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Cutaneous Fibropapillomas and Renal Myxofibroma in a Green Turtle, *Chelonia mydas*

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ABSTRACT: A debilitated 7 kg juvenile green turtle (*Chelonia mydas mydas*) with multiple ulcerated and infected cutaneous fibropapillomas was clinically evaluated and found to have a nonregenerative anemia, hypoproteinemia, hypoalbuminemia and several electrolyte abnormalities. Surgery was performed to remove the larger tumors. The turtle did not eat postsurgically, and an attempt was made to place a pharyngostomy tube utilizing endoscopy. Edematous esophageal papillae, the angulation of the gastroesophageal junction, and a S-shaped configuration of the esophagous prevented successful placement of the tube. The animal was found dead the next day and necropsied. Multiple large white firm nodules were seen bulging from both kidneys. Microscopic examination of the nodules resulted in a diagnosis of renal myxofibroma.

Key words: Fibropapilloma, myxofibroma, green turtle, *Chelonia mydas*, case history.

Green turtle fibropapilloma (GTFP) is a significant disease afflicting the green turtle (*Chelonia mydas*) in Florida and Hawaii. The disease was first observed over 50 yr ago (Lucke, 1938; Smith and Coates, 1938). Fifty-seven percent of green turtles collected in the Indian River Lagoon System of east central Florida (USA) between 1985 and 1986 had GTFP. Prior to 1982, this disease was not observed in any of the collected turtles (Jacobson et al., 1989). Up to 10% of nesting female green turtles tagged in Hawaii had GTFP (Balazs, 1986).

The cause of GTFP remains unknown. In the original descriptions, leeches were commonly found on these growths and spirorchid digenean (*Learedius learedi*) eggs were often seen within capillaries in the fibrous portion of the tumors (Lucke, 1938; Smith and Coates, 1938). In a recent study, no digenean eggs were observed in any of 28 biopsies examined from fibropapillomas of six Florida green turtles sampled over a 6 mo period (Jacobson et al., 1989). Thus, it seems unlikely that spirorchid eggs are the cause of GTFP. Instead, since adult spirorchid digeneans are located within the heart and aorta (Greiner et al., 1980) it appears that eggs that are released into the vascular compartment simply collect within dermal capillaries of pre-existing tumors.

From a comparative standpoint, a viral etiology seems to be the most likely cause of GTFP of green turtles. Herpesvirus (Raynaud and Adrian, 1976; Jacobson et al., 1989), poxvirus (Hirth et al., 1969; Pulley and Shively, 1973), and papillomavirus (Jacobson et al., 1989) have all been either associated with or found to be a cause of papillomas and fibropapillomas in reptiles, birds and mammals. Molecular studies utilizing low stringency southern blot hybridization and a reverse southern blot failed to demonstrate papillomavirus DNA in any tumors of a series of samples of GTFP (Jacobson et al., 1989). In the same study, ultrastructural examination of multiple biopsy specimens failed to demonstrate herpesvirus, poxvirus or papillomavirus. However, particles with electron dense centers and measuring 155 to 190 nm were seen within intracytoplasmic vacuoles in the stratum basale. The exact nature of these particles remain unknown and further studies are needed to elucidate the cause of this disease.

A 7 kg male juvenile green turtle, *Chelonia mydas mydas*, with multiple cutaneous fibropapillomas was presented to the University of Florida College of Veterinary Medicine (Gainesville, Florida 32610, USA), for clinical evaluation. The turtle was collected near Marathon, Florida (24.5°00′S, 81°00′E) on 24 October 1986 and was maintained for approximately 1

TABLE 1. Hematologic and serum biochemical findings in a debilitated green turtle with cutaneous fibropapillomas and three clinically healthy green turtles.

Evaluation	Determinant	Debilitated turtle	Normal (SD)
Hematology	WBC (μl)	13,800	12,066 (874)
	RBC ($\times 10^6$ μl)	0.15	0.61 (0.23)
	Hemoglobin (g/dl)	2.4	10.1 (3)
	PCV (%)	8.0	36.00 (7)
	MCV (fl)	498.0	590.00
	MCHC (g/dl)	30.00	28.06
	Differential		
	Heterophils (%)	71	88 (2)
	Lymphocytes (%)	20	7 (2)
	Monocytes (%)	3	5
	Eosinophils (%)	3	—
	Basophils (%)	3	—
	Fibrinogen (mg/dl)	100	—
Serum biochemistry	ALP (U/liter)	6	25.7 (11)
	ALT (U/liter)	26	7.0 (2.6)
	AST (U/liter)	756	155 (59)
	Na (mEq/liter)	115	150 (4)
	K (mEq/liter)	3.7	4.1 (0.1)
	Cl (mEq/liter)	74	108 (5)
	BUN (mg/dl)	58	26 (25)
	Total protein (g/dl)	1.1	4.2 (0.9)
	Albumin (g/dl)	0.3	1.6 (0.6)
	Globulin (g/dl)	0.8	2.6 (0.5)
	Calcium (mg/dl)	3.4	7.3 (0.5)
	Pi (mg/dl)	5.2	8.0 (1.2)
	Cholesterol (mg/dl)	55	201 (110)
	Glucose (mg/dl)	101	102 (27)
	Uric acid (mg/dl)	0.6	1.5 (0.6)
	Total bilirubin (mg/dl)	1.1	4.2 (0.9)

yr in a large cement pond provided with an open-flow system with the adjoining ocean. Several species of locally collected marine fish were kept in the same tank. The diet consisted of a mixture of fish, including *Hemiramphus brasiliensis* and *Agonostomus monticola*.

Physical examination revealed the turtle was severely debilitated. The carapace and plastron had multiple cracks, were soft, and covered with algae and numerous barnacles. Cutaneous growths ranging in size from 1 cm × 1 cm to 10 cm × 10 cm, consistent with green turtle fibropapilloma (Jacobson et al., 1989) were located on multiple areas of the plastron, carapace, skin and left cornea. A blood sample obtained from the jugular vein and submitted for a complete blood count and serum chemical profile revealed a severe nonregenerative anemia, hypoproteinemia, hypoalbuminemia, elevated serum aspartate aminotransferase, hypocalcemia, hyponatremia, and hypochloremia when compared to blood values of three clinically healthy green turtles collected by one of the authors (ERJ, Table 1). Several oocysts of an unidentified coccidian-like protozoan were found on examination of a rectal wash specimen. Because of the extent of involvement, a decision was made to remove the largest fibropapillomas.

Preoperatively, the turtle was treated with 300 ml of lactated ringer's solution subcutaneously and by intracoelomic infusion, and intramuscularly with trimethoprim/sulfadiazine (30 mg/kg, Tribrissen 24%, Coopers Animal Health, Inc., Kansas

City, Missouri 64108, USA); Praziquantel (Droncit® 15 mg/kg, 56.8 mg/ml, Mobay Corporation, Animal Health Division, Shawnee, Kansas 66201, USA); Injacom 100 + B (0.05 ml/300 g Roche Chemical Division, Hoffman, La Roche, Inc., Nutley, New Jersey 07110, USA); and iron dextran (5 mg/kg, 100 mg/ml, The Butler Company, Columbus, Ohio 43228, USA). The turtle was restrained, intubated and anesthetized with 2% halothane in 1 liter of oxygen. The turtle was manually ventilated four times/min. Several of the largest fibropapillomas were surgically removed and the skin was closed with 2.0 Clear Monofilament Polyglyconate (Maxon®, Davis and Geck, D+G Monofil Inc., Manati, Puerto Rico 00701, USA) suture material in a simple interrupted pattern. Complete recovery from anesthesia was prolonged, taking approximately 12 hr.

Postoperatively, supportive care was continued and the turtle was offered mullet, smelt, mackeral, spinach, endive and a vitamin/mineral supplement. Due to a poor appetite, attempts were made to pass a feeding tube into the stomach. This was unsuccessful. The turtle continued to lose weight and an attempt was made to place a pharyngostomy tube for purposes of enteral alimentation. A similar anesthetic protocol as previously described was used. Again the tube could not be inserted into the stomach. A fiberoptic endoscope was passed down the esophagus, revealing severely edematous esophageal papillae preventing the passage of the endoscope. Recovery from anesthesia was uneventful, and the turtle was returned to its tank. It was found dead in the tank the next morning and was examined at necropsy.

Grossly, the plastron and carapace were thin and soft. The shell was easily cut with a scalpel blade. This was attributed to chronic debilitation and anorexia. The coelomic cavity was filled with approximately 1 liter of clear fluid. The stomach was filled with undigested plant material. The cranial pole of each kidney contained a white, firm nodule which comprised approximately one-eighth of the entire kidney (Fig. 1). On cut section the nodule was homogenous in color and texture. Several smaller white foci were scattered throughout the kidneys. The intestinal, esophageal, and gastric wall were edematous. No other gross abnormalities were seen. Multiple tissues from all major organ systems including the cutaneous fibropapillomas and the renal nodules were fixed in neutral buffered 10% formalin, embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin. Sections of fibropapillomas and renal nodules were also stained by Masson's trichrome method and by the Alcian blue method (pH 2.5). Representative histological specimens from this case are deposited in the Registry of Comparative Pathology (Armed Forces Institute of Pathology, Washington, D.C. 20306; accession number 2221382).

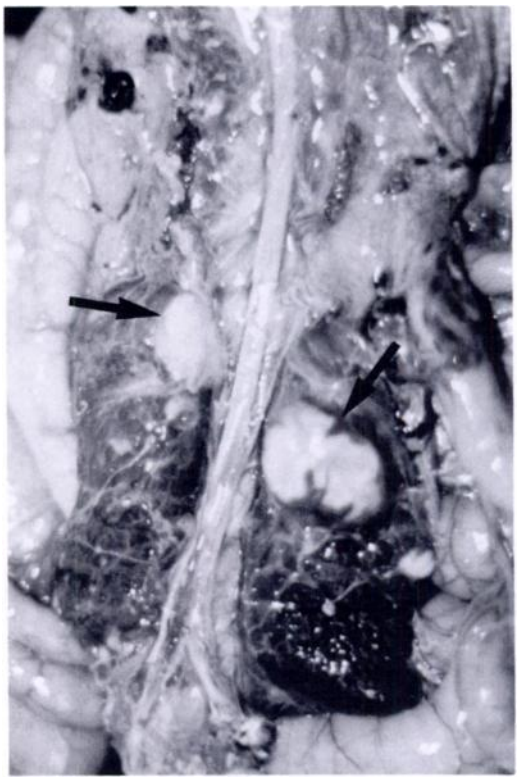

FIGURE 1. Kidneys of a green turtle demonstrating nodules within the cranial poles (arrows).

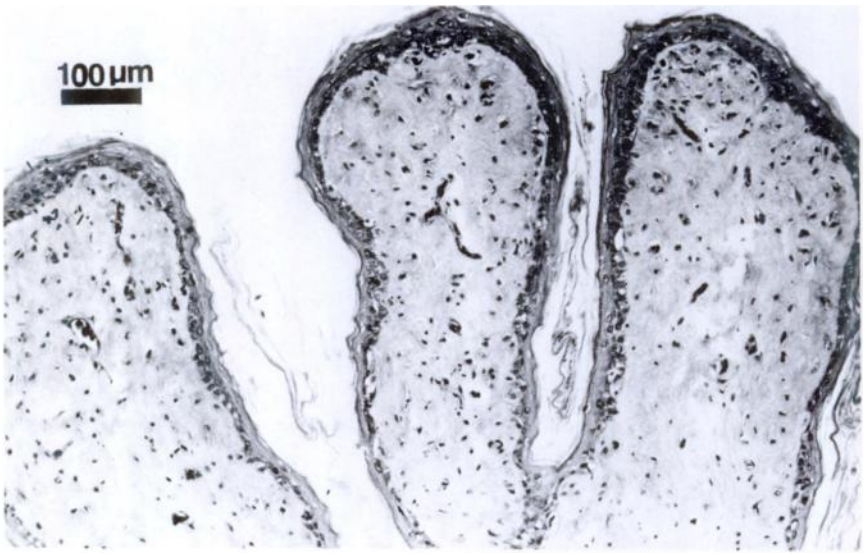

FIGURE 2. Photomicrograph of a cutaneous fibropapilloma of a green turtle. The tumor is papilliferous and composed of a thickened fibrocellular dermis lined by mildly hyperplastic epithelium. H&E Stain.

Histologically, these fibropapillomas consisted of verrucous masses composed of well-differentiated proliferating fibroblasts with moderate amounts of intervening collagen. The stratified squamous epithelium was mildly hyperplastic and minimally hyperkeratotic; no intranuclear inclusions were seen (Fig. 2). The white nodules seen grossly in the kidney were composed of spindle-shaped cells with oval nuclei within a ground substance ranging from regularly arranged eosinophilic staining collagen to more randomly oriented cells within a lightly basophilic staining myxomatous ground substance (Fig. 3). At the surface of the kidney, the nodules were covered by the renal capsule. In most areas, the collagenous tissue was sharply demarcated from adjacent normal appearing renal tissue. Normal appearing tubules were scattered throughout the proliferating connective tissue.

Sections of fibropapillomas and renal nodules stained by the Masson's trichrome method indicated that most of the connective tissue was collagen. By the Alcian blue method, both the dermal component of the fibropapilloma and sections of renal nodules stained light blue, and indicated the presence of mucopolysaccharides. Because of these features, the renal nodule was interpreted to be a myxofibroma. Histologically, the renal myxofibroma and dermal component of the fibropapillomas were indistinguishable.

Digenean eggs were observed not only in the dermis of the fibropapillomas, but also in sections from a variety of organs including lung, liver, kidney, spleen and submucosa of intestinal tract. The inflammatory response was moderate and was composed of mostly mononuclear cells. The digenean eggs probably contributed to the debilitated condition of the turtle.

Praziquantel has been recommended for treating reptiles with trematode infections (Jacobson, 1987) and was administered to this turtle. However, at necropsy, the primary site of infection of adult spirorchid digeneans, the heart and great vessels (Greiner et al., 1980), was not examined in detail and it is unknown whether this drug was efficacious.

There is only one previous report documenting internal fibrous nodules in a green turtle with cutaneous fibropapillomas (Schlumberger and Lucke, 1948). In one of three green turtles examined, mul-

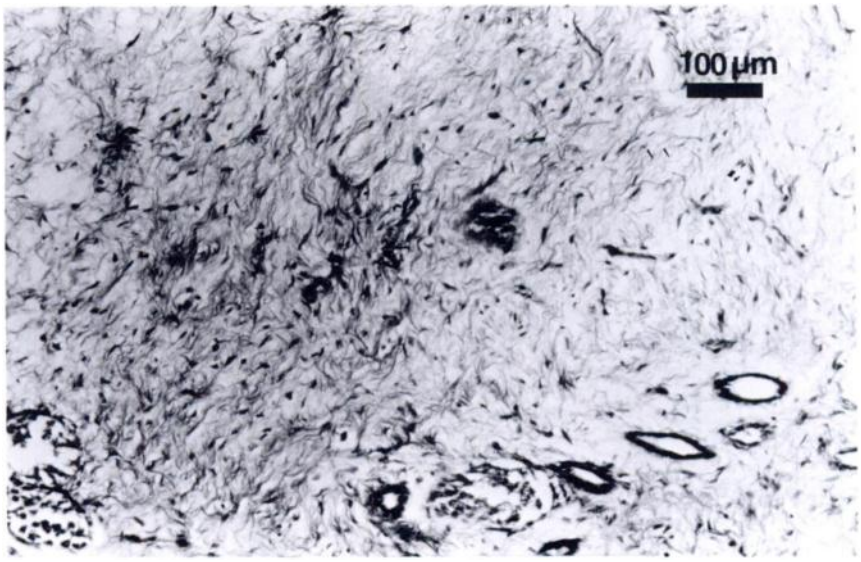

FIGURE 3. Photomicrograph of a renal nodule from a green turtle. Interlacing bands of collagenous connective tissue are seen throughout the mass. In some areas the collagenous stroma is rather loosely arranged. Masson's trichrome stain.

tiple nodules, histologically compatible with the dermal component of the cutaneous fibropapillomas were found in the lungs. However, renal fibroma has not been reported previously in green turtles. Since thorough necropsies are not consistently performed on dead green turtles with this disease, many cases of systemic involvement are most likely missed.

Renal fibromas have been reported in other species. Of 44 cases of renal tumors examined in dogs, four fibromas were reported (Picut and Valentine, 1985). The gross and microscopic appearance of these tumors showed some similarities to that of the green turtle. In humans, renal fibromas are benign tumors which are common incidental findings at necropsy (Xipell, 1971). In both humans and dogs, the tumors occur in mature individuals, while the green turtle of the present report was a juvenile.

Since the cutaneous lesions were considered to be benign based on morphological criteria, the renal lesions may have been primary lesions, independent of the cutaneous disease. Assuming there is an infectious etiologic basis for GTFP, they may have resulted from a septicemia or viremia. Leeches or trematodes could possibly act as vectors for the causative agent. Poxviruses, spread by biting arthropods, not only cause severe cutaneous fibromas and fibropapillomas in squirrels (*Sciurus* spp.) and rabbits (*Sylvilagus* spp.), but visceral lesions resembling generalized metastases as well (O'Conner et al., 1980). In white-tailed deer (*Odocoileus virginianus*) and European elk (*Alces alces*) with cutaneous fibromas and fibropapillomas, pulmonary fibromas can be occasionally found (Koller and Olson, 1971; Sundberg and Lancaster, 1988). The presence of viral DNA has been confirmed in lung tumors of European elk (Moreno-Lopez et al., 1986). In green turtles, identification of an infectious agent and development of molecular probes or specific antisera will be needed to determine the relationship between renal and skin tumors.

Compared to values for clinically healthy green turtles, the hematologic and serum chemical abnormalities in the green turtle were indicative of a debilitated condition. The turtle had a severe nonregenerative anemia, hypoproteinemia, hypoalbuminemia, hypocalcemia, hyponatremia and

hypochloremia, elevated serum aspartate aminotransferase and blood urea nitrogen. Probably, the cutaneous fibropapillomas, spirorchidiasis, coccidiosis and renal myxofibromas all contributed to the death of the turtle. Also, fibropapillomas growing from the conjunctiva and corneal surface as seen in this turtle may have resulted in an avisual animal which will slowly starve to death (Jacobson et al., 1989).

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