# SHELL DISEASE IN RIVER COOTERS (PSEUDEMYS CONCINNA) AND YELLOW–BELLIED TURTLES (TRACHEMYS SCRIPTA) IN A GEORGIA (USA) LAKE

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ABSTRACT:   A disfiguring shell disease was detected in river cooters (*Pseudemys concinna*) and yellow-bellied turtles (*Trachemys scripta*) from Lake Blackshear, Georgia (USA). The turtles used were part of a mark-recapture study conducted from September 1991 to June 1993. Histologic changes on four turtles included acute segmental necrosis of the epidermis, followed by ulceration, necrosis of the underlying dermis and dermal bone, and exaggerated remodeling of bone. Additional findings included visceral inflammatory lesions and bacterial infection, sepsis, and marked trematode ova granulomatosis. The cause of the shell lesions was not determined.

*Key words:*   Shell disease, river cooter, *Pseudemys concinna*, yellow-bellied turtle, *Trachemys scripta*.

## INTRODUCTION

Very little is known about diseases of the shell of wild turtles, although there are several accounts in the literature of infectious shell disease in captive turtles. Hunt (1957), Frye (1991) and Migaki et al. (1984) noted that most necrosis of turtle shells was of mycotic origin. Shell rot due to *Mucorales* spp. (Hunt, 1957), as well as *Fusarium* spp., *Geotrichum* spp., *Trichosporon* spp., and *Coniothyrium* spp. (Austwick and Keymer, 1981) has been reported. These genera generally are considered saprophytes, and likely colonize secondarily to other shell damage or infect animals already weakened by some predisposing factor (Austwick and Keymer, 1981). Hunt (1957) found that in some cases algae caused shell disease without previous damage. The Gram-negative bacterium, *Baneckea chitinovora*, causes a chronic ulcerative shell disease in captive turtles (Wallach, 1976). An idiopathic shell disease of wild river cooters (*Pseudemys concinna*) and yellow-bellied turtles (*Trachemys scripta*) from a lake in Georgia (USA) has recently been described (Lovich et al., 1996). We describe the histogenesis of these shell lesions in conjunction with additional laboratory findings.

## MATERIALS AND METHODS

Since November 1984, personnel from the Georgia Department of Natural Resources (GDNR) have been investigating a necrotizing and proliferative shell disease problem of turtles in Lake Blackshear, Georgia (USA; 31°50′N, 83°55′W). Through a collaborative effort by the GDNR and the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia, Athens, Georgia, a preliminary study was performed in 1985 which was designed to determine the geographic extent of the problem and to obtain specimens for laboratory analysis. From this preliminary work, two important facts emerged: first, the problem appeared to be confined to the central portion of Lake Blackshear. Second, although at last ten species of turtles occur in the lake, only the two most herbivorous species appear to be affected: river cooters and yellow-bellied turtles (Parmenter and Avery, 1989; Ernst and Barbour, 1989; Lovich et al., 1996). The unsightly appearance of the shell lesions in affected turtles has generated considerable public concern, especially regarding water quality and status of food and sport fish in the lake. Because of these problems, turtles were examined to establish a basic understanding of the pathogenesis and etiology of the shell disease in Lake Blackshear.

The turtles used in this study were part of a mark-recapture study conducted on Lake Blackshear from September 1991 to June 1993. In this study, 310 (76%) of 410 turtle captures (173 *Trachemys scripta* and 237 *Pseudemys concinna*) had evidence of shell lesions. Two adult male river cooters (turtles 1 and 2) and

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two adult male yellow-bellied turtles (turtles 3 and 4) captured from Lake Blackshear were received alive at the Veterinary Medical Teaching Hospital, University of Florida, Gainesville, Florida (USA). The turtles were subsequently housed in heated, partially flooded dog kennels and fed a finely chopped mixture of spinach, broccoli, squash, apple, and monkey chow (Ralston Purina, St. Louis, Missouri, USA). Physical exams were performed on all turtles. Blood was collected in lithium heparin microtainer tubes by cardiac puncture for plasma biochemical analyses (Express 550 Chemistry analyzer and 664 Fast Four System, Ciba Corning, Oberlin, Ohio, USA) and complete blood counts (CBC) (turtles 1 to 3). Total and differential white blood cell counts were performed manually (Campbell, 1988). Erythrocyte counts and hemoglobin measurements were performed on a Coulter Counter ZBI Model MHR (Coulter Corporation, Miami, Florida). For microbial culture of blood, 4 ml was collected aseptically from the heart of all four turtles and divided equally into two bottles containing 20 ml of broth medium. One bottle containing *Brucella* broth (Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) was maintained in an anaerobic environment at 37 C, while the second bottle containing Columbia broth (Becton Dickinson Microbiology Systems) was vented and maintained in an aerobic environment at 37 C. Bacteria were identified by RapID ANAII System (Innovative Diagnostic Systems, Inc, Norcross, Georgia) or API 20 E System and apiNFT System (bioMerieux Vitek Inc, Hazelwood, Missouri). Turtles 1 to 3 were euthanized 1 day after submission. Turtle 4 was observed for 2 mo prior to euthanasia. All turtles were euthanized with sodium pentobarbital (Anthony Products, Arcadia, California, USA) overdose administered intravenously in the dorsal cervical sinus. No other treatments were administered to any of the turtles.

Complete gross and microscopic examinations were performed on all turtles. Sections of heart, greater vessels, trachea, lung, liver, spleen, kidney, thyroid, adrenal, pancreas, testes, esophagus, stomach, duodenum, jejunum, ileum, colon, urinary bladder, skeletal muscle, eye, and brain were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin (H&E). Liver was also stained with the Prussian blue method for iron. In addition, fresh specimens of carapace and plastron were sectioned at 2 mm thickness, and fixed in 10% buffered formalin for 48 to 72 hr. The specimens then were decalcified in a solution of formic acid (23%), sodium citrate (1.6%), and distilled water (76%). Decalcifying specimens

were radiographed periodically, and removed from the decalcification solution when mineralized bone was no longer apparent radiographically, usually after 4 to 6 days. Generally, shells with necrotic lesions took less time to decalcify than viable shell. Decalcified specimens of shell were rinsed in tap water for 24 hr, then embedded in paraffin, sectioned, and stained with H&E as described for other tissues. Sections of shell lesions were also stained using Giemsa, Fontana-Masson, Fite's acid-fast, Brown and Brenn, Gomori's methylamine silver (GMS), and periodic acid-Schiff's (PAS) (Luna, 1960).

The ultrastructure of an acute epidermal lesion from the plastron of turtle 1 was examined using transmission electron microscopy. After a focus of acute epidermal necrosis was identified in one of the histologic sections, a 2 × 2 mm area of the paraffin block surrounding the lesion was excised. The specimen was deparaffinized in xylene, placed into two changes of phosphate buffer and then immersed in Trump's fixative (McDowell and Trump, 1976) overnight. Afterwards, the specimen was post-fixed in 2% osmium tetroxide (Ted Pella, Inc., Redding, California), dehydrated through a graded concentrations of alcohols and acetone, and embedded in Spurr's embedding media (Spurr, 1969). Ultrathin sections were placed on 100-mesh Formvar® carbon-coated grids (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA), stained with 5% aqueous uranyl acetate and Reynold's lead citrate (Ted Pella, Inc.), and examined with a Hitachi H-7 electron microscope at 75KV (Hitachi 87000, Hitachi Instruments, Dansbury, Connecticut, USA).

## RESULTS

With the exception of shell lesions on the carapace and plastron, all turtles were otherwise normal on physical examination. *Bacteroides* sp. was isolated from the blood of all four turtles. Additional isolates from blood included *Morganella morganii* (turtle 1) *Aeromonas hydrophila* (turtles 1 and 3) and a second *Bacteroides* sp. (turtles 2 and 3). Mild hypoglycemia was detected in turtle 2 (51 mg/dl). Turtles 2 and 3 were judged to have moderate leukocytosis (20,100/μl and 17,100/μl, respectively) and basophilia (8,990/μl and 5,730/μl, respectively). All turtles had low numbers of unclassified dark, spherical, 1 to 2 μm cytoplasmic bodies within erythrocytes. All remaining hematological and serum chem-
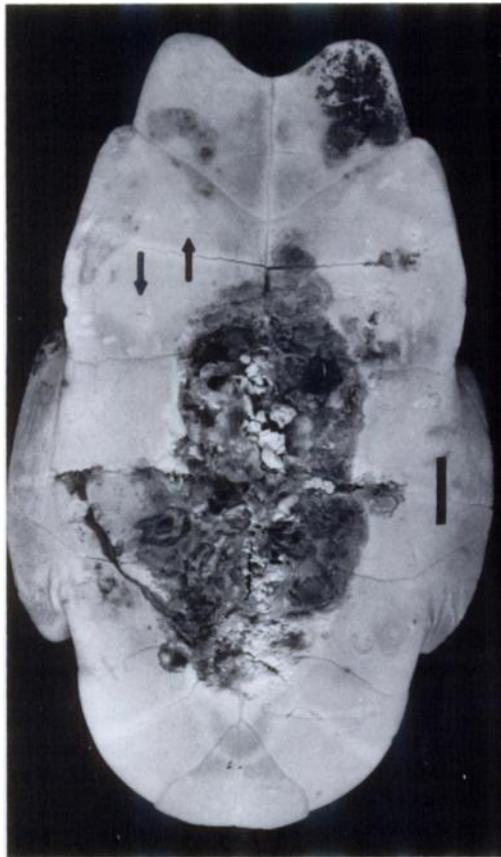
FIGURE 1.    River cooter with severe scute disfigurement, ulcers and nodular irregularities in chronic shell lesion, and small foci of pale tan discoloration involving the scutes of the plastron in acute shell lesion (arrows). Bar = 2 cm.
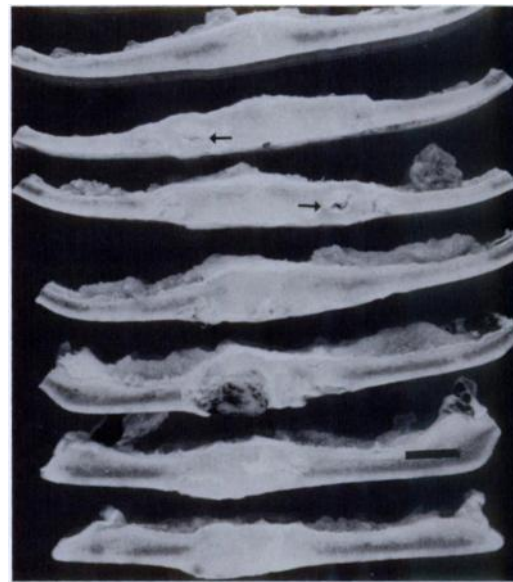


FIGURE 2.    Sequential transverse sections of plastron from a river cooter showing necrotic regions, sclerosis of dermal bone and inclusion cysts (arrows). Bar = 1 cm.

istry values were judged to be within normal limits.

Shell lesions, hepatocellular hemosiderosis, trematode ova granulomata and intestinal parasitism were detected in all turtles. In addition, cholangitis (turtles 1 to 3) and pancreatitis (turtles 2 to 4) were also detected. Widely varied lesions were detected in the plastron and carapace of all turtles. Depending on the stage of development, lesions were characterized by softening of the outer layer of keratin and pale tan discoloration or flaking of the scute, extensive ulceration with undermining of the epidermis and dermis resulting in exposure of the outer surface of dermal bone, serpiginous disfigurement of the scutes, and deep pitting or raised coalescing nodules of dermal bone sometimes covered by intact but disfigured scutes. On cut surfaces of shell with chronic lesions, raised regions were confluent with localized thickenings of dermal bone. This thickened bone varied from firm and white to soft and tan or brown. Sections adjacent to pitted regions had marked undermining of viable soft tissue and bone by the developing ulcer, resulting in cavitating pseudocyst formations. (Figs. 1 and 2). Firm white coalescing nodules occupied up to 50% of the pancreas in three of the turtles. Low numbers of helminths resembling *Serpinema* sp. and *Spiroxys* sp. nematodes were detected in the stomach of one turtle. Low to moderate numbers of parasites resembling *Neoechinorhynchus* sp. acanthocephalans were detected in the small intestine of all turtles. No adult spirorchid trematodes were identified in any of the turtles.

Compared to normal plastron (Fig. 3), affected shell without obvious gross lesions had occasional segmental foci of acute coagulative necrosis of epidermis, with affect-
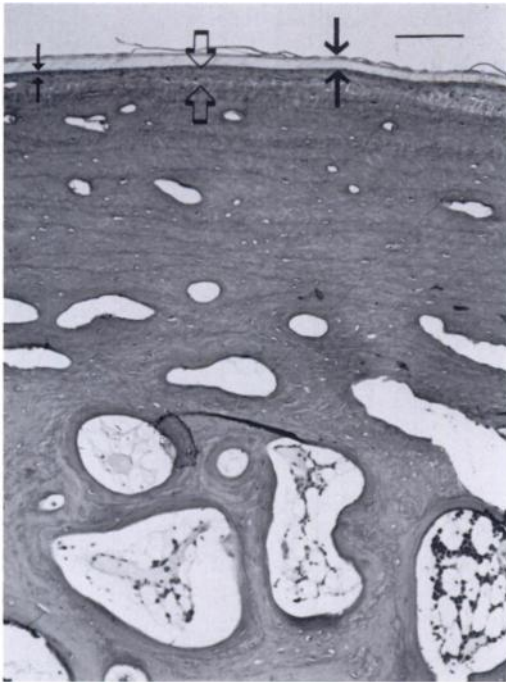
FIGURE 3. Normal histology of plastron from a river cooter showing keratin layer (large black arrows), epidermis (small black arrows), dermis (open arrows), dermal cortical and trabecular bone and marrow spaces containing hematopoietic cells. Note smooth margins of bone around marrow spaces and general absence of osteoblasts and osteoclasts. H&E. Bar = 100 μm.

ed epidermis covered externally by intact keratin (Fig. 4). Microorganisms, including virus, were not detected by electron microscopic examination in the area of acute epidermal coagulative necrosis. The epidermis in this area was mostly one cell wide, as opposed to two to three layers of cells in nonnecrotic areas. Ultrastructural alterations appeared to be limited to the nuclei, which were characterized by dispersion of chromatin, fragmentation of the nuclear membrane, and loss of nucleoli. Some areas contained only clear spaces with nuclear remnants. Multifocally, the epidermal basement membrane was minimally thickened, and frequently small clear clefts separated the basement membrane from the overlying degenerative or necrotic epithelium. The adjacent connective tissue of the superficial dermis con-

sisted of collagen with loosely arranged (edematous) areas. There was a reduction in the number of fibroblasts and an absence of melanocytes.

Sections corresponding to macroscopic areas of shell thinning or softening were characterized microscopically by thinning and fragmentation or lifting of keratin from the epidermal surface, accompanied by full thickness epidermal necrosis and ulceration, edema or rarefaction of the dermis, and moderate numbers of acidophilic granulocytes in the margins of unaffected dermis (Fig. 5). Dermal pegs contiguous with necrotic cortical dermal bone were characterized by diffuse necrosis of stromal cells. Numerous osteoclasts lined the inner scalloped surfaces of necrotic dermal cortical bone. Pitted foci had extensive ulceration of the epidermis, hyperplasia of epidermis at the ulcerated interface and variable evidence of partial or complete epidermal reepithelialization, with undermining and extrusion of necrotic soft tissue and bone by the newly formed epidermis (Figs. 6 and 7). Viable marrow spaces adjacent to these areas often had moderately increased numbers of acidophilic granulocytes and granulocytic progenitors.

Raised nodular regions of the shell detected macroscopically had microscopic evidence of chronicity. Cortical bone was replaced by broad anastomosing trabeculae of woven or lamellar bone surrounding fibrotic marrow spaces, accompanied by thickening and fibrosis of the dermis, and irregular thickening of the epidermis (Fig. 8). In some large nodular regions in which the surface of the shell was covered by intact but deformed scutes, dermal bone contained cysts lined by thick cornified epithelium containing luminal keratin, necrotic bone and cellular debris (Fig. 8). Ulcerated and necrotic regions in all but the acute areas of necrosis occasionally were lined superficially by pleomorphic fungal elements, pleomorphic Gram-positive and Gram-negative bacteria, and branching chains of PAS- and GMS-positive beaded
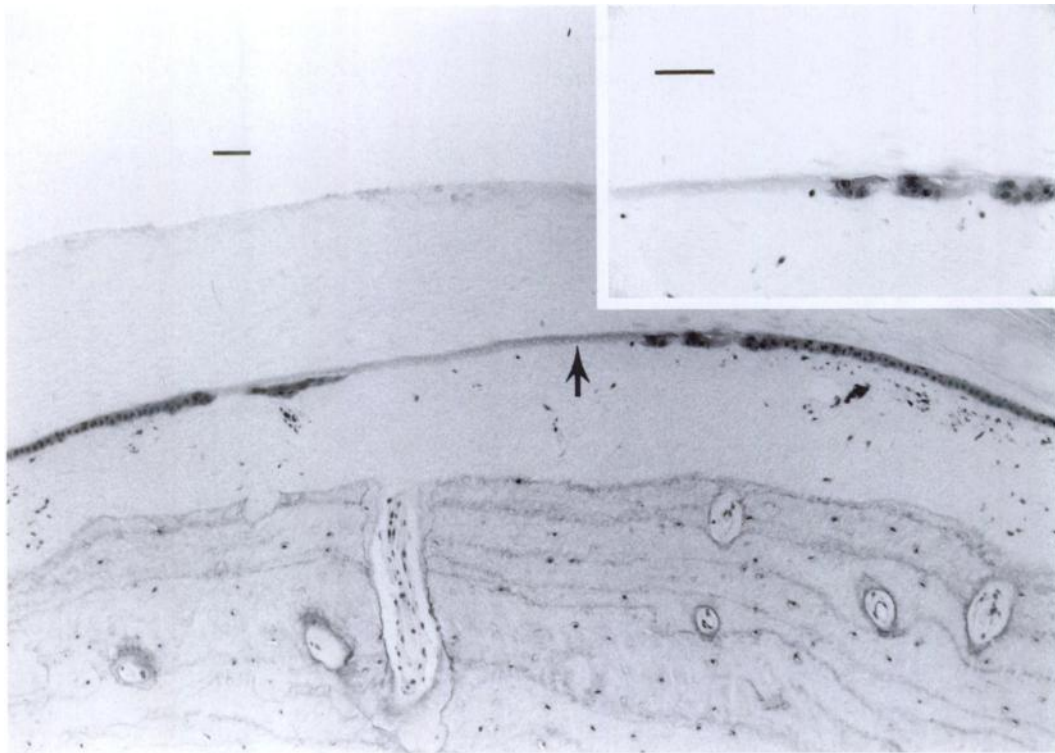
FIGURE 4.    Acute segmental epidermal coagulative necrosis at bridge of plastron (arrow) from a river cooter. Note intact keratin layer and absence of inflammation. H&E. Bar = 100 μm. Inset: Early thickening of epidermis at margins of necrotic focus. Uranyl citrate and lead citrate. Bar = 100 μm.
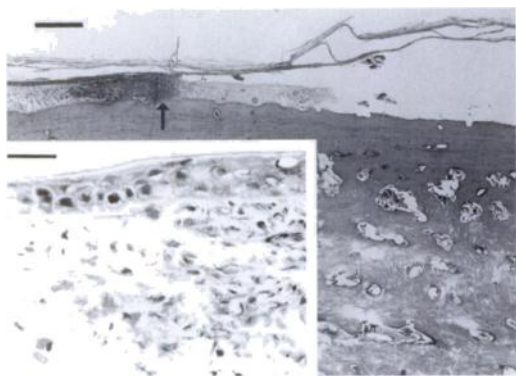
or filamentous coccobacillary microorganisms. These organisms also were inconsistently seen within the lumina of cysts, admixed with cellular debris or necrotic bone. Rarely, unclassified algae and mites



FIGURE 5.    Fragmentation and lifting of keratin, necrosis and loss of the epidermis and dermis, margination of the necrotic dermis by cellular infiltrates (arrow), and osteoclasis of dermal cortical bone in a shell lesion from a river cooter. Note scalloped margins of bone lining marrow spaces indicative of osteoclasis. H&E. Bar = 300 μm Inset: Cellular infiltrate of granulocytes admixed with cell debris. H&E. Bar = 100 μm.
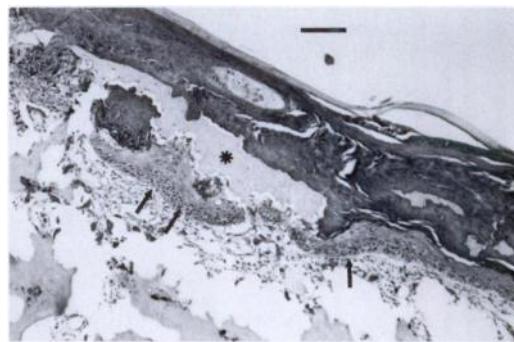


FIGURE 6.    Near complete re-epithelialization (arrows) of ulcerated region with undermining of necrotic dermis and necrotic dermal bone (asterisk) in a shell lesion from a river cooter. Process is occurring beneath intact keratin at margin of ulcer. H&E. Bar = 200 μm.
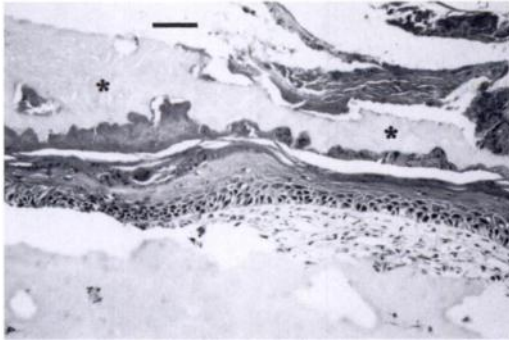
FIGURE 7. Complete re-epithelialization and hyperplasia of the epidermis, with extrusion of necrotic bone (asterisks) in a shell lesion from a river cooter. H&E. Bar = 100 μm.
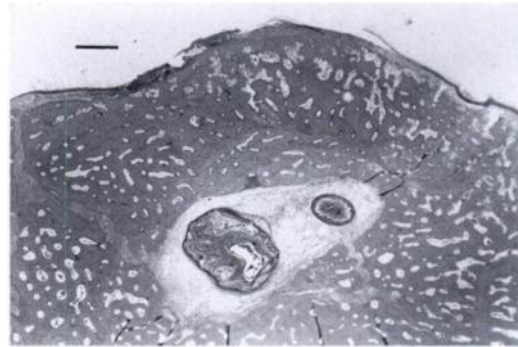


FIGURE 8. Plastron from a river cooter with chronic, quiescent lesion. Note nodular irregularity associated with marked osteosclerosis and loss of demarcation of cortical and trabecular bone. Smooth surfaces of bone lining marrow spaces indicate cessation of remodeling activity. Focal epidermal inclusion cyst surrounded by dense fibrous tissue and inactive bone. Reulceration of epidermis. H&E. Bar = 60 μm.

were detected in the outer keratin layers of some sections. Low numbers of trematode ova were detected within small blood vessels and lymphatics in marrow spaces and dermis in a few areas of affected shell. Lesions in the shell of the turtle held for observation prior to necropsy had the entire spectrum of chronic lesions, but no evidence of acute epidermal necrosis.

Consistent microscopic changes also were detected in the viscera of the turtles. The most severe changes occurred in the pancreas of three turtles. These lesions were characterized by severe, necrotizing pyogranulomatous inflammation centered around cores of eosinophilic granular material, necrotic cells, clusters of pleomorphic Gram-negative and Gram-positive bacteria, and occasional small clusters of trematode eggs. A degenerating parasite resembling a trematode was within the core of a pancreatic granuloma in one turtle. The remaining pancreatic tissue had variable degrees of hyperplasia of ducts, with periductular fibrosis and inflammation. All turtles had moderate to marked hepatocellular hemosiderosis and multifocal, mild cholangitis comprised of low to moderate numbers of lymphocytes and macrophages surrounding slightly fibrotic bile ducts. Rarely, inflamed bile duct lumina contained trematode ova. Trematode ova were detected in low to moderate numbers within small blood ves-

sels and lymphatics of all viscera, including brain and uveal tract. Inflammation, comprised of macrophages and multinucleated giant cells directed at these ova, was minimal and occasionally ova were not associated with inflammation. The trematode ova were ovoid, unioperculate and ranged from 40.0 μm long × 25.0 μm wide to 61.0 μm long × 32.0 μm wide. All turtles had mild multifocal infiltrates of lymphocytes and acidophilic granulocytes in the lamina propria of the small intestine.

## DISCUSSION

Although the macroscopic appearance of the shell lesions varied considerably, we propose that there was a spectrum of histologic alterations that could be placed into chronological order. The initial lesion appeared to be acute segmental necrosis of the epidermis. At this early stage, the keratin layer was of average thickness and appeared to be intact. Epidermal necrosis was followed by ulceration, dermal necrosis and migration of acidophilic granulocytes, presumably heterophils, to the margins of necrotic foci in the dermis. As cellular elements of dermal projections into the underlying cortical bone became necrotic, blood supply to the cortical bone

apparently became compromised, resulting in necrosis and marked osteoclasis of the peripheral cortical bone. In severe, chronic, ulcerated lesions, the viable epidermis adjacent to necrotic foci became hyperplastic and the associated dermis became thickened and fibrotic. In an infolding fashion, this regenerating, hyperplastic epidermis undermined the necrotic bone and dermis, eventually extruding the necrotic tissue to the surface of the shell. Also in chronic lesions, the trabecular bone became thickened and the demarcation between cortical and trabecular bone became less distinct. Occasionally, undermined re-epithelialized areas adjacent to healing ulcers became circumscribed and entrapped by the fibro-osseous repair process, resulting in a form of intra-osseous epidermal inclusion cyst. These microscopic changes resulted in the thickened, roughened, and ulcerated appearance of the shell at the gross level.

The segmental nature of the early lesions may have resulted from vascular lesions and subsequent infarct; however, vascular necrosis, vasculitis, vascular thrombosis and hemorrhage were not significant features of any of the examined specimens of affected shell. While trematode ova were common in visceral tissues, they were rarely detected within shell lesions. Although shell cultures were not performed, all examined turtles in the study had bacterial septicemia based on blood culture. In addition, three turtles had severe pancreatic lesions that contained bacteria. Toxins released from bacteria in the circulation could provide a source for the acute epidermal lesion. Fatal septicemia with skin ulceration and necrosis caused by *Citrobacter freundii* has been reported in captive *Chrysemys* sp. (Murphy and Collins, 1983). Plastron ulceration and granulomatous inflammation of the spleen, liver, and skin caused by *Mycobacterium* sp. have been described in chelonians (Rhodin and Anver, 1977). We interpret these observations as an association between sepsis, visceral bacterial inflamma-

tory disease, and shell lesions in chelonians. In dogs and humans, necrotizing lesions of the epidermis have been associated with severe pancreatic and hepatic lesions, although the cause for this phenomenon is unknown (Gross et al., 1993).

Based on these observations, the shell lesions in the river cooters and yellow-bellied turtles could be caused by a toxic insult to the epidermis subsequent to a systemic toxin or visceral infectious disease. The absence of acute lesions in the untreated turtle held 2 mo for observation following removal from the lake, the occurrence of lesions in two herbivorous species, and the confinement of the problem to one lake also support the possibility of a nutritional, toxic or infectious problem in the lake. An external caustic insult to the epidermis is a less likely possibility, since the keratin layer was mostly intact and histologically normal in acute lesions. A more diffuse distribution of lesions over the plastron and carapace would be expected if exposure to a caustic environmental substance were the cause of the lesion.

Although shell lesions were not cultured, microorganisms were not detected within the acute lesions by light or electron microscopy. Pleomorphic fungi and bacteria were inconsistently detected within superficial or necrotic regions of chronic shell lesions and within epidermal inclusion cysts. Rarely, unclassified algae and mites were detected in the outer layers of keratin in some chronic lesions. These organisms have also been found in the epidermis of green turtles (*Chelonia mydas*) with fibropapillomas but their pathologic significance is unknown (Aguirre et al., 1994). In a preliminary study of 35 affected turtles from the lake, a variety of microorganisms were isolated inconsistently from the shell lesions. These included *Aeromonas hydrophila*, *Enterobacter* sp., *Corynebacterium* sp., *Morganella morganii*, *Micrococcus* sp., *Alcaligenes flourescens*, *Pseudomonas flourescens*, *Neisseria flourescens*, *Brevibacterium acetylicum*, and *Actinobacter woffi*. Fungal isolates includ-

ed *Penicillium* sp., nonpathogenic phyco-mycetes and nonpathogenic *Aspergillus* sp. (E. W. Howerth, unpubl.). Based on our findings, the bacteria, fungi, algae, and mites detected within the shell lesions probably were superficial contaminants and opportunistic pathogens that colo-nized the devitalized shell lesions. The le-sions induced by trematode ova were con-sistent with spirorchidiasis, which is con-sidered to be a debilitating disease in ma-rine (Wolke et al., 1982) and freshwater (Ward, 1921) chelonians. The shell lesions of this report have not been described as a manifestation of spirorchidiasis in turtles. The presence of bacteria, fungi, algae, mites and trematode ova probably exac-erbated the severity of shell lesions, but we could not determine that any one of these organisms was the primary cause. Consis-tent bacterial, fungal, and algal coloniza-tion and occasional trematode ova-induced lesions were seen in the skin and shell le-sions of turtles exposed to diesel fuel (M. M. Garner, unpubl.).

Hepatocellular hemosiderosis was a prominent finding in the livers of all tur-tles. This was most likely due to iron se-questration associated with chronic inflam-matory disease or increased dietary iron rather than increased erythrocyte turnover (Kelly, 1993); the turtles were not anemic based on hematocrit, erythropoiesis was not prominent in the marrow and extra-medullary hematopoiesis was not detected outside the bone marrow of any of the tur-tles. Because the turtles were not anemic, the unclassified erythrocyte intracyto-plasmic bodies detected in all turtles had little or no pathological significance. Sim-ilar appearing bodies have been reported for the desert tortoise (*Gopherus agassizii*) and appear to be degenerating organelles (Alleman et al., 1992). The presence of mild lymphohistiocytic cholangitis was at-tributed to trematode ova within bile ducts and ascending infection or inflammation originating in the pancreas and small in-testine.

Turtles 2 and 3 had an apparent mod-erate leukocytosis consistent with an in-flammatory or stress leukogram; however, normal physiologic reference ranges for river cooters and yellow-bellied turtles have not been published. In addition to changes associated with illness, it is likely that hematological parameters are influ-enced by age, reproductive status, and sea-sonal events such as hibernation (Dres-sauer, 1970). Therefore, variation in he-matological parameters in this study were interpreted conservatively. Plasma glucose values in turtles 2 and 3 may have reflect-ed the low range of normal values or mild hypoglycemia associated with fasting or clinical disease. We judged that the re-maining hematological and serum chem-istry valuer were within normal limits, based on similar species (Steen, 1996).

The exact cause of shell lesions in the turtles from Lake Blackshear is uncertain. Based on necropsy findings, the most plau-sible explanation is that the lesions are in-dicative of an underlying visceral disorder. Internal lesions were numerous in these turtles and included bacterial infection, trematode endoparasitism, hepatocellular hemosiderosis and possibly pancreatitis. Although histologic changes indicative of immune-suppression such as lymphoid de-pletion and marrow hypoplasia were not detected, impairment of the immune re-sponse would explain why severe bacterial and parasitic lesions had developed in these turtles. Confinement of this problem to a single lake is evidence that environ-mental factors in the lake have a role in the etiopathogenesis of the lesions. Expo-sure to sublethal levels of various organic and inorganic substances can suppress nonspecific disease resistance factors in fish and lead to disease from organisms usually considered saprophytic or oppor-tunistic (Overstreet and Howse, 1977; Sin-dermann, 1979). Additional studies should include complete bacterial and viral isola-tion attempts from shell and viscera, com-plete ultrastructural characterization of the pathologic changes in the shell, assessment of the immune response of affected tur-

tles, reproductive potential of affected turtles, health status of other species of reptiles, fish, birds and mammals in the lake, and comprehensive analysis of potentially toxic or immunosuppressive trace minerals and organic compounds in the lake and in affected turtles.

## ACKNOWLEDGMENTS

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