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COMMUNICATIONS

Pathology of Fibropapillomatosis in Olive Ridley Turtles *Lepidochelys olivacea* Nesting in Costa Rica

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Abstract.—Fibropapillomatosis (FP) is a neoplastic disease that primarily affects green turtles *Chelonia mydas* in epidemic proportions worldwide. Although several infectious agents (herpesvirus, retrovirus, and papillomavirus) have been associated with the condition, the etiologic agent has not been isolated or characterized. Recently, FP has been reported in other sea turtle species including confirmed cases in loggerhead turtles *Caretta caretta* in Florida and field observations in olive ridley turtles *Lepidochelys olivacea* in the Pacific coasts of Mexico and Costa Rica. Skin and tumor specimens were collected from 72 olive ridley turtles nesting in Ostional Wildlife Refuge, Costa Rica, between July and September 1997. In all, 50 tumor biopsies were examined from 25 of the affected turtles. In addition, six biopsies were examined from five turtles that did not have visible masses and served as controls. Grossly, masses were 25 mm or less in diameter, white to gray, smooth to verruciform, raised tumors of the integument of the neck and flippers. Histologically, 42 of 50 were diagnosed as fibropapillomas and eight were classified as chronic active dermatitis and not tumors. Twenty of 42 fibropapillomas were in stages of regression and 9 of the remaining 22 tumors had histological changes that suggested early degeneration within the tumor. During field surveys based on gross lesions, prevalences of 1–10% have been reported in this nesting population. This is considered the first histopathologic confirmation of FP in olive ridley turtles.

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Fibropapillomatosis (FP) is a disease characterized by multiple cutaneous and internal tumors, ranging from 0.1 cm to more than 30 cm in diameter, that has primarily been reported in green turtles *Chelonia mydas* worldwide (Jacobson et al. 1989; Balazs and Pooley 1991; Aguirre et al. 1994; Herbst 1994; Williams et al. 1994; Adnyana et al. 1997). The disease has a circumtropical distribution and has been observed in all major oceans; where present, prevalence varies among locations, ranging from as low as 1.4% to as high as 90% (Herbst 1994). Several infectious agents have been identified as associated with the tumors, including retroviruses (Casey et al. 1997), herpesviruses (Jacobson et al. 1991; Herbst et al. 1995; Quackenbush et al. 1998; A. A. Aguirre and T. R. Spraker, unpublished data), and papillomaviruses (Y. Lu, University of Hawaii, unpublished data), yet the primary etiologic agent remains to be proven.

The olive ridley turtle *Lepidochelys olivacea* is the most abundant species of marine turtle in the world as a result of continuous massive nestings ("*arribadas*") and their recent protection. The largest nesting aggregation in the world occurs in the Orissa, India, followed by three major sites in the eastern Pacific off the coasts of Mexico and Costa Rica. As many as 100,000 females may nest in one *arribada* in Ostional, Costa Rica. The species is classified as endangered by the IUCN Red Data Book (Pritchard 1997).

Causes of disease or natural mortality are mostly unknown for olive ridley turtles. The first report of fleshy tumors on the head, neck, and front flippers of nesting females occurred in Costa Rica in 1982 (Cornelius and Robinson 1983). The first suspected case of FP in the Ostional olive ridley turtle population was reported during the *arribada* of October 1987. A nesting female was photographed demonstrating grossly multiple cutaneous tumors with the largest measuring 30 mm in diameter. Since that time, and based on field observations, both the prevalence and the size of skin tumors in individual animals have increased. The objective of the present study was to describe the histopathology of skin masses surgically removed from adult female olive ridley turtles nesting at Ostional Wildlife Refuge and to determine whether or not they were fibropapillomas.

Methods

An intense field survey was undertaken on Ostional beach between July and September 1997. Turtles were sampled during the *arribadas* occurring during those months. Turtles were measured and tagged following techniques previously described (Lutz and Musick 1997). All turtles were thoroughly examined for the presence of FP and a description of mass size, number, and location was recorded. Biopsies were collected from fibropapillomas of neck and flippers with either a disposable 6-mm Baker's dermal punch (Webster, Inc., Sterling, Massachusetts) or by excisional biopsy. Pigmentation, surface, attachment, and location of growths were recorded. In addition, normal skin biopsies were taken from the left brachial and neck regions of clinically healthy turtles, which served as controls. Skin and tumor areas were thoroughly prepared with Betadine solution before surgical excision. A topical double antibiotic ointment was applied postbiopsy with wounds left open for drainage. All masses and skin biopsies were immersed in 10% neutral phosphate-buffered formalin. In the laboratory, tissues were embedded in paraffin, sectioned to 5–6-μm thick, and stained with hematoxylin and eosin, Masson's trichrome stain, and Gomori methenamine–silver nitrate stain for the identification of fungi.

Results

Field Observations

An average of 300,000 turtles nest at Ostional each month. It is calculated that approximately 6–10% of these nesting females present cutaneous FP with 1% being severely affected. During the most recent surveys performed by two of us (A.C. and L.d.T.), 30 of 300 (January 1998) and 5 of 125 (February 1998) turtles were grossly identified with FP. Field observations provided evidence that the size and number of tumors has increased on individual turtles among seasons. For example, between 1991 and 1993, the average size of tumors found was 10 mm in diameter with an average number of three per turtle. In 1997, the average size of tumors observed was 25 mm in diameter, with the largest measuring 140 mm, and the average number of tumors per turtle was more than 20 (A. Chaves and L. du Toit, unpublished data).

Gross Pathology

Skin and tumor specimens were collected from 72 nesting female olive ridley turtles for histopathologic and virologic studies; from these, 50 tumor biopsies were examined from 25 of the affected turtles. In addition, six biopsies were examined from five turtles that did not have visible masses and served as controls. Mean ± SE curved carapace length for all 30 females with and without FP was 68.2 ± 0.5 cm (range, 63–73 cm) and curved carapace width averaged 73 ± 0.5 cm (range, 67–77 cm); they appeared strong, in good body condition, and were actively depositing eggs. All turtles were lightly affected, demonstrating single or multiple masses measuring from 1 to 34 cm, except for two turtles that were severely affected with FP. Tumors were observed in the front flippers (25 turtles), neck (6 turtles), carapace margins (6 turtles), eyes (3 turtles), and seams and scutes (2 turtles). Grossly, tumors were from 5 mm to several cm in diameter, white to gray (pigmented), smooth to verruciform, well circumscribed, and pedunculated masses on the integument of the neck and front flippers. Both of the heavily affected turtles presented multilobulated, cauliflower-like masses, some with ulcerated or necrotic tissue. None of the turtles examined presented tumors in the rear flippers or the tail area.

Histopathology

Epidermis of skin biopsies (5) from control turtles was 5–8 cells in thickness and had no epidermal or dermal pathology (Figure 1). In all, 50 tumor biopsies 30 mm or less in diameter were examined histologically from 25 other turtles. Numerous histological features of the epidermis, dermis, and subcutaneous tissues were evaluated

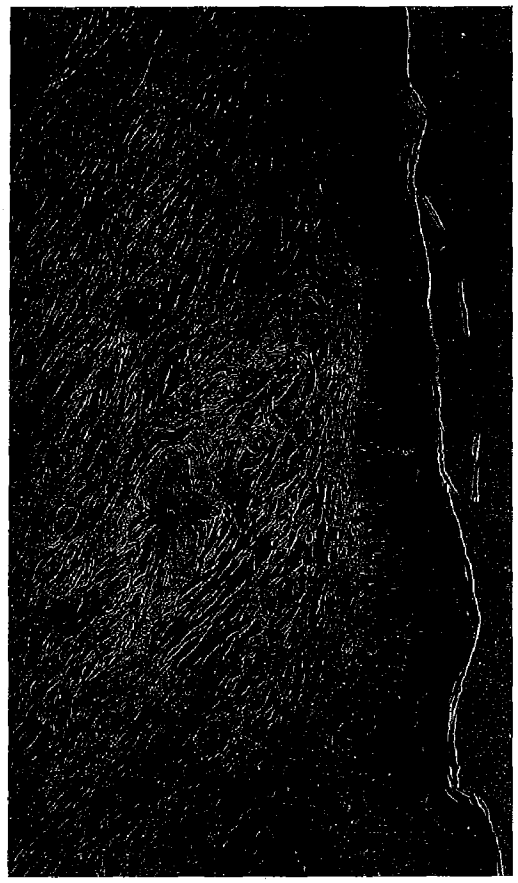

FIGURE 1.—Light micrograph of normal skin stained with hematoxylin and eosin from olive ridley turtle nesting in Ostional Wildlife Refuge, Costa Rica. Observe the uniformity in the thickness of the epidermis approximately 6–7 cells thick. The thin layer of superficial keratin, the interweaving of mature collagen of the dermis, and pigmentation are normal. Bar = 333 μm.

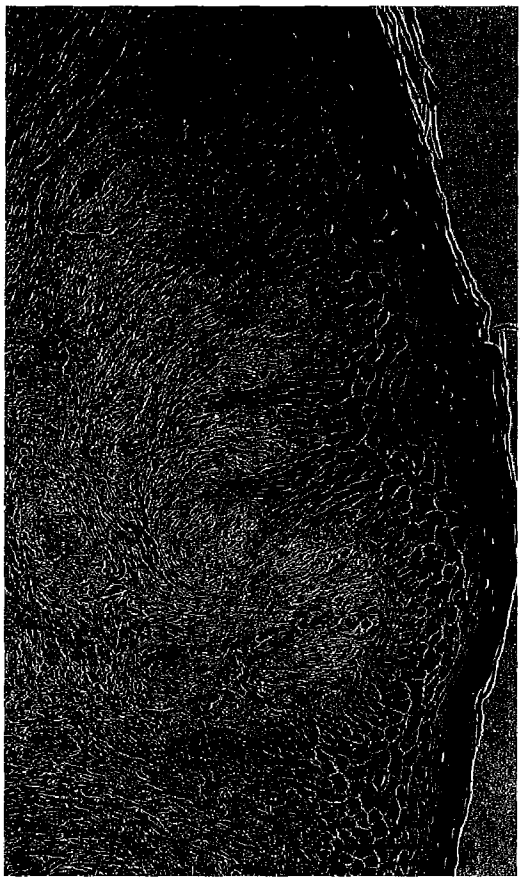

FIGURE 2.—Light micrograph of cutaneous fibropapilloma tissue section stained with hematoxylin and eosin from olive ridley turtle. The epidermis is characterized by mild orthrokeratotic hyperkeratosis and moderate acanthosis. The dermis is composed of spindle cells forming haphazard fascicles. Bar = 133 μm.

(Tables 1, 2). In all, 42 of 50 masses were diagnosed as fibropapillomas based on criteria previously published (Jacobson et al. 1989; Aguirre et al. 1994). Eight of the 42 masses that were grossly classified as FP were histologically diagnosed as small foci of chronic active dermatitis and not tumors. These areas of chronic active dermatitis were characterized by mild acanthosis (hyperplasia of the stratum spinosum) and orthrokeratotic hyperkeratosis (thickening of the stratum corneum) but not pseudoepitheliomatous hyperplasia. The epidermis covering the tumors was characterized by acanthosis from 10 to 15 cells in thickness. A mild to moderate degree of orthrokeratotic hyperkeratosis and a mild to moderate degree of pseudoepitheliomatous hyperplasia characterized by elongation of rete ridges interdigitating with the underlying neoplastic connective tissue of the dermis were observed (Figure 2). Mild intercellular (spongiosis) and intracellular edema were identified within the epidermis. In areas of extensive spongiosis a mild degree of cytoplasmic vacuolar degeneration was observed. Mild individual cellular necrosis and degeneration associated with a mild infiltration of lymphoid cells were observed in the stratum basale. Mitotic figures were not observed. Small to extensive areas of necrosis associated with underlying inflammation were found within the epidermis. Margination of chromatin with intranuclear inclusion bodies was not found except in one tumor that had a few squamous epithelial cells that contained intranuclear bodies of viral

TABLE 1.—Histopathological changes of the epidermis observed in 42 integumental fibropapilloma biopsies from olive ridley turtles nesting at Ostional, Costa Rica.

Histopathological change	Number (%)
Papillar pattern	19 (45)
Linear pattern	21 (50)
Acanthosis	41 (98)
Orthrokeratotic hyperkeratosis	39 (93)
Pseudoepitheliomatous hyperplasia	39 (93)
Intercellular edema	23 (38)
Intracellular edema	13 (31)
Cytoplasmic vacuolar degeneration	13 (31)
Individual cell necrosis	2 (5)
Necrosis (full depth) with underlying inflammation	6 (14)
Lymphocytic infiltration, st. basale	35 (83)
Bacteria on surface of epidermis	33 (79)
Fungus and algae on surface of epidermis	0 (0)
Mites or amphipods on surface of epidermis	30 (71)

inclusions. Infiltration of lymphoid cells was commonly observed within the stratum basale.

The portion of the tumor within the dermis predominated in most tumors and was characterized by a proliferation of plump spindle-shaped cells of fibroblastic or mesenchymal origin (Figure 3). Two patterns of growth were observed. Papillary, the most common pattern, was characterized by papillary projection of epidermal and fibroblastic tissue over a fibrovascular stalk or core in larger tumors. The second pattern, linear, was a more flattened pattern of dermal proliferation that appeared to be just beneath the epidermis in smaller tumors. This linear pattern was also present around the edges of most papillary tumors.

Three patterns of cellular architecture of the fibroblastic portion of the tumors were observed in-

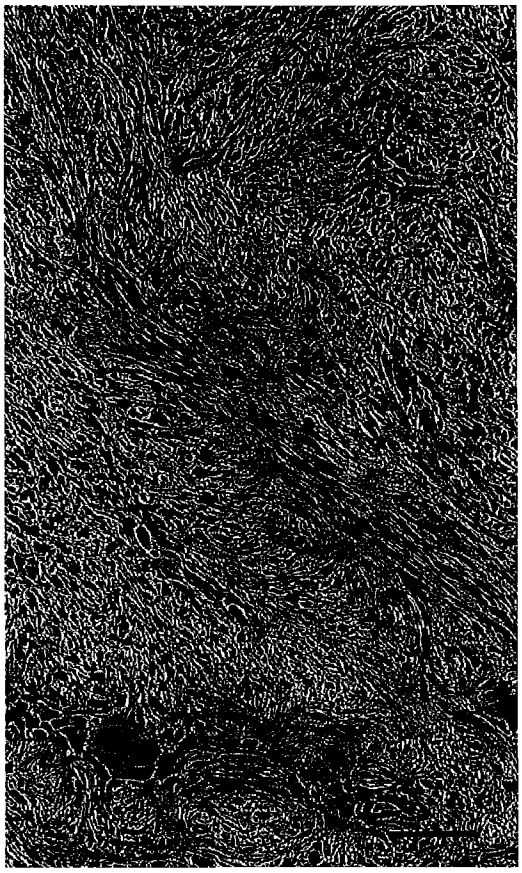

FIGURE 3.—Light micrograph of fibroblastic portion of a cutaneous fibropapilloma from olive ridley turtle. This portion of the tumor is composed of plump spindle cells forming a relatively haphazard arrangement of fascicles. The degree of cellularity is moderate. Bar = 66 μm; stained with hematoxylin and eosin.

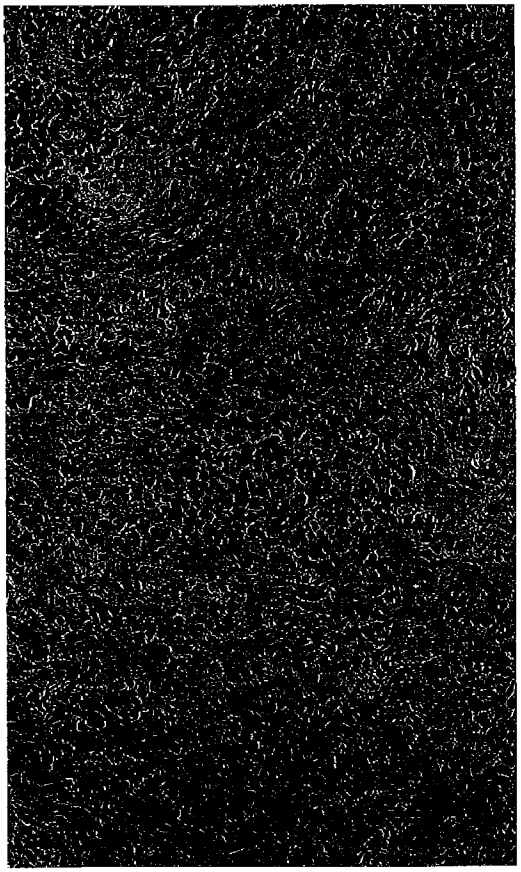

FIGURE 4.—Light micrograph of fibroblastic portion of a cutaneous fibropapilloma, interpreted as regressing, from olive ridley turtle. Note the extensive inflammation characterized by lymphoid infiltration with loss of neoplastic fibroblastic tissue. Bar = 66 μm; stained with hematoxylin and eosin.

cluding a relatively haphazard arrangement of whorls and fascicles of proliferating cells, sheets of cells, and interweaving bundles of spindle-shaped fibroblastic cells. In some areas the interweaving bundles were thin and tightly interwoven, whereas in other areas the interweaving fascicles were in wide bands. Often all three patterns could be found in the same tumor, especially if it was 15 mm or larger in diameter. The degree of dermal cellularity was relatively low in most tumors and moderate in approximately one-third. Mitotic figures were not observed. A mild to moderate degree of neovasculation was found in all tumors. These vessels were often cuffed by a thin layer of lymphocytes. Melanin was found to some degree in most tumors.

Masson's trichrome stain verified the presence of collagen in tumors. Granulomas surrounded by minimal inflammation and containing blood trematode eggs were occasionally observed within tumors. Keratine "pearls" were characterized by small circular foci of keratin surrounded by a thin layer of epidermal cells. The margins of most tumors (83%) showed evidence of spread by expansion and not infiltration into surrounding tissues. Twenty of 42 tumors demonstrated an immunologically mediated reaction characterized by marked neovascularization and inflammation associated with extensive lymphoid infiltration and loss of fibroblastic tissue (Figure 4). Four of these biopsies were collected from sessile, smooth, pedunculated tumors. Nine of the remaining 22 tumors presented early changes of degeneration. Changes in the underlying dermis or subcutis were characterized by mild to moderate lymphoid cuffing of vessels. Occasionally granulomas containing spirorchid trematode eggs were observed within the underlying subcutaneous tissues. Fibroblastic hyperplasia was a feature of the underlying connective tissue (Table 2).

TABLE 2.—Histopathological changes of the dermis and subdermis–subcutis observed in 42 integumental fibropapilloma biopsies collected from olive ridley turtles nesting at Ostional, Costa Rica.

Histopathologic change	Number (%)
Dermis	
Fibroblastic proliferation patterns	
Haphazard	19 (45)
Sheets	13 (31)
Interweaving bundles	3 (7)
Cellularity of tumor	
Low	28 (67)
Moderate	13 (31)
Neovascularization in tumor	
Low	14 (33)
Moderate	27 (64)
Vessels cuffed with lymphocytes	36 (86)
Foci lymphocytic inflammation	32 (76)
Pigment, melanin	24 (57)
Granulomas containing parasitic ova	7 (17)
Keratin pearls	2 (5)
Margins spread by expansion	35 (83)
Regression	20 (48)
Subdermis–subcutis	
Vessels cuffed with lymphocytes	33 (79)
Granulomas containing trematode eggs	6 (14)

Discussion

The present study reports the first histopathologic confirmation of FP in olive ridley turtles. Smith and Coates (1938) first reported FP on a green turtle captured at Key West, Florida, in 1934. A detailed description was made suggesting a possible viral etiology. The same year a brief report followed from the Tortugas (Lucké 1938:92–94). Then, Schlumberger and Lucké (1948) first described internal tumors from specimens collected at the Dry Tortugas.

Since the early 1980s, FP has reached epidemic proportions throughout the world. Detailed histopathologic description of cutaneous FP has been made for green turtles in the Caribbean (Williams et al. 1994), Florida (Jacobson et al. 1989), the Hawaiian Islands (Aguirre et al. 1994), and Indonesia (Adnyana et al. 1997). Gross evidence of FP has been also reported in loggerhead turtles (*Caretta caretta*), olive ridley turtles (*Lepidochelys olivacea*), Kemp's ridley turtles (*L. kempii*), and flatback turtles (*Natator depressus*) (Limpus and Miller 1994). From these, histopathologic confirmation of FP has occurred for loggerheads and a stranded Kemp's ridley turtle from Florida (J. C. Harshbarger, George Washington University Medical Center, unpublished data).

The basic histological features of FP in olive ridley turtles presented many similarities to the ones described in Hawaiian green turtles (Aguirre et al. 1994). One major difference, however, was that 48% of the tumors (20 of 42) from the olive ridleys had extensive areas of lymphocytic inflammation within fibroblastic tumor tissue, and nine of the remaining tumors had histologic changes of inflammation with mild degeneration within the tumor. This is in comparison to 2% (1 of 52) of the tumors from juvenile green turtles similarly collected during field situations in the Hawaiian Islands (Aguirre et al. 1994, 1998). These histological changes suggest regression. Similar structural changes have been described

in canine cutaneous histiocytomas that included necrosis of tumors with marked limphocytic infiltration (Pulley and Stannard 1990). One other factor that may play a role in the state of regression is the possible viral etiology of FP. It is common to find inflammation in the virus-induced papillomas of young cattle, horses, and dogs. This inflammation is believed to be associated with regression (Schneider 1978). If these tumors in nesting olive ridley turtles were in stages of regression, this may explain why 8 of the 50 surgically removed masses that grossly were suspected to be tumors were instead areas of inflammation (chronic dermatitits). Another marked difference observed between turtles of Costa Rica and the Hawaiian Islands was related to age and sex of turtles collected. Generally, turtles captured in Hawaii were juvenile or subadult turtles (40–80 cm in standard carapace length) of both sexes. During the Costa Rican study, only nesting females were sampled. Adult turtles may be more resistant to FP or might have survived the infection with their tumors in stages of regression. Further controlled studies will answer some of these questions.

The appearance of this disease in olive ridley turtles constitutes an added threat to sea turtles of the Pacific. The disease has been reported in other olive ridley populations, even though encouraging signs of recovery of the species have been observed worldwide. Biopsy specimens from six olive ridley turtles from Ostional were subjected to molecular study using the seminested PCR (polymerase chain reaction) assay with degenerate primers. A herpesvirus DNA polymerase sequence was identified in four tumors (Quackenbush et al. 1998). Further studies are underway including efforts to isolate and characterize the etiologic agent to provide possible insights for the epidemiology, control, treatment, and prevention of this disfiguring and debilitating disease.

Acknowledgments

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References

Adnyana, W., P. W. Ladds, and D. Blair. 1997. Observations of fibropapillomatosis in green turtles (*Chelonia mydas*) in Indonesia. Australian Veterinary Journal 75:737–742.

Aguirre, A. A., G. H. Balazs, B. Zimmerman, and T. R. Spraker. 1994. Evaluation of Hawaiian green turtles (*Chelonia mydas*) for potential pathogens associated with fibropapillomas. Journal of Wildlife Diseases 30:8–15.

Aguirre, A. A., T. R. Spraker, G. H. Balazs, and B. Zimmerman. 1998. Spirorchidiasis and fibropapillomatosis in green turtles from the Hawaiian Islands. Journal of Wildlife Diseases 34:91–98.

Balazs, G. H., and S. G. Pooley, editors. 1991. Research plan for marine turtle fibropapilloma. NOAA Technical Memorandum NMFS-SWFSC-156.

Casey, R. N., and five coauthors. 1997. Evidence of retrovirus infections in green turtles *Chelonia mydas* from the Hawaiian Islands. Diseases of Aquatic Organisms 31:1–7.

Cornelius, S. E., and D. C. Robinson. 1983. Abundance, distribution, and movements of olive ridley sea turtles in Costa Rica, III. U.S. Fish and Wildlife Service, Endangered Species Report 13, Albuquerque, New Mexico.

Herbst, L. H. 1994. Fibropapillomatosis of marine turtles. Annual Review of Fish Diseases 4:389–425.

Herbst, L. H., and five coauthors. 1995. Experimental transmission of green turtle fibropapillomatosis using cell-free tumor extracts. Diseases of Aquatic Organisms 22:1–12.

Jacobson, E. R., and seven coauthors. 1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). Journal of Comparative Pathology 101:39–52.

Limpus, C. J., and J. D. Miller. 1994. The occurrence of cutaneous fibropapillomas in marine turtles in Queensland. Pages 186–188 *in* R. James, compiler. Proceedings of the Australian marine turtle conservation workshop. Queensland Department of Environment and Heritage and The Australian Nature Conservation Agency, Brisbane, Australia.

Lucké, B. 1938. Studies on tumors in cold-blooded vertebrates. Carnegie Institution, Annual Report of the Tortugas Laboratory, Washington, D.C.

Lutz, P. L., and J. A. Musick, editors. 1997. The biology of sea turtles. CRC, Marine Science Series, Boca Raton, Florida.

Pritchard, P. C. H. 1997. Evolution, phylogeny, and current status. Pages 1–28 *in* P. L. Lutz and J. A. Musick, editors. The biology of sea turtles. CRC, Marine Science Series, Boca Raton, Florida.

Pulley, L. T., and A. A. Stannard. 1990. Tumors of the skin and soft tissue. Pages 23–82 *in* J. E. Moulton, editor. Tumors in domestic animals, 3rd edition. University of California Press, Los Angeles.

Quackenbush, S. L., and ten coauthors. 1998. Three closely related herpes viruses are associated with fibropapillomatosis in marine turtles. Virology 246: 392–399.

Schlumberger, H. G., and B. Lucké. 1948. Tumors of fishes, amphibians, and reptiles. Cancer Research 8:657–753.

Schneider, R. 1978. General considerations. Pages 1–15 *in* J. E. Moulton, editor. Tumors in domestic animals, 2nd edition. University of California Press, Los Angeles.

Smith, G. M., and C. W. Coates. 1938. Fibro-epithelial growths of the skin in large marine turtles, *Chelonia mydas* (Linneaus). Zoologica (New York) 23:93–98.

Williams, E. H., Jr., and ten coauthors. 1994. An epizootic of cutaneous fibropapillomas in green turtles *Chelonia mydas* of the Caribbean: part of a panzootic? Journal of Aquatic Animal Health 6:70–78.