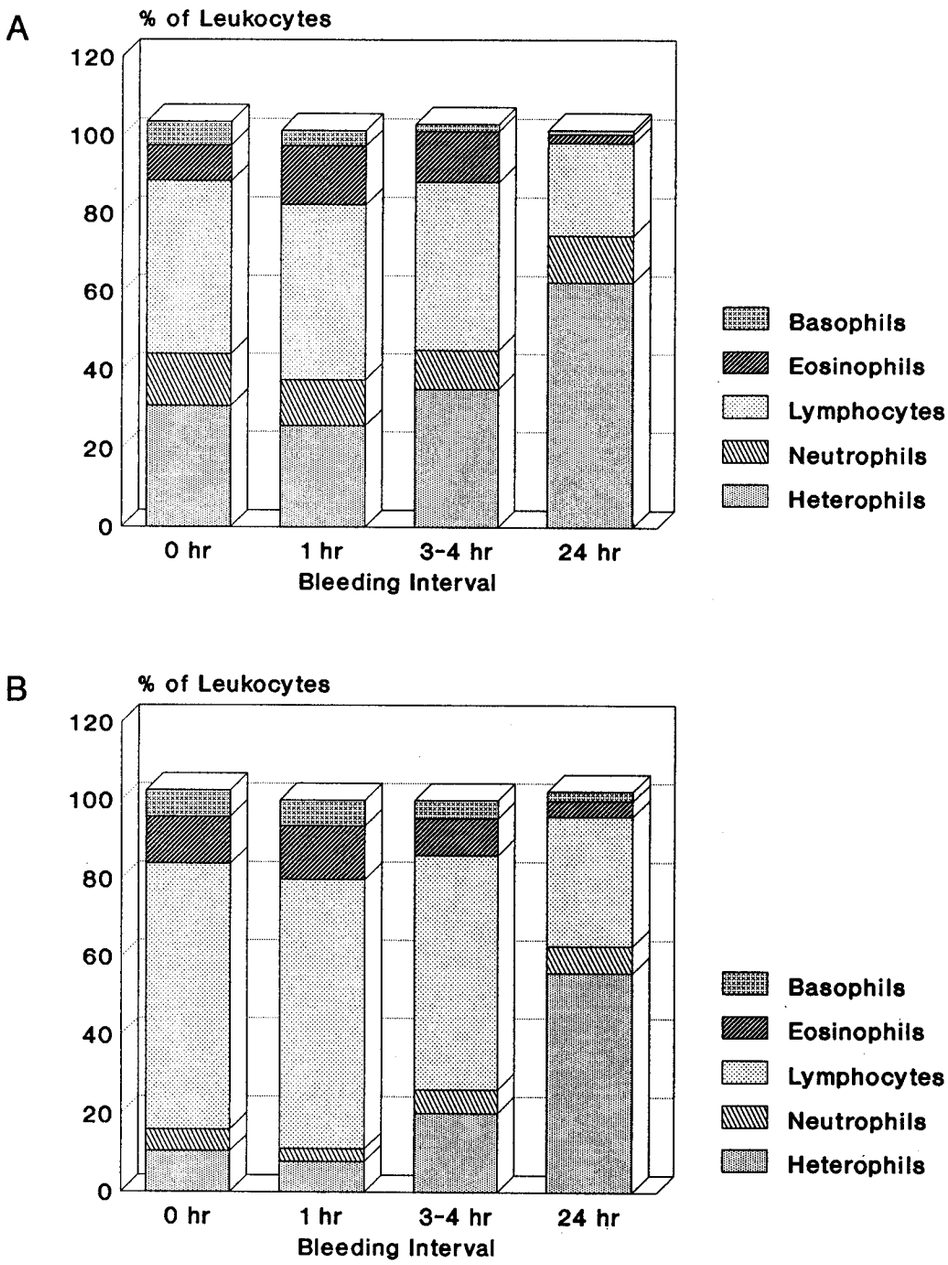



Figure 5. Mean percentages for different types of leukocytes at four bleeding intervals for juvenile green turtles (Chelonia mydas) with (A) and without (B) fibropapillomas captured at Kaneohe Bay, Island of Oahu, Hawaii, September 1993.

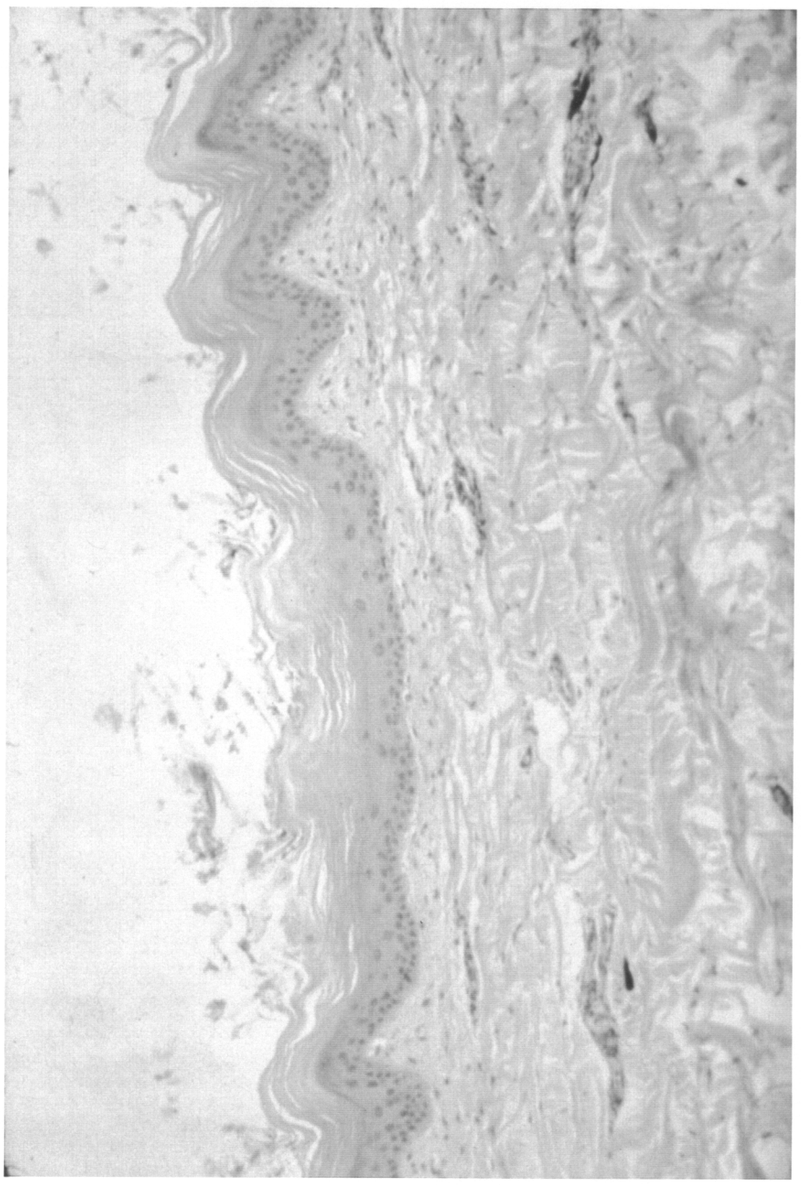


Figure 6. Normal skin of a juvenile green turtle (Chelonia mydas). H & E Stain, x 10.

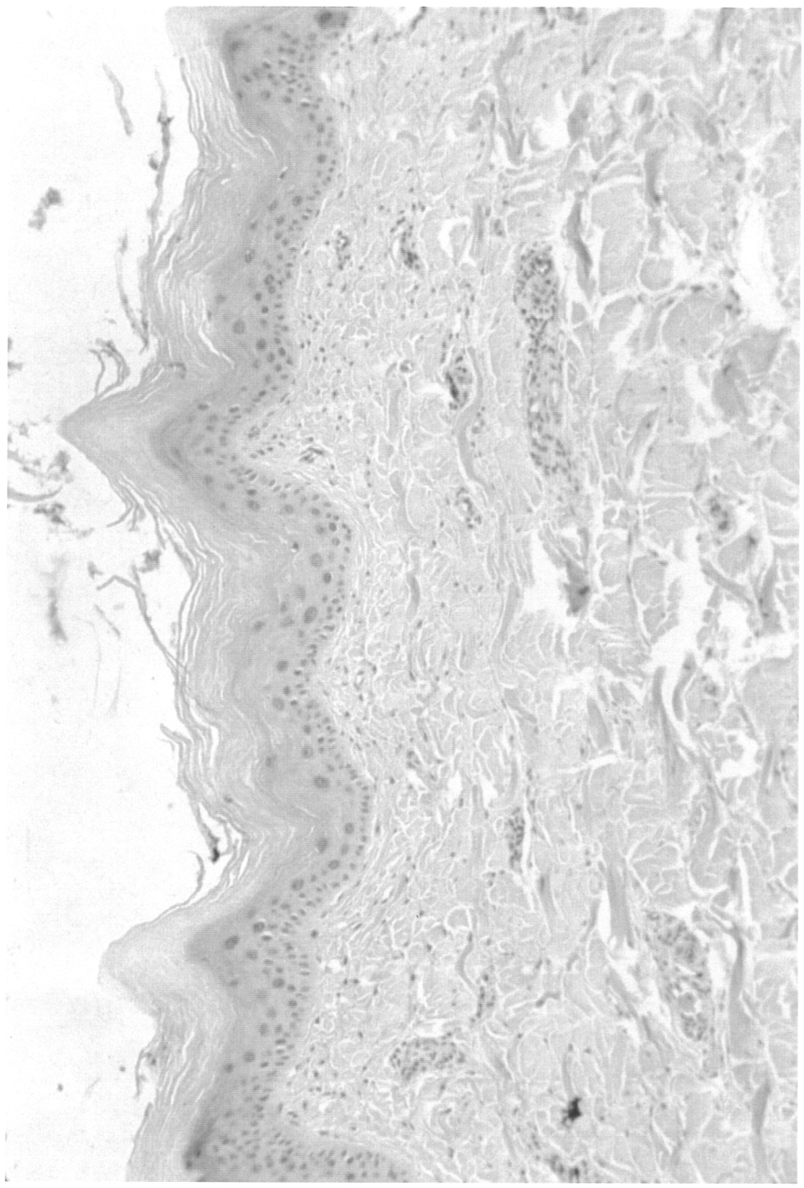


Figure 7. Mild focal dermatitis with lymphocytic cuffing of vessels in normal epidermis of a juvenile green turtle (Chelonia mydas). H & E Stain, x 10.

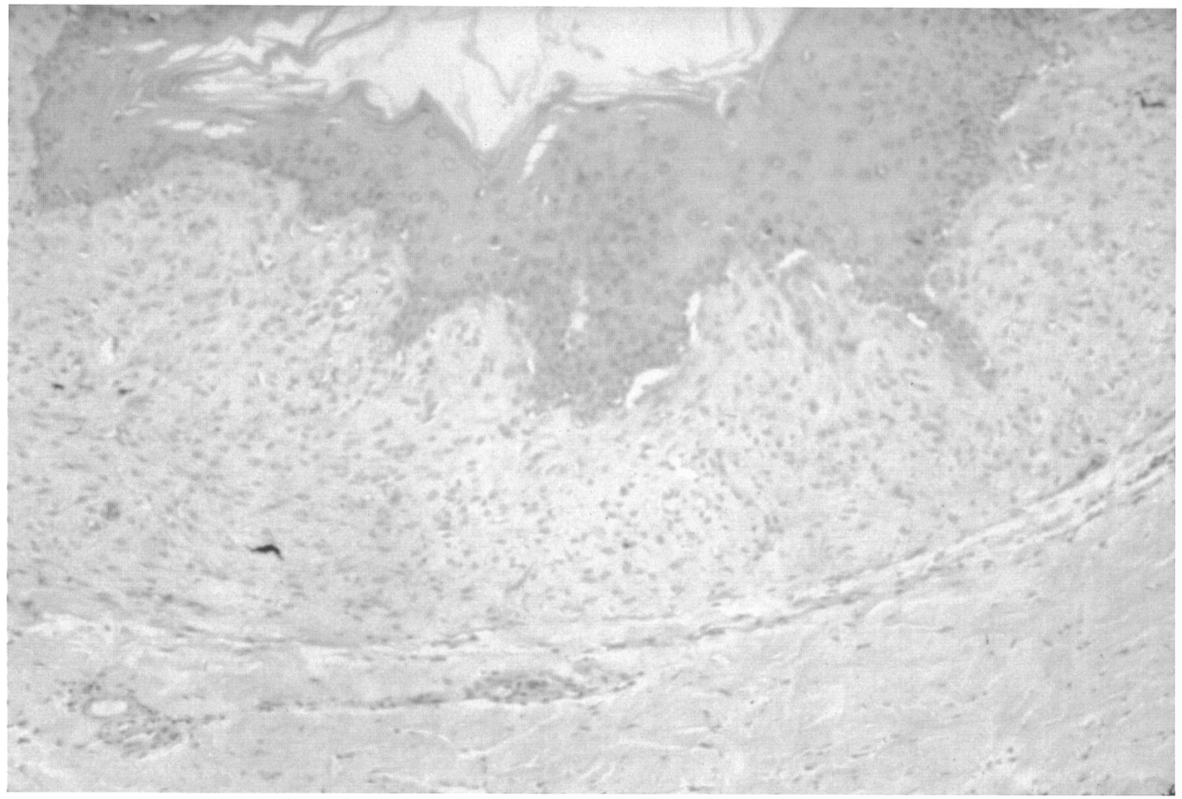


Figure 8. Flat sarcoid described in early growths within the dermis presenting early pseudoepitheliomatous hyperplasia, and acanthosis of the epidermis of a juvenile green turtle (Chelonia mydas) fibropapilloma. H & E Stain, x 10.

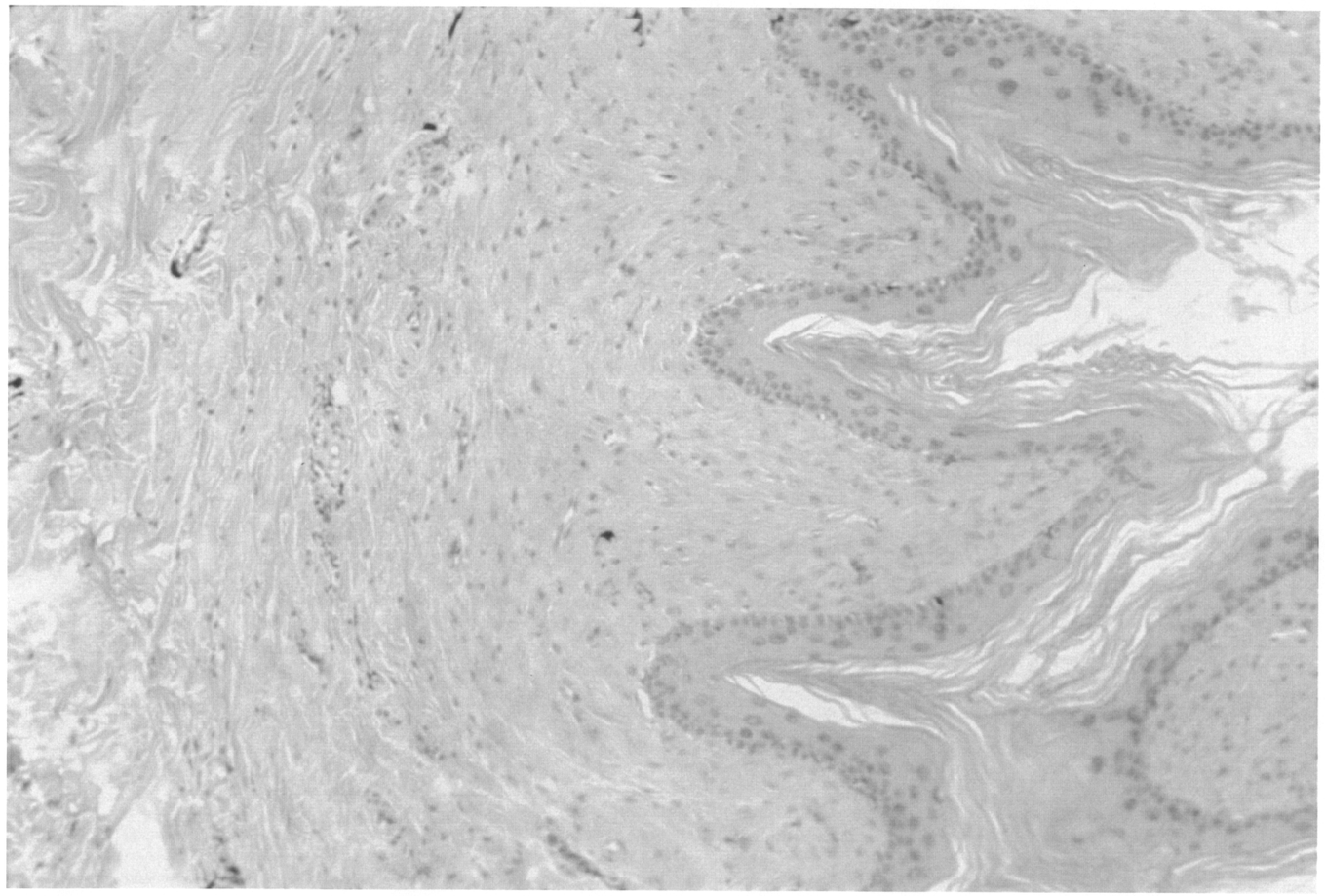


Figure 9. Pseudoepitheliomatous hyperplasia and papillary projections of squamous epithelial cells, and mesodermal proliferation invading normal tissue of a juvenile green turtle (Chelonia mydas) fibropapilloma. H & E Stain, x 10.

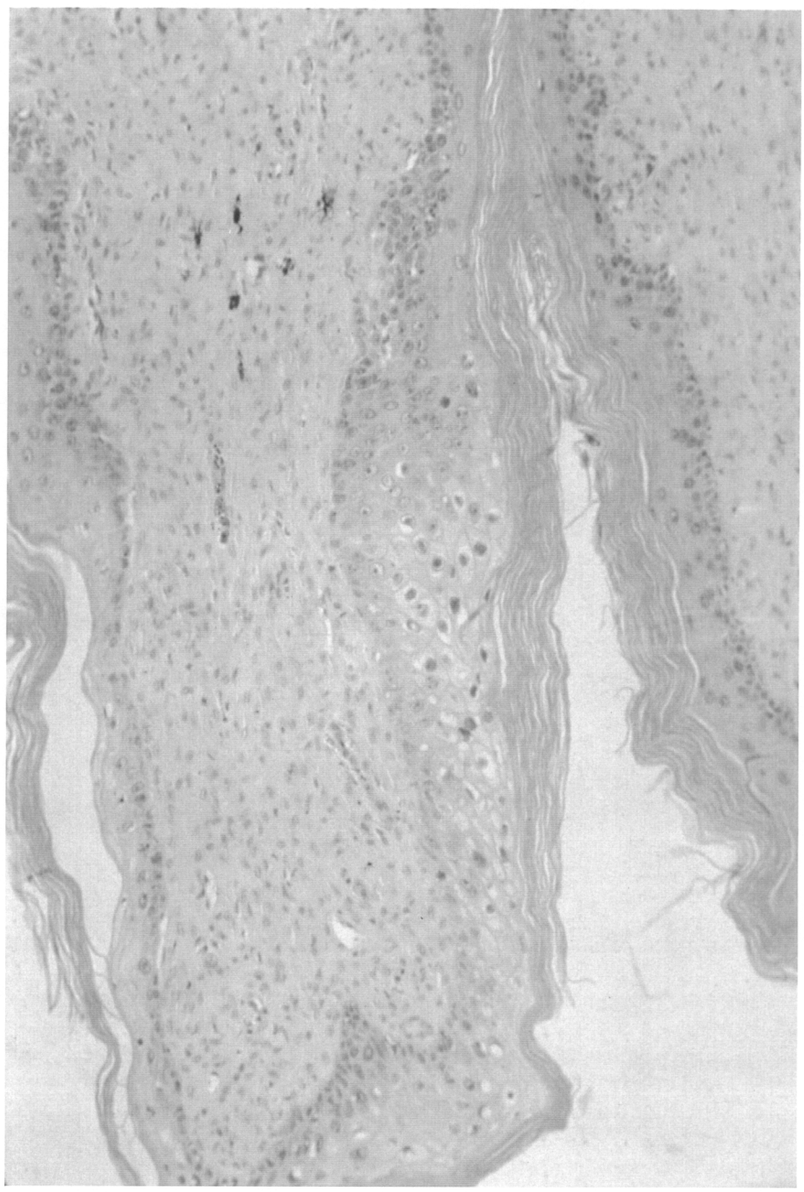


Figure 10. Early ballooning degeneration, inflammatory reaction, and nuclear necrosis of stratum basale of epidermis of a juvenile green turtle (Chelonia mydas) fibropapilloma. H & E Stain, x 10.

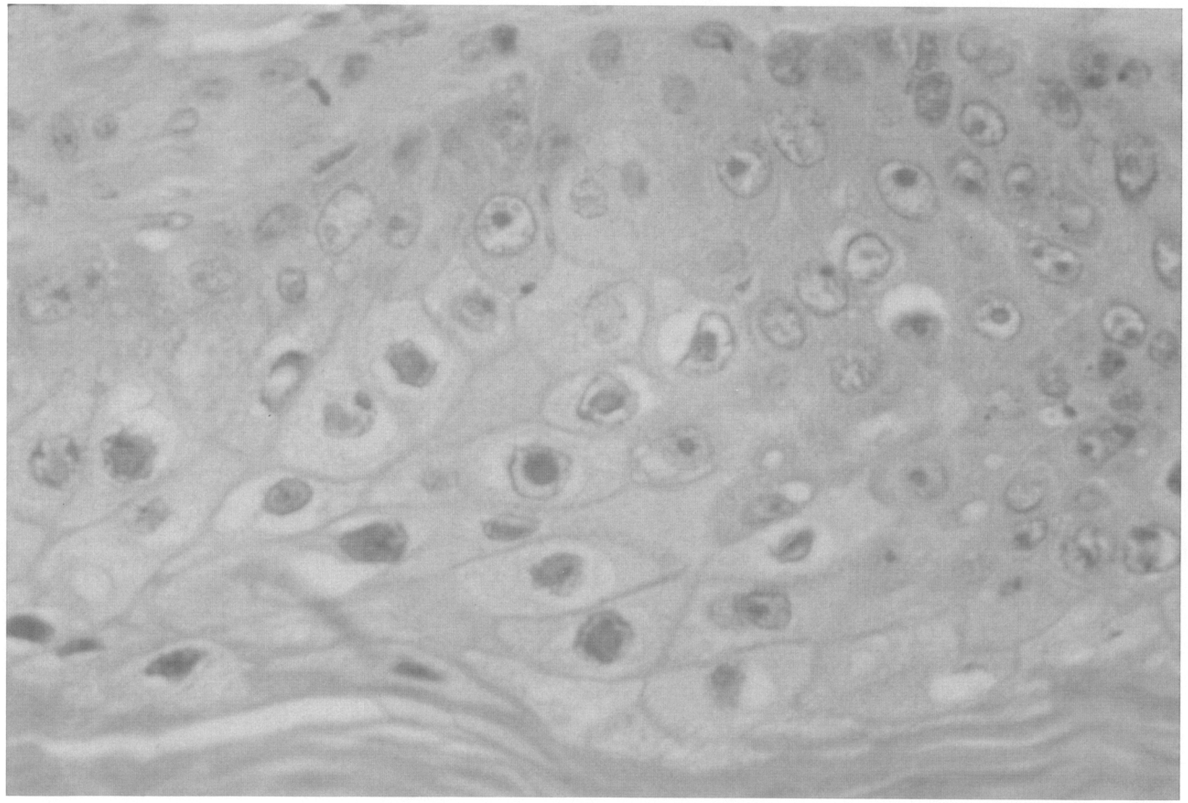


Figure 11. Unidentified structures similar to intranuclear inclusions showing chromatin margination by large basophilic bodies within hypertrophied nuclei of epidermal cells of a juvenile green turtle (Chelonia mydas) fibropapilloma. H & E Stain, x 40.

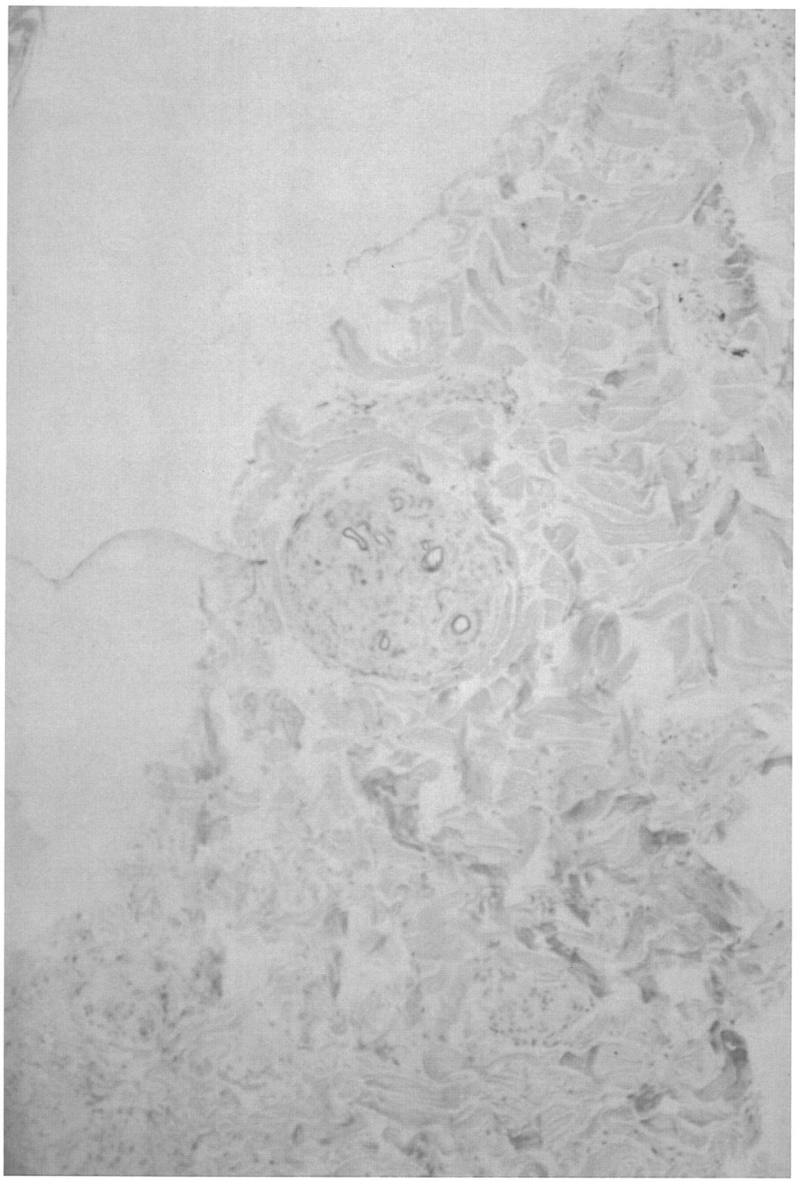


Figure 12. Spirorchid trematode ova surrounded by a granuloma in the epidermis of a juvenile green turtle (Chelonia mydas) fibropapilloma. H & E Stain, x 10.

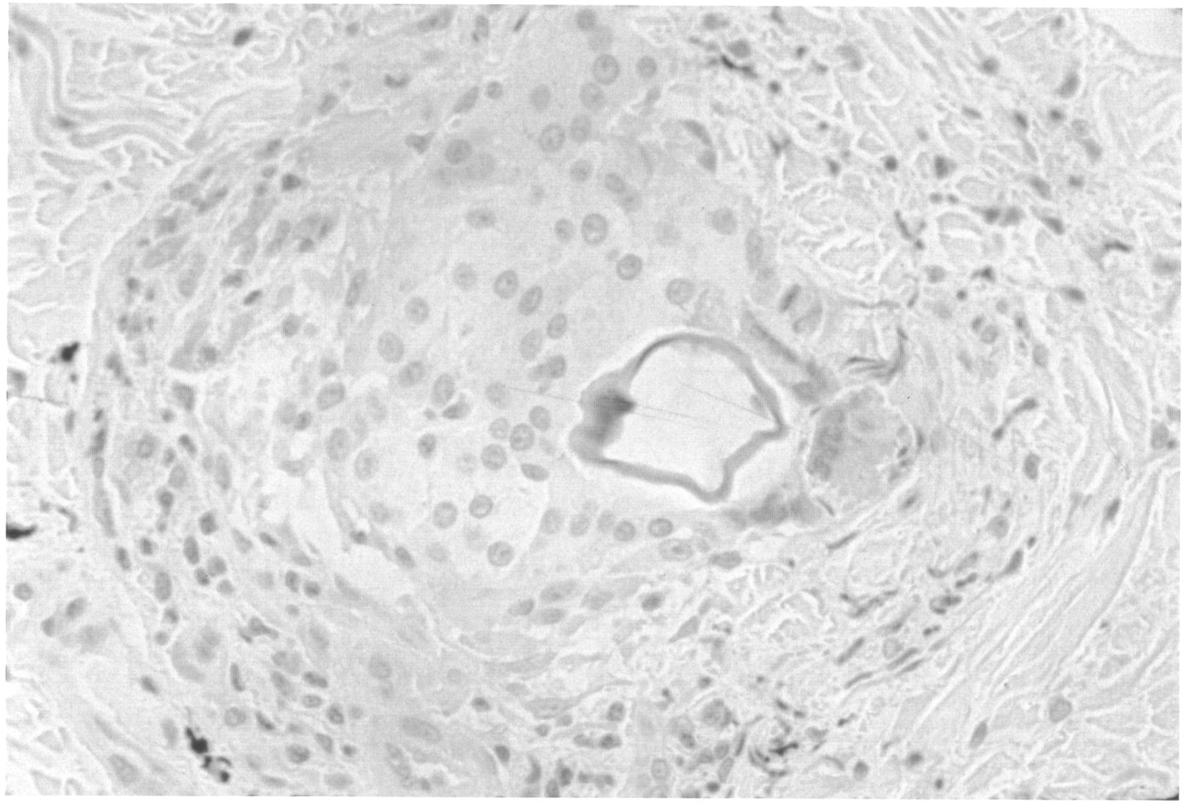


Figure 13. Inflammatory response around a spirorchid trematode egg primarily formed by lymphocytes, plasma cells and heterophils in the epidermis of a juvenile green turtle (Chelonia mydas) fibropapilloma. H & E Stain, x 40.