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FIBROPAPILLOMATOSIS OF MARINE TURTLES

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Abstract. Cutaneous fibropapillomatosis in green sea turtles, *Chelonia mydas* (GTFP), was first reported over 50 years ago. In the last decade, GTFP has emerged as a significant worldwide epizootic with prevalences as high as 92% in some green turtle populations. Lesions similar to GTFP have been observed in other marine turtle species including olive ridleys, *Lepidochelys olivacea*, flatbacks, *Natator depressus*; and loggerheads, *Caretta caretta*, but disease in these species occurs at lower frequencies and is less well documented. The etiology of GTFP is unknown, and a variety of hypotheses concerning the possible etiology and pathogenesis of GTFP have been proposed and are discussed in this paper. Possible etiologies include viruses, metazoan parasites, ultraviolet radiation, and chemical carcinogens. Recent evidence from controlled transmission experiments implicates a filterable infectious agent as the primary etiology of GTFP. A herpesvirus has been identified in some lesions but has not been isolated and cultured; consequently, Koch's postulates have not yet been fulfilled for this agent. The epizootiology and pathogenesis of GTFP are poorly understood. Epizootiologic evidence, while limited to a few field studies, suggests that environmental conditions in certain nearshore marine habitats favor a high prevalence of disease expression. The possibility that immune system modulators play a role in the persistence and severity of this disease is discussed. Detailed investigations of the epizootiology of GTFP must await identification of the etiologic agent and development of specific diagnostic tests. In addition, until immune function tests can be developed and validated for free-ranging turtles, hypotheses about the role of immune system dysfunction in GTFP epizootics cannot be tested.

Keywords. Reptiles, Sea turtles, Fibropapilloma, Disease, Neoplasia

INTRODUCTION

Reports of neoplasia in chelonians are uncommon (1-4). For example, reports tabulated by Machotka (4) cover 25 different neoplastic conditions in 19 turtle species. Of these, cutaneous papillomas, fibromas, and fibropapillomas in green turtles, *Chelonia mydas* (Fig. 1), are most frequently documented in the literature. These three proliferative lesions are the hallmarks of the disease referred to as green turtle fibropapillomatosis (GTFP). The documented increases in GTFP prevalence in certain areas and the apparent spread of GTFP to locations where it has not been observed previously make GTFP the most significant neoplastic disease of reptiles.

Heightened awareness following published reports of fibropapillomatosis in green turtles has led to increased anecdotal reports of fibropapillomas in other sea turtle species. Fibropapilloma-like lesions have been observed by field workers in loggerhead turtles, *Caretta caretta*, from Indian River Lagoon (Llewellyn Ehrhart, University of Central Florida, Orlando, FL 32816, personal communication), Florida Bay (Barbara Schroeder, Florida Marine Research Institute, Tequesta Field Laboratory, Tequesta, FL 33469, personal communication), Florida Keys (Herbst, personal observation), and Australia (5), in Olive Ridley turtles, *Lepidochelys olivacea*, from the Pacific coast of Costa Rica (Any Chaves, Universidad de Costa Rica, San Jose, Costa Rica, personal communication; Pamela Plotkin, Texas A&M University, College Station, TX 77843, personal communication), and in flatback turtles, *Natator depressus*, from Australia (5). The anecdotal reports are supported by photographic documentation of lesions in loggerheads (Fig. 2) and olive ridleys (Fig. 3) only; to date, only loggerheads have been confirmed to have fibropapillomas by histological examination of lesion biopsies (6). A case of nasal papilloma has been reported from an aquarium housed hawksbill, *Eretmochelys imbricata*, but disease has not been documented in free-ranging turtles (6). Taken together, these findings raise concerns about the impact of these diseases on sea turtle conservation.

The purpose of this paper is to compile what is currently known about fibropapillomatosis in ma-

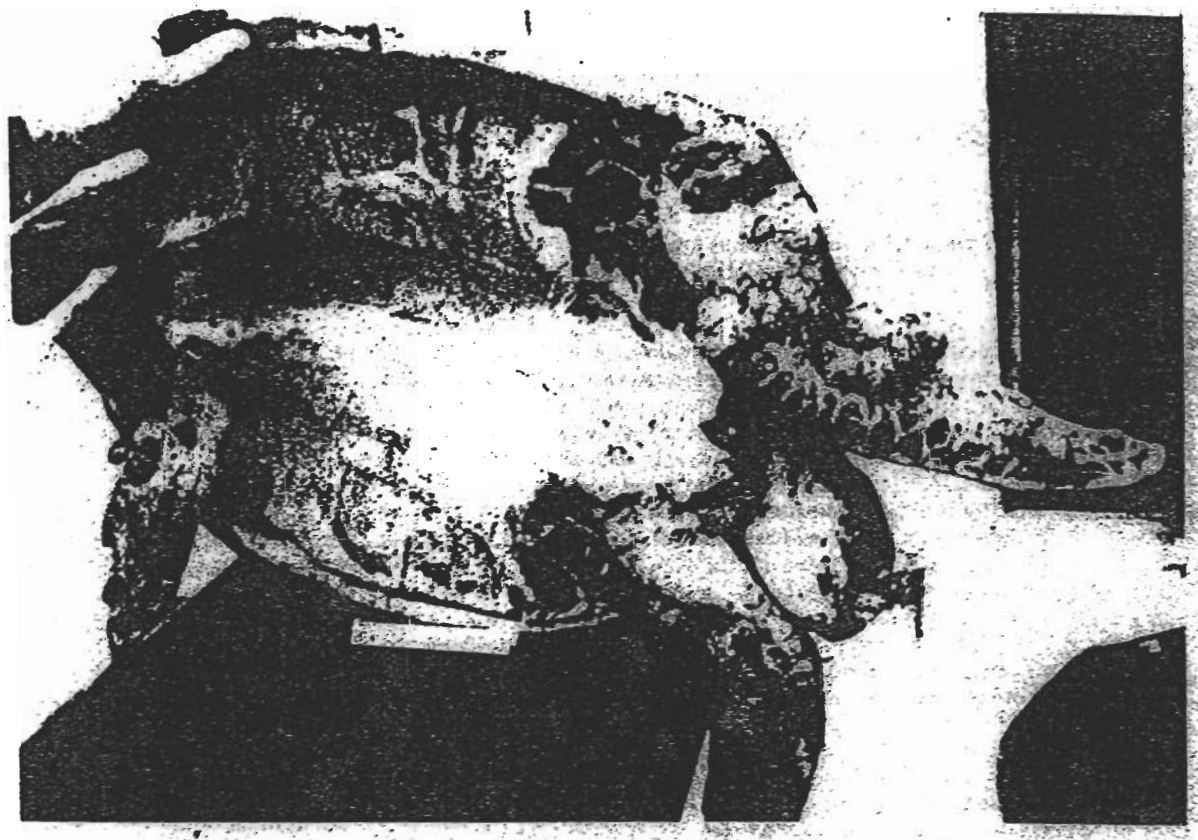

Fig. 1. A juvenile green turtle, *Chelonia mydas*, with cutaneous fibropapillomatosis. This turtle stranded in December 1993 near Key West, Florida, in severely debilitated condition as evidenced by the sunken plastron. Multiple fibropapillomas were found on the neck, front and rear flippers, axillary and inguinal areas, perineum, and covering both eyes.

rine turtles and to weigh critically the evidence for or against various hypotheses regarding the etiology and pathogenesis of these diseases. This paper also outlines the important questions that need to be addressed if we are to understand and monitor the impact of these diseases on worldwide marine turtle populations. Because of the lack of information on fibropapillomatosis in other species of marine turtles, the following discussion refers to fibropapillomatosis in green turtles unless otherwise specifically indicated.

Historical perspective

Cutaneous papillomas, fibromas, and fibropapillomas were first described by Smith and Coates in 1938 at the New York Aquarium in a captive green turtle, *Chelonia mydas*, that had been captured near Key West, Florida 2 years previously (7). Two other green turtles and two loggerheads that were housed with this animal did not have lesions. Subsequently, Smith and Coates observed fibropapillomas in 3 of 200 free-ranging green turtles (27–91 kg) captured off of Key West (7). That same year, Lucké described similar tumors from a green turtle caught off of Cape Sable, Florida (8). Masses were located on the tail, flippers, axillae, neck, eyelids, and corneas. Schlumberger and Lucké (9) subsequently described fibropapillomas from three Florida green turtles, and found numerous fibrous masses within the lungs of one turtle. In 1958, Hendrickson noted the occasional occurrence of fibrous masses on nesting females in Sarawak and Malaya (10). The first confirmed case of GTFP in Hawaii occurred in 1958 in a juvenile green turtle captured by local fisherman in Kaneohe Bay, Oahu (11). A survey of local fishermen conducted by Balazs (11) suggests that GTFP was rare to nonexistent prior to this. Since this first report, green turtles with fibropapillomas have been reported with increasing frequency from Hawaii (11, George Balazs, National Marine Fisheries Service, Southwest Fisheries Center, Honolulu, HI 96822, personal communication). In 1980, an outbreak of fibropapillomatosis occurred in a breeding group of adult green turtles at Cayman Turtle Farm, Ltd. Grand Cayman, British West Indies (12,13). The outbreak began in wild caught

Fig. 2. A juvenile loggerhead turtle, *Caretta caretta*, with multiple cutaneous fibropapillomas. This turtle was captured, tagged, and released in Florida Bay. (Photograph courtesy of Barbara Schroeder, Florida Marine Research Institute, Florida Department of Environmental Protection, Tequesta, Florida.)

adults but subsequently developed over several years in farm raised turtles as well. Ehrhart, Sindler, and Witherington (14) documented the first cases of GTFP in the Indian River Lagoon, Florida, in 1982. Netting surveys within the northern portion of the Indian River Lagoon system (Mosquito Lagoon) had been conducted since 1977 without encountering any green turtles with fibropapillomas. However, when the study area was shifted to the central portion of the system (Indian River) in 1982, affected turtles were encountered immediately. A review of late 19th century accounts of the Florida east coast green turtle fishery and of reports on Indian River Lagoon turtles published between 1978 and 1983 failed to yield any record of GTFP prior to this (14,15). Continued monitoring at this site since 1982 has revealed GTFP prevalences of about 50%.

PATHOLOGY

Gross description

Cutaneous fibropapillomas of green turtles are single to multiple raised masses ranging from 0.1 cm to greater than 30 cm in diameter. Individual masses may be either verrucous or smooth and either sessile or pedunculated. Large cutaneous masses are often ulcerated and necrotic. Cutaneous fibropapillomas are usually found on the soft skin but may be found anywhere on the turtle's body, including carapace and plastron. Common sites for GTFP are the flippers, neck, chin, inguinal and axillary regions, and tail base (Fig. 1). The conjunctiva (especially bulbar conjunctiva) is a common site for GTFP, and masses may grow over the cornea (7,8,13,16) (Fig. 4). Tumor pigmentation is usually related to the pigmentation of the skin at the site of origin. The gross appearance of cutaneous fibropapillomas in loggerheads and olive ridleys is similar to GTFP (Figs. 2 and 3).

Visceral nodules have been reported on green turtles with cutaneous fibropapillomatosis (9,17–19). Schlumberger and Lucké (9) discovered numerous spherical 3–5 cm masses in the lungs of one green turtle. Norton, Jacobson, and Sundberg (17) observed multiple firm white nodules in both kidneys from a juvenile green turtle with extensive cutaneous fibropapillomatosis collected in the Florida

Fig. 3. A nesting adult female olive ridley turtle, *Lepidochelys olivacea*, with multiple fibropapilloma-like masses on the left front flipper. This photograph was taken during an *arribada* at Playa Nancite, Guanacaste, Costa Rica. (Photograph courtesy of Pamela Plotkin, Texas A&M University, College Station, TX 77843.)

Keys. Jacobson, Buergelt, Williams, and Harris (18) examined two turtles with GTFP and, in one animal, found several discrete firm, white foci up to 1 mm diameter on the surface of one kidney and multiple discrete 1–4 cm diameter nodules in the other. They also found similar nodules 1–2 cm diameter within both lungs. Approximately 17% (9 of 52) of the green turtles with severe cutaneous fibro-

Fig. 4. A juvenile green turtle, *Chelonia mydas*, with extensive bilateral ocular fibropapillomatosis (left eye shown). Masses originate from bulbar and palpebral conjunctiva, limbus, and cornea.

papillomatosis presented for necropsy at a rehabilitation center have been found to have similar internal nodules (Richie Moretti and Tina Brown, The Turtle Hospital, Marathon, FL 33050, personal communication). Williams et al. (19) found lung and kidney nodules in 41% (7 of 17) of the green turtles examined from Puerto Rico. Visceral masses range from 0.1 cm to over 20 cm in diameter. These nodules are usually smooth, firm, and white but some may be gelatinous and translucent. Nodules are embedded within tissue parenchyma but often bulge from the surface and may even be pedunculated (Fig. 5). Most visceral nodules appear to be well demarcated from surrounding tissue but some may have irregular borders suggesting infiltration of surrounding stroma. This is especially true in the kidney where there is a large amount of fibrous stroma (Fig. 6). The organs commonly affected in a sample of 13 green turtles with cutaneous and visceral masses were lungs (77%), kidneys (69%), heart (38%), gastrointestinal tract (31%), and liver (23%). Nine turtles (69%) had nodules in more than one organ system. Visceral fibromas have not been described in any other sea turtle species.

Histologic description

Jacobson et al. (13) described the histologic and ultrastructural appearance of normal green turtle skin to provide a basis for comparison with fibropapillomas. Normal skin has a relatively thin epidermis four to seven cells thick (Fig. 7). Epithelial cells are flattened at the surface, nuclei become pyknotic and are lost, and cells become covered with a layer of keratin-like material approximately one- to two-thirds the thickness of the cell layer. The skin surface pattern varies from smooth to spiked. In normal skin, the epidermis is separated from dermis by a basement membrane adjacent to which the dermis is organized into a thin layer corresponding to the papillary layer of mammals. Occasional papillary projections into the epidermis are seen. The papillary layer consists of thin collagenous bundles, small mononuclear cells, small blood vessels, and chromatophores. Below the papillary layer, the

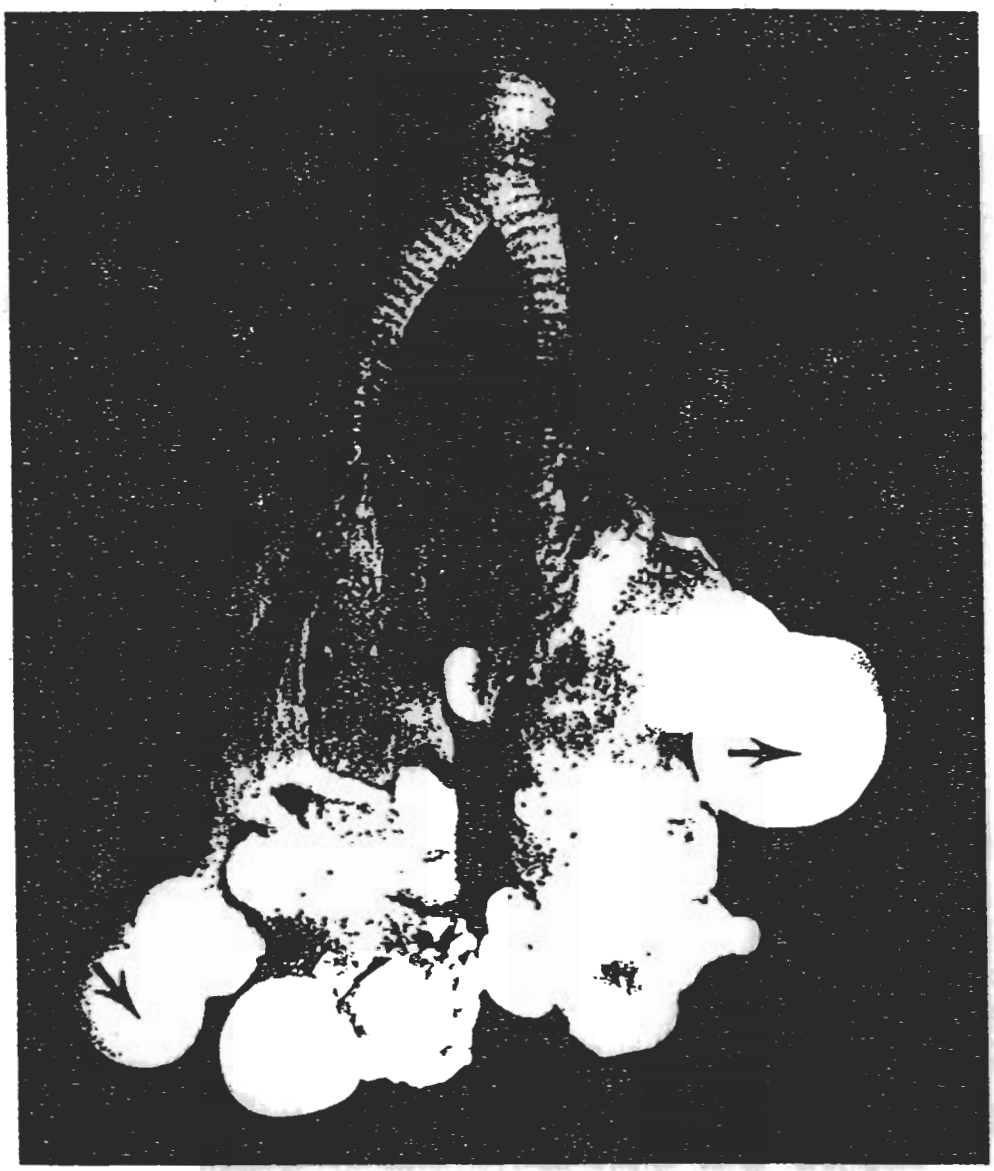

Fig. 5. Lungs from a juvenile green turtle, *Chelonia mydas*, with extensive cutaneous fibropapillomatosis. Multiple nodules ranging from 0.2 to 5 cm in diameter are found in both lungs. Pulmonary nodules are well demarcated, smooth, and either firm and white, or gelatinous and translucent. Masses arising deep within the lung parenchyma bulge from the surface while those originating near the pleural surface appear pedunculated. Gelatinous translucent (myxomatous) nodules (arrow) appear to arise from firm white (fibromatous) nodules.

reticular layer consists of large collagen bundles, fibroblasts, and blood vessels (13).

Histologic descriptions of cutaneous GTFP appear in several publications (6–9,13,16,17,19–21). The characteristic description of cutaneous GTFP is that of papillary epidermal hyperplasia supported on broad fibrovascular stromal stalks (Fig. 8). The ratio of epidermal to dermal proliferation varies among lesions. Lesions that are comprised primarily of proliferating epidermis with little or no underlying dermal involvement are properly called papillomas while those lesions predominantly comprised of proliferating dermal components with relatively normal epidermis are called fibromas (Fig. 9). Those masses in which both tissues are hyperplastic are termed fibropapillomas (Fig. 8). Several authors have postulated that there is a developmental progression from papilloma (early lesions) through fibropapilloma to fibroma (chronic lesions) (6,8,13).

Orthokeratotic hyperkeratosis was observed in

Fig. 6. Kidneys from a juvenile green turtle, *Chelonia mydas*, with extensive cutaneous fibropapillomatosis. Multiple irregularly shaped firm white nodules (arrows) ranging from 0.5 to 3 cm in diameter are found in both kidneys. Although well demarcated from renal parenchyma, the nodules often extend into the fibrous stroma of the kidney.

all studies and ranged from none to marked in different fibropapilloma sections (6–9,13,16,17,20,21). Epithelial cells in hyperplastic areas tend to be hypertrophied (13,16). The degree of epidermal hyperplasia in GTFP varies from mild to moderate (7–15 cells thick) on skin to extensive (up to 30 cells thick) on some conjunctival and palpebral masses (13,16). Fibropapillomas with extensive epithelial hyperplasia often exhibit anastomosing rete ridges extending deep into the dermis.

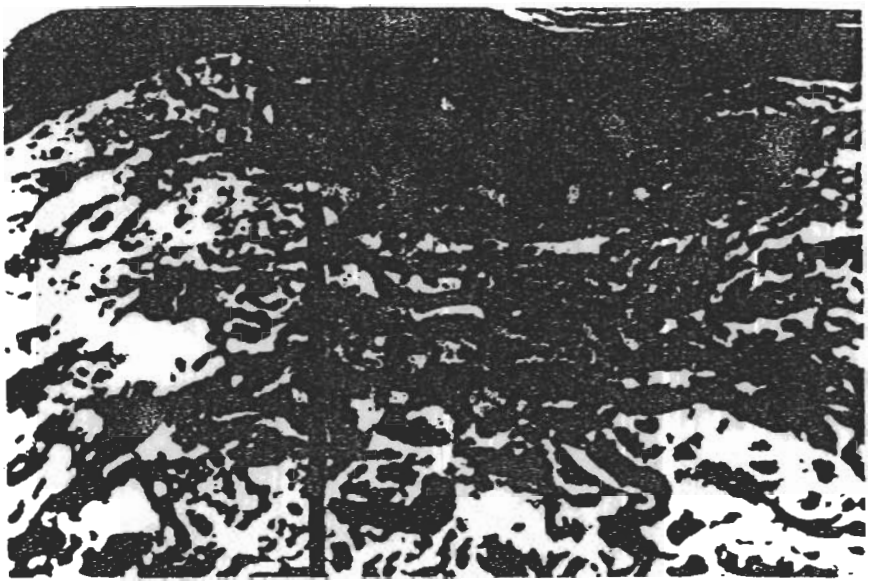

Fig. 7. Normal skin from a green turtle, *Chelonia mydas*. The epidermis is four to seven cells thick and covered by a thin keratinized layer corresponding to the stratum corneum. The dermis is organized into a thin papillary layer (p) consisting of thin collagen bundles, small mononuclear cells, small blood vessels, and chromatophores and a reticular layer (r) consisting of large collagen bundles, fibroblasts, and blood vessels. H&E, original magnification × 160. (Reprinted with permission from [13].)

The fibrovascular stroma contains numerous well-differentiated fibroblasts arranged in a ground substance containing compact bundles of collagen fibers. Fibroblasts and collagen bundles tend to be haphazardly arranged, are more numerous than in normal dermis, and are denser near the basement membrane (13,16). Various amounts of collagen and mucopolysaccharide ground substance have

Fig. 8. Fibropapilloma from a green turtle, *Chelonia mydas*, showing typical arborizing pattern of papillary epidermal hyperplasia supported by fibrovascular stroma. H&E, original magnification × 35.

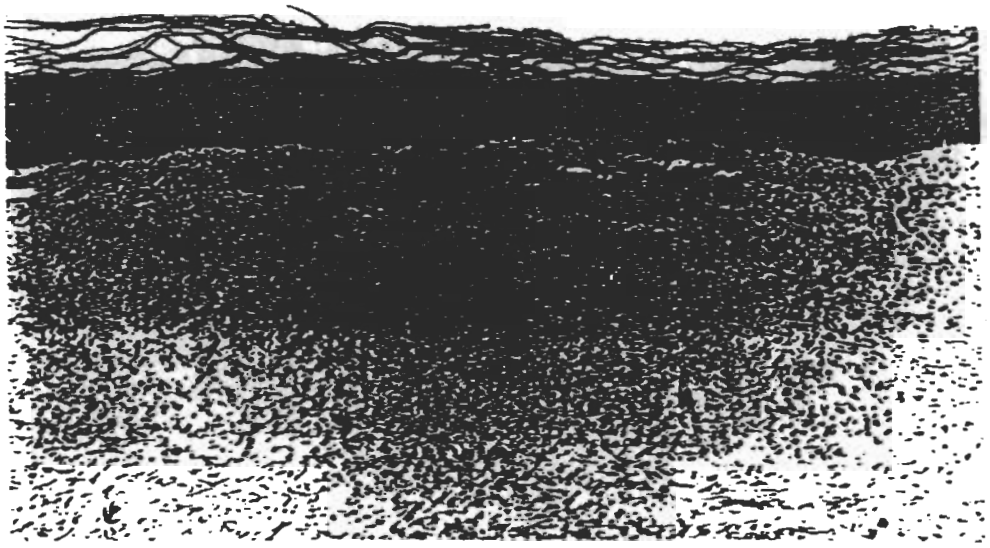

Fig. 9. Fibroma from a green turtle, *Chelonia mydas*, showing extensively proliferating dermal component covered by mildly hyperplastic epidermis. Only a small fraction of the fibrovascular component of this mass is shown. H&E, original magnification × 87.

been demonstrated in cutaneous tumors by trichrome and alcian blue staining (17). Nerves and numerous small blood vessels are found within the stroma. Fibropapillomas examined in several studies show no malignant or anaplastic changes and few mitotic figures (7,19).

In what they interpreted as the earliest lesions, Jacobson et al. (13) observed epidermal hyperplasia and multifocal areas of ballooning degeneration in the stratum basale (Fig. 10). The papillary layer of the dermis consisted of numerous fibroblasts and compact bundles of collagen fibers, and perivascular cuffs of lymphocytes and plasma cells were seen throughout the dermis.

Differences in tumor pigmentation are due to the distribution of melanophores within the tissues. Highly pigmented masses contained diffusely distributed melanophores within the dermis and between epidermal cells. In addition, melanophores accompany blood vessels within the masses and impart a grey-black streaky appearance to the stroma of some lesions even if the epithelium is unpigmented (7).

Brooks, Ginn, Miller, Bramson, and Jacobson (16) described a series of fibropapillomas from four eyes of three juvenile green turtles from the Florida Keys. Masses arose from the cornea, limbus, conjunctiva, or mucocutaneous junction of the eyelids. Eyelid, conjunctival, and limbal masses tended to be polypoid or pedunculated with a high degree of arborization while corneal lesions examined from two eyes tended to be sessile and multinodular. Histologically, ocular masses were typical of cutaneous GTFP, composed of hyperplastic epithelium overlying a well-vascularized fibrous stroma. Epithelial types that were observed in various lesions ranged from cornified or uncornified stratified squamous epithelium to epithelia containing numerous goblet cells. Two corneal masses were examined and found to have different histological patterns. One mass had mild epidermal hyperplasia of stratified squamous epithelium (up to 15 cells thick). Normal corneal epithelium was gone, and the fibroblastic mass blended with normal stroma. The second corneal lesion had marked epidermal hyperplasia (up to 40 cells thick) forming numerous endophytic nodular masses and anastomosing cords. This lesion was invasive of the cornea and sclera, causing obliteration of corneal stroma and Descemet's membrane. Epithelial necrosis with occasional ballooning degeneration of cells in the wing cell layer were seen in some sections (16).

Other histologic features have been described in some fibropapillomas but are not found consistently, so their significance is unclear. They may be incidental findings. Trematode eggs surrounded by epithelioid macrophages and multinucleate giant cells were observed within the dermal capillaries of

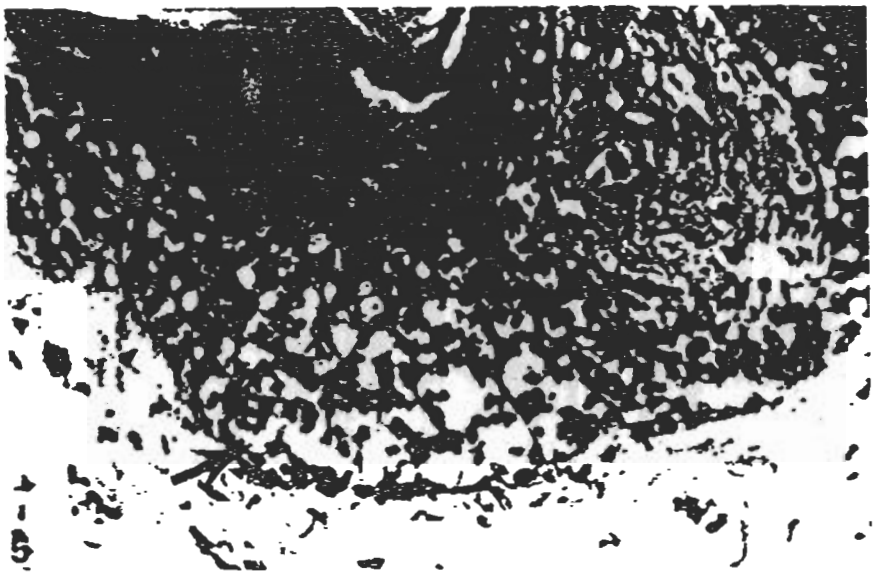

Fig. 10. Papilloma from a green turtle, *Chelonia mydas*. Ballooning degenerative changes are seen in cells within the stratum basale (arrow) in what was interpreted by Jacobson et al. (1989) as the "earliest" GTFP lesion. H&E, original magnification × 400. (Reprinted with permission from [13].)

some fibropapillomas (6,13,16–21) (Fig. 11). In some lesions containing eggs, eosinophilic granulocyte infiltrates were also observed (20). Similar trematode egg granulomas were observed in normal dermis and other tissues (17,21). In some fibropapilloma specimens, epithelial cells in the stratum spinosum and outer layers of epidermis were hypertrophic and vacuolated. In these areas, amphophilic intranuclear inclusions were sometimes observed (13,21). Lymphocytic perivascular infiltrates (Fig. 12) were described in several studies (7,13). Cleft formation at the dermal-epidermal junction (Fig. 13) has also been noted in several fibropapillomas examined by Jacobson et al. (13). Surfaces of tumors are colonized by a variety of bacteria, fungi, algae, and invertebrates including mites (21). Jacobson et al. (18) found eosinophilic intranuclear inclusions containing herpesvirus-like particles within some superficial epidermal cells undergoing intracytoplasmic vacuolation and ballooning degeneration (Figs. 14–16).

The lung nodules described by Schlumberger and Lucké (9) were composed of dense fibrous tissue covered by ciliated columnar epithelium and were compatible with the dermal component of cutaneous masses. The renal nodules described by Norton, Jacobson, and Sundberg (17) were sharply demarcated from surrounding renal tissue and covered by a renal capsule at the kidney surface. Normal renal tubules were found scattered throughout the proliferating connective tissue. Histologically, these nodules were composed of spindle-shaped cells with oval nuclei arranged within a matrix ranging from regularly arranged eosinophilic collagen fibers to lightly basophilic myxomatous ground substance. These myxofibromas contained both collagen and mucopolysaccharides and were indistinguishable from the dermal component of cutaneous lesions. Lung and kidney nodules examined by Jacobson et al. (18) were composed of fibrous tissue and contained trematode eggs. Some lung nodules examined histologically by this author demonstrate a progression from myxomatous to fibromatous ground substance as lesions mature (Figs. 5 and 17).

Clinical course, morbidity, and mortality

Cutaneous fibropapillomas can become large enough to interfere with locomotion and are easily entangled in discarded line. Ocular fibropapillomas (Fig. 4) may occlude vision, and those invading the cornea may cause secondary panophthalmitis with destruction of the globe (16). Visceral fibromas grow by expansion within the stroma of the affected organ and eventually disrupt normal organ functions. Cardiac dysfunction, buoyancy problems and respiratory compromise, hydronephrosis, and gastrointestinal obstruction have all been observed or suspected causes of death in affected turtles (Herbst, personal observation; Balazs, personal communication; Moretti and Brown, personal communication).

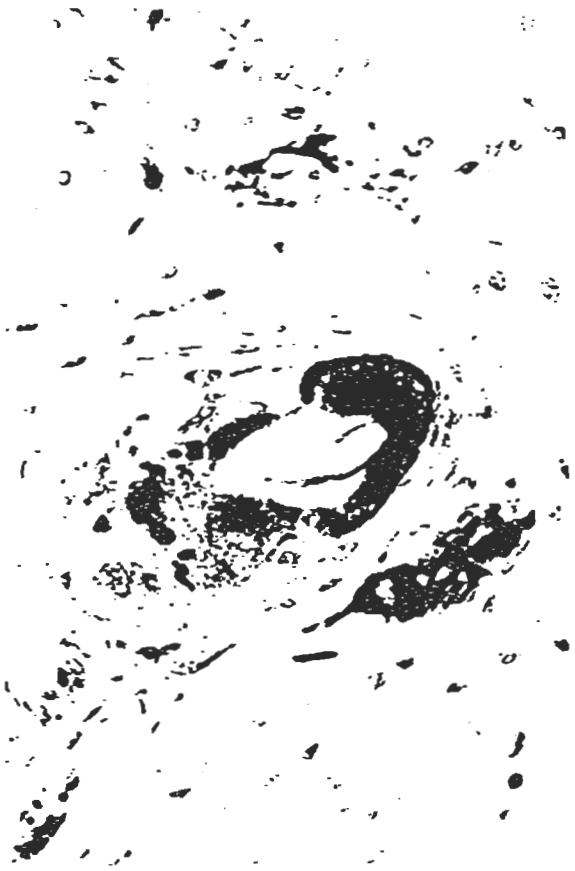

Fig. 11. GTFP. Spirorchid trematode egg lodged within a blood vessel and engulfed within a multinucleate macrophage giant cell. H&E, original magnification × 350.

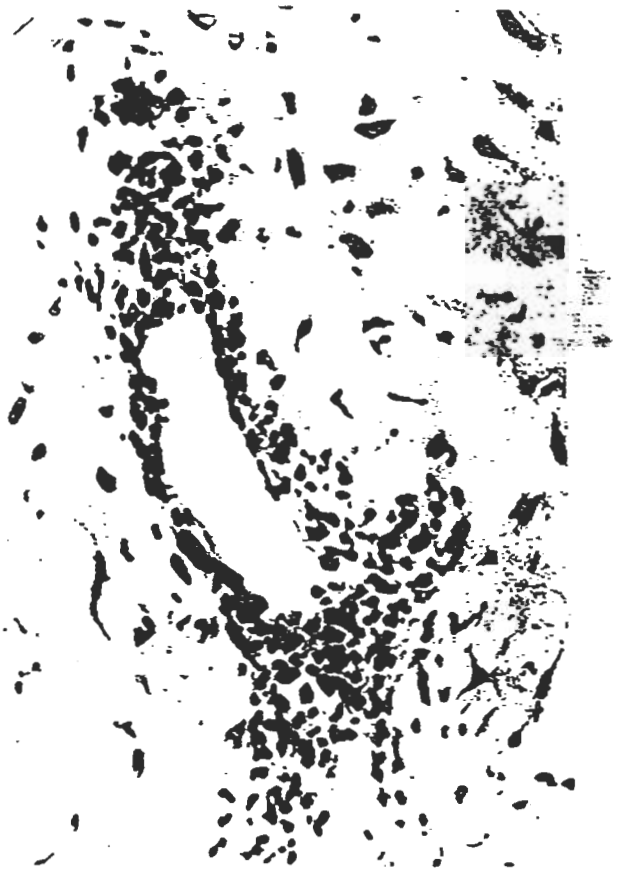

Fig. 12. GTFP. Perivascular mononuclear cell infiltrate is observed in many GTFP sections. H&E, original magnification × 350.

Many green turtles with multiple cutaneous fibropapillomas become severely debilitated (Fig. 1). Blood chemistries and blood cell counts of severely affected green turtles confirm a general pattern of debilitation (17,22). Abnormalities include nonregenerative anemia, hypoproteinemia, electrolyte imbalances, uremia, and elevations in liver enzymes (17). The cachexia may be caused by any combination of the following: inability to locate, ingest, or digest food; excessive energy demands for growth by proliferating tumors; increased energetic costs for locomotion; the physiological effects of certain cytokines such as tumor necrosis factor, mediated by the immune system; and/or concurrent disease such as spirorchidiasis. Whatever the mechanism(s), a number of animals become sufficiently debilitated by GTFP to strand (11,23). In one rehabilitation center about 50% of green turtles that were still alive at stranding died despite extensive rehabilitation efforts (Moretti and Brown, personal communication).

The duration and course of clinical GTFP are poorly understood, primarily because individual turtles with fibropapillomas of known duration have not been available for longitudinal studies. A few green turtles have been held in captivity long enough to provide some generalizations about the clinical course of the disease. Jacobson et al. (13) held six immature turtles with multiple cutaneous GTFP in captivity for several months. Some tumors on some animals decreased in size while others increased in some animals when examined 4 months after capture. Ehrhart et al. maintained three green turtles with GTFP in captivity for approximately 3 months (14,15). During that time one animal lost several tumors, a second gained eight new tumors, and the third exhibited no changes. In these holding experiments, the length of time that the animals had the disease prior to capture is unknown. Field mark and recapture studies also indicate a variable clinical course. In these studies recapture rates are generally low and there is no control over the time interval between capture and recapture. For exam-

Fig. 13. GTFP. Cleft formation at the dermo-epidermal junction. There is separation of the epidermis from the superficial dermis and eosinophilic material accumulated within the cleft. H&E, original magnification × 87.

ple, of 56 green turtles recaptured in the Indian River, 7% had tumors when first marked but had none at recapture, 14% contracted tumors between first capture and recapture, 38% had lesions both times, while 41% were free of lesions both times (15). These data, while limited in number, support the conclusion that the clinical course is prolonged and that some individuals may spontaneously recover from disease. Studies of the temporal patterns of progression and regression of experimentally transmitted GTFP in captive turtles are needed.

EPIZOOTIOLOGY

Epizootiology is the study of the temporal and spatial patterns of disease expression in animal populations and includes efforts to identify etiology, describe incidence and prevalence, morbidity and mortality, routes of natural exposure or transmission, and the conditions that lead to disease outbreaks (epizootics). The number of turtles that develop GTFP in the wild over time (incidence) and the proportion of affected turtles that develop severe disease and die (morbidity and mortality) are unknown. These data are desperately needed if we are to understand the full demographic impact of GTFP on wild turtle populations.

Epizootiologic studies of GTFP are hampered by several factors. First, there are no diagnostic tests to detect exposure and early (preclinical) disease because the etiologic agents(s) has not been identified. Thus all prevalence data are based on observation of gross cutaneous tumors. Second, because green turtles are migratory and long lived, taking between 20 and 50 years to reach sexual maturity (24–26), it is difficult to sample certain life history stages such as the posthatching pelagic phase, and it is impossible to conduct longitudinal studies of cohorts. Third, attempts to correlate disease prevalence with assorted biotic and abiotic factors are limited by the geographic scale over which field surveys need to be conducted. In most areas of the world, turtle populations are monitored poorly due to limited human and financial resources. Consequently, surveillance for GTFP and monitoring of potentially relevant biotic and abiotic factors has been limited.

Finally, even in well-monitored sites, sampling methodologies introduce biases that affect prevalence estimates. Most field studies are conducted on feeding grounds or nesting beaches; therefore, it is not surprising that postpelagic juveniles and adult females are overrepresented in the prevalence estimates. Nesting beach surveys underestimate the true prevalence of GTFP in adult females because debilitated turtles are less likely to nest and are therefore not sampled (5). Field surveys and fisheries that employ tangle nets tend to select for larger turtles because the small turtles are not caught in the mesh. Surveys based on stranded sea turtles may overestimate the prevalence of severe debilitating disease. Cold stunning events and sampling methods that use direct in-water captures provide the least biased population samples. The reader is asked to keep these caveats in mind when evaluating the information presented below.

Geographic distribution

Fibropapillomatosis in green turtles has been reported from around the world (Fig. 18), including the Atlantic (Florida, Bahamas, Brazil), the Caribbean (Cayman Islands, Puerto Rico, Virgin Islands, Barbados, Venezuela, Colombia, Nicaragua, Costa Rica, Panama, Belize), and Indo-Pacific (California, Hawaii, Australia, Sri-Lanka, Seychelles, Sarawak, Malaya, Japan) (5,10,11,13,19,27–30, Karen Bjorndal and Alan Bolten, University of Florida, Gainesville, FL 32611, personal communication; Jean Mortimer, University of Florida, Gainesville, FL 32611, personal communication;

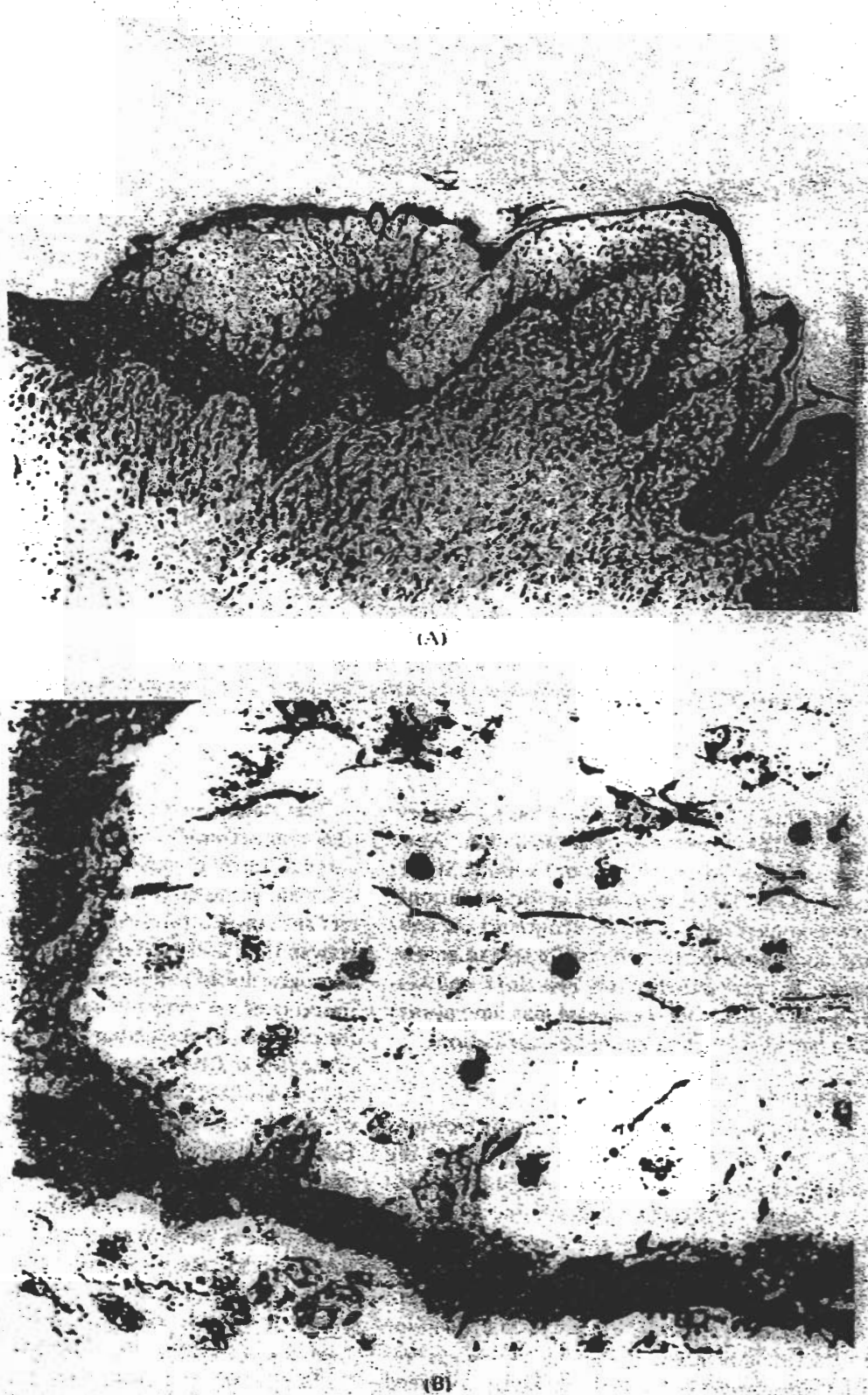

Fig. 14. Fibropapilloma from a green turtle, *Chelonia mydas*. (A) Focal area of ballooning degeneration in the epidermis. H&E, original magnification × 75. (B) Higher magnification showing epidermal cells containing intranuclear inclusions. H&E, original magnification × 750. (Reprinted with permission from [18].)

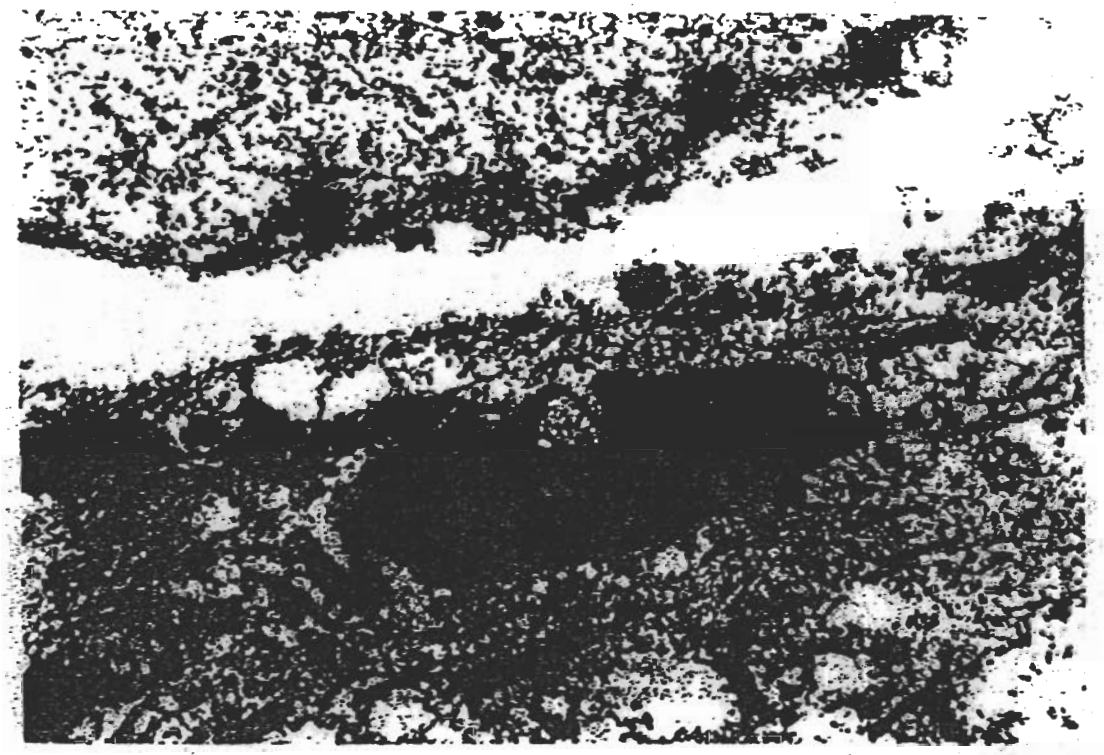

Fig. 15. Viral particles in a GTFP. Low magnification electron micrograph showing intranuclear (I) and intracytoplasmic (C) viral particles within epidermal cells. Original magnification × 29,575. (Reprinted with permission from [18].)

Cynthia Lageaux, University of Florida, Gainesville, FL 32611, personal communication; Anne Meylan, Florida Marine Research Institute, St. Petersburg, FL 33701, personal communication). There are insufficient data to reconstruct the temporal and spatial pattern of disease spread among regions. The early reports from Florida (7) and Malaysia (10) suggest that the disease may have always had a worldwide albeit sporadic distribution.

Prevalences

The prevalence of GTFP varies among locations and from year to year. Table 1 summarizes the available prevalence data from several field studies. A survey conducted by Smith and Coates in 1938 of turtles captured in the Key West, Florida fishery found a low prevalence (1.5%) (7). Most population monitoring, however, has been conducted since 1975. Monitoring in most areas prior to 1982 found little or no disease, but then prevalences rose rapidly in the 1980s and have remained high. This may reflect in part an increased awareness of the disease, but most probably reflects an increase in the prevalence and severity of GTFP over time.

Prevalences in well-monitored feeding ground sites range from 0% in Anagua, Bahamas (Bjorndal and Bolten, personal communication), Bermuda (Meylan, personal communication), and offshore reef sites in Australia (5) to 92% in Kaneohe Bay, Hawaii (11). Large differences in prevalence among demographically matched populations may be found over very short distances (<1 km) as seen, for example, by comparing the prevalence of GTFP in the Indian River (about 50%) with that from the near-shore Sabellarid worm reef on the ocean side of the barrier island at Wabasso Beach (0%) (15, Ehrhart, personal communication). In general, there appears to be an association of high GTFP prevalences with in-shore marine habitats. There also appears to be a positive association between human activity (agriculture, industry, urban development) in the catchment area of near-shore waters and GTFP prevalence, although the information on human impacts in most areas is limited and anecdotal.

Prevalence data from other species with fibropapilloma-like lesions are scant. Adult female olive ridley sea turtles nesting at Playa Nacite and Playa Ostional, Costa Rica, have a very low prevalence of fibropapilloma-like lesions (Chaves, personal

Fig. 16. Viral particles in a GTFP. High magnification electron micrograph of mature particles with typical herpesvirus morphology within the cytoplasm of an epidermal cell. Original magnification × 91,975. (Reprinted with permission from [18].)

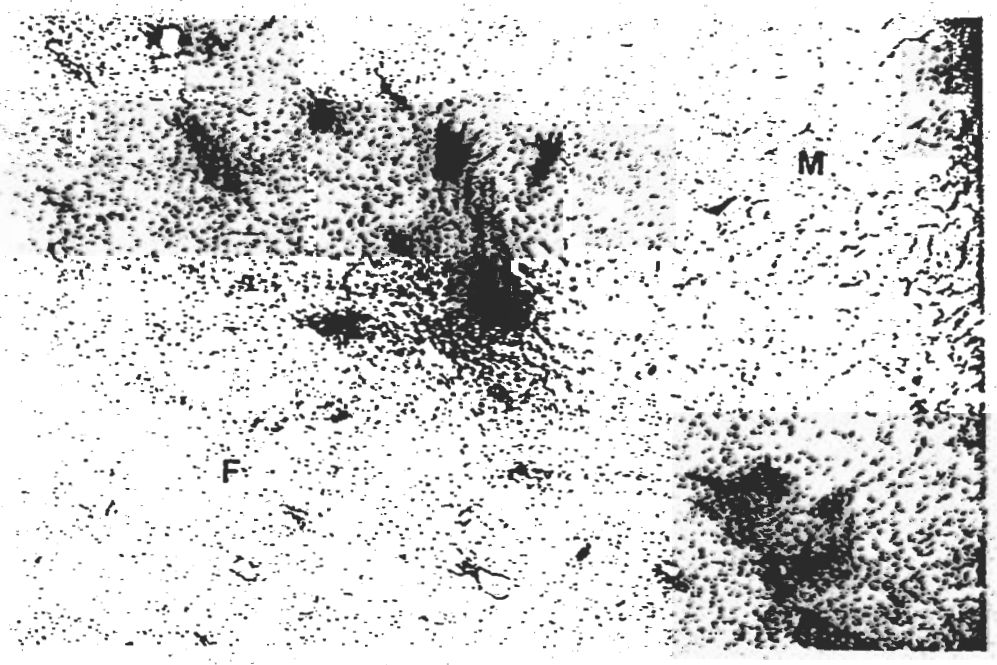

Fig. 17. Pulmonary nodule from a green turtle, *Chelonia mydas*. This section illustrates the transition from collagenous extracellular matrix in a fibrous nodule (F) to a more myxomatous extracellular matrix in an adjacent myxoma (M). H&E, original magnification × 35.

Table 1. Prevalences of GTFP in free-ranging green turtles

Locality	Turtle habitat	Impact of human activity	Prevalence of GTFP (%)	Sample period	Sample method	Reference
Western Atlantic/Gulf of Mexico/Caribbean						
Florida, Gulf Coast			50	1992 (1st observation, 1985)	Stranding recovery	23
Florida, Florida Bay	Seagrass flats	Agriculture, urban	70	1990–1993	Hand capture, net	Schroeder[a]
Florida, Florida Keys	Reef/seagrass flats		1.5	1938	Fishery survey	7
Florida, Florida Keys			20–60	1980–1990	Stranding recovery	23
Florida, Atlantic Coast			10	1980–1990	Stranding recovery	23
Florida, Wabasso Beach	Ocean, inshore worm reef		0	1988–1993	Net	15, Ehrhart[a]
Florida, Mosquito Lagoon	Lagoon, seagrass flats	Relatively protected natural area	0	1975–1981	Net	14,15
			0	1977, 1981	Cold stun recovery	
			29	1985	Cold stun recovery	
			1.6	1990	Cold stun recovery	
Florida, Indian River	Lagoon, seagrass flats	Agriculture, urban, industry	20–61	1982–1993	Net	15, Ehrhart[a]
Bermuda	Reef/seagrass flats	Minimal (pristine)	0	1968–1993	Net	Meylan[a]
Bahamas, Anagua	Tidal bay/seagrass flats	Minimal (pristine)	0	1974–1993	Hand capture	Bjorndal and Bolten[a]
Nicaragua, Porta Cabesas	Reef/seagrass flats	Minimal	<5	1993	Fishery survey	Lageaux[a]
Panama, Bocas del Toro, Chiriqui Lagoon	Lagoon, seagrass flats	Minimal	35	1989–1993	Net	Meylan[a]
Puerto Rico			17	1988–1992 (1st observation, 1987)	Stranding recovery	23
Barbados, Barclay's Park	Nearshore feeding ground	Agriculture	90	1990 (1st observation, 1982–1983)	Net, fishery	29
Mid-Pacific Region						
Hawaiian Islands			26–60	1983–1993	Stranding recovery	11, Balazs[a]
Hawaii, Kiholo Bay	Bay/reef/algae flats	Minimal	0	1987–1990	Hand capture	11

Hawaii, Punaluu Bay	Bay/reef/algae flats	Minimal	1	1976-1993 (1st observation, 1984)	Hand capture	11, Balazs[a]
Molokai, Palaau	Reef/algae flats	Agriculture (minimal)	0	1982-1985 (1st observation, 1985)	Net	11, Balazs[a]
			1-53	1987-1993		
Oahu, Kaneohe Bay	Bay/reef	Urban	49-92	1989-1991 (1st observation, 1958)	Hand capture	11
Oahu, Waikiki Beach	Reef	Urban	9	1990-1993	Hand capture	31
French Frigate Shoals	Nesting beach		7-12	1988-1992	Beach survey	11, Balazs[a]
Pearl/Hermes Reef	Reef	Minimal (pristine)	0	1982-1987	Hand capture	11
Midway Island	Reef	Urban (military infrastructure)	0	1969-1978 (1st observation, 1990)	Hand capture	11
Western Pacific						
Australia, Torres Strait	Reef	Pristine	0	1977-1980	Fishery survey	32
Australia, Heron Island and Wistari Reefs	Outer-barrier reef	Pristine	0	1968-1992	Hand capture	5
Australia, Clack Island Reef	Inner-shelf reef	Pristine	0	1988-1990	Hand capture	5
Australia, Hazelwood Island Reef	Inner-shelf reef	Pristine	0	1989	Hand capture	5
Australia, Green Island Reef	Inner-shelf reef reef	Pristine	0	1988-1990	Hand capture	5
Australia, Shoal Water Bay	Inshore seagrass flats	Relatively protected natural area	2-3	1988-1990	Hand capture	5
Australia, Repulse Bay	Inshore seagrass flats	Agriculture	0-22	1988-1990 (1st observation, 1989)	Hand capture	5
Australia, Moreton Bay	Inshore seagrass flats	Urban, industry	8	1990	Hand capture	5
Indian Ocean						
Seychelles	Nesting beach		0	1981-1992	Beach survey	Mortimer[a]
Aldabra Island	Feeding ground	Minimal (pristine)	0	1981-1992	Hand capture	Mortimer[a]

[a]Personal communication.

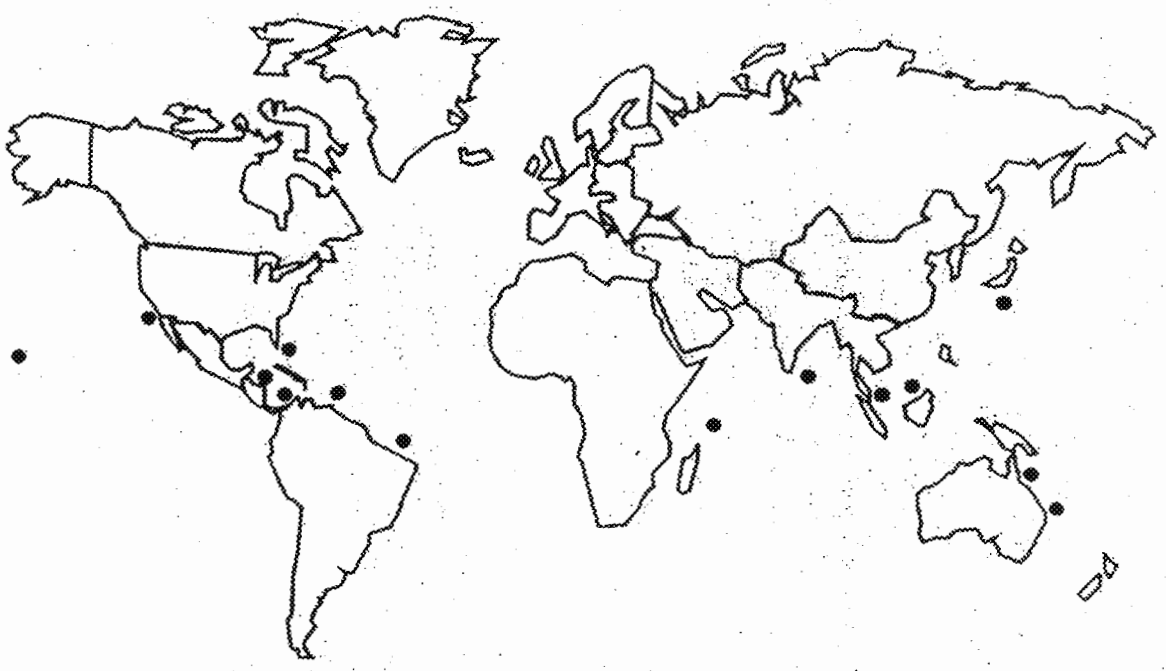

Fig. 18. Global distribution of GTFP. Localities where GTFP has been reported are marked in black dots. Although monitoring is sporadic in most areas, GTFP has been reported from every major ocean basin in which green turtles, *Chelonia mydas*, are found.

communication). Juvenile and adult loggerheads sampled by in-water capture in Florida Bay have about a 7% prevalence of fibropapilloma-like lesions (Schroeder, personal communication), while in the Indian River only two loggerheads with fibropapillomas have ever been caught out of hundreds netted since 1982 (Ehrhart, personal communication).

Seasonality

There is a seasonal pattern in the prevalence of GTFP among stranded green turtles in Florida with more affected turtles stranding in the winter months (Wendy Teas, Southeast Fisheries Science Center, NMFS, Miami, FL 33149, personal communication). Anecdotal reports indicate that tumors grow rapidly in summer and are quiescent in the winter in response to water temperature (Moretti and Brown, personal communication). Thus tumors may grow rapidly in summer with warm water temperatures and may reach a size that is debilitating by autumn. The onset of colder water temperatures in winter may further stress GTFP affected turtles sufficiently to cause the winter stranding peak.

Demographic distribution

GTFP appears to affect certain age and size classes of turtles more than others. GTFP is rare (0–12%) among nesting adult females and lesions tend to be focal and mild (Balazs, personal communication; Ehrhart, personal communication), although these are underestimates of the true prevalence in the adult population (see above). In Hawaiian feeding ground sites, intermediate-sized turtles (40–90 cm carapace length) were most commonly and most severely affected (11). Ehrhart (15) and Schroeder (personal communication) found similar results in Indian River and Florida Bay, respectively. Turtles between 10 and 30 kg in weight were more likely to have GTFP, and the disease was more severe than in larger or smaller size classes (15). Similarly, among stranded turtles in Florida, 93–98% are between 30 and 69.9 cm carapace length (Teas, personal communication). Few pelagic juvenile green turtles have ever been examined to provide any insight about this life history stage. However, the consensus is that GTFP develops after juveniles have migrated into near-shore waters. Juvenile green turtles enter near-shore feeding grounds after 2–5 years of pelagic existence. Newly arrived juveniles are recognized by their small size (<40 cm straight carapace length, <5 kg weight) and the lack of epibiota (algae, bryozoans, leeches, etc.) on their carapaces as seen in older resident turtles (5,15). GTFP has never been observed in any of these new arrivals (5,15,33). Although small green turtles <5 kg are underrepresented in net surveys, 145 cold-stunned turtles

were collected in the Mosquito Lagoon, Florida, in 1985 and provided a relatively unbiased sample of the juvenile turtle population (34). Twenty-eight percent of the cold-stunned turtles in this sample were recent recruits (<5 kg), and none of these turtles had GTFP even though the prevalence of GTFP in the overall sample was 29%.

There are several explanations for the lack of clinical GTFP among small juvenile green turtles that have recently immigrated to near-shore feeding grounds from the pelagic environment. One is that affected pelagic stage juveniles do not survive long enough to recruit to feeding grounds. Survivorship of healthy turtles through the 2–5 years of pelagic existence is already so low that it is unlikely that diseased turtles would survive. The observation that postpelagic stage juveniles develop the disease in near-shore feeding grounds can be explained by two hypotheses. One hypothesis is that while exposure to the etiologic agent(s) takes place in the pelagic zone, the disease has a long latent period that results in clinical disease developing only in older juveniles after they have migrated to neritic feeding grounds. The second hypothesis is that exposure to the etiologic agent(s) occurs after juveniles are recruited to near-shore feeding grounds. The wide variation in prevalences of disease among size/age matched populations of juvenile green turtles from different near-shore sites lends support to the second hypothesis. If the cause of GTFP were encountered in the pelagic zone and the pelagic juveniles assorted randomly among near-shore sites, then one would expect the distribution of GTFP prevalences among near-shore sites to be more uniform than is observed. Ehrhart's field study provides the most convincing data because a high prevalence site (Indian River Lagoon) is separated from a zero prevalence site (worm reef) by less than 1 km distance, the size distributions of turtles from the two sites are matched so that they represent turtles of the same age, and there is documented movement of turtles from the ocean side into the lagoon but not vice versa (15, Ehrhart, personal communication).

Epizootiologic associations

Anecdotal reports indicate that GTFP is more prevalent in near-shore habitats with poor water exchange (lagoons, bays) and most prevalent in those habitats that are impacted by human activities including agricultural, industrial, and urban development (see Table 1). Although more careful and objective documentation of human impacts is needed, there is a suggestion that environmental degradation may play a role in disease expression.

Factors in certain marine environments may provide conditions adequate for either infectious or noninfectious disease. Certain sediment types may accumulate chemical contaminants and combined with low flushing rates could increase the level of exposure to chemical carcinogens or immunotoxins. More variable water temperatures in shallow embayments could affect the rate of fibropapilloma proliferation, pathogen replication, immune system function, and/or toxin metabolism. For example, thermal stress has been shown to exacerbate herpesvirus infection in hatchling green turtles (35,36). Some sites may provide an optimum biotic and abiotic environment for survival and transmission of an infectious etiologic agent. Disease transmission would be enhanced by high population densities of vectors or intermediate host species, sediment types favoring pathogen survival outside the host, and low flushing rates. Some marine sites may attract a high density of susceptible turtles, which would facilitate the transmission of pathogens in a density dependent fashion, as has been shown for horizontally transmitted damselfish neurofibromatosis (37) and the herpesvirus of Lucké's renal adenocarcinoma (38,39). In addition, congregation of turtles from many different breeding stocks into common feeding grounds may allow the exchange of many diseases, including GTFP. Finally, in some marine habitats, green turtles may be rendered more susceptible or less able to recover from an infectious disease due to stressors that result in immune dysfunction (to be discussed later). These hypotheses provide the framework for studies to determine the etiology and understand its pathogenesis.

ETIOLOGY

The etiology of green turtle fibropapillomatosis is unknown but is under intensive investigation. Plausible hypotheses concerning the etiology of GTFP can be generated using two compatible approaches: (1) epizootiology and (2) comparative pathology. Epizootiologists study the patterns of disease occurrence within and among populations and propose mechanisms and identify factors that could explain the observed prevalence patterns. Comparative pathologists review available information about the causes of similar disease processes in other species and establish a list of potential etiologies that can be investigated experimentally. Neither approach can prove etiology. Demonstration of etiology requires rigorous experimentation to fulfill Koch's postulates. This is most easily accomplished with diseases caused by infectious agents. However, disease expression may depend on a variety

of host-related, pathogen-related, and environmental factors (40,41). Fulfillment of Koch's postulates for a neoplastic disease, which may be caused by a single agent or by a complex interaction of multiple etiologic agents, may be impossible. The criteria for inferring etiology based on epidemiologic associations are discussed in detail by Foster, Bernstein, and Huber (42).

Histologically, GTFP is characterized by benign proliferation of epidermal cells and well-differentiated dermal fibroblasts. Thus GTFP has features in common with benign proliferative skin lesions found in other vertebrates such as papillomas, fibromas (43), and aberrant wound healing phenomena such as keloidosis (44) and exuberant granulation tissue (45). Consequently, without knowing the etiology and pathogenesis of the lesion, it is difficult to classify GTFP as either a neoplastic or hyperplastic condition (46). Neoplasia, by definition, involves a permanent change in the cell's genotype leading to relatively unregulated proliferation and differentiation. Hyperplasia, on the other hand, represents a proliferation of normal tissue beyond the usual amount for a site, but the hyperplastic cells are genotypically normal and are responding to some stimulus. Hyperplasia is generally not progressive and proliferation ceases when the inciting stimuli are removed (47).

The general result of decades of research into the causes of neoplasia is that neoplasia may result from any of a variety of derangements at any point in the complex signaling and control network of normal cellular proliferation and differentiation. The pathogenesis of neoplasia may involve multiple cumulative steps (48,49). Unregulated proliferation may result from only a few steps, whereas multiple sequential events may be necessary for progression to malignancy. In this light, numerous etiologic agents may, independently or in concert and by a variety of different mechanisms, result in the same metabolic derangement(s) leading to neoplastic transformation (50). Thus, identifying a single etiology for any particular type of proliferative lesion may be difficult if not impossible. Potential causes of neoplastic and hyperplastic proliferative lesions in other vertebrate species include abiotic agents (ultraviolet light, chemical contaminants) and infectious biological agents (viruses, bacteria, metazoan parasites), with or without predisposing heritable genetic conditions. The following sections review and discuss evidence for or against the involvement of each of these factors in the etiology and pathogenesis of GTFP.

Environmental factors

Ultraviolet light. Smith and Coates (7) were the first to suggest a role for solar radiation in the pathogenesis of GTFP. Fifty years later there is mounting concern that ozone depletion is causing an increase in ultraviolet-B (290–320 nm) irradiation (51) and that this may be having pervasive effects in aquatic ecosystems (52) and on animal health (53). UV-B produces direct DNA damage by pyrimidine dimer formation (54). This may lead to mutation in cellular oncogenes and the development of neoplasia (55). UV-B also causes immunosuppression in experimental animals (56–59). The proposed mechanism involves pyridine dimer formation (60,61) and/or a *trans* to *cis* isomerization of urocanic acid in the skin following UV-B absorption (59,62). For example, trout exposed to levels of UV-B radiation within the ambient range recorded for midlatitudes developed skin damage and became immunosuppressed, as evidenced by a high incidence of fungal skin infections (63).

Increased UV-B exposure could occur in the shallow inshore waters where green turtles feed. However, GTFP prevalence varies too greatly over very short distances (as in Ehrhart's study area) for UV-B to be a major factor in disease expression. The role of UV-B in modulating the immune system of turtles deserves further investigation.

Chemical contaminants. A variety of chemical compounds have been demonstrated to cause benign fibro-epithelial proliferation and to have mutagenic and carcinogenic properties under experimental conditions (64,65). The list of compounds is extensive but they seem to act by either of two basic mechanisms of action: (1) direct nucleic acid damage leading to genetic mutation (initiators) and (2) cellular damage or irritation leading to proliferation (promoters). As mentioned earlier, chemical effects may be one of many mechanisms involved in multistep carcinogenesis.

The involvement of chemical contaminants in naturally occurring neoplastic disease of lower vertebrates has been documented best in fish. The prevalence of liver pathology, including liver neoplasia in brown bullheads, *Ictalurus nebulosus*, was higher at contaminated sites than at relatively clean sites in several North American lakes and rivers (66–68). Disease prevalence was correlated with contaminant levels in fish in one study (67) and with sediment levels in another (66). Neoplastic lesions were induced experimentally by treating bullheads with sediment extracts (66). Similar associations between

hepatic neoplasia, polluted sites, and sediment contaminant concentrations have been found for mummichogs, *Fundulus heteroclitus*, in Chesapeake Bay (69) and various bottom fish in Puget Sound (70). A similar association has been found between contaminated sites, *in vitro* mutagenesis of water and sediment extracts from those sites, and the prevalence of pigment cell neoplasia (Chromatophoromas) in croakers, *Nibea mitsukurii* (71,72). In addition, experimental application of chemical carcinogens elicited the tumors in these fish. In most of these studies, polycyclic aromatic hydrocarbon (PAH) concentration was a major factor in the association between disease prevalence with contaminant levels. Similarly, PAHs have been implicated in the pathogenesis of cutaneous neoplasia in tiger salamanders, *Ambystoma tigrinum*, from a polluted pond (73,74). Cutaneous papillomas have been experimentally induced in lizards, *Lacerta agilis*, with dimethyl benzanthracene (75).

Chemical contaminants may also play a role in the pathogenesis of certain neoplastic diseases by disrupting immune functions that would otherwise allow the host to eliminate transformed cells. The effects of various immunotoxins have been reviewed by Dean, Cornacoff, and Luster (76). The effects of various chemical compounds on immune function in fish has also been reviewed (77–79) and experimentally demonstrated in some species (80). Contaminants may also disrupt the immune system indirectly by disrupting neuroendocrine functions (81).

The role of chemical contaminants in GTFP is unknown. As mentioned previously, prevalence data from some field studies suggest that high GTFP prevalence is associated with marine habitats that have been impacted by human activity, including agriculture, industry, and urban development (Table I). However, few data are available comparing contaminant levels in these marine systems with others where GTFP is less prevalent. Similarly, data on contaminant levels in green turtle tissues are scant (82–87). While Hall, Belisle, and Sileo (84) found significant amounts of hydrocarbons in two green turtles that stranded after a major oil spill, most surveys of organochlorine and polychlorinated biphenyl residues in green turtle tissues including muscle and liver (85); liver and fat (86); liver, kidney, and fat (87); and eggs (82,83) have yielded relatively low levels, often below the limits of detection of the methods.

There are many problems when trying to relate contaminant levels to disease prevalence. First, the biologic effect (toxicity) of any particular residue level in green turtles is unknown. Second, surveys of residue levels are usually limited to those chemicals that persist in the environment or bioaccumulate, while a particular toxic effect such as genetic damage, in a multistage carcinogenesis model, can result from transient exposures. Thus, exposure to a potent chemical carcinogen may occur transiently in a completely different habitat from that being monitored. Third, toxic effects may not be direct as in some experimental models but may involve complex interactions with other abiotic and biotic factors. Thus, fulfilling the criteria for implicating chemical contaminants as primary etiology of GTFP or as cofactor could be extremely difficult (42). Nevertheless, there is a need to conduct further toxicological studies. Specifically, there is a need to collect data on the water, sediment, and turtle tissue burdens of several classes of chemical contaminants (including known chemical carcinogens and immunotoxins) from several carefully matched marine sites with different prevalences of disease. In addition, controlled experiments involving exposure of turtles to water or sediment extracts from high and low prevalence areas will be necessary in order to clearly demonstrate a contaminant effect in the etiology and pathogenesis of this disease.

Infectious disease

The epizootiologic patterns observed among free-ranging green turtle populations including the sudden appearance of GTFP at new geographic sites, variation in prevalence over relatively short distances, and temporal variation within a locality are compatible with an infectious etiology. The observation that some animals recover from GTFP is also compatible with an infectious disease. In addition, the most plausible explanation for the appearance and spread of GTFP among captive green turtles is that an infectious agent is the primary cause of GTFP. A GTFP outbreak documented at Cayman Turtle Farm, Grand Cayman, in 1980 began in wild caught adults and subsequently developed in captive reared turtles over several years. Once eliminated, GTFP has not recurred at Cayman Turtle Farm despite little change in husbandry conditions (12,13). A similar outbreak occurred in a head starting facility in the Florida keys among 2-year-old captive reared green turtles that had been held in a pond where GTFP-affected turtles were rehabilitated and possibly had direct contact with affected turtles (88).

Viruses. A number of virus families (Papovaviridae, Herpesviridae, Adenoviridae, Poxviridae,

Retroviridae) are known to induce proliferative and/or neoplastic lesions. Papillomaviruses (papovaviridae) are the documented cause of papillomas, fibromas, and fibropapillomas in many mammalian and avian species (89) and are associated with malignant neoplasia as well (90,91). Among reptiles, a papillomavirus has been described from a hyperplastic skin lesion of five Bolivian side-necked turtles, *Platemys platycephala* (92), and papovavirus-like particles have been observed in papillomas of green lizards, *Lacerta viridis* (93,94). A polyomavirus (papovaviridae) of hamsters produces benign cutaneous neoplasia in these rodents (95), but other polyomaviruses of rodents and primates do not produce disease in their natural hosts (96). Herpesviruses have been associated with cutaneous papillomas and/or fibromas in green lizards, *Lacerta viridis* (93); african elephants, *Loxodonta africana* (97); carp, *Cyprinus carpio* (98,99); and several salmonids (100–104). Poxviruses are responsible for fibroepithelial proliferative lesions in squirrels (105,106), rabbits (107,108), and primates (109). Retroviruses have been associated with or proven the cause of dermal sarcomas in walleyes, *Stizostedion vitreum* (110); lip fibromas in angelfish (111); neurofibromas in damselfish, *Pomacentrus partitus* (112); and fibromas and sarcomas in a variety of mammalian species including cats (113,114) and primates (115,116). The molecular mechanisms of virus-induced proliferation and oncogenesis vary, but all in some way disrupt the cells' signal transduction network (117–119). Certain viruses produce proteins that bind to and activate cellular receptors or function like activated receptors. For example, the E5 protein of certain papillomaviruses binds and activates the PDGF receptor (120–122). The adenovirus E1B protein and the papillomavirus E6 protein bind and inactivate cell cycle checkpoint protein p53 (117,119). Similarly, adenovirus E1A protein, polyomavirus T antigen, and papillomavirus E7 target the cell cycle control protein pRB (retinoblastoma gene product). Poxviruses may produce epidermal growth factor (EGF)-like peptides (123), and a retrovirus (simian sarcoma virus) produces a PDGF-like peptide (*sis*) (117). Certain herpesviruses may express a protein (ribonucleotide reductase) with protein kinase activity similar to receptor kinases (124).

Histologic Evidence for Viruses. The perivascular lymphocytic infiltrates observed in some GTFPs are consistent with, although not specific for, virus infection. Smith and Coates (7) failed to find virus-like inclusions within the tumors that they examined. Jacobson et al. (13) examined tumors from six turtles from Florida and one turtle from Hawaii by light and electron microscopy. In some sections, cells in the stratum spinosum and outer layers of the epidermis were hypertrophic and vacuolated, and amphophilic intranuclear inclusion bodies suggestive of herpesvirus infection were occasionally seen. Ultrastructural examination revealed mild acanthosis (three to six cells thick) and intracytoplasmic vacuoles containing 150–170 nm diameter granules of varying electron densities were described within the superficial epidermis but not identified (13). Aguirre, Balazs, Zimmerman, and Spraker (21) described basophilic intranuclear inclusions in several lesions that they suspected to be nucleoli but also considered compatible with viral inclusions. However, viral particles were not found within these inclusions when examined by electron microscopy. They also observed intracytoplasmic electron dense granules approximately 150 nm in diameter that were morphologically similar to viral particles. These, however, were found in both normal and GTFP epithelium. These intracytoplasmic granules are now generally accepted to be mucin granules that are produced and secreted by normal turtle keratinocytes as they differentiate (13,21,125).

A herpesvirus has been conclusively demonstrated in some fibropapillomas taken from two juvenile green turtles housed in the same rehabilitation facility in the Florida Keys (18). In 3 fibropapillomas examined from one turtle and 1 of 14 tumors examined from a second turtle, focal areas of ballooning degeneration of superficial epithelium were found to contain eosinophilic intranuclear inclusions (Fig. 14). Electron microscopy (Figs. 15 and 16) demonstrated the presence of particles within the nucleus conforming in size and morphology (icosahedral 77–90 nm diameter) with immature herpesvirus and intracytoplasmic particles conforming with mature enveloped herpesvirus (110–120 nm diameter). This agent was not successfully isolated and cultured.

Molecular Evidence for Viruses. Jacobson et al. (13), in their survey of fibropapillomas from one Hawaiian and six Florida green turtles, examined paraffin embedded sections for the presence of papillomavirus group-specific structural antigens using peroxidase-antiperoxidase immunohistochemistry. Total DNA extracted from portions of these same tumors was probed under low stringency conditions with bovine papillomavirus type 2 virion DNA. Finally, a reverse Southern blot was performed in which radiolabeled tumor DNA from two turtles was allowed to hybridize with blots containing 25 different cloned papillomavirus genomes (6 bovine,

7 human, and dog, rabbit, coyote, mouse, rat, and parrot papillomaviruses). These screenings for papillomavirus yielded negative results (13). Similarly, Marc Van Ranst (Einstein Medical College, Bronx, New York, personal communication) performed low stringency southern blot analysis on DNA extracts of 11 tumors collected from a single immature green turtle from the Florida Keys. Probes included full genomic DNA from human papillomaviruses HPV-1, HPV-2, and HPV-5, bovine papillomavirus BPV-1, canine oral papillomavirus, and pygmy chimpanzee papillomavirus PCPV-1. Results were negative.

Preliminary experiments have also been conducted using the polymerase chain reaction (PCR) and degenerate PCR primers for conserved sequences in the E1 and L1 mammalian papilloma virus genes. These primers failed to amplify any sequences in 11 GTFP biopsies from one green turtle (Van Ranst, personal communication).

Experimental Evidence for Viruses. The most direct way to demonstrate the infectious nature of a neoplastic disease is to conduct transmission studies with cell-free preparations of tumor tissue. Recently, Herbst, Jacobson, Moretti, Brown, and Klein (126,127) demonstrated that fibropapillomas can be produced in experimental turtles by injecting cell-free homogenates of cutaneous green turtle fibropapillomas. Four replicate transmission experiments were set up using a separate wild caught GTFP affected donor and four unexposed captive-reared yearling green turtles for each replicate. GTFP homogenates were inoculated by intradermal injection or skin scarification into several anatomic sites on each recipient. Sham treated sites received sterile saline inoculations. In addition, four captive-reared turtles were kept as untreated controls. Fibropapillomas developed at one or more inoculation sites in turtles from three replicate transmission experiments. Transmission was successful with both 0.45 μm filtered and unfiltered homogenates. In the fourth replicate experiment and in the control group, no tumors developed after 6 months of observation. Histologic findings within experimental lesions were consistent with GTFP. These limited experimental results suggest that a subcellular infectious agent is present within some fibropapillomas at some stage in their development and is the etiology of GTFP.

Assessment of the Evidence for a Viral Etiology. Surveys of fibropapilloma biopsy specimens by light and electron microscopy for virus particles, by antibody and nucleic acid probes for viral antigens, and viral DNA have been limited and largely unsuccessful. With the exception of the herpesvirus described by Jacobson et al. (18), no virus particles have been observed in tumors and no viral antigens or viral DNA have been associated with lesions. The experimental evidence of Herbst et al. (126,127) provides the clearest indication that an infectious subcellular agent, most probably a virus, is the etiology of GTFP. The agent responsible for positive experimental transmission has not yet been identified.

The herpesvirus identified by Jacobson et al. (18) has not been successfully isolated and cultured. Therefore, the experiments to fulfill Koch's postulates with this agent cannot be conducted. Koch's postulates have been fulfilled for several herpesvirus-induced skin tumors of fish (98,100–104). In addition, herpesviruses are the cause of other forms of neoplasia including Marek's disease in chickens (128), Lucke's renal adenocarcinoma in frogs (38,39), lymphoma in spider monkeys, owl monkeys, and marmosets infected with *Herpesvirus saimiri* type 2 (129), and Burkitt's lymphoma in humans infected with Epstein–Barr virus (130). However, herpesviruses also have a propensity for colonizing tumors and tissues of debilitated animals, and thus the association of herpesvirus with GTFP may represent a secondary infection. One example is the herpesvirus that causes gray patch disease in young green turtles, which causes similar skin lesions (acanthosis, hyperkeratosis, and ballooning degeneration) (131). As a further illustration of the problem of assigning causation, Raynaud and Adrian (93) found three distinct virus types in papillomas of green lizards, *Lacerta viridis*, including herpesvirus, papovavirus, and reovirus-like particles, whereas Cooper, Gschmeissner, and Holt (94) found papovavirus-like particles in one papilloma from the same species. None of these associations can be interpreted as causative without isolation and culture of each agent separately followed by controlled transmission experiments.

Preliminary searches for an association of papillomavirus antigens and DNA with GTFP yielded negative results. However, the data are insufficient to rule out papillomavirus involvement. First, papillomaviruses are extremely diverse, and it is not unlikely that a reptilian papilloma virus would fail to react with mammalian and avian probes and antisera (132). Second, papillomavirus virion production occurs only in the most superficial terminally differentiated epidermal cells of a permissive host species (133). Papilloma virus also infects nonpermissive tissues and nonpermissive species and may cause hyperplastic or neoplastic lesions in the absence of virion production (133,134). Equine sar-

coids, for example, are fibromatous tumors that are believed to be caused by bovine papilloma virus infection. Intact or partial viral genomes are found within sarcoid fibroblasts as unintegrated episomes (135–137), but infectious virion production never occurs. Thus, tumors could be inclusion body and virus particle negative and, consequently, viral structural antigen free. Third, virion production occurs only in the most superficial epithelium after a prepatent period (138), and the productive phase may be very limited. Thus, fibropapillomas may vary in maturity, and when producing papillomavirus particles the relevant superficial keratinizing epithelial cells may be lost with minor trauma, sample handling, and processing. Finally, the expression of many poikilothermic viral diseases may be modulated by temperature and other environmental factors. For example, Lucké's renal adenocarcinoma in leopard frogs, *Rana pipiens*, is caused by a herpesvirus. Intranuclear viral inclusion bodies and infectious viral particles are produced in tumors only at low environmental temperatures (38,39). Higher temperatures (20–22 °C) cause the virus to enter a cellulolytic phase followed by a quiescent phase in which virion production ceases and tumors are not infectious (39,139,140). Similar processes may be occurring in green turtle fibropapillomas.

The molecular approaches have been limited to papillomaviruses. Consequently, the role of oncogenic retroviruses has not been investigated. As with the papillomaviruses, retroviruses may cause neoplasia without ever developing a patent life cycle in the host (117). In the absence of electron microscopic evidence for virus production and shedding from the tumor, it is difficult to implicate retrovirus as an etiology. Detection of integrated retroviral genomes (provirus) within the green turtle genome will require specific molecular probes. Such molecular probes will be unavailable until the agent is identified and portions of its genome sequenced. Until then it is unlikely that nonspecific retroviral probes would yield conclusive results given the ubiquity of endogenous retroviral sequences in vertebrates (141).

Bacteria. Chronic bacterial infections may induce proliferative lesions in some tissues. For example, intracellular *Campylobacter*-like organisms are associated with proliferative enteritis in ferrets, hamsters, and swine (142,143). An invasive spirochaete has been observed in papillomatous foot lesions in cattle, but experiments to fulfill Koch's postulates have not yet been conducted (144). Numerous bacteria species have been cultured from the surfaces of cutaneous green turtle fibropapillomas (21). Bacteria are not seen within intact GTFP lesions however, and little inflammation is observed within tumors unless the surface is ulcerated. Moreover, the transmission experiments using 0.45 μm filtered tumor homogenates rule out the involvement of most bacteria species (126,127).

Metazoan parasites. An association between parasites and neoplasia has been made in several species. Dogs infected with the nematode, *Spirocerca lupi*, which encysts in the esophagus, may develop fibrosarcoma at the site (145). Rats with tapeworm, *Cysticercus* (*Taenia*), cysts were reported to develop hepatic carcinomas with high frequency (146). *Schistosoma mansoni* infection in humans has been associated with bladder cancer. The pathogenesis is associated with egg shedding (147). Studies of the pathogenesis of *Schistosoma mansoni* indicate that fluke eggs antigens can elicit a fibrotic response in the host (148–150). This phenomenon has supported the hypothesis that spirorchid trematode eggs may induce fibromas by similar mechanisms (46). Associations of tumor with spirorchid trematode eggs and marine leeches have also led to speculation that these organisms may be involved in the etiology and pathogenesis of GTFP.

Evidence for a Metazoan Parasite Etiology. Marine turtles are host to a variety of digenetic trematode species. At least 12 species of spirorchid trematodes have been described in the green turtle (151). Their natural history is very similar to *Schistosoma* in that adult worms inhabit the vascular system and eggs must migrate through tissues to reach an outlet to the environment. The association of fluke egg deposition with fibropapillomatosis was first noted by Smith and Coates (20), who found eggs of *Haplotrema constrictum* in sections of over half of 230 fibropapillomas that they examined from Florida green turtles. Benign papillomatous lesions in the gallbladder of green turtles have been described in association with eggs of *Rhytidodoides similis* (family: Rhytidodidae) (152).

Jacobson et al. (13) did not find trematode ova in any sections of 28 tumor biopsies collected from 6 Florida green turtles but eggs were present in tumor sections from 1 Hawaiian turtle. However, Norton et al. (17) and Jacobson et al. (18) found eggs in many sections of tumors from 3 Florida turtles, Williams et al. (19) found eggs in fibropapillomas examined from 39 Caribbean green turtles, and Aguirre et al. (21) found eggs in biopsy sections from 8 of 10 Hawaiian turtles affected with GTFP. More recently, trypsin digestion of whole fibropapillomas has led to recovery of trematode ova from all fibropapillomas examined from both Hawaiian and Florida green turtles (Murray Dailey, Califor-

nia State University, Long Beach, CA 90840, personal communication; Ellis Greiner, University of Florida, Gainesville, FL 32610, personal communication). Thus, trematode ova are probably present in most fibropapillomas but not always found in tissue sections.

Spirorchid trematode ova have been found in 16 of 21 (76%) wild green turtles, 3 of 10 (33%) oceanarium-reared green turtles, and 3 of 102 (2.9%) farmed green turtles from Queensland, Australia, while the prevalence of fibropapillomatosis was 0% (32,153). Pathological lesions associated with egg deposition in tissues of sea turtles have been described (32,153–157). Microscopically, lesions are characterized by mononuclear cell infiltrate and granulomatous inflammation with Langhans giant cells, vasculitis and perivasculitis, and fibrosis (Fig. 11). In turtles with GTFP, such lesions are found within tumors as well as in otherwise normal tissues (17,21).

Herbst et al. (127, 158) conducted preliminary experiments in which aliquots of 50 viable spirorchid trematode eggs from each of 2 species (*Learedius learedii* and an unidentified species) were injected into several intradermal and subcutaneous sites in 3 recipient green turtles. Neither of these turtles developed cutaneous GTFP after more than 1 year of observation. While these preliminary experiments did not vary dosages of eggs or frequencies of injection, the results suggest that spirorchid trematode eggs do not play a direct role in the etiology of GTFP lesions. Similarly, in two experimental turtles that developed GTFP after receiving tumor homogenates, neither animal was found to harbor adult spirorchids or their eggs in any tissue, and when tumor homogenates from these trematode-free turtles were used in a transmission experiment both recipient turtles developed tumors.

Finally, an argument has been put forward that external parasites may have some role in the pathogenesis of fibropapillomatosis. Nigrelli (159) and Nigrelli and Smith (160) found leeches, *Ozobranchus branchiatus*, infesting the folds of papillomas and suggested that the leeches may act as vectors of the causative agents. Most authors agree, however, that leeches do not cause tumors directly although hirudinin secretion may cause some increased vascularization at leech attachment sites (151,160).

Assessment of Evidence for a Parasite Etiology. The significance of metazoan parasites in the etiology of GTFP remains unclear. Smith and Coates (20) concluded that the trematode eggs were incidental, tending to accumulate passively in the microvasculature of tumors. Similarly, other authors have also tended to discount the eggs as primary causes (13,151). On the other hand, some authors have characterized cutaneous fibromas from green turtles as a hyperplastic response to trematode eggs (46). The occurrence of trematode egg-induced lesions in otherwise normal tissues and in green turtles that do not have GTFP argues against a direct hyperplastic/tumorigenic effect. The experimental evidence (127,158) also does not support a direct role for metazoan parasites as the etiology of GTFP, although the egg dose and dose rate may have been insufficient to elicit a response. Indirect roles for metazoan parasites in the pathogenesis or epizootiology of the disease remain as distinct possibilities. Parasites may serve as vectors for an infectious agent, as was suggested in one epizootic in captive turtles (88). Both trematode and leech infestations may severely debilitate the host (32,153, 155,156,161), so that the immune system cannot effectively respond to the GTFP agent. Spirorchid egg deposition within tumor vasculature may trigger a chronic inflammatory and immune response that could result in either a continuing hyperplastic response within tumors or eventual tumor rejection. The effect of concurrent parasitic diseases on the pathogenesis of GTFP deserves further investigation.

Genetic factors

Neoplastic transformation often involves the accumulation of multiple genetic changes within the cell. Familial patterns of neoplastic disease arise from a heritable (germline) genetic lesion that renders individuals more susceptible to disease development following subsequent somatic cell genetic damage. For example, a germline loss of function mutation in a tumor suppressor locus would predispose an individual to neoplasia following any event that disables the remaining functional allele (162–164). Familial patterns of neoplasia are well documented in humans. Examples include Li-Fraumeni syndrome, Wilm's tumors, retinoblastoma, and neurofibromatosis (163,164). Laboratory mice show extensive strain variation in susceptibility to experimental tumorigenesis (165). A well-documented example of genetic susceptibility to neoplasia among lower vertebrates is found in certain platyfish, *Xiphophorus maculatus/Xiphophorus helleri*, hybrids, which have high rates of spontaneous and ultraviolet light-induced melanoma due to loss of a functional tumor suppressor gene (166–168). Heritable defects in DNA repair mechanisms could also render individuals more prone to neoplasia as is the case in xeroderma pigmentosa (169,170). The familial pattern of epidermodysplasia verruciformis is be-

lieved to involve heritable defects in cellular immune function and an inability to eliminate papillomavirus infection (171,172). In rabbits, certain major histocompatibility loci have been associated with the regression or progression to malignancy of Shope papillomavirus-induced tumors (173). In addition, individuals of some species have genetic predispositions to exuberant hyperplastic responses to wounding, e.g. keloidosis in humans (44) and "proud flesh" in horses (45), that can resemble benign neoplasia.

The possibility that some green turtles have a genetic predisposition to develop GTFP must be considered. However, there is no evidence that this is the case because genealogical studies in this species are impractical and methods to distinguish susceptible from resistant individuals are unavailable. Although some histological features of GTFP resemble granulation tissue, an aberrant wound response is unlikely. The author has conducted wounding experiments in both healthy and GTFP-affected green turtles while obtaining multiple skin biopsies for other purposes. Both GTFP-affected and -unaffected green turtles have normal wound healing responses. The anecdotal reports that GTFP lesions tend to occur at sites that are vulnerable to wounding (mating scars, bites, entanglements, etc.) can be explained equally well by exposure to an infectious agent.

PATHOGENESIS

Pathogenesis is the developmental process of a disease. To understand pathogenesis both etiology and host response must be identified (174). There are numerous questions regarding the pathogenesis of GTFP that cannot be answered adequately until an etiologic agent is identified and available for experimentation or until diagnostic reagents and techniques become available for exploring the molecular basis of the lesion. Among these are: Is GTFP a hyperplastic or neoplastic process? If GTFP is a neoplastic disease, which cells are transformed and what is the molecular mechanism of transformation? Is there progression from benign to more malignant disease? Are visceral nodules part of GTFP and do they represent multicentric or metastatic disease? What is the mechanism of tumor regression? Is the immune system involved in tumor rejection and how? Does immune dysfunction play a role in the pathogenesis of GTFP?

Is GTFP hyperplasia or neoplasia?

Histologically, fibropapillomas are characterized by proliferation of apparently normal epidermal and/or dermal elements. GTFP cells are well differentiated and show no anaplastic characteristics. Growth is slow with few mitotic figures seen with light microscopy. Flow cytometric analysis of GTFP cell DNA content shows them to be diploid (Elliott Jacobson, University of Florida, Gainesville, FL 32610, personal communication). As previously discussed, these characteristics are consistent with either hyperplasia or benign neoplasia. It is important to know if GTFP is a neoplastic disease because the approaches to prevention and control as well as the prognosis for success depend on this information. There is a need to conduct *in vitro* and *in vivo* testing of both epidermal and fibroblast cell lines derived from GTFP tumors to resolve these questions.

The key criteria distinguishing neoplastic cells from normal cells undergoing hyperplasia are that the neoplastic (transformed) phenotype is irreversible and involves independence from regulation. Neoplastic cells continue to proliferate in an environment that would normally inhibit proliferation. *In vitro*, neoplastic cells may exhibit one or more of the following phenotypic characteristics which represent different pathways of regulation: (1) anchorage independence, (2) loss of contact inhibition, (3) reduced serum dependence, and (4) immortality. *In vivo*, neoplastic cells should cause tumors in the appropriate host. On the other hand, activated normal cells (hyperplastic), while exhibiting morphological changes associated with rapid proliferation similar to neoplastic cells, are highly regulated by exogenous factors, respond appropriately to these stimuli, and become inactive when the stimuli cease. In culture, cells derived from hyperplastic lesions should be phenotypically normal.

If GTFP represents true neoplasia, the molecular basis of the lesion needs to be identified. If hyperplasia, the causative stimulus needs to be identified. Work in this area is only just beginning, and toward this end several workers have successfully established fibroblast and epithelial cell cultures derived from GTFP (175,176, Herbst et al., unpublished). Mansell, Jacobson, and Gaskin (175) described the *in vitro* morphology and growth characteristics of GTFP-derived fibroblasts but failed to establish matched normal fibroblast lines for comparison. In addition, no attempt was made to demonstrate transformed phenotype in these cell lines. Simpson, Jacobson, and Balazs (176) were the first to attempt to demonstrate *in vivo* tumorigenicity of GTFP-derived cell lines in irradiated anoles (177) and in normal immature green turtles. Neither experiment demonstrated tumorigenicity *in vivo*. While these

preliminary data suggest that GTFP cells are not transformed, it remains possible that *bona fide* GTFP neoplastic cells failed to become or remain established in culture. It is also possible that the majority of cells within a fibropapilloma are not neoplastic but hyperplastic. Many of the cells, comprising the bulk of a tumor, may be responding to paracrine proliferative signals generated by a few neoplastic cells (178).

Does GTFP progress to malignancy?

Cutaneous GTFP is characterized by self-limiting fibro-epithelial tumors that may eventually regress if the turtle does not become debilitated and die due to impaired vision, swimming ability, or other organ function. However, in some cases some tumors may behave more aggressively and slowly invade tissues such as the cornea (16) and carapace (Herbst, personal observation). Smith and Coates (20) described an eyelid mass from one green turtle that exhibited adenomatous changes suggestive of early malignancy. Whether these cases represent malignant transformation is unclear. Because the tumors are composed of fibroblasts and mesenchymal cells, these cells are able to proliferate and expand throughout the stromal component of an organ, in their normal location, without "invading" across basement membranes or along blood vessels. Whether internal mesenchymal tumors are the result of metastasis (which is characteristic of malignancy) or multiple independent oncogenic events also remains to be determined.

Relationship between visceral and cutaneous tumors

Visceral fibromas and myxofibromas have been reported only in green turtles with extensive cutaneous GTFP lesions. There are relatively few opportunities to necropsy green turtles with healthy integuments to search for internal nodules. However, no internal nodules were found at necropsy in over 100 cutaneous GTFP-free green turtles taken in the Nicaraguan fishery in 1993 (Lageaux, personal communication). The association of visceral tumors with cutaneous GTFP suggests that they arise from the same pathologic process. The distribution of internal tumors primarily to lungs and kidney supports hematogenous spread of neoplastic cells or an oncogenic pathogen. Given the evidence for an infectious etiology presented above, the pathogenesis of visceral lesions may involve viremia followed by multifocal transformation of susceptible cells. Internal fibromas are also found in deer (179) and european elk (180) with papillomavirus infections. Whether visceral tumors arise in synchrony with cutaneous fibropapillomas or develop only in turtles with chronic skin lesions is an important question. Further investigation of the relationship of internal tumors to cutaneous GTFP will have to await the development of molecular probes.

Mechanism(s) of tumor regression?

Spontaneous regression of fibropapillomas has been observed in some field studies (15, Meylan, personal communication; Balazs, personal communication). Ehrhart found that of 25 recaptured green turtles that had fibropapillomas on first capture, about 16% were free of GTFP on subsequent recapture (15). In captivity, one turtle that was given an autogenous tumor graft, subsequently showed tumor regression (Moretti and Brown, personal communication). The mechanism of spontaneous GTFP regression needs to be determined.

Environmental factors may play a major role in the progression or regression of tumors in poikilothermic species. For example, the prevalence of papillomas of Japanese newts, *Cynops pyrrhogaster*, varies seasonally with highest prevalences found in the autumn (181). These papillomas begin to regress spontaneously at certain temperatures (4 or 25–30 °C) but progress at others temperatures (10 and 13 °C) (182). They also regress in response to experimental doses of ultraviolet radiation (183). Herpesvirus expression and tumor growth rates of Lucké's renal adenocarcinoma of leopard frogs, *Rana pipiens*, vary with season and temperature. Virion production ceases and tumors grow more rapidly and metastasize more frequently at warm temperatures in the summer (38,39). Retrovirus-induced dermal sarcoma of walleyes, *Stizostedion vitreum*, also shows seasonal variation with highest prevalences in the spring followed by spontaneous tumor regression in summer (184,185). Tumor growth is also influenced by temperature (186). Similarly, retrovirus-induced northern pike lymphosarcoma is effected by temperature (187) and shows seasonal regression (188). On the other hand, spontaneous regression is not seen in bicolor damselfish, *Pomacentrus partitus*, afflicted with damselfish neurofibromatosis (189).

Environmental mediation of tumor regression may occur as a direct effect on tumor virus replication causing a switch from cell proliferative to cytolytic phases, a direct anti-proliferative effect on neoplastic cells, or as an indirect consequence of environmental modulation of the immune system.

Role of the host immune system in regression

Many viral tumors of homeothermic vertebrates, including warts and fibropapillomas caused by papillomaviruses (43,138), and fibromas caused by poxviruses (109), also undergo spontaneous regression. Cell mediated immune responses are thought to be important in spontaneous recovery from these diseases (190). Regression of human warts is associated with cell mediated immune response (172,191,192) as is regression of bovine fibropapillomas (193) and Shope papillomas in rabbits (173,194). Possible mechanisms include cell mediated cytotoxicity and/or cytokine mediated suppression of papilloma cell proliferation (195–197). In several species, antiviral and anti-tumor cell antibodies are produced (198–205). The role of humoral immune response in tumor rejection is thought to be minimal, although an arthus-like reaction characterized by thrombosis within blood vessels of regressing human papillomas has been described (199,200).

Important questions regarding the pathogenesis of GTFP are whether or not and how the host immune system responds to the etiologic agent and/or neoplastic cells. Neoplastic tissues do not necessarily elicit host immune responses. To elicit an immune response, fibropapillomas must express abnormal or novel antigens to which the host has not been tolerized, such as virus structural antigens, nonstructural viral antigens, abnormal host cellular antigens, and host cellular antigens that are normally expressed only in immunologically privileged sites being expressed at inappropriate sites or times during ontogeny. Fibropapillomas resulting from derangement of the intracellular signaling network caused by spontaneous or chemically induced mutations or by retroviral integration into the genome may not express novel antigens. On the other hand, fibropapillomas that are caused by an infectious agent are likely to express antigens against which an immune response can be mounted.

Evidence for immune system involvement in GTFP

There is some histologic evidence that the green turtle's immune system is involved in the pathogenesis of GTFP. Perivascular lymphocyte infiltrates have been observed in many tumors (7,13,16) but whether these cells were responding to spirorchid trematode eggs or other secondary pathogens is unknown (see below). Cleft formation at the dermoepidermal boundary has been observed in several cutaneous GTFP biopsies (13). These clefts (Fig. 13) resemble those that develop in certain autoimmune skin diseases of mammals such as pemphigus and systemic lupus erythematosus, which are characterized by immunoglobulin deposition within specific layers of the skin (206). This phenomenon may represent part of the green turtle immune response to abnormal (infected) basal epidermal cells. A similar process involving acantholysis and cleft formation between basal and suprabasal cells has been described in regressing bovine fibropapillomas (193).

Histopathological studies of the immune response to fibropapillomas are confounded by the presence of secondary infections by opportunistic pathogens and skin surface commensals following trauma to the tumor surface, and by the accumulation of spirorchid trematode eggs within tumors. These foreign organisms elicit inflammatory and immune responses that can make interpretation of tumor immunopathology difficult. The availability of relatively uncontaminated experimentally induced fibropapillomas, fibropapilloma cell lines, and reagents to detect green turtle antibody classes and immunocytes will make studies of the immunopathogenesis of GTFP practical.

Role of immune dysfunction in GTFP pathogenesis

Studies of virus-induced papillomas and fibropapillomas in human and other vertebrates indicate that, while immune suppression is not a necessary prerequisite for viral infection, disease tends to be more persistent and severe (207–210) and more likely to progress to malignancy (211) in those individuals with compromised cellular immune function. A genetic defect in cell mediated immune function may permit papillomavirus infection to persist in patients suffering from epidermodysplasia verruciformis (171,172).

Failure of the turtle's immune system to either recognize or to eliminate the relevant pathogen(s) and/or transformed fibropapilloma cells may be a major factor in the development of severe persistent fibropapillomatosis in green turtles. Many factors may impair the ability of the green turtle immune system to cope successfully with disease-producing agents. The reptilian immune system, as in other poikilotherms, is influenced by both season and temperature (212–215). Seasonal changes in immunologic status may be modulated by neuroendocrine mechanisms associated with corticosteroid and reproductive steroid hormone levels (214,215). One hypothesis for the seasonal stranding pattern seen in GTFP-affected green turtles predicts that, given a certain lag time for an effective immune response to develop, individuals infected

late in the fall are unable to eliminate tumors as their immune system competence declines with falling water temperatures. On the other hand, rapid tumor growth in the summer may itself debilitate animals sufficiently so that by winter, they are unable to survive.

Other stressors that influence immune function either directly or through neuroendocrine mechanisms that may be important in marine environments with high GTFP prevalences are extremes of salinity, ultraviolet irradiation, chemical contaminants, disturbance by human activities (boat traffic, fishing, acoustic disturbances), food availability (nutritional deficiency), crowding and social interactions, and concurrent diseases such as parasitism. Any combination of these factors may modulate the green turtle's immune system in ways that abrogate an effective response to fibropapillomatosis.

Implicating immune system dysfunction in the pathogenesis of GTFP will be difficult because few turtle-specific immunological reagents are available, immune function assays have not been validated in green turtles, and normal reference ranges have not been established. Immune system studies in sea turtles are in their infancy. Collins (216) reviewed the available information on turtle immunology and provided initial anatomic descriptions of green turtle lymphoid tissues. The immunoglobulin classes of green turtles have been described (217,218), and some preliminary investigations of cellular immune functions in green turtles have been conducted (219). Studies have been initiated to meet the need for green turtle–specific immunological reagents (220). Eventually, systematic surveys that compare variation in immune function parameters of apparently healthy turtles among populations with high and low GTFP prevalences using these reagents could test the hypothesis that immunological dysfunction has rendered some green turtle populations more susceptible to severe GTFP. If immune dysfunction is found to play a role in GTFP pathogenesis, then it will become important to identify those factors responsible for immunomodulation in those populations.

CONCLUSION

This paper has brought together the available information about GTFP and has pointed out the gaps in our understanding of this important disease of free-ranging green turtles. A major question concerns the impact of GTFP on the long-term survival of green turtle populations. According to demographic models, large juvenile and adult turtles have the highest reproductive value and changes in survivorship among these two classes have more impact on population trends than changes in egg, hatchling, or small juvenile survivorship (221). Because GTFP primarily effects large juveniles and, to a lesser extent, adult green turtles, it poses a significant threat to the long-term survival of this endangered species. Accurate estimates of mortality due to GTFP are needed to further assess the impact of this disease. It will also be important to be able to monitor green turtle populations for exposure to the agent(s) responsible for fibropapillomatosis and to estimate the percentage of the population that is exposed. Prevalence estimates currently rely on identification of turtles with visible cutaneous masses, and this underestimates the true prevalence of infection/exposure in the population. Specific and practical diagnostic assays are needed for conducting epizootiologic surveys to estimate the numbers of susceptible animals that become infected and either never develop clinical disease or having developed clinical disease, recover. Diagnostic tests will also be needed in studies designed to elucidate natural routes of transmission and to identify natural reservoirs in the environment.

Prerequisite to the development of these diagnostic tools is the identification and description of the etiologic agent(s). Now that transmission experiments have implicated a filterable infectious agent as the cause of GTFP, efforts to identify and culture it must be continued. Once identified, isolated, and cultured, the putative agent(s) must be tested in transmission studies to fulfill Koch's postulates.

Investigation of pathogenesis, including progression, regression, dissemination to other organs, and host immune response are needed as soon as the materials are available to conduct the studies. Experimentally induced fibropapillomas will facilitate these studies. Establishment of *bona fide* GTFP cell lines including development of methods and criteria to distinguish them from normal cell lines would be extremely valuable in studies of the molecular pathogenesis of this disease and in *in vitro* studies of green turtle immune responses to GTFP.

An understanding of the factors that may have rendered apparently healthy turtles more susceptible to fibropapillomatosis and allowed this disease to become pandemic will be critical to the long-term management of sea turtle stocks. This cannot be accomplished without concurrent studies aimed at improving our understanding of the function of the green turtle's immune system.

Finally, there is a need to better characterize cutaneous fibropapillomatosis in other species of marine turtles. The questions regarding epizootiology,

etiology, and pathogenesis are the same for these species and, as progress is made with GTFP, it is hoped that these questions will become easier to answer.

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