FIBROPAPILLOA FILE
COLLECTED BY G. BALAZS
1980s - 1990s

HAWAIIAN GREEN TURTLES ARE EXPERIENCING A DISABLING, LIFE-THREATENING tumor disease which has reached epidemic proportions off the island of Molokai, where no cases of the disease had been reported prior to Oct85. George W. Boehlert, director of the Honolulu Laboratory of the Natl. Marine Fisheries Service's Southwest Fisheries Science Center, said that the disease was diagnosed in more than half of the green turtles captured and examined at Palaau, a shallow reef area off Molokai, during a recent survey by George H. Balazs, leader of the Honolulu Laboratory's Marine Turtle Research Program. Some stydy sites, however, are still free of the disease: There has been no sign of the disease in hundreds of turtles examined over the past four years at Kiholo Bay, Punaluu, and Puako on the island of Hawaii.

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NOAA CONCLUDED THAT THE "MASSIVE" ATMOSPHERIC PLUMES APPEARING periodically on satellite pictures as eruptions from the East Siberian Sea, are in reality clouds, not streams of methane gas escaping from the sea floor as some scientists previously believed. Analysis of data and air samples taken during a research aircraft penetration of the plume showed that it had originated two miles above Bennett Island in the East Siberian Sea, not at the surface. The investigation was conducted by Russell Schnell of the Natl. Oceanic & Atmospheric Administration's Climate Monitoring & Diagnostics Laboratory in Boulder CO.

Schnell headed a month-long study of the Bennett Island plumes which involved a U.S. scientist flying in a Russian research aircraft in an area that until now had been closed to all but Soviet military and scientific personnel. Bennett Island is 400 miles north of the Siberian coastline in the Arctic. "While it apeared from satellite pictures the plumes were streaming directly out of the ice cover around Bennett Island," Schnell said, "on site there was no evidence of them until you were about 1,000 feet altitude over the island."

Based on geological information, historical records, satellite analyses and knowledge of hydrocarbon reservoirs, scientists had speculated that Arctic warming may have caused methane hydrates, trapped in permafrost at the bottom of the sea, to melt and vaporize into plumes streaming up from the ocean floor. But analysis of scores of air samples collected from inside and around the plume by Anthony Hanson of the Univ. of California's Lawrence Berkeley Laboratory did not support the methane theory. Edward J. Dlugokencky, who did the analyses at the Boulder laboratory, said the methane concentrations, as well as carbon dioxide levels, in the samples were consistent with normal Arctic air.

Schnell likened the Bennett Island plume phenomenon to mountain wave clouds occasionally seen streaming eastward from along the Front Range of the Rocky Mountains. Those plumes generally extend only 10 miles or less, whereas the Bennett Island plumes persist for several hundred miles across the Arctic. Several days after the plume penetration by Hanson they were able, by using meteorological data, to forecast the appearance of another plume within three hours accuracy.

***

Another tumor-inflicted turtle found but expert doesn't see an epidemic

KAHULUI — A large green sea turtle weakened by tumorous body growth and unable to swim out to sea was found at Baldwin Park Thursday, the second such find in three weeks.

A veterinarian was expected to examine the 150-pound turtle this morning and determine whether the animal would survive shipping to Honolulu for research and convalescence.

The animal was covered with the fibropapilloma tumors that have plagued green sea turtles in Hawaii and other regions in recent years.

Another large sea turtle suffering from the disease was found stranded on Baldwin Beach several weeks ago. It was covered with fist-size tumors and was either unable or unwilling to swim back out to sea, said George Balazs, marine turtle researcher with the National Marine Fisheries Service.

Balazs said the recent finds don't translate into an epidemic.

"It's a regular occurrence on Maui," he said. "I would guess there are far more incidents than just the ones you hear about."

Balazs said scientists do not know what causes the disease, although a higher incidence occurs in or near bays next to urban areas.

Kahului Bay apparently is notorious for its tumorous turtles. In 1990, 80 percent of the turtles seen there were reported having fibropapillomas — the highest percentage in the state, he said.

Such turtle strandings are common at Hilo and Kaneohe bays and other urbanized bays around the state.

"But I've tagged hundreds of turtles over the years on the Kona coast, for example, and I've never seen fibropapillomas there," the scientist said.

Maui News - 3-6-92

HAWAII TRIBUNE HERALD
JUN 22 1992

Tumors epidemic among Molokai turtles

HONOLULU (AP) — Disabling tumors that lead to death among Hawaiian green sea turtles have reached epidemic proportions in a population living in a shallow reef area off Molokai, according to a federal marine scientist.

More than half the turtles captured and examined at Palaau in a recent survey were affected, said George Boehlert, director of the Honolulu Laboratory.

No cases of the disease were reported before 1985 at Molokai, where the second case was found in 1987 and where its incidence has risen about 5 percent annually since, Boehlert said in a news release last week.

The number of tumor-infected turtles found statewide has been increasing, particularly at several points off Oahu, while several study sites off Hawaii remain tumor-free, he said.

Scientists do not know what causes the disease known as fibropapilloma, or how it spreads, but possibilities include pollutants and blood parasites specific to sea turtles, according to Boehlert.

It causes tumors that grow up to 12 inches across. The tumors block breathing and eating when growing in turtles' mouths, throats and nasal passages, impair vision when growing in the eyes and impair swimming when growing on the jaws, necks, tails and flippers.

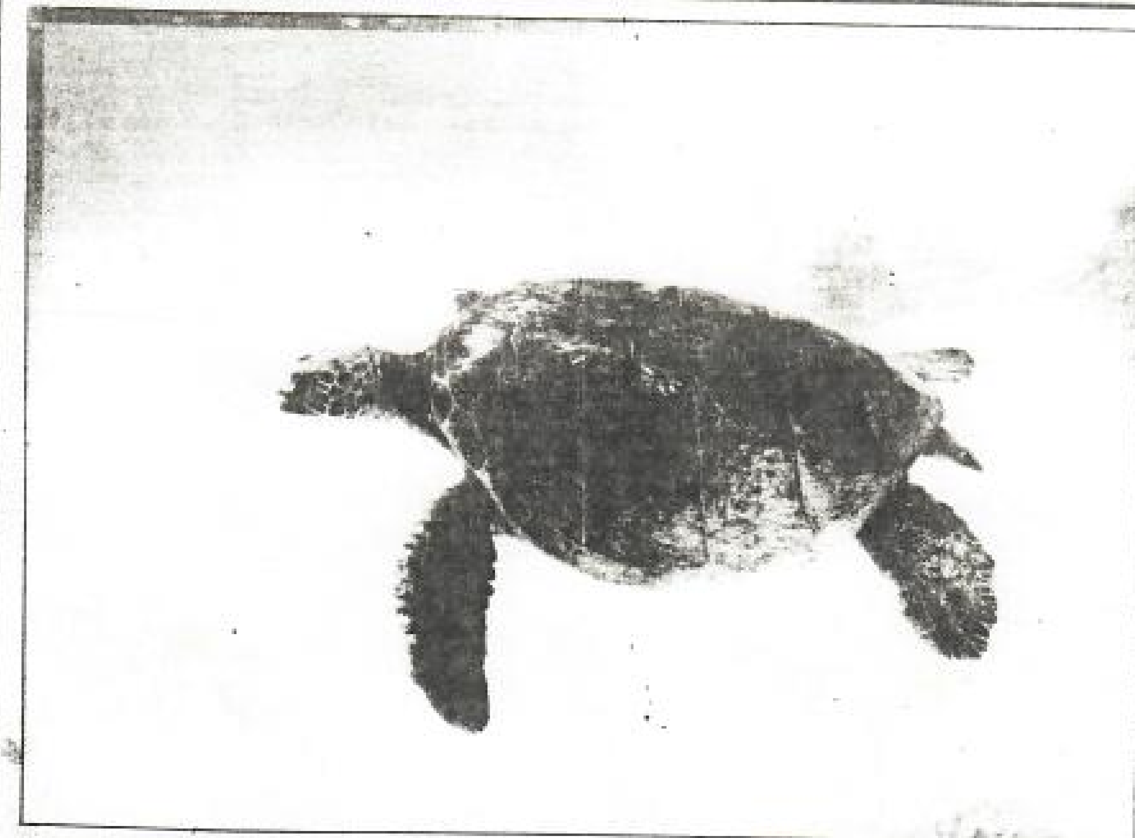
Another survey is planned in mid-July by George Balazs, leader of the laboratory's Marine Turtle Research Program.

The Honolulu Laboratory is agency within the National Oceanic and Atmospheric Administration's National Marine Fisheries Service.

In related news, sea turtle research is being conducted off Waikiki in a joint project of the National Marine Fisheries Service and Atlantis Reef Divers.

Nineteen turtles have been spotted and tagged to date through the program through which researchers are tracking the turtles' habits and trying to determine the number of turtles in the area.

Different species of sea turtles are variously listed as threatened or endangered.



SEA TURTLES THREATENED — Disabling tumors have reached epidemic proportions in green sea turtles in a reef area off Molokai.

Study finds epidemic of tumors in green sea turtles off Molokai

By the Associated Press

HONOLULU — Disabling tumors that lead to death among Hawaiian green sea turtles have reached epidemic proportions in a population living in a shallow reef area off Molokai, according to a federal marine scientist.

More than half the turtles captured and examined at Palaau in a recent survey were affected, said George Boehlert, director of the Honolulu Laboratory.

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The Honolulu Laboratory is an agency within the National Oceanic and Atmospheric Administration's National Marine Fisheries Service.

Mystery tumors threaten world's green sea turtles

OCEAN WATCH
By Susan Scott

THE green sea turtles of the world are in trouble.

The incidence of tumors growing in and on these reptiles is increasing so rapidly that turtle researchers fear the worst: This could be the final blow to animals already threatened by extinction.

The urgency of the problem prompted the Honolulu Laboratory of the National Marine Fisheries Service to hold a workshop on it last December. Scientists from across the country discussed their research and devised strategies.

The results were recently published in a fisheries service memorandum called Research Plan for Marine Turtle Fibropapilloma. The 118-page report includes everything people know about the disease.

Turtle tumors are not new.

The first confirmed cases date to the 1930s in Florida and to 1958 in Hawaii.

But the recent rise in the number of turtles with this disease is alarming.

In 1983, the year a local network was started to monitor beached turtles, 31 percent of stranded turtles in Hawaii had tumors.

In 1990, 53 percent had tumors.

Turtles that have tumors so large and debilitating that they drift onto beaches to die are a pathetic sight.

Typically, the tumors grow around the eyes and in the flipper folds until the animals can't see or swim.

Sometimes the tumors, which can be 12 inches across, grow in mouths and internal organs.

The sight of a severely affected turtle is one you'll never forget.

But the most disturbing aspect of this disease is the number of unknowns that surround it.

Researchers don't know the cause of the tumors, how they are spread, or the impact they have on turtle or human populations.

And a worried public doesn't know what, if anything, is being done about it.

The recent meeting of 12 researchers is a group effort to address these unknowns.

Two of those people, Drs. Alvin Smith and Douglas Skilling of Oregon State University's School of Veterinary Medicine, intend to use their special skills at identifying uncommon viruses to help the find the cause of this disease.

In the research plan, these scientists write, " . . . this study would demonstrate to a concerned public that there is a strong interest in the scientific community and strong commitment by the responsible federal agencies to solve problems that further threaten endangered or depleted species."

The commitment may be there, but the money is not.

The research plan to find the cause of turtle tumors recommends a 5-year schedule of activities at an estimated cost of $2.7 million.

To act on any of the resulting information would require additional funding.

Right now, this research plan does not have a source of money, which is a serious missing link in this chain of problem-solving.

When the money will come, and how much, is a mystery, just like the tumors themselves.

Here are some interesting clues about this disease I found while reading the research plan:

■ Location: Epidemic proportions in Hawaii and Florida. Sightings near some Caribbean islands, Panama, Belize, Australia, Malaysia and Japan.

■ Turtles afflicted: So far, only greens. However, two people have reported tumors on olive ridleys in the Eastern Pacific.

■ Possible causes: Virus, parasitic worm, pollutants, changes in natural environment, weakened immune systems, stress, introduced seaweed, ticks on nesting beaches.

■ Major constraints: Inadequate funding, highly mobile subjects, lack of a large population of experimental animals, complexity of factors in this disease, reptile diseases perceived as relatively unimportant.

■ How you can help: If you find a dead or dying turtle, with or without tumors, report it to 548-5918 (state) or 943-1240 (federal).

Write or call your government representatives and urge them to help fund this research plan.

Let them know that we residents of Hawaii love our sea turtles and want them to make it through this tumor crisis.

Susan Scott is a marine science writer and author of Oceanwatcher, a guide to Hawaii's marine animals. Her Oceanwatch column appears Monday in the Star-Bulletin.

ACCESSION SHEET

RTLA : 5291 Date Rec'd :02/27/91 Date Acc :02/27/91
Contrib. No. :F0149030

Reprint No. :

Contributor :BALAZS, G.H.

Common name:Green sea turtle

Phylum :Chordata
Class :Reptilia
Order :Testudines
Family :Cheloniidae
Genus :Chelonia
Species :mydas
Strain :

Continent :Oceanica
Country : State :
City/CO. : Habitat:M
Locality :Johnston Atoll: Turtle captured in coastal waters of north shore of Johnston Island
Date Coll :01/26/91 -

Fixative :Formalin Killed :
Age : Sex : Stage :Subadult (possibly)
Material :A firm, nodular, marbled black and white mass (approximately 6.7 cm x 4.8 cm x 3 cm)

Gross Description
A firm, nodular, marbled black and white mass (approx. 6.7 cm x 4.8 cm x 3 cm) from a green sea turtle; carapace 70 cm L. Contributor: "Tumor removed by physically twisting it off during capture struggle. Seven tumors total were noted on the turtle during the brief time it was being restrained by skin divers underwater. Turtle swam off after struggling free."

Letter date:02/27/91 Report date:

Enclosure :

Comments :Johnston Is. is site of a nerve gas/chemical munitions destruction facility that started operating 6 mos. ago

The above information is how your specimen is entered in our data base. If you have any additions or corrections please provide them to the Registry of Tumors in Lower Animals.

National Museum of Natural History · Smithsonian Institution
WASHINGTON, D.C. 20560 · TEL. 202- 357-2647; FAX: 202-357-3043

27 February 1991

Dr. George H. Balazs
Honolulu Laboratory
Southwest Fisheries Center
National Marine Fisheries Service, NOAA
U.S. Department of Commerce
2570 Dole Street
Honolulu, Hawaii 96822-2396

Dear Dr. Balazs:

Thank you very much for contributing a nodular mass from a green sea turtle specimen to the Registry of Tumors in Lower Animals for histologic processing, examination and evaluation. We have accessioned this tissue (your F0149030) as RTLA 5291 and are enclosing a copy of the Accession Sheet for your records. Would you please proofread the Accession Sheet and let us know of any corrections or additions.

Dr. Harshbarger will send you a set of the histoslides and his opinion of this case in due course. Meanwhile, he sends you his best personal regards.

Sincerely,

Phyllis M. Spero

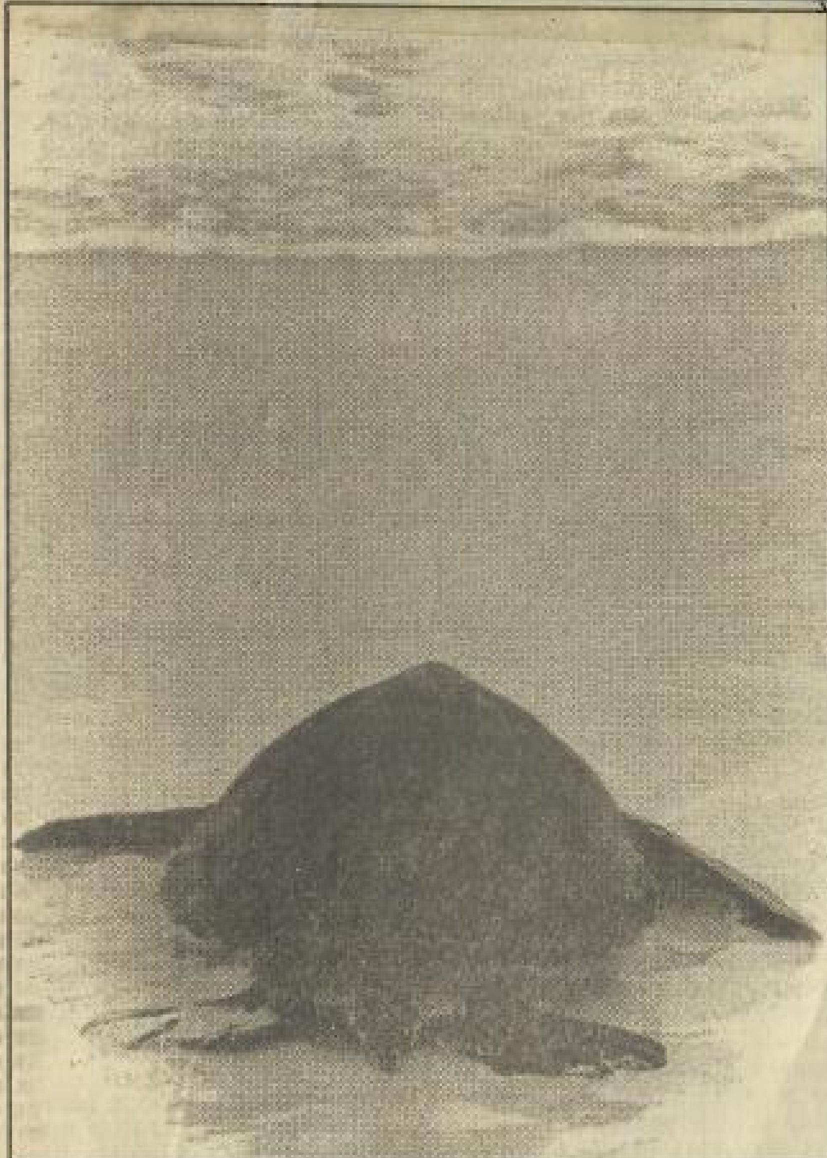
Phyllis M. Spero
Registrar/Museum Specialist
Registry of Tumors in Lower Animals

Enclosure: Accession Sheet for RTLA 5291

SUNDAY, April 12, 1992
Sunday Star-Bulletin & Advertiser
D1

Health/Science

ENVIRONMENTAL UPDATE



Advertiser photo by Bruce Asato

A turtle heads for surf on an Oahu beach.

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night.

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THE MEDICINE

served Canada well for eight years as a platform from which to practice science and assert national sovereignty over the far reaches of the sparsely populated Northwest Territories.

It was also a place for rugged individualists and frontier characters to get away from it all, brave wind-chill readings

... one of the last residents who came aboard Feb. 25.

It will take about eight to 10 trips with a heavy-lift helicopter in the summer to save the bigger equipment. And workers will have to drive two bulldozers over the icepack to a nearby island to await removal by ship in the summer.

The ice island broke into

EARTHWEEK: A DIARY OF THE PLANET

Droughts

... now mark the steep edge. One of the ice pieces carried off the permanent weather station, which can be seen frozen in place over a mile to the west across the desolate white icepack.

"We were hoping the ice island would get caught up in the Arctic gyre," said Jim Godden, 38, base manager for Po-

clockwise circle.

"But it got part way out into the Arctic Ocean, and then it got sucked into the straits because of the strong currents off Cape Isachsen. The minute it started heading south, people lost interest. There's not much sense in spending money to study places we already know about."

Turtles: Tumors still a problem

FROM PAGE 1

areas, like Kaneohe Bay, half the turtles had fibropapillomas, but other areas, like south Molokai and West Hawaii, remained virtually free of them through the 1980s.

George Balasz, a National Marine Fisheries Service researcher, said it's gotten so bad that in some areas, it is considerably easier to find a diseased turtle than to find a healthy one.

Researchers have found similar differences off the East Coast. More than half the young turtles in Florida's Indian River Lagoon System had tumors, but in the Atlantic Ocean a few miles away, no tumors were found.

Jacobson said the situation is repeated worldwide: Turtles in the open sea tend to be far less likely to have tumors than those in shallow, nearshore waters.

Researchers have not identified a pest or poison or pollution source that might cause the tumors. They haven't identified virus or bacteria sources. And they have not been able in the laboratory to infect clean turtles using tissue from sick turtles, so they still don't know how the animals get the tumors.

Researchers across the country and at international institutions are studying the problem.

A research plan published last year by the National Marine Fisheries Service cited several reasons for learning more, beyond attempting to control the impact on the threatened sea turtles themselves.

■ They could represent a reaction to a toxic pollutant in the ocean that we haven't yet identified.

■ There is the potential humans could suffer health hazards associated with them.

■ The visitor industry could be affected if people entering the state's waters repeatedly see turtles carrying ugly tumors.

The pale tumors can appear on the face, in the eyes, under the flippers, in the lungs and elsewhere. They can grow to more than 10 inches in diameter, and can block vision, impede swimming or even choke turtles to death if they grow in their mouths or throats. Many animals have washed up on beaches dying of starvation, too blind to find food, or incapable of swallowing it.

Researchers have tried removing tumors surgically or with lasers, but they often grow back. There are a few cases of tumors disappearing on their own, but none of these have been seen in Hawaiian turtles.

Scientists have found blood fluke eggs in some of the tumors, but not in others. They have found a herpes virus in some tumors, but have not been able to infect other turtles and have them develop tumors.

One problem with the disease is that there is so little money available to study it. Jacobson said that if you added up all the money available nationwide for the last six years for the study of fibropapilloma in sea turtles, it might add up to $40,000.

"We certainly are being hampered by that. Funding is not that great and it may never be. We're going to have to peck away at it and see what we can come up with," he said.

Tumors, Anemia Could Sink Gree

by Leticia Q. Pineda

Green sea turtles were once an abundant species. They have been killed for food, sport, trophies, and by plastic debris they ingested or became entangled in. In 1978, the United States placed green sea turtles on the threatened species list. Hawaii followed suit and also designated them fully protected.

However, green sea turtles from parts of Hawaii, Florida, and the Caribbean now face another threat from within their own bodies. An incurable disease is reducing the turtle population even further.

The scientific name of the disease is fibropapilloma. This "elephant-man like" disease causes tumors to grow on the turtles' soft skin, turning them into grotesque husks. A turtle can be so covered with tumors that they lose their sight and mobility in the sea. Internal tumors can also block air and food passages, drowning or starving a turtle.

"Since a tumor on a green sea turtle is highly supplied by blood, it has extra demands on its vascular system. One would expect all organs to work harder," George Balazs, zoologist and leader of the marine turtle research at the National Marine Fisheries Service (NMFS) Honolulu Lab explained. This would, in turn, likely cause the turtles to have a difficult time diving and holding their breath.

Besides the tumors, scientists have found other irregularities. Affected turtles are anemic compared to normal turtles and have lower serum globulin values.

Sexual maturity for a green sea turtle is reached on the average at around 25 years old. Because of this delayed sexual maturity, more turtles may be dying of the disease than breeding. Thus, scientists race against time to find both cause and cure.

Although occasional cases of fibropapilloma were first reported in 1938 in the Florida Keys, experts do not know why or how the disease has spread so far in the past few years. According to Balazs, of 113 dead turtles known stranded in the Hawaiian islands reported to NMFS in 1989, 56 had tumors.

Recently, a small amount of green turtle feces were found on the beaches of Kualoa Bay. Last year, hundreds of pellets washed ashore in the same area prompting the state Department of Health to close the beach. Balazs gave the department information from parasitologists Dr. Robert Desowitz from Leahi Hospital and Dr. Murray Dailey of University of California at

An autopsy by NMFS revealed that this frozen ma
North Shore had 41 tumors, many of which were

Long Beach who said "humans are not at risk of parasites and diseases specific to reptiles."

The strongest theory for the cause of the disease blames a virus, possibly aggravated by water pollution from chemical runoffs. This comes from studies of other papillomas-type tumors found in other animals, most of which are caused by viruses.

However, a virus is not always the culprit in papilloma diseases. Dailey contends eggs from parasitic flatworms from marine snails may be the cause of fibropapilloma. Much of the time, autopsies reveal that diseased turtles have a large number of internal parasites. The question exists, though, whether parasites play a role in causing tumors or arrive after the formations.

Scientists do not know how, and even if, diseased turtles can contaminate each other. Balazs and his colleagues have found many sea turtles in Molokai and Oahu with early signs of the tumors. Turtles that are strong enough to swim are tagged and released. Weaker turtles near death are cared for or used for scientific studies.

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n Sea Turtle Population Comeback

aturing male Hawaiian green sea turtle found off the
embedded with leech eggs.
—Leticia Q. Pineda photo

Most experimental treatments for diseased turtles that has been tried involve the physical removal of the tumor. Former Honolulu Zoo veterinarian, Patrick Leadbeater spent hours surgically removing tumors from a diseased turtle only to have the tumors grow back months later. Cryogenics (freezing a tumor) and infusion of dye into the tissue are examples of other techniques tried on individual turtles.

Even if such treatments did work, it would not have any great practical value. "Our principal concern is not to treat individual turtles. We wish to find the underlying factors that cause the disease to be prevalent—growing and spreading—among turtles in the wild," said Balazs.

Much of the research is to keep track of the scope and magnitude of the disease. Without such observational research, scientists would never know turtles in Molokai were also affected, said Balazs.

NMFS gathers more data on diseased green sea turtles through periodic trips to their habitats. Turtles are tagged with a permanent ID number and a return mailing address stapled to the edge of the flipper.

Through constant observation, NMFS scientists hope to collect statistics on, for example, whether turtles' conditions worsen or improve. So far the evidence suggests that the turtles worsen.

Because the disease is so widespread in Florida—experts say 50% of turtles in the Keys have it—scientists are working on the problem together.

Dr. Elliot T. Jacobson, professor of veterinary medicine at the University of Florida, visited Hawaii last year to collect Hawaiian green sea turtle tumor samples and he still keeps in weekly contact with NMFS.

An invitational planning meeting is cited for early December in Hawaii to establish research strategy. So far, five mainland scientists are slated to attend.

People can aid in the NMFS research by reporting green sea turtles that are unable to swim. Make reports to NMFS by calling 943-1221. In the evenings and on weekends, call the state Conservation and Resources Enforcement hotline, 548-5918.

Although this disease affects only green sea turtles in specific areas, "It may very well be reflective of other troubles in the ecosystem," Balazs said.

Saiki's Bill *(Continued from page 1)*

part of our interdependent ocean environment," Saiki said. "The traditions and culture of Hawaii and Guam place great emphasis on the care of the land and the surrounding waters. Hawaii and Guam are best equipped to provide oversight for these islands."

According to Saiki, some of the richest stores of minerals like cobalt and manganese nodules lie in the ocean floor within the exclusive economic zones (EEZs) of these islands.

She cites the importance of proper management, because destruction of the ocean floor anywhere close to the islands will be detrimental to Hawaii.

William Paty, chairman of the Hawaii Board of Land and Natural Resources, says that a limited entry program that sets rules and regulations with respect to the exclusive economic zones of the six islands will be the most effective form of management.

Nevertheless, Paty admits that trying to manage six islands thousands of miles apart will be a challenge.

"It is not an easy thing to do," said Paty. "Although the plan sounds good on paper, developing something on the islands will be difficult."

If Saiki's bill is passed, scientists and politicians can effectively study the economic, environmental, and strategic benefits for Hawaii.

The six islands have unlimited potential for marine research, wildlife preservation, and mineral findings.

"Given the transportation development that we have, over time, it may be ill advised to turn our backs on the opportunity to try jurisdiction," said Paty.

"This legislation is needed today for its impact tomorrow," said Saiki. "As the Congress considers a greater role for states in the management of their own EEZs, we need to be sure that the Pacific possessions are managed by the people with the greatest stake in the Pacific's future."

Wholphin Update

The baby wholphin, a cross between a wholphin and bottlenosed dolphin, born at Sea Life Park on July 19 has died. (See Makai, August 1990)

"At this time, there aren't any specific reasons known for her death," said Carrie Cushman, park public relations manager.

Tissue samples have been sent to the mainland for analysis to determine the cause of death.

Hanauma Bay Lecture Series
Fall 1990

Sponsored by the Hanauma Bay Educational Program

Witches Brew Hike, Toilet Bowl Hike
Place: Hanauma Bay Educational Desk
Time: 8:30-11 a.m
Date: September 8

Hawaiian Coastal Plants and Hike
(Field trip to Sandy Beach)
Place: Hanauma Bay Educational Desk
Time: 9-11 a.m.
Date: September 17
Speaker: Ray Tabata,
UH Sea Grant Marine Specialist
*Transportation to Sandy Beach not available

Underwater Photography of Hanauma Bay
Place: Hawaii Kai Public Library
Time: 7-9 p.m.
Date: September 18
Speaker: D.R. Schrichte

Seabirds and Marine Turtles
Place: Hawaii Kai Public Library
Time: 7-9 p.m.
Date: September 25
Speakers: Craig Rowland and Mike Moser,
U.S. Fish and Wildlife

Fishes of Hanauma Bay
Place: Hanauma Bay Educational Desk
Date: September 22
Time: 9-10 a.m.
Speaker: Ellyn Tong

Geology of Hanauma Bay
*Place: Hanauma Bay Educational Desk
Time: 8-11a.m.
Date: October 6
Speaker: Dr. George Walker

Witches Brew Hike, Toilet Bowl Hike
Place: Hanauma Bay Educational Desk
Time: 8:30 a.m.
Date: October 20
Speaker: Alan Hong

Mythology and Legends of Hanauma Bay
Place: Hanauma Bay Educational Desk
Time: 9-10 a.m.
Date: November 10
Speaker: Mike Markrich

Water Safety at Hanauma Bay
Place: Hanauma Bay Educational Desk
Time: 9-10 a.m.
Date: November 17
Speaker: Alan Hong

Call Allen Tom at 956-2870 for more information.

End of the Road

On Sunday July 1, John Quincy Adams, Brian Keaulana, and Dennis Gouveia of Water Patrol Incorporated, along with five of their associates completed what is believed to be the first-jet ski trip around the entire island of Oahu.

The purpose of the sometimes grueling trip was to discover the limitations and practicalities in using jet skis for water rescue work in Hawaii's waters.

"Through this exercise, we hope to have demonstrated the potential of the jet ski as a very safe and valuable tool in water patrol and rescue work," said Adams.

What's Happening

by Walter Ritte, Jr.

Turtles

What's hapening...to our turtles? **Tumors Continue to Spread in Hawaiian Green Turtles** was the headline in the Hawaii Wildlife Newsletter. George Balaz, of NOAA Fisheries, writes..."the incidents of fibroapapellomas, a life threatening tumor, has substantially increased in an important aggregation of the Hawaiian Green Trutle, which resides along the southern coast of the island of Moloka'i. Prior to 1985 there were no known cases of the tumors in this area. In the last harvest, March 1990, 38.5% of the turtles had tumors.

What can we do to help protect these turtles? Turtles were once a traditional Hawaiian food until greed wiped them out and the Federal Government stepped in to protect them under the Endangered Species Act. Now a mysterious disease on our reefs? Call the Feds and demand an answer: 943-1221, or call the State Office at 548-6550. Only through public pressure will government get us an answer.

Hawaiians

Homesteaders have taken a long over due stand to Pono their wai (take care of their water). They have asked the Federal Government to step in because the state, including DHHL have not acted in their best interest. Hawaiians are different from the "general public," they have rights beyond the general public. They are having a difficult time getting government to recognize and deal with Hawaiian Rights. Their water rights over the Kualapu'u aquifer is a good example. Δ

Balazs

MAUI NEWS
FEB. 18 1987

Turtles dying

HONOLULU — An alarming number of Hawaiian green sea turtles are being found washed up on beaches, distressed and weakened by tumors, some scientists report.

George Balazs, sea turtle specialist at the National Marine Fisheries Service's Honolulu Laboratory, said tumor cases in the sea turtles have grown from six or seven a year from 1982 to 1984 to 18 in 1985 and 31 last year.

Balazs said Hawaiian green sea turtles, an endangered species, face many survival problems including predators and human debris floating in the ocean.

But, he said, the tumor problem is so serious it has been given the highest priority for research.

WHAT DO WE KNOW ABOUT GREEN TURTLE

FIBROPAPPILOMA

1. Both juvenile and adult green turtles are susceptible.

2. Lesions are predominantly cutaneous and periocular. However, one green turtle with tumor nodules in kidneys was found.

3. The lesions are consistent with fibropapillomas of mammals and birds.

4. The epidemiology suggests an infectious disease agent. From a comparative perspective, a viral agent seems most probable. However, pollutants may affect the expression of the disease.

5. Histologic examination of the earliest recognized lesion revealed ballooning degenerative changes in epidermal cells in the stratum basale. By electron microscopy, cells undergoing ballooning degeneration contained membrane bound vacuoles. Vacu9les developed in the cytoplasm and were released into the extracellular spaces. Vacuoles were also seen in the dermis and occasionally, particles with electron-dense centers and measuring 155 to 190 nm were seen in these vacuoles. Although the specific identity of these particles remain unknown, their morphology is consistent with members of the family Retroviridae.

6. Spirorchid trematode eggs were not seen in any of 28 biopsy specimens ~~of~~ fibropapillomas from 6 affected green turtles. This paprasite is most likely not of significance in the pathogenesis of green turtle fibropapilloma.

7. Fibropapillomas are potentially life-threatening to affected green turtles.

GREEN TURTLE FIBROPAPILLOMA: FUTURE RESEARCH

1. Continue monitoring population in Indian River Lagoon System.
2. Toxicologic (pollutant) survey needs to be conducted comparing affected and normal green turtles in the Indian River Lagoon System.
3. Studies determining genetic prediposition to the disease should be conducted.
4. Continued electron microscopic studies with the earliest recognized lesions need to be conducted.
5. Tissue culture studies need to be continued.
6. Further challenge studies should be conducted.
7. Further molecular studies demonstrating the presence or absence of exogenous DNA or RNA should be performed.
8. An anti-green turtle globulin needs to be prepared to survey for the presence of turtle immunoglobulin in the earliest lesions.

SUMMARY

Six juvenile green turtles (Chelonia mydas) with multiple cutaneous fibropapillomas, were evaluated over a 6 month period. Histologically, fibropapillomas consisted of minimally to moderately hyperplastic epidermis overlying a thickened hypercellular dermis. In the earliest lesions, ballooning degeneration was present predominantly in the stratum basale where rete ridges extended into the dermis; aggregates of mixed inflammatory cells were present around dermal vessels. As the lesions matured, they developed an arborizing, papillary pattern. More mature lesions had a less verrucous, often ulcerated surface, with the dermis composed primarily of large collagenous fascicles and relatively few fibroblasts. While numerous trematode eggs were present within dermal capillaries of a histologically similar biopsy specimen from an Hawaiian green turtle, no trematode eggs were observed in any of 28 biopsies examined from the 6 Florida green turtles in this study. Low stringency Southern blot hybridization and a reverse Southern blot failed to demonstrate papillomavirus DNA in any of the samples extracted. Ultrastructural evaluation of the earliest lesions demonstrated membrane bound intracytoplasmic vacuoles within epidermal cells in the stratum basale. Similar vacuoles were also observed in the epidermal intercellular spaces and within the dermis. Occasionally, particles with electron dense centers and measuring 155-190 nm were observed in these vacuoles.

Add Rand

6th Annual Workshop on
Sea Turtle Biology and Conservation
19-21 March 1986
Waverly, Georgia

INCIDENCE OF FIBROPAPILLOMAS IN HAWAIIAN GREEN TURTLES

Literature citations

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March 1986

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A-10 Honolulu, December 18, 1983 The Sunday Star-Bulletin & Advertiser

False nestings, tumo[r]
new green sea turtle

By Jan TenBruggencate
Advertiser Kauai Bureau

LIHUE — There are several strange things happening with Hawaii's endangered green sea turtles, including false nesting attempts on Oahu and Kauai during the past two years.

Fisheries biologist Don Heacock, of the state Division of Aquatic Resources, this summer got a call that a turtle had tried to nest on a small sand beach on the island's southern coast. The turtle, a big one, had come ashore as many as five times and twice left great cavities 4 feet across, and piles of sand as if it had buried eggs in a nest it had dug.

It was interesting for several reasons. The beach had no history of turtle nesting. And, while an occasional Hawksbill turtle nest is located in the main Hawaiian islands, the green sea turtle hasn't been known to nest here in some 40 years.

Until last year, when one laid eggs, and the eggs hatched, on a Kahuku beach on Oahu. And this year on a little beach at Kaneohe Marine Corps Air Station, there was a false nesting. A turtle had made tracks like it had laid eggs, but after waiting past the incubation period, officials dug it up and found no nest.

The Kauai nesting site was carefully left undisturbed, but weeks after the two-month incubation period was past, Heacock went digging. There were no eggs. Another false nest.

Heacock and federal wildlife biologist George H. Balazs, of the National Marine Fisheries Service, said they don't know what it means. Maybe the false nests were left by sick turtles. Maybe the turtles dug and found the beaches unsuited for nesting. There's not enough evidence to tell, they said.

But with one successful nesting and two false nests on the main islands, there's a suggestion that the turtles are regaining some interest in the Islands after not having nested here for decades.

Helicopter pilots on Kauai also have noted recently that large green turtles have been spotted basking on isolated beaches of the Na Pali Coast. Twenty years ago, such basking was common. Lately it's been rare.

Balazs said it may occur in winter when the high surf disturbs the ocean bottom where turtles normally rest, so they haul up on shore. It's clear that the presence of curious humans can quickly drive them back into the sea, he said.

The nestings, false and real, and the renewed basking may be hopeful signs, but biologists are disturbed by another apparent trend. There are increasing reports of tumors on Hawaiian green sea turtles.

Balazs said it could be that there is simply better reporting of turtle biology now and it could be that there is an alarming increase in the number of turtles affected by tumors. In any case, for many individual turtles, the tumors can end up being fatal.

Heacock said he recently received a report of a green sea turtle floundering in the Hanapepe area. He found growths on its head had almost entirely blocked its vision.

Balazs said the tumors are found generally around the head, and sometimes come directly out of the eye. Sometimes they're small, but he has seen one as large as a football growing from a turtle's neck.

Scientists have studied them and found they're not malignant and do not appear to be caused by a virus.

"We've found out what they are not caused by, but we haven't found what they are caused by," Balazs said.

Similar tumors have been reported in green turtles in other areas, but the problem seems worse in the Hawaiian green sea turtle population, he said.

The National Marine Fisheries Service and the state Division of Aquatic Resources welcome reports concerning the turtles.

There's still a great deal of mystery surrounding green sea turtles, called honu by Hawaiians and *Chelonia mydas*. The scientific community doesn't know much about where they go for the first few years of their lives, about what they eat or how quickly they grow.

Scientists are wary of estimating turtle populations in even the vaguest terms. They can't even guess at total population. The breeding population, males and females, runs perhaps 1,000 to 1,500, according to Balazs.

But that doesn't mean that many are breeding each year. Males may breed annually, but females sometimes do so every two years, every three years, every six years and sometimes maybe only once in a lifetime.

When they do breed, the big females may be 11 to 59 years old and have shells a meter long. They lay clutches of eggs in the sand that run from 40 to 140 eggs, averaging about 100. And while most lay only one or two clutches in a season, some go as many as six.

As recently as 40 years ago, the Hawaiian green sea turtles were known to nest on Kauai, Lanai and Oahu, but, for many years, their only nesting colony has been on the sand spits of French Frigates Shoal, some 400 miles from Kauai in the Northwestern Hawaiian Islands.

They still migrate hundreds of miles and feed around the main islands, though. The turtles were taken commercially until 1974, when they were put under government protection.

...s provide mysteries

Two green sea turtles and a friend bask on a beach in the Leeward Islands.

Animal hospital

CHINA POST (TAIPEI, TAIWAN) 4-4-94

Topless bar turns turtle haven

By Jane Sutton
Reuter

Marathon, Florida — The building that once housed the only topless bar in the island fishing community of Marathon now serves as a hospital for the growing number of endangered sea turtles afflicted with a deadly tumor disease.

The Hidden Harbor Environmental Center, formerly Fanny's bar, is believed to be the only hospital in the world devoted exclusively to ailing sea turtles.

Its founders, charter boat captain Tina Brown and hotelier Richard Moretti, have been rescuing stranded sea turtles in the middle Florida Keys since 1985.

They opened the non-profit hospital two years ago while struggling to treat a growing epidemic of cauliflower-like tumors that blind the giant turtles and immobilize their flippers.

"It's a very graphic, nasty looking disease," said Brown. "The tumors usually affect their eyes first so they can't see to eat."

All five U.S. sea turtle species, which can each weigh up to 400 pounds (181 kilograms), are already endangered or threatened. Recent studies by the Florida Department of Natural Resources indicate 50 percent of the sea turtle population in the Florida Keys have the tumor disease, called fibropapilloma.

It was first seen among green sea turtles and is spreading rapidly among loggerheads and other turtle populations in the Atlantic, Caribbean, Pacific and Indian Oceans.

Studies of the Hidden Harbor patients, carried out by Dr. Elliot Jacobson of the University of Florida, have shown the tumors are caused by a virus. Biologists believe pollution is weakening the turtles' immune systems, making them suddenly more vulnerable.

By the time tumor-stricken turtles are brought to the center, they are usually starving. Brown and Moretti fatten them up with fish, squid and turtle chow, then haul them into the center's operating room on golf carts.

Dr. Lisa Bramson, a volunteer veterinarian from Key West, rolls them onto a reconfigured gynecological examination table and surgically removes the tumors.

The turtles recuperate in a saltwater swimming pool for a year, then are released if they regain their health. Relapses are frequent, and about 40 percent have inoperable internal tumors and die anyway, Brown said.

The center has treated hundreds of turtles for other ailments as well. Sea turtles are notorious for raiding lobster traps and getting their flippers tangled in trap lines.

"They crunch right through the wooden traps to get the lobsters and eat the lobsters whole in the shell," Brown said.

Others have lost flippers to sharks and boat propellers, but can be released once the infected wounds heal and they learn to swim and steer with the three remaining flippers. "Like handicapped people, they learn to use what they have," Brown said.

Those with propeller gashes often have air trapped between the layers of their shells. The bubbles make them swim lopsidedly, rubbing callouses on the soft skin around the shell.

Brown and Moretti patch the broken shells with fiberglass, and attach one-pound (0.45-kilogram) weights to compensate for the air bubbles. That allows them to submerge and swim normally, and the callouses disappear.

The turtle tenders hope to attempt a cornea transplant on a female loggerhead who survived eye tumor surgery but was left blinded.

"If I can get her one good eye I could let her go," Brown said.

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration

L?@?*Z

1/2/85

To : George

From: Bill

I called this guy today to discuss this. I think we should try to get someone to do a bioassay on the stuff, probably in a mouse, to see if it is toxic. If so, then we could go on with more expensive assays to see exactly what the agent is .

Lets talk about it when you get settled.

ASBESTOS ? #30 + TAX

ORGANOCHLORINES ?

#150 BROAD SPECTRUM

TRANSMITTAL FORM CD-82A (10-87)
PRESCRIBED BY DAO 214-2 ☆U.S. G.P.O. 1982-564-008/1147

INDUSTRIAL ANALYTICAL LABORATORY, INC.

MARK R. HAGADONE, M.S.
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TELEPHONE
(808) 947-5402

American Bio-Science Laboratories 4400 KALANIANAOLE HWY., HONOLULU, HI 96821 (808)735-1702

Patient: TURTLE,MALE

To: GILMARTIN

Account #: 99998

Date Received: 11/29/84

BSL Spec #: K34087

Date Collected:

Page: 1

AGE:

SEX:MALE

Test	Result	Units	Reference Range	
F OCCULT BLOOD	NONE DETECTED		NONE DETECTED	J

NAT'L MARINE FISHERIES SERVICE
DEC 4 9 42 AM '84
HONOLULU LABORATORY

Laboratory Report

Date Reported: 11/30/84

LAURENCE MCCARTHY, M.D., Director

Account #: 99998

NAT MARINE FISH SER
2570 DOLE ST
HON. HI 96822

Patient: TURTLE,MALE

Date Rec'd.: 11/29/84

BSL Spec #: K34087

A

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American Bio-Science Laboratories

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An AIHA / NIOSH Accredited Industrial Hygiene Laboratory
Certification No. 154

27 December 1984

National Marine Fisheries
2570 Dole Street
Honolulu, Hawaii 96822

ATTENTION: Mr. William Gilmartin

Dear Mr. Gilmartin:

Per my telephone conversation with Mr. George Balazs, I have agreed to provide you with a quick investigation at no charge into the chemical nature of the brown-to-black debris found in the autopsied turtle's gut. Initial examination undertaken utilizing both microscopy and simple chemical techniques demonstrated that the substance was primarily water soluble in nature. This rules out material such as petroleum hydrocarbons from oil spills (corroborated by TLC work accomplished by the U.S. Coast Guard) and/or some of the more complex assortment of toxic organic matter. From microscopic analysis it appears that the debris was made up largely of fish scales, oil globules, a considerable amount of inorganic carbonate, numerous fibers, grass trichomes, what appear to be salt crystals, and various other inorganic matter. The pH of the mixture was neutral, ruling out highly alkaline or acidic substances. Limited infrared spectroscopy accomplished on the washed and dried solids isolated from this material demonstrated a primarily inorganic mixed carbonate/silicate composition. A strong odor of hydrogen sulfide was no doubt due to microbiological action on undissolved foodstuff.

It is our opinion that this material is primarily inorganic in nature and probably not responsible for the turtle's death. However, we have not ruled out the possibility of toxic heavy metals -- lead, arsenic, barium, etc. -- analysis for which could be accomplished with a heavy metal screen for the eight most likely candidates for approximately $210.

I hope this brief survey will be of some help to you. Should you wish to pursue the course of action described above, please don't hesitate to contact us at your earliest convenience.

Sincerely,

Mark R. Hagadone
Vice President
INALAB

2-19

H_2S – released from petroleum products when contacted with acid.

T.L.C. OF TURTLE INTESTINE CONTENTS

1. TURTLE SAMPLE HAD A DEFINITE HYDROGEN SULFIDE SMELL.
2. UPON CENTERFUGING THE SAMPLE AT 2200 RPM. FOR 10 MINUTES THERE WAS NO SEPERATION NOTICIBLE IN THE SAMPLE
3. UPON ADDING CaSO4 TO REMOVE THE H2O NO FOAMING OF THE SAMPLE WAS NOTICED.
4. THE METHANOL AND .4 ACETIC ACID PHASE AND VORTEXING IT FOR 1 MINUTE THE SAMPLE TURNED GRAY AND DID NOT SETTLE UNTILL IT WAS CENTERFUGED FOR 10 MINUTES AT 2200 RPM.
5. THE RESULTING OIL /METHANOL PHASE WAS LIGHT AMBER IN COLOR AND HAD NO HYDROGEN SULFIDE SMELL.
6. THE METHANOL PHASE WAS THEN EXPOSED TO 10.0 ACETIC ACID/METHANOL FOR 15 MINUTES.
7. THE SAMPLE WAS THEN EXPOSED TO HEXANE/METHANOL TO MAKE THE SAMPLE MIGRATE ACROSS THE SILICA GEL PLATE. THE MIGRATION WAS UNIFORM AND TOOK 45 MINUTES. THE 5 COMPONENT TEST DYE DEVELOPED INTO 5 PARTS AND THE SAMPLE DID NOT MIGRATE AT ALL.
8. THE TURTLE SAMPLE HAD A pH OF 6.5-7 WHICH IS NEUTRAL.

J. R. SPEIR
FOIL TECHNICIAN

INLAB
947-5402
→ MIKE HARRIS
MARK HADDENDON

King - Kalaulau
1523

cariae developing in bivalves. The Acanthocolpidae and Allocreadiidae, as thus restricted, are regarded as separate but fairly closely related groups, possibly more so than any two subfamilies hitherto allocated to the family Allocreadiidae (sensu lato).

There seems good reason to support Hopkin's (1941) allocation of trematodes having cotylomicrocercous cercariae to a separate family, Opecoelidae, and Manter's (1940) suggestion that the Gyliauchenidae is more closely related to the Lepocreadiinae than to the amphistomes. Superfamily relationships of the Allocreadiidae, Acanthocolpidae, Opecoelidae, Lepocreadiidae, and Gyliauchenidae are not apparent from the fragmentary knowledge of life histories and embryology, particularly of marine forms.

15. *Notes on Embryonating Eggs of* Zygocotyle lunata *and on Their Preparation for Cytological Study.* GABRIEL C. GODMAN AND CHARLES H. WILLEY, New York University.

Difficulty encountered from mold and bacteria on shells of embryonating eggs of *Zygocotyle lunata* is effectively overcome by keeping living Ostracoda with the eggs. After several washings, 30 to 50 eggs are placed in a covered Petri dish with from 10 to 15 ostracods which may be obtained from aquaria or cultured easily in water containing decaying lettuce. They hold and rotate the eggs with their appendages, effectively scraping off the fungi and leaving them clean and undamaged. The Ostracoda must be replaced and the water changed about every 5 to 7 days, since most of the crustacea die within that period. Ova of *Z. lunata* prepared for sectioning in early stages of development collapse immediately in most common fixatives (Bouins, Helly's, Zenker's, Gibson's). In Flemming's fluid they suffer no change of form, penetration has occurred within 10 minutes and fixation is complete within 30 minutes. Since collapse or rupture occurs in the usual series of alcohols, successful dehydration was accomplished only by the capillary-wick technique of McClung, by which a very gradual instillation of alcohol occurs. They collapse and rupture in pure dioxan, buckle slowly in 50 per cent dioxan, and its gradual addition offers no advantages over alcohol. Clearing must be accomplished slowly with xylol or chloroform by the capillary-wick technique. Gradual infiltration with the rubber-paraffin mixture of Hance is of value in reducing to a minimum the shattering of the shell that occurs in pure paraffin.

16. *Parasites of the Green Turtle,* Chelonia mydas *(L.), with Special Reference to the Rediscovery of Trematodes Described by Looss from this Host Species.* ROSS F. NIGRELLI, New York Aquarium.

Looss (1899, 1902) reported some 24 species of trematodes from *Chelonia mydas*. The following were recently recovered from the viscera of 50 turtles: (Pronocephalidae) *Cricocephalus albus* (Kuhl and Hasselt, 1822) and *C. megastomus* Looss, 1902 (stomach); *Pronocephalus obliquus* Looss, 1901 and *Glyphicephalus lobatus* Looss, 1901 (intestine); *Pyelosomum cochlear* Looss, 1899 (urinary bladder). A new species of *Charaxicephalus* Looss, 1901 was found in the stomach. No forms of *Pleurogonius* Looss, 1901 were present. *Deuterobaris proteus* (Brandes, 1891), *Octangium sagitta* (Looss, 1899), *Angiodictyum parallelum* (Looss, 1901) and *Polyangium linguatula* (Looss, 1899), all Angiodictyidae, were found in the large intestine. *Microscaphidium* Looss, 1900 is not represented. A specimen of *Schizamphistomoides spinulosum* (Looss, 1901) Stunkard, 1925 (Paramphistomidae) was recovered from the large intestine. Of the intestinal distomes reported by Looss, *Enodiotrema megachondrus* (Looss, 1899) (Plagiorchiidae) was the only one found. Other distomes present in our collection are as follows: (Rhytidodidae) *Rhytidodoides intestinalis* Price, 1939, *R. similis* Price, 1939, and a new species of *Rhytidodoides* Price, all from gall bladders. The family Spirorchidae Stunkard, 1921, is represented in this collection by *Learedius learedi* Price, 1934, and *L. similis* Price, 1934, from the heart and visceral vessels. Large numbers of leeches, *Ozobranchus branchiatus* (Menzies, 1791), were found associated with fibro-epithelial tumors on eyelids. Heavy infections of *L. learedi* and *R. similis* occurred in 65 per

Journal of Parasitology
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Program Abstract of 17th Annual meet

cent of the organs examined. In four cases, a papillomatous disease of the gall bladder (Smith, Coates and Nigrelli, 1941) was found associated with heavy infections of *R. similis.*

17. *Studies on Host-parasite Reactions. V. The Integumentary Type of Strigeid Cyst.* GEORGE W. HUNTER, III, Wesleyan University, AND WANDA SANBORN HUNTER, University of California at Los Angeles.

The integumentary type of cyst characteristic of *Crassiphiala bulboglossa* and *Neascus rhinichthysi* is described from the yellow perch, *Perca flavescens,* and the black-nosed dace, *Atratulus atronosus,* respectively. In both cases special connective tissue stains were used. An inner hyaline cyst is elaborated by the parasite while the host responds by producing a connective tissue cyst containing melanin and melanin-bearing cells resembling melanophores. These outer host cysts produced by the fish are, in general, less dense than those noted in the case of parasites penetrating to the deeper layers of the host, as *Uvulifer ambloplitis.*

18. *The Incidence of* Trichomonas foetus *in Wisconsin Cattle.* BANNER BILL MORGAN, University of Wisconsin.

Examinations were made of the uteri from 560 Wisconsin dairy cows obtained from a local packing plant. Of the 560 uteri examined 416 were pregnant, 110 normal non-pregnant, 31 non-pregnant with pyometra, and 3 pregnant with pyometra. From the total number of uteri examined 8 or 1.4 per cent were positive for trichomonads. Of the 8 cases, the amniotic fluid of an apparently healthy seven-months foetus (approximate age) was teeming with *T. foetus.* From the 31 non-pregnant uteri with pyometra 5 were positive and from the 3 pregnant uteri with pyometra containing macerated foeti 2 were positive. The incidence of *T. foetus* from the 33 uteri with pyometra was 21 per cent.

Amniotic fluid, occasionally allantoic fluid, swabs from the mouth of each foetus, and exudate from uteri with pyometra were inoculated into culture media. The material was incubated at 30° C for 18 hours and examined for trichomonads. After the first examination the cultures were incubated at 37° C for 18 hours and examined again. The culture material was the all-autoclaved medium of Schneider (unpublished thesis). Occasionally egg slants layered with saline, Ringer's, or saline and serum were used.

Four cases of trichomoniasis have been positively diagnosed out of 28 herds representing a total number of approximately 500 dairy cows for an incidence of 0.8 per cent. This is a preliminary report, but the above figures indicate that trichomoniasis, at least in Wisconsin, is an important problem in the dairy cattle industry.

19. *The Effects of Sulfaguanidine on Experimental Bovine Coccidiosis.* DONALD C. BOUGHTON, U. S. Regional Animal Disease Research Laboratory, Auburn, Alabama.

In one experiment 9 calves were inoculated simultaneously with approximately 50 million sporulated oöcysts of mixed types from one large suspension. Four of the 9 received no drug. All of these developed clinical coccidiosis, *Eimeria bovis* being the dominant species. One was sacrificed when moribund 15 days after inoculation. The remaining three died on the 2[illegible]th, 21st, and 27th days, respectively. Five calves were given at least 21 consecutive daily doses of sulfaguanidine at the rate of 0.1 gm per 2.2 pounds of body weight beginning 2 days after inoculation. The 5 treated calves survived, did not develop clinical coccidiosis, and discharged very few *E. bovis* oöcysts during the month following inoculation. In a second experiment 12 calves were given a similar inoculation of infective oöcysts from a second suspension. Three calves received no drug. Sulfaguanidine was given to the remaining 9 calves at the rate of 5.0 gms per day for 8 consecutive days as follows; three calves, at the time of inoculation; 3 calves, mid-way in the 3-week incubation period; and 3 calves, toward the end of the incubation period. All calves receiving the early and mid-way treatment and 2 of the 3 untreated calves developed

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7 July 1986

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Dear George:

Thank you for your communications of 14, 19, 20, and 23 May including the manuscript, "Fibropapillomas in Hawaiian Green Turtles". I am enclosing a copy of the manuscript with my editorial suggestions, corrections, and possible additions. Use them at your discretion. Also enclosed is a quick and dirty photo of a trematode ovum in a tumor and a copy of the paper by Greiner et al in case you do not have it.

Best regards,

John

John C. Harshbarger, Ph.D.
Director
Registry of Tumors in Lower Animals

Enclosures

5/86

FIBROPAPILLOMAS IN HAWAIIAN GREEN TURTLES

Neoplasms identified by the Registry of Tumors in Lower Animals (RTLA) as fibropapillomas are being commonly found on green turtles in the Hawaiian Islands. Up to 10% of the nesting females tagged each year at the principal breeding colony of French Frigate Shoals have these epithelial growths ranging from a few millimeters to 30 cm in diameter. They most frequently occur on the neck, eyes, flippers, jaw, tail, and sometimes even in the mouth. In Hawaii fibropapillomas have been recorded in turtles as small as 45 cm juveniles to adult males and females over 85 cm. However, thus far none have been found in turtles 35-45 cm, the minimum size range at which recruitment to benthic habitat takes place in Hawaii.

During 1985, 35% of the 51 stranded green turtles examined throughout Hawaii had fibropapillomas. Local divers and fishermen regularly report seeing afflicted turtles in coastal foraging pastures and underwater sleeping areas. A considerable increase in such sightings is believed to have occurred over the past 20 years.

Fibropapillomas in green turtles were first described in the scientific literature almost 50 years ago by Smith and Coates (1938). At that time, 3 out of 200 green turtles (27 to 91 kg) examined at Key West, Florida, had fibropapillomas. During the same year, Lucke (1937/38) mentioned that green turtles in the Dry Tortugas "not infrequently suffer from papillomatous neoplasms which may attain so great a size as seriously to interfer with their locomotion." Hendrickson (1958) stated that "occasional" green turtles nesting in Sarawak, Malaysia had ulcerated fibromas on their "throat and neck." Since the RTLA began accepting specimens in 1965 (Harshbarger 1974), fibropapillomas from green turtles have been verified from the Florida Keys (RTLA Accession No. 12 & 651) ~~and~~ Hawaii (RTLA 121, 1767, 1774, 1856, 1883, 2097 and 3572) and the Cayman Turtle Farm (RTLA 3099). ~~Several neoplasms from the Cayman Turtle Farm have recently been submitted by Jack Frazier, but as yet have not been cataloged or histologically identified.~~ Jacobson (1981) reported that a "slowly increasing incidence of papillomatosis" was being studied in a breeding group of green turtles at the Cayman farm. These growths were first noted in wild captured adults but later developed in farm reared turtles. According to Jim Wood, the problem no longer exists, although several juvenile turtles released from the farm and recaptured a year or two later and found to have growths. In east central Florida, immature green turtles have been reported by Lew Ehrhart to show a high incidence of these fibrous growths during recent years where none had been seen since tagging studies began there in 1977. Pat Wells, working in the Florida Keys has found similar growths on five of nine stranded green turtles handled during the first 3 months of 1986. Previously, only two cases of tumors had been reported out of six strandings of green turtles seen since 1982.

Thus far, no other species of sea turtle except for the green turtle has been documented to have fibropapillomas, although Jack Frazier and Tom Fritts have seen what were believed to be these growths in olive ridleys in the eastern Pacific.

Fibropapillomas in Hawaiian green turtles can result in reduced vision, disorientation, blindness, physical obstruction to normal swimming and feeding, and an apparent increased susceptibility to parasitism by the marine leech, *Ozobranchus branchiatus*. Our observations also suggest that fibropapillomas can cause severe emaciation, increased predation by tiger sharks and humans, and probably a reduced ability to successfully migrate and breed. Entanglement in fishing line and other gear also appears to be more likely in afflicted turtles.

The etiology of fibropapillomas in green turtles remains unknown. Possible causes suggested in the literature include an immune response to trematode ova, secretion of hirudin by marine leeches, virus, excessive solar radiation, chemical pollutants that (impact on the surface (Fred ? pers. comm.) or) impair the immune system, stress, and a genetic predisposition to neoplasia. Biopsy material from fibropapillomas on two Hawaiian green turtles ~~were~~ was sent to the RTLA for electron microscopy, but no virus was found. Leeches would naturally gravitate to the relatively rich source of blood provided by the well vascularized lesions rather than initiating them.

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The growth rate of fibropapillomas has been documented in two Hawaiian green turtles. A nesting female with no signs of neoplasia when first seen was recovered 3 years later in an emaciated state with a large fibropapilloma along the dorsal base of its tail. Another apparently healthy tagged turtle, an adult male, had numerous growths, including a large mass in the axilla, when seen again just 2 years later. The fact that relatively small turtles in Hawaii can be heavily afflicted with fibropapillomas indicates that growth can occur fairly fast under certain conditions.

The experimental treatment of fibropapillomas in Hawaii has included surgical removal and strangulation. Neither procedure proved satisfactory due to the large number of growths often present, their highly vascular nature when large, and the apparent presence of nerve bundles that transmit pain, especially in growths associated with the eyes. There is no evidence

The only substantive evidence for any of the suggested causes is the consistent presence of ova from digenetic blood flukes (Family Spirochidae) within the fibrotic portion of the lesions. Smith and Coates (1939) originally reported ova in over half of the 250 fibropapillomas examined from at least six turtles. At least seven of the ten RTLA cases contain ova (RTLA 12, 121, 1767, 1774, 2097, 3099, and 3572), including specimens from Florida, Hawaii, and the Cayman Islands' turtle farm. The Cayman Islands' turtles are heavily infested with the cardiovascular fluke, Learedius learedi, (Greiner et al, 1980) and some of their shed ova lodge in dermal capillaries (Jacobson, 1980). The incidence of fibropapillomas gradually increases in the captive animals, suggesting an infective process. In histological sections, the ova generally appear as oval, yellowish/brown, acellular capsules containing undifferentiated cells. Host response consists of a capsule of epithelioid macrophages surrounded by fibrosis. If trematode ova are in fact the etiologic agents, then the lesions represent a nonneoplastic host response, characterized by an exuberant, cutaneous, foreign body fibrosis associated with papillary epidermal hyperplasia.

to suggest that fibropapillomas in Hawaiian turtles may spontaneously cease or diminish.

The Hawaiian population of green turtles is geographically isolated and relatively small, consisting of only a few hundred females nesting annually at French Frigate Shoals. A recovery team has recently been appointed to formulate a plan to rehabilitate the population. The occurrence of fibropapillomas is viewed as one of several problems that urgently need to be addressed through additional research. In general, the problems of disease and natural predation are two areas where basic data are frequently lacking and more attention is needed for the development of effective management plans. Workers are therefore encouraged to document any occurrence of neoplasia found in sea turtle populations by submitting preserved tissue to the RTLA, Smithsonian Institution, Room W216, Washington, D.C. 20560.

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probably not regular food items in the diet of the size of bass which are usually collected in surveys (Clady, 1976, Am. Midl. Nat. 91:453–459). We speculate that *Textrema hopkinsi* was absent from this survey because the probable molluscan host (*Amnicola* spp.) is not commonly found in reservoirs. *Bothriocephalus* sp. has only been reported from Sam Rayburn Reservoir in Texas and is probably not an important parasite of bass in this state.

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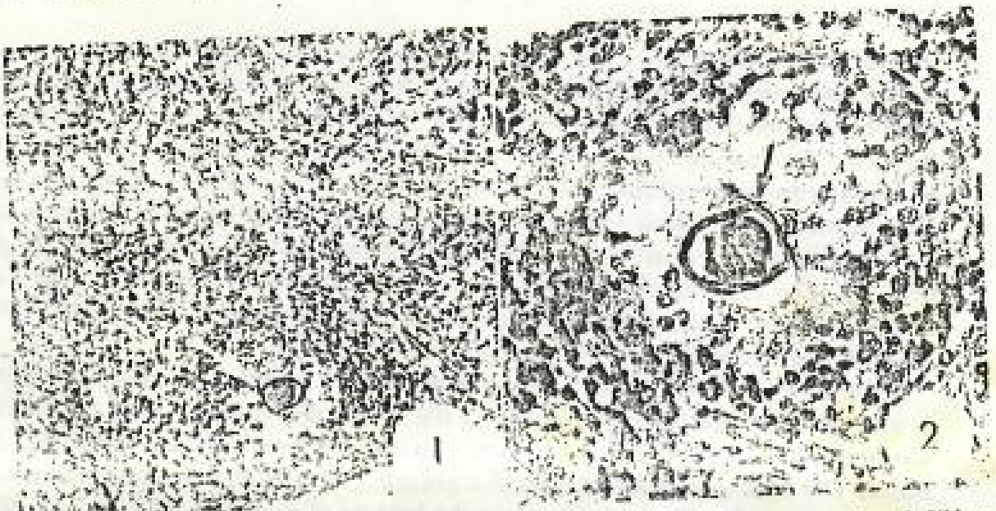
Proc. Helminthol. Soc. Wash.
47(1), 1980, p. 142–144

Research Note

Helminths of Mariculture-reared Green Turtles (*Chelonia mydas mydas*) from Grand Cayman, British West Indies

Ten mariculture-reared green turtles (4 to 4.5 years of age) were examined at necropsy during December 1978 at the Cayman Turtle Farm, Grand Cayman, British West Indies. These were selected at random from a holding tank containing several hundred turtles and included eight males weighing from 45 to 147 lb ($\bar{x}$ = 83 lb) and two females (both 105 lb). Each of the following organs was placed into an individual container in which it was either macerated or cut open. Spleens, gonads, kidneys, and livers were macerated; urinary and gall bladders, the airways of the lungs and tracheae, the major vessels attached to the heart, and the heart chambers were cut open. The gastrointestinal tract was first subdivided into esophagus/stomach, small intestine, and large intestine and then each section was slit lengthwise. The surfaces of the organs cut open were examined visually and then washed through a series of 50- and 100-mesh screens. The remaining washed material from each organ was then examined for helminths under a dissecting microscope. All helminths recovered were relaxed in cold tap water, fixed in AFA and stained with Harris' hematoxylin. Kidney tissues and the proximal portions of the aortic trunk were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 7 μm and stained with hematoxylin and eosin.

Four of the 10 turtles harbored digenetic flukes. A single specimen of *Pleurogonius* sp. (Pronocephalidae) was collected from the small intestine of one turtle. This genus contains 13 species that have been described or reported from *C. mydas* and our specimen most closely resembles *P. mehrai* Ruiz 1946. Previous reports of *P. mehrai* include those from green turtles in Trinidad (Gupta, 1962, Can. J. Zool. 39:293–298) and India (Mehra, 1939, Proc. Natl. Acad. Sci. India



Figures 1, 2. Pathology associated with apparent *Learedius learedi* eggs in green turtles. 1. Kidney tissue showing inflammatory reaction to the presence of *L. learedi* egg (at tip of arrow) (×140). 2. Section of *L. learedi* egg (at tip of arrow) in kidney tissue (×370).

9:99–130). The other fluke recovered was *Learedius learedi* Price 1934 (Spirorchiidae) reported previously from a green turtle in the U.S. National Zoo. It has also been found in *C. mydas* in Panama (Cabellero et al., 1955, An. Inst. Biol. Nac. Univ. Mex. 26:149–191) and in Florida (Nigrelli, 1941, J. Parasitol. 27(suppl.):15–16). Twenty-five (three to 15 per host) specimens of *L. learedi* were present in the heart and (or) associated major vessels of four of 10 turtles necropsied. Since the heart and associated major vessels were treated as one entity in the initial examinations, hearts and the major vessels from 10 additional turtles were examined after separating the vessels from the heart. All 10 of these were infected with *L. learedi*, ranging from one to 49 ($\bar{x}$ = 14) flukes per host. A total of 141 flukes was collected, of which 12 were found in the vessels and 129 in the chambers of the heart. Upon histopathological evaluation, in one animal there was found a focus of increased cellularity in the endothelial-intimal layer of the aorta comprised mostly of mononuclear cells. Whether or not this lesion was associated with fluke attachment is unknown.

Due to the location of the adult *L. learedi* in the heart and associated major vessels, its eggs probably would be found in a variety of tissues. Since the eggs must first leave the circulatory system and then eventually leave the host, organs such as the lung, liver, and kidney would be the most accessible for completion of the cycle. The eggs of *L. learedi* are morphologically similar but smaller than those of a related fluke, *Hapalotrema constrictum*, reported by Smith and Coates (1939, Zoologica [N.Y.] 24:379–383) in association with fibroepithelial tumors on green turtles in Florida waters. Their diagnosis of *Hapalotrema* was based only on eggs; adults were not found. *Hapalotrema* adults have been reported from *C. mydas* in India, but have also been reported from the Atlantic loggerhead (*Caretta caretta*) in Florida waters, and it is probable that other marine turtles are hosts for these flukes as well.

Kidneys examined showed a range in lesions from a mild multifocal interstitial infiltrate of mononuclear cells to a much more severe chronic inflammatory reaction consisting of tubular and glomerular necrosis with diffuse interstitial infiltrate of mononuclear cells and fibrosis (Fig. 1). Tissues from several green turtles had what appeared to be trematode eggs surrounded by discrete chronic granulomatous reactions consisting of epithelial cells, multinucleated giant cells, and mononuclear cells. These eggs were seen as oblong homogenous yellow capsules surrounding a central area of undifferentiated cells measuring 43 μm by 34 μm (Fig. 2). The eggs of other spirorchiids which occur in freshwater turtles are known to cause similar tissue damage (Goodchild and Dennis, 1967, J. Parasitol. 53:38–45; Halliman et al., 1971, J. Parasitol. 57:71–77).

Since these turtles had been in the same holding tank for the past 18 months, it was probable that they had acquired the flukes during this period, suggesting infected snails and (or) cercariae were entering the tank with the incoming seawater. The tank was examined for the presence of potential intermediate hosts. Three species of snails were found on the bottom of the tank and were identified as *Nodolittorina muricatus*, *Strombus gallus*, and *Diodora listeri*. Although several living representatives of these snails were placed in seawater in containers overnight, no cercariae emerged.

Representative specimens of the flukes have been deposited in the U.S. National Parasite Collection, Beltsville, Maryland (USNM Nos. 75099 and 75100) and in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska (No. 20909).

We wish to thank Dr. Fred Thompson of the Florida State Museum for identifying the snails and the Cayman Turtle Farm, Ltd., for financial and logistical support for this study. Florida Agricultural Experiment Stations Journal Series No. 1769.

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Proc. Helminthol. Soc. Wash.
47(1), 1980, p. 144–147

Research Note

Helminths of the Ring-Necked Pheasant, *Phasianus colchicus* (Gmelin) (Phasianidae), from the Texas Panhandle

The ring-necked pheasant, *Phasianus colchicus* (Gmelin), was first established in the Texas Panhandle about 1940 (Jones and Felts, 1950, Texas Game and Fish 8:4–7). The first hunting season was established in 1958 (Guthery et al., 1980, U.S. For. Serv. Gen. Tech. Rep., in press), and this species has since become a primary resident gamebird of the region. Because of the complete dearth of information on the helminth fauna from pheasants in the Southwest, the present study was initiated.

Smith, G. M. and C. W. Coates

The Occurrence of Trematode Ova, Hapalotrema constrictum (Leared), in Fibro-Epithelial Tumors of the Marine Turtle, Chelonia mydas (Linnaeus).

Zoologica (New York), 24(3): 379-382. 5 plates. 1939.

1222 SMITH, G. M., and C. W. COATES. The occurrence of trematode ova, Hapalotrema constrictum (Leared), in fibro-epithelial tumors of the marine turtle, Chelonia mydas (Linnaeus). *Zoologica [New York]* 24(3): 379-382. 5 pl. 1939.—The presence of parasitic ova of a blood fluke, *H. constrictum*, has been noted in many of the growths occurring in the marine turtle. This neoplastic disease in the turtle is characterized by multiple cutaneous growths which may at times involve the eyelids and cornea. In 2 turtles examined by the authors, no trace of these ova were discovered in heart, liver, spleen, kidneys, nor in any of the numerous cutaneous tumors nor in those of the eyelid and cornea, and it is therefore considered that the presence of parasitic ova, as such, is not related probably to the [illegible] cause of the disease.—C. W. Coates.

6th Annual Workshop on
Sea Turtle Biology and Conservation
19-21 March 1986
Waverly, Georgia

INCIDENCE OF FIBROPAPILLOMAS IN HAWAIIAN GREEN TURTLES

By

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ABSTRACT

Neoplasms identified by the Registry of Tumors in Lower Animals (RTLA) as fibropapillomas are being commonly found on green turtles in the Hawaiian Islands. From 5 to 10% of the nesting females seen each year at French Frigate Shoals have these epithelial growths which range from a few millimeters to 30 cm in diameter. They most frequently occur on the neck, eyes, flippers, jaw, tail, and sometimes even in the mouth. Fibropapillomas have been recorded in turtles from 45 cm juveniles to adult males and females over 85 cm. However, thus far none have been found in the 35-45 cm range, the minimum size at which recruitment to benthic habitat takes place in Hawaii.

During 1985, 29% of the 56 stranded turtles examined throughout Hawaii had fibropapillomas. Local divers and fishermen regularly report seeing green turtles with "tumors" in coastal foraging pastures and at underwater sleeping areas. A considerable increase in these sightings is believed to have occurred over the past 20 years.

Fibropapillomas in green turtles were first described in the scientific literature in the late 1930's. Three out of 200 green turtles (60 to 200 lb) examined in 1937 at Key West, Florida, had these growths. Since the RTLA (located at the Smithsonian Institution) began accepting specimens in 1965, fibropapillomas from green turtles have been verified from the Florida Keys (RTLA 12 & 651) and Hawaii (RTLA 121, 1767, 1774, 1856, 1883 & 2097). Several neoplasms from Cayman Turtle Farm have recently been submitted but as yet have not been cataloged or histologically identified. Immature green turtles in east central Florida have been reported by Lew Ehrhart to recently show a high incidence of similar fibrous growths. Thus far, no other species of sea turtle except for the green turtle has been documented with fibropapillomas.

Fibropapillomas on Hawaiian green turtles can result in reduced vision, disorientation, blindness, physical obstruction to normal swimming and feeding, and an apparent increased susceptibility to parasitism by the marine leech, <u>Ozobranchus branchiatus</u>. Our available evidence also suggests that fibropapillomas can cause severe emaciation, increased predation by tiger sharks and humans, and probably a reduced ability to successfully migrate and breed. Entanglement in fishing line and other gear also appears to be more likely in turtles afflicted with these growths.

The etiology of fibropapillomas in green turtles remains unknown. Possible causes that have been suggested include an immune reaction to trematode ova, secretion of hirudin by marine leeches, virus, excessive solar radiation, chemical pollutants that impair the immune system, stress, and a genetic predisposition to neoplasia. Biopsy material from fibropapillomas on two Hawaiian green turtles were sent to the RTLA for electron microscopy, but no virus was found.

The growth rate of fibropapillomas has been documented in two Hawaiian green turtles. A nesting female with no signs of neoplasia when first seen was recovered 3 years later in an emaciated state with a large fibropapilloma along the dorsal base of its tail. Another apparently healthy tagged turtle, an adult male, had numerous growths, including a large mass in the axilla, when seen again just 2 years later. The fact that relatively small turtles can be heavily afflicted with fibropapillomas indicates that growth can occur fairly fast under certain conditions.

The experimental treatment of fibropapillomas in Hawaii has included surgical removal and strangulation. Neither procedure proved satisfactory due to the large number of growths often present, their highly vascular nature when large, and the apparent presence of nerve bundles that transmit pain, especially in growths associated with the eyes. There is no evidence to suggest that fibropapillomas in Hawaiian turtles may spontaneously cease or diminish.

The Hawaiian population of green turtles is geographically isolated and relatively small, consisting of only a few hundred females nesting annually at French Frigate Shoals. A recovery team has recently been appointed to formulate a plan to rehabilitate the population. The occurrence of fibropapillomas is viewed as one of several problems that urgently need to be addressed through additional research.

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INCIDENCE OF FIBROPAPILLOMAS IN HAWAIIAN GREEN TURTLES

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KAILUA TURTLE

National Museum of Natural History · Smithsonian Institution
WASHINGTON, D.C. 20560 · TEL. 202· 381-5936

29 July 1977

Dr. George H. Balazs
Hawaii Institute of Marine Biology
University of Hawaii at Manoa
Post Office Box 1346
Coconut Island
Kaneohe, Hawaii 96744

Dear Dr. Balazs:

Thank you very much for sending the Registry six pieces of tissue consisting of rugose growths attached to the skin of the lower jaw, right limb, and surrounding both eyes of a Chelonia mydas specimen (our accession number RTLA 1767). These growths grossly resemble fibropapillomas that previously have been seen on green sea turtles, which have been associated with leeches, algae, or other foreign material. Microscope slides and a diagnosis will be sent as soon as possible.

We would appreciate receiving a series of color transparencies at your convenience.

Sincerely yours,

Phyllis M. Schellenger

Phyllis M. Schellenger
Registrar
Registry of Tumors in
Lower Animals

Enclosures - Activities Reports

12 December 1977

1856

TEAR OUT ACCESSION SHEET

PLEASE SUBMIT TO:

Dr. John C. Harshbarger
Registry of Tumors in Lower Animals
National Museum of Natural History
Room W216-A
Smithsonian Institution
Washington, D.C., 20560, USA

DATE RECD. ________
CONTRIBUTOR'S NO. ________
RTLA NO. ________
USNM NO. ________
NIH HISTOPATH NO. ________

COMMON NAME green turtle
FAMILY cheloniidae
PHYLUM chordata
GENUS chelonia
CLASS reptilia
SPECIES mydas
ORDER chelonia

CATEGORY: NEOPLASM X INFLAMMATION ___ INFECTION ___ PARASITIC ___ TOXIC ___
DEVELOPMENTAL ___ TRAUMATIC ___ NORMAL ___ OTHER ___

CONTRIBUTOR (NAME & ADDRESS):
George H. Balazs
Hawaii Institute of Marine Biology
P.O. Box 1346
Kaneohe, HI 96744

COLLECTOR (NAME & ADDRESS):
same

ITEMS SUBMITTED (QUANTITY):

GROSS MATERIAL 3 bags containing a total of 6 specimens
PHOTOGRAPHS color transparencies of the live animal available upon request
SLIDES ________
REPRINTS ________
BLOCKS ________
OTHER ________

ORIGIN OF ANIMAL: MARINE X FRESHWATER ___ ESTAURINE ___ TERRESTRIAL ___

WHERE COLLECTED Kaneohe Bay, Island of Oahu, Hawaii

DATE COLLECTED 30 Nov 77 SEX unk AGE unk STAGE OF LIFE CYCLE immature

HOW KILLED barely alive when frozen FIXATION 10% formalin

GROSS DESCRIPTION Extensive tumors present on immature green turtle (straight carapace length-18 3/4 ; straight carapace width- 14 3/4; weight 27 lbs) found floating at the surface, weak, very little life. Animal was subsequently frozen while still alive. Dissection revealed that the stomach and intestinal tract were full of food (benthic algae). This is interesting in view of the fact that both eyes appear to be covered over with tumors. The three bags of material submitted contain the following: (1) two complete front flippers with associated pectoral musculature; (2) complete pelvic section, including both hind flippers and tail; (3) head and neck, heart, and section of lung.

MICROSCOPE DX ________

COMMENTS (e.g., possible exposure to chemical pollutants or infectious agents, incidence and duration of this condition in the population, etc.)- unknown

(Please use reverse side, if more space is needed)

TUMORED TURTLE
1958 JOHN NAUGHTON
AND FRIENDS KANEOHE BAY

FEB 1958

1/25/58

Turtle taken off Kekepa Islet (Turtle Rock), Kaneohe Bay. J. Naughton

October 23, 1974

Dr. John C. Harshbarger, Director
Registry of Tumors in Lower Animals
Smithsonian Institution
Washington, D. C. 20560

Dear Dr. Harshbarger:

You may recall that I corresponded with you several months ago concerning the incidence of tumors in green sea turtles. I have only recently returned from French Frigate Shoals (23^{o} 45' N 166^{o} 10' W) where studies were conducted on the nesting colony for the second consecutive season. Approximately 10% of the adults observed (including both sexes) were seen to have tumors. Sizes ranged from 2 cm in diameter on up to approximately 30 cm. Of particular interest was what appeared to be a relatively high incidence of growths which originated from the eye. I have enclosed a photograph which displays one such animal (with my compliments for your reference collection). The area of growth did not originate from the iris or pupil and, therefore, appeared to present only a physical barrier to normal vision. I am particularly interested in this aspect from the standpoint of long range migration as the animals in question were apparently able to successfully complete their voyage to the atoll.

I would very much like to obtain a copy of the Registry's most recent activities report. Unfortunately, this report, which you mentioned in your letter of April 24th, was never received.

I have enclosed a paper which describes a portion of my green turtle work during 1973.

Best wishes and Aloha!

Sincerely,

GEORGE H. BALAZS
Jr. Marine Biologist

GHB:ec

Enclosures (2)

National Museum of Natural History · Smithsonian Institution
WASHINGTON, D.C. 20560 · TEL. 202- 381-5936

10 August 1979

Dr. George H. Balazs
Hawaii Institute of Marine Biology
University of Hawaii at Manoa
P. O. Box 1346
Coconut Island
Kaneohe, Hawaii 96744

Dear Dr. Balazs:

We appreciated receiving the two growths removed from the ventral neck region of a green turtle, Chelonia mydas, specimen (your tag number 2828; our RTLA 2097). Dr. Harshbarger will be in touch regarding his opinion of this case as soon as possible.

Thank you very much for your continued interest in the Registry.

Sincerely,

Phyllis M. Schellenger

Phyllis M. Schellenger
Registrar
Registry of Tumors in Lower Animals

P.S.

Please send color transparencies at your convenience.

TEAR OUT ACCESSION SHEET

PLEASE SUBMIT TO:

Dr. John C. Harshbarger
Registry of Tumors in Lower Animals
National Museum of Natural History
Room W216-A
Smithsonian Institution
Washington, D.C., 20560, USA

DATE RECD. ______
CONTRIBUTOR'S NO. ______
RTLA NO. ______
USNM NO. ______
NIH HISTOPATH NO. ______

COMMON NAME green turtle
FAMILY Cheloniidae
PHYLUM Chordata
GENUS Chelonia
CLASS Reptilia
SPECIES mydas - Hawaiian population
ORDER Chelonia

CATEGORY: NEOPLASM x INFLAMMATION ___ INFECTION ___ PARASITIC ___ TOXIC ___
DEVELOPMENTAL ___ TRAUMATIC ___ NORMAL ___ OTHER ___

CONTRIBUTOR (NAME & ADDRESS):
George H. Balazs
UNIVERSITY OF HAWAII
Hawaii Institute of Marine Biology
Coconut Island • P. O. Box 1346 • Kaneohe, Hawaii

COLLECTOR (NAME & ADDRESS):
same
UNIVERSITY OF HAWAII
Hawaii Institute of Marine Biology
Coconut Island • P. O. Box 1346 • Kaneohe, Hawaii 96744

ITEMS SUBMITTED (QUANTITY):

GROSS MATERIAL one jar-numerous sections
PHOTOGRAPHS ______
color SLIDES available upon request
REPRINTS ______
BLOCKS ______
OTHER ______

ORIGIN OF ANIMAL: MARINE x FRESHWATER ___ ESTAURINE ___ TERRESTRIAL ___

WHERE COLLECTED Trig Island, French Frigate Shoals, Northwestern Hawaiian Islands

DATE COLLECTED 17 June 79 SEX male AGE unknown STAGE OF LIFE CYCLE sexually mature- adult

HOW KILLED ______ FIXATION 5% GlutCHO in buffer (supplied by your lab)

GROSS DESCRIPTION small neoplasms (2) growing on skin of ventral neck region; growths were completely removed and sectioned ; no bleeding occurred

Tag 2828

MICROSCOPE DX ______

COMMENTS (e.g., possible exposure to chemical pollutants or infectious agents, incidence and duration of this condition in the population, etc.)

(Please use reverse side, if more space is needed)

National Museum of Natural History • Smithsonian Institution
WASHINGTON, D.C. 20560 • TEL. 202- 381-5936

14 May 1979

Dr. George H. Balazs
Hawaii Institute of Marine Biology
University of Hawaii at Manoa
P. O. Box 1346
Coconut Island
Kaneohe, Hawaii 96744

Dear Dr. Balazs:

Linda, Phyllis, S. C. (Chang), and I were delighted to meet you.

The tissue mass, associated with the intestinal mesentery of your hawksbill turtle, Eretmochelys imbricata, (RTLA 2042) that we received in January 1979, is composed almost entirely of mature fat cells. These cells are in a honeycomb arrangement. Their nuclei are flattened against the cell wall due to the build-up of stored fat in the cytoplasm. The fat was dissolved by alcohol during histologic processing leaving a cytoplasm composed largely of empty storage vesicles. Neoplastic fat tissue is usually so well differentiated that it is difficult to distinguish a lipoma from normal fat. In this case it looks normal, it occurred in a normal location, and since the turtle was feeding well on fish, the mass probably represents normal fat body rather than a lipoma. A set of microslides from the three tissue blocks is being sent.

I am also sending the fourth microslide (RTLA 1774.3) from a series of eight tissue blocks from a Chelonia mydas. Microslides from RTLA 1774.1, 1774.5, and 1774.6 had been sent earlier. The tissue in the remaining blocks from this case, RTLA 1774.2, 1774.4, 1774.7, and 1774.8, are so friable I doubt if we will ever get decent microslides. The external lesions in this case are fibropapillomas as I pointed out in my letter of 6 March 1978. The lung lesions, seen clearly in one of the two microslides from RTLA 1774.1 are fibromas. Hans G. Schlumberger and Balduin Lucké (Tumors in Fishes, Amphibians and Reptiles, 1948, Cancer Res., 8:657-753) noted (on page 702) similar lung lesions, on green sea turtles with integumentary fibropapillomas, which they thought were more likely to have originated in situ than to represent metastases from integumentary tumors. All tumors examined in your case, RTLA 1774, have a benign look and I believe as Schlumberger and Lucké did that they arose de novo -- perhaps from a virus.

Finally, three microslides and several electron microscope photographs of the fibropapillomas from another Chelonia mydas specimen (RTLA 1883) are being sent. The EM did not show virus. As discussed and at your convenience, we need glutaraldehyde-fixed material from fresh normal skin, and very small early lesions in both skin and lungs for further study. For best fixation make one dimension of each piece less than 2 mm thick. Glutaraldehyde is being sent -- keep refrigerated until ready -- then use 10 to 20 times the volume of fixative per volume of tissue. If the items have not arrived safely, please let us know and we will send another set.

Best personal regards,

John

John C. Harshbarger, Ph.D.
Director
Registry of Tumors in Lower Animals

National Museum of Natural History · Smithsonian Institution
WASHINGTON, D.C. 20560 · TEL. 202- 357-2647

17 October 1980

Dr. George H. Balazs
Assistant Marine Biologist
Hawaii Institute of Marine Biology
University of Hawaii at Manoa
P. O. Bcx 1346
Coconut Island
Kaneohe, Hawaii 96744

Dear George:

Thank you for sending the article mentioning the Registry of Tumors in Lower Animals: Shadduck, John A. and James B. Murphy. 1979. Suggestions for the Post-Mortem Examination of Reptiles. Herp Rev., 10: 113-115. I would not have see it otherwise.

We have just processed more of your green sea turtle fibropapillomas for electron microscopy but unless we find something virus-like, we will defer future effort to my friend, Dr. Elliott Jacobson, University of Florida, and his collaborator, Dr. Harry Haines of Miami, who are attacking the problem with a high level of commitment and sophisticated experimental virology. They are working closely with the commercial green turtle rearing project in the Cayman Islands, which is having a high prevalence of fibropapilloma in their turtles and it seems to spread as if it is caused by an infectious agent.

Best personal regards,

John

John C. Harshbarger
Director
Registry of Tumors in Lower Animals

February 11, 1980

Dr. John C. Harshbarger, Director
Registry of Tumors in Lower Animals
Smithsonian Institution, Rm. W216A
Washington, D. C. 20560

Dear John:

Many thanks for your letter of 6 February 1980 describing the results of RTLA 2097. I was particularly interested to learn that no virus had thus far been found in this specimen. From your letter, I received the impression that this information had already been communicated to me at an earlier date, however, this has not been the case. Perhaps a letter to me was lost in the mail during recent months. Nevertheless, the cause of these fibropapillomas in Hawaiian (and other) green turtles remains unclear, and I truly wish that there was something I could do to help resolve the situation.

In the event that it has not been brought to your attention, I have a recent article from Herpelogical Review which mentions your program.

Sincerely,

GEORGE H. BALAZS
Assistant Marine Biologist

GHB:ec

Enclosure

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Southwest Fisheries Center
Honolulu Laboratory
P. O. Box 3830
Honolulu, Hawaii 96812

January 28, 1982 F/SWC2:GHB

Sea Turtle Stranding Report

Date of stranding - October 24, 1981

Location - Kailua Beach, Island of Oahu, State of Hawaii (approximately 21°24'N 157°43'W)

Species - Chelonia mydas (green turtle)

Straight carapace length- 60.5 cm

Condition - Barely alive

Observation and remarks - Many fibropapillomas were present around the eyes and junctions of flippers to the body. Nodules varied in size from a few millimeters to 6-7 cm. Some were ulcerated and necrotic. Many unidentified small ecoparasites were attached to the epidermis of these nodules.

The major visceral organs appeared grossly normal. Ascitis was minimal. The stomach and intestines were full of algal food material. Pulmonary abscesses and necrotic foci were noted by light microscopy in sections of the liver and kidney. Vibrio parahemolyticus and Acinetobacter lwoffi were isolated from a lung swab.

Person that found the turtle - Robert Bourke

Person that conducted examination - Dr. Thomas Sawa
Chief, Veterinary Laboratory
Department of Agriculture
State of Hawaii

Persons submitting this report to SEAN - William G. Gilmartin and George H. Balazs
NMFS, Honolulu Laboratory
P. O. Box 3830
Honolulu, HI 96812

VL-3
?/81

TMR file
60.5 cm

State of Hawaii
Department of Agriculture
DIVISION OF ANIMAL INDUSTRY
Veterinary Laboratory

#7272

Date: December 3, 1981

Accession No.: 81-10-153

Dear Doctor: --------

The specimen that you submitted on October 28 described as approximately

? 2-year-old female Green Sea Turtle, National Marine Fisheries Center, Kewalo Basin

has been examined. The observations and remarks are:

The turtle was found beached on Kailua on Saturday, October 24th. There were many skin nodules and it was listing to the right in the tank. Euthanasia was by decerebration.

Many skin nodules, varying from few mm in diameter to 6-7 cm were seen around the eye, including the eye-lids virtually closing the eyes, neck and junctions of flippers to the body. Some of the nodules were ulcerated and necrotic. Many unidentified small flukes were found attached to the epidermis of these nodules. The internal portion of the nodules was soft, pliable, whitish and appeared fibrous.

The major visceral organs appeared grossly normal. Ascitis was minimal.

There was much brownish "algae" in the stomach and much intestinal contents.

By light microscopy, although grossly normal, pulmonary abscesses and necrotic foci in sections of the liver and kidney sections were noted.

Vibrio parahemolyticus and *Acinetobacter lwoffi* were isolated from a lung swab. Gut contents were negative for parasites.

Except for the obvious anemia, the results of the differential picture of the hemogram was difficult to interpret due to my inexperience in this particular species. The results of the Ca - P - mg blood levels are skewed, probably not real.

The skin nodules were fibromas, but whether or not they were induced by the flukes were undetermined. However, the sites of the fluke attachments involved only the epidermis; whereas the nodule involved only the dermis. Therefore, it would appear that the fibroma was not induced by chronic irritation (fluke).

Thomas R. Sawa

THOMAS R. SAWA, DVM, Ph.D.
Chief, Veterinary Laboratory

cc: Division Head
Chief, LDC
Dr. W. Gilmartin ✓
Dr. R. Nakamura
Dr. J. Brock
Mr. Bob Burke

Table 11.--Histology report by Grant N. Stemmermann, M.D.,[1] for a green turtle found dead at Kawela Bay, Oahu, on March 28, 1985.

Lungs:	There is marked enlargement of the alveolar spaces, associated with greatly increased amounts of smooth muscle in the alveolar walls. The alveolar septa contain numerous embryonated helminthic eggs, most of which are surrounded by multinucleated histiocytes. The pulmonary arteries, especially the large- and medium-sized vessels, show extensive intimal thickening by smooth muscle. This almost obliterates the lumens of some vessels.
Liver:	The lobular architecture is fairly well preserved, although there are large numbers of hemosiderin-laden histiocytes through all portions of the lobules. Ova similar to those in the lungs are found within the lobules, but are fewer in number and are associated with a less conspicuous host reaction. A single, fairly large segment of the portal vein has a markedly thickened intima and is surrounded by clusters of round cells.
Small intestine:	There is too much autolysis to distinguish much cytologic or even histologic detail in the mucosa. However, the configuration of the villi suggest that there has been marked mucosal damage and inflammation, associated with the accumulation of many round cells in the lamina propria. The mucosa and submucosa contain occasional helminthic ova. They are larger than those in the liver and lungs.
Kidney:	The configuration of the flomeruli, some in the process of subcapsular development, suggest that this animal is not yet mature. Present in both the capsule and the interstitium of the cortex are occasional ova similar to those in the liver and lung. The renal artery is markedly thickened in its intimal aspects and also shows distinct endothelial hyperplasia. There is a single glomerulus that shows cellular infiltration, adhesions between capillary and capsule and basement membrane thickening. This glomerulus is surrounded by a dense cuff of round cells. There is one embryonated ovum within a distal convoluted tubule. This egg is not associated with either cell reaction or tubular damage.

Table 11.--Continued.

Conclusions:	This animal apparently succumbed to the effect of cardiovascular fluke infection (Diginea: Spirorchidae). The most extensive tissue damage was found in the lungs, which showed extensive interstitial fibrosis and severe pulmonary sclerosis consistent with debilitating right heart failure; and of the small gut where there was extensive mucosal necrosis and ulceration. The latter would have been inconsistent with adequate nutrition. Renal involvement was associated with focal embolic glomerulonephritis. The presence of embryonated eggs in undamaged distal convoluted tubules suggests that some eggs may be passed in the urine.

---

[1] 46-458 Haiku Plantation Drive, Kaneohe, HI 96744.

Department of Zoology
University of Canterbury Christchurch 1 New Zealand

25 Nov. 1985.

HAPALOTREMA

Linda D. Banish,
National Marine Fisheries Service,
Southwest Fisheries Centre,
Honolulu Laboratory,
P.O. Box 3830, Honolulu,
Hawaii 96812.

Dear Linda,

Thank you for your letter of 15th October and the specimens you sent. This is our end-of-year exam marking season, and I have been rather preoccupied with that lately. My apologies for taking so long to reply to you. I still haven't spent as much time on your specimens as I would like, but I thought I should get this letter away now, or Christmas might come and go before you heard from me.

I have had a quick look at the cestode cysts (?) At this time, I don't think they are cysts. I dissected one out, and couldn't be sure what it was. I should get more time in the next few weeks to examine the material more carefully.

The fluke was a lot easier. It is a member of the genus Hapalotrema (family Spirorchiidae). I have enclosed a photocopy of a drawing of this type of beast. The specimen you sent lacked an anterior end. By the look of it, when you tried to remove it from the artery, it tore just behind the ventral sucker, with which it was probably clinging to the artery wall. The posterior end was also bent over and obscured some of the details there. The specimen was quite well fixed and easy to work with. Hapalotrema and its relatives that live in the blood vessels seem to be very pathogenic in sea turtles, much as schistosomes are in mammals. They have a snail intermediate host. Cercariae released from the snail penetrate directly (as far as we know) into the turtle. growing on algae ?

Your method of fixing seems O.K. The general rules I tell people are that a) formalin is generally better than alcohol as a fixative if the specimens are to be used for later preparations (whole mounts or sections) and b) hot fixatives generally produce a satisfactory specimen if the parasites are still alive. Cold fixatives do not kill the specimens quickly enough to prevent their contracting strongly when the fixative comes into contact with them. In the case of flukes, specimens held flat, or very slightly compressed during fixation, are easier to work with later. Their internal organs are easily seen. However, a lot of people tend to over-flatten flukes when they try to do this. The result is a squashed, distorted specimen. Only experience will tell you how much pressure can be applied. I make mistakes myself, so, if I have a few specimens, I often flatten one or two, and fix the rest with hot (70°C) formalin. That way I hope to get something useful. If in doubt, simply fix with hot formalin and don't worry about trying to flatten the specimens.

As far as references etc. go, I have sorted out a few that might be of use. If I think of any more, or come across useful ones, I'll let you know. That's it for the time being. Sorry this has been rather rushed. I wish you interesting finds in future necropsies! Best wishes

David

David Blair

JAMES CHOW

SHIPPED 3/28/86

TEAR OUT ACCESSION SHEET

PLEASE SUBMIT TO:

Dr. John C. Harshbarger
Registry of Tumors in Lower Animals
National Museum of Natural History
Room W216-A
Smithsonian Institution
Washington, D.C., 20560, USA

DATE RECD. ______
CONTRIBUTOR'S NO. ______
RTLA NO. ______
USNM NO. ______
NIH HISTOPATH NO. ______

COMMON NAME Green Turtle
FAMILY ______
PHYLUM ______
GENUS Chelonia
CLASS ______
SPECIES mydas
ORDER ______

CATEGORY: NEOPLASM X INFLAMMATION ___ INFECTION ___ PARASITIC ___ TOXIC ___
DEVELOPMENTAL ___ TRAUMATIC ___ NORMAL ___ OTHER ___

CONTRIBUTOR (NAME & ADDRESS): G.H. BALAZS

COLLECTOR (NAME & ADDRESS): G.H. BALAZS

NATIONAL MARINE FISHERIES SERVICE
HONOLULU LABORATORY
P. O. BOX 3830
HONOLULU, HAWAII 96812

ITEMS SUBMITTED (QUANTITY):
GROSS MATERIAL 2 JARS
SLIDES ______
BLOCKS ______
OTHER ______

ORIGIN OF ANIMAL: MARINE X FRESHWATER ___ ESTUARINE ___ TERRESTRIAL ___
WHERE COLLECTED HALEIWA, IS. OF OAHU, HAWAII

DATE COLLECTED 3-26-86 SEX F? AGE ______ STAGE OF LIFE CYCLE IMMATURE
HOW KILLED ______ FIXATION 10% BUFFERED FORMALIN

GROSS DESCRIPTION CURVED CARAPACE LENGTH - 73.5 cm
SMALL PAPILLOMAS ON EYES, NECK AND FLIPPERS. SIMILAR(?) GROWTH FOUND IN THE MOUTH BLOCKING GLOTTIS. TURTLE FOUND IN A LETHARGIC STATE FLOATING AT THE SURFACE. IT DIED A FEW HOURS LATER IN CAPTIVITY. TUMORS COLLECTED AND PRESERVED SHORTLY AFTER DEATH. 1 JAR OF TUMORS FROM THE MOUTH

MICROSCOPE DX 1 JAR OF TUMORS FROM THE EYES, NECK & FLIPPERS

COMMENTS (e.g., possible exposure to chemical pollutants or infectious agents, incidence and duration of this condition in the population, etc.)
SUBSAMPLES FROM EACH JAR ARE ALSO BEING SENT TO DR. FRED SMITH AT THE UNIVERSITY OF GEORGIA. VOLUME OF PRESERVATIVE REDUCED FOR SHIPPING

(Please use reverse side, if more space is needed)

MARCH 1986

6TH ANNUAL WORKSHOP ON
SEA TURTLE BIOLOGY AND CONSERVATION

Wednesday (19 March 1986)

3:00 pm - 10:00 pm	Registration
6:00 pm - 8:00 pm	Cookout
8:00 pm - 10:00 pm	Social hour and Poster Session

Thursday (20 March 1986)

8:00 am - 12:00 noon Registration

8:15 am - 8:30 am Announcements

8:30 am - 10:00 am SESSION I "NESTING BEACH MANAGEMENT"- E. Possardt, Chr.

Paper Presentations

X 8:30 am Production and success of the loggerhead and green turtle nesting colony at Melbourne Beach, Florida in 1985.
Blair Witherington and Llewellyn M. Ehrhart. Florida Central U.

X 8:45 am A local government's response to lighting disorientation of hatchlings: an oceanfront lighting ordinance.
Teresa A. Kramer, Envir. Resource Specialist. Brevard County, Florida.

X 9:00 am Turtle nest screening at Canaveral National Seashore.
Richard Bryant, Park Ranger, Canaveral NS, Florida.

X 9:15 am The value of hatcheries.
C. Robert Shoop, U/Rhode Island

X 9:30 am Assessment of 1985 predator control project along Kennedy Space Center Beach.
Jane Provancha, Bio-2, Kennedy Space Ctr., FL

X 9:45 am Panel Discussion on Nesting Beach Management

Earl Possardt, Ross Witham, Lew Ehrhart, Sally Murphy

10:15 am - 10:45 am Coffee Break

Thursday, March 20 continued

10:45 am - 12:00 noon SESSION II "BEACH NOURISHMENT" - Ross Witham, Chr.

Paper Presentations:

10:45 am Activities on major nesting beaches: a growing problem.
Paul W. Raymond, NMFS, St. Petersburg, FL

11:00 am Sea turtle protection, 1985, in conjunction with the South Beach nourishment project.
Richard Wolf, Chief Environmental Officer, City of Boca Raton, FL

11:15 am Effects of sand compaction on nesting sea turtles.
Dave Nelson, US Army Waterways Experiment Station, MS.

11:30 am Panel Discussion on Beach Nourishment

Ross Witham, Nora Murdock, Richard Wolf, Dave Nelson, Paul Raymond, Ed Standora

12:00 noon - 1:00 pm Lunch

1:30 pm - 3:00 pm SESSION III "KEMP'S RIDLEY" - Peter Pritchard, Chr. (Jack Woody)

Paper Presentation

1:30 pm Analysis of eight years work in Rancho Nuevo.
Rene Marquez M.

1:45 pm Progress in the head-start phase of Kemp's ridley sea turtle recovery.
Charles W. Caillouet, Jr. NNMFS, Galveston, TX

2:00 pm Disbribution and frequency of Kemp's ridley strandings along the Atlantic & Gulf coast, US (1980-85). Barbara Schroeder, NOAA/NMFS, Miami

2:15 pm Kemp's ridley activities at Padre Island, Texas.
Milford Fletcher, NPS

2:30 pm Panel Discussion on Kemp's Ridley

Peter Pritchard, Charles Caillouet, Rene Marquez, Jack Woody, Larry Ogren

3:00 pm - 3:30 pm Coffee Break

Thursday, March 20, continued

3:30 pm - 5:00 pm SESSION IV "TED IMPLEMENTATION" - Tom Murphy, Chr.

Paper Presentations

X 3:30 pm Status of the TED.
Chuck Oravetz, NMFS, St. Petersburg, FL

X 3:45 pm Commercial fishermen's view of the turtle problem and the use of TEDs.
David L. Harrington, Sea Grant, GA

X 4:00 pm The conservation communities' view.
Michael Weber, CEE

X 4:15 pm FWS viewpoint.
Jack Woody, FWS, Albuquerque, NM

X 4:30 pm Panel Discussion on TED Implementation

Tom Murphy, Jack Woody, Chuck Oravetz
Joe Webster, David Harrington,
Michael Weber

6:00 pm - 7:00 pm Dinner

7:00 pm - 8:00 pm SPECIAL SESSION "SEA TURTLE RESEARCH & CONSERVATION IN COSTA RICA", Karen Bjorndal, chr.

7:00 pm Status of marine turtles on the Pacific American coast.
Douglas C. Robinson, Universidad de Coasta Rica

7:15 pm The first step of the WIDECAST Carribean Action Plan in Costa Rica
Ana C. Chaves Q.

7:25 pm Sex determination, by temperature, in the olive ridley turtle (Lepidochelys olivacea) in Ostional Costa Rica.
Adrian Ugalde

7:35 pm Potential significance of nesting solitary turtles, Lepidochelys olivacea, on the Pacific coast of Costa Rica.
Juan Carlos Castro

7:45 pm Hatching success in the natural nests of the olive ridley at Ostional Beach, Costa Rica.
Mario Alvarado

8:00 pm - 10:00 pm Social Hour and Poster Sessions

Friday (21 March 1986)

8:15 am - 8:30 am Announcements

8:30 am - 10:00 am SESSION V "STRANDINGS AND SITINGS" - Douglas Beach, Chr.

Paper Presentations

8:30 am Sea turtles in northern waters; insights through the study of stranded sea turtles. Robert Prescott, Wellfleet Bay Wildlife Sanctuary, So. Wellfleet, MA

8:45 am Cold Stunning of Kemp's Ridley Sea Turtles in Long Island and Possible Implications. Sam Sadove, Marine Mammal Stranding Center. and Ann Meylan.

9:00 am Frequency and distribution of strandings and sitings along New Jersey coast and Delaware Bay. Bob Schoelkopf, Delaware DNR

9:15 am Florida sea turtle stranding, 1980-84. Walter Conley, FL DNR, St. Petersburg, FL

9:30 am Panel Discussion on Strandings and Sitings

Douglas Beach, Robert Prescott, Fred Berry, Bob Schoelkopf, Jack Musick, Walter Conley

10:00 am - 10:30 am Coffee Break

10:30 am - 12:00 noon SESSION VI "TELEMETRY" - George Balazs, Chr.

Paper Presentations

10:30 am Preliminary results of satellite telemetry and sub-adult loggerhead movements. Richard Byles, VIMS, Gloucester Point, VA.

10:45 am A telemetric study of leatherback sea turtle behavior in Narragansett Bay, RI. J. Keinath

11:00 am Movements of translocated loggerhead turtles using radio telemetry. Tom Murphy, SCWMRD, Green Pond, SC.

11:15 am Panel Discussion on the value of Telemetry in Recovering Sea Turtle Populations

George Balazs, Tom Murphy, Richard Byles, J. Keinath, NMFS rep., Ed Standora

Friday, March 21 continued

12:00 noon - 1:00 pm Lunch

1:30 pm - 4:00 pm SESSION VII "POPULATION BIOLOGY" - Nat Frazer, Chr.

Paper Presentations

1:30 pm Population sizes, growth rates and movements of green turtles and hawksbills in the USVI.
Ralf Boulon, Jr. US Virgin Islands

1:45 pm Growth rates of immature green turtles and loggerheads on the southern Bahamas.
Karen Bjorndal & Alan Bolton, U/FL, Gainesville

2:00 pm The reproductive characteristics of loggerhead turtles at Wassaw Island, GA. 1973-85.
Joe Hendricks, Mercer U., Macon, GA. and Savannah Science Museum, Savannah

greater photo
(1) loggerhead straight on
(2) Tracked turtle back to the beach

2:15 pm Age, growth, survival and mortality in loggerhead turtles estimated from tag-recapture experiments.
Tyrrell Henwood, NOAA/NMFS Pascagoula, MS

2:30 pm Incidence of Papillomatosis in Hawaiian Green Turtles.
George Balazs, NOAA/NMFS, Honolulu, HI

2:45 pm A preliminary investigation of Papillomatosis in green turtles.
Llewellyn Ehrhart, U/Central Florida, Orlando

3:00 pm - 3:30 pm Coffee Break

3:30 pm Panel Discussion on Population Biology

Nat Frazer, Ralf Boulon, Karen Bjorndal, Lew Ehrhart, Tyrrell Henwood, George Balazs

4:15 pm - 5:30 pm SESSION VIII - SUMMARY - Jack Woody chr.

Fred Berry, Nat Frazer, Tom Murphy, Douglas Beach, Jack Woody, Ross Witham, Peter Pritchard, George Balazs.

POSTERS

Wednesday 8:00 pm - 10:00 pm

"Awareness of Protection"	Bruce Jaildagian Greenpeace, Jacksonville, FL
"Nest-site Selection by Loggerheads: Is Sand Temperature the Only Important Cue?"	Tom Mann Clemson U., S. C.
"T. E. D."	Chuck Oravetz & Paul Raymond NOAA/NMFS, St. Petersburg, FL
"The Conservation Practices of Biologically Sacrificing Sea Turtles"	Craig Smith, Lyle Kochinsky Robert Steinborg Eco-Search, Inc., Dania, FL
"Leatherback Sea Turtles: Tag Use, Tag Loss"	Karen Eckert U/Georgia, Athens, GA
"Harnessing the Leatherback Sea Turtle for Research"	Scott Eckert U/Georgia, Athens, GA

Thursday 8:00 pm - 10:00 pm

"The Release of Sea Turtles by Cayman Turtle Farm"	Fern Wood Cayman Turtle Farm, BWI
"Age and Growth Studies on Loggerhead Sea Turtles in Chesapeake Bay"	Ruth Klinger VIMS, Gloucester Point, VA
"Blood Chemistry and Health of Sea Turtles in Virginia"	Sarah Bellmund VIMS, Gloucester Point, VA
"The Relationships of Bodyform to Locomotion in Sea Turtles"	Jeanette Wyneken U/Illinois, Urbana, IL
"Sperm Storage in Turtles"	Jess Jones U/Cincinati, Ohio
"Preliminary Results from Heron Island Australia Sea Turtle Rodeo" (with VCR tape)	Dave Owens Texas A&M U, College Sta., TX
"Sea Turtle Nest Protection"	Mary Adele Donnelly CEE, Washington, DC
"Florida Light Campaign"	Mary Adele Donnelly CEE, Washington, DC

TMR FILE

TURTLE ISLANDS NATIONAL PARK
BOX 768, SANDAKAN, SABAH, MALAYSIA.
CABLES "NATAPARK", SANDAKAN. TEL. 2188

PAR AVION AIR MAIL

Your Ref:-

Our Ref:- ECPRO/11/4/38

Date: 3rd April, 1986

Mr. George Balazs,
Zoologist,
NOAA, National Marine Fisheries Service,
SWFC Honolulu Laboratory F/SWC2,
2570 Dole Street,
Honolulu, HI 96822-2396,
U.S.A.

Dear George,

Many thanks for your letter of 25th March, 1986 and note that the turtle is an Olive ridley. It is the first time that this species has been recorded in Sabah - at Pulau Selingaan - Turtle Islands Park.

In Sarawak, it is found in small numbers on the Turtle Islands. It is also common on the east coast of West Malaysia mainly in Terengganu, Kelantan and Pahang. It is rare on the West Coast. Harvestors on the East Coast had reported a decline in Ridley numbers. In Indonesia, it nests in several places, but not in large concentrations. In general, populations are said to have declined.

You are welcome to keep the photographs. If enlargements, turn out to be clear, I will send you another for record purposes. Presently, we are awaiting the fate of the nest in the hatchery. Will keep you informed of the outcome.

In 1965, when I first started visiting Pulau Selingaan, one nesting Green turtle was observed with a large mass growing on the the first and second neural scutes. As attempts at removal of the mass for laboratory examination caused the animal great pain, it was permitted to return to the sea. It is the only animal, I have seen with this type of affliction.

I am afraid conferences are presently out because of government financial constraints. It is indeed a great pity that in this part of the world there is no exposure to other professionals and their views on related projects.

With all good wishes.

Regards.

Yours sincerely,

Stanley

G. S. de Silva.

PAR AVION

AIR MAIL

LEMBAGA PENGUASA AMANAH
TAMAN NEGARA SABAH

Mr. George Balazs,
Zoologist,
NOAA, National Marine Fisheries Service,
SWFC Honolulu Laboratory F/SWC2,
2570 Dole Street,
Honolulu, HI 96822-2396,
U.S.A.

← Second fold here →

Sender's name and address: Turtle Islands National Parks
P. O. Box No: 768 /Sandakan, 90008
Sabah, East Malaysia

← To open cut here

'TULIP' Air Letter

AN AIR LETTER SHOULD NOT CONTAIN ANY ENCLOSURE: IF IT DOES IT WILL BE SURCHARGED OR SENT BY ORDINARY MAIL.

Papillomas & C. mydas

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Southeast Fisheries Center
Miami Laboratory
75 Virginia Beach Drive
Miami, FL 33149-1099

27 May 1986 F/SEC11:BAS

MEMORANDUM FOR: Files

FROM: F/SEC11 - Barbara A. Schroeder B.A. Schroeder

SUBJECT: Summary of Marquesas Keys Marine Turtle Field Work (12-16 May 1986)

A preliminary assessment of marine turtles of the Marquesas Keys was coordinated by the U.S. Fish and Wildlife Service and conducted from 13-15 May 1986. The field party consisted of: Earl Possardt (USFWS), Lew Ehrhart (UCF), Larry Ogren (NMFS), Barbara Schroeder (NMFS), Ken Dodd (USFWS) and Deborah Holle, Kip Watson and Tom Wilmers (all USFWS/Key Deer NWR).

The Marquesas Keys, under the jurisdiction of the U.S. Fish and Wildlife Service as part of Key West National Wildlife Refuge, are located approximately 19 miles due west of Key West, FL, on the western side of Boca Grande Channel. The Marquesas are made up of a ring of keys which form a central lagoonal area 2-3 miles wide. Netting and sighting activities were conducted around the perimeter of the keys and inside the lagoon. Field work was based out of Key West using boat launching ramps at the U.S. Navy Base Trumbo Annex and Garrison Bight Marina. Two boats (a 17' and 23' Mako) were provided by the refuge. Large mesh tangle nets (16" stretched on the diagonal) were used for three separate sets in waters of varying depths (2' - 10'). Nets were tended continuously.

The nine sightings and one turtle capture on 13 May were evidence that the Marquesas provide suitable habitat for green turtles (C. mydas). Grass beds are extensive around the Marquesas perimeter and especially within the lagoon. Unfavorable weather conditions, which resulted in poor sighting conditions, and strong currents which hampered the nets fishing properly probably contributed to the lack of sightings or captures on 14 and 15 May. I learned from a fisherman at the marina on 15 May that he had seen 2-3 loggerheads (C. caretta) and 6-7 greens on 14 May inside the lagoon. I suspect this was during the early morning hours before the winds picked up. Additional netting and sighting work will be necessary to determine such things as extent of usage of this area, species and size composition, and seasonal usage patterns.

In addition to the netting and sighting work, the beaches were examined as potential nesting habitat. There appear to be two major sandy beach areas on the west and north sides of the Marquesas. Most of the beach is extremely narrow, the high tide line often at the vegetation edge. The contribution of the Marquesas beaches as nesting grounds are undoubtedly minimal. My field notes are summarized as follows:

13 May 1986

0912: depart Key West
1000: arrive Marquesas

* Set #1: 24·33.2'N 82·10.0'W (see chart), running west from and perpendicular to the shoreline

1012: begin deploying first net
1020: net set, 190 yards out
1134: begin deploying second net, continued running due west (added on to first net)
1147: 260 additional yards out (= 450 yards total)
1515: begin pulling net in
1607: net in, no turtle captures, two rays (Dasyatis sayi) and one permit (Trachinotus falcatus) caught and released, large manta ray in area hit net but did not entangle, water temperature 27.0·C, air temperature 30.5·C (1345 hrs)

(1030-1400): second boat running inside lagoon sighted 2 C. mydas in AM and 7 C. mydas in PM, all estimated to be in the 4.5 kg to 14 kg range, one turtle captured by diving off boat, processed and released (data attached), waters calm and clear in lagoon

1610: depart Marquesas
1705: arrive Key West

14 May 1986

0930: depart Key West
1051: arrive Marquesas

* Set #2: 24·34.2'N 82·07.6'W (see chart), running approximately NE/SW (inside lagoon)
1315: begin deploying net
1330: net set, 260 yards out
1540: begin pulling net in
1603: net in, no turtle captures, no other species captured, water temperature 28.5·C, (1600 hrs)

(1230 - 1500): second boat exploring lagoon area for turtle sightings, no sightings made, water choppy and difficult to see into because of windy conditions.

1645: depart Marquesas
1750: arrive Key West

15 May 1986

0800: depart Key West
0908: arrive Marquesas

* Set #3: 24·33.9'N 82·07.9'W (see chart), running approximately ENE/WSW (inside lagoon)

0913: begin deploying net
1000: net set, 260 yards out; strong current running east and pulling float line down, added extra floats to keep top line up
1500: begin pulling net in
1525: net in, no turtle captures, one ray (*Dasyatis sayi*) caught and released

(1100 - 1430): explored lagoon and Marquesas SE perimeter for turtle sightings, no sightings made, water very choppy again due to continued windy conditions.

1530: depart Marquesas
1639: arrive Key West

NMFS/SEFC
COOPERATIVE MARINE TURTLE TAGGING PROGRAM
TAGGING DATA (HEADSTARTED, REHABILITATED, NETTED, OTHER RELEASE)

Tag Number(s) (list all tag #'s and letter prefix): NNW 724 / NNW 725 / (inconel tags)

Species: C. mydas

Date Released: 13 May 1986

Describe release location (include lat/long if available and include county):
Florida, Monroe County, Key West National Wildlife Refuge, Marquesas Keys, inside lagoon, 24° 34.0'N 82° 08.0'W

Describe source of eggs and year hatched (headstarts) or original stranding or capture location (include county) AND date (rehabs, netted, etc.)
caught 13 May 1986 same location described above

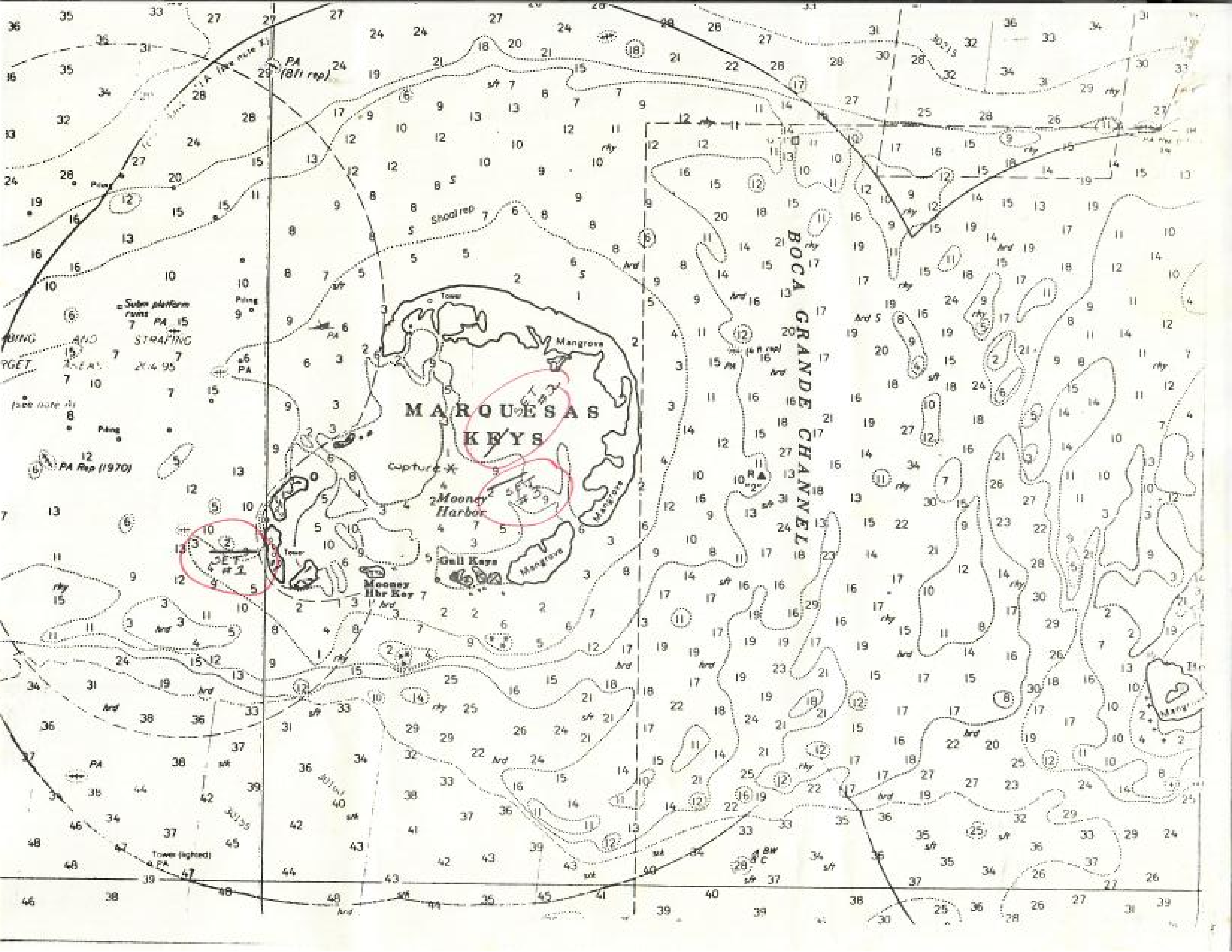
Describe type of gear in use when turtle was captured (if applicable)
caught by hand, dove off boat (Limpus' rodeo method)

Carapace length straight line: 49.5 cm ______ in — Plastron: 39.6 cm
Carapace width straight line: 40.6 cm ______ in — Head Width: 7.2 cm; Body Depth: 17.6 cm
Carapace length over curve: ______ cm ______ in — Greatest Carapace Length: 49.7 cm
Carapace width over curve: ______ cm ______ in

Weight: 13.6 kg ______ lbs

Additional remarks or data (use back if necessary) papillomas: left side of neck; (LR) trailing edge; left inframarginal seam and marginal area; (LF) at flipper insertion; (RF) at insertion and trailing edge; perianal area / all small, first two described were the largest, overall condition of turtle looked very good, unusually sharp marginals from rear flippers back!!

Organization Tagging (please include a phone number)
cooperative effort NMFS/UCF/USFWS


MARQUESAS KEYS
BOCA GRANDE CHANNEL
Mooney Harbor
Gull Keys
Mooney Hbr Key
Mangrove
Capture
Shoal rep
Subm platform ruins
STRAFING
PA Rep (1970)
PA (81 rep)
Tower (lighted)

June 30, 1986 F/SWC2

Dr. Fred Smith
Department of Anatomy
and Radiology
University of Georgia
Athens, Georgia 30602

Dear Fred,

Your letter of May 23rd was forwarded to me at French Frigate Shoals where I have been tagging nesting green turtles for the past month. I am now back in Honolulu trying to catch up on all of the correspondence and other paper work that was waiting for me.

It was disappointing to learn that the RFA from the National Cancer Institute excluded reptiles, hence any possiblity of using designated funds for the papilloma problem in green turtles. Do you have plans to submit the unsolicited application that the program administrator indicated would be welcomed to compete for general funds? I hope that such research will be in line with the new goals currently being established by your department. You can continue to count on my support in whatever capacity that you feel may be appropriate. I will send you more samples as they become available.

Other than John Harshbarger and Elliot Jacobson, I am not aware of anyone else who is interested in pursuing the papilloma problem from the standpoint of cause and prevention. A copy of my response to Dr. Jacobson has been enclosed for your information, along with a more recent letter he sent me. As tumor material becomes available, I plan to send him samples in the EM preservative he described.

I do look forward to hearing from you again when your departmental policy is crystalized.

Sincerely,

George H. Balazs
Zoologist

Enclosure

bc: Balazs
Larry Ogren
HL

The University of Georgia
College of Veterinary Medicine
Athens, Georgia 30602

Department of Anatomy and Radiology
(404) 542-3221

May 23, 1986

George H. Balazs
National Marine Fisheries Service
Southwest Fisheries Center
Honolulu, HI 96812

Dear George:

I am sorry to be so uncommunicative, but I have been sitting in a holding pattern here in our department. We are working on "Departmental Goals" and I am in much the same boat as Elliott Jacobson with regard to my department head. We are not making much progress about getting our goals set so I will at least get you caught up on progress with the papilloma project.

The tissues you have sent have been in good shape each time. The fixation and specimen size has been excellent. The papillomas from the last turtle were very interesting because there were cryptic areas in some of the specimens (external and oral samples) which contained glandular elements within the epithelium. The epithelial cells deep within these crypts contained what appeared to be a mucoid material. I will do some special stains to confirm what the material is.

I believe the glands just represent squamous metaplasia, but their presence indicates to me that the cause of the papillomas may be acting more on the external surface of the animal (like a pollutant) rather than from within (like a trematode egg). However, consider that no more than a hunch at this point.

Now about the $$$. The RFA from the National Cancer Institute was limited to finfish and shellfish and was not appropriate for reptiles according to the program administrator. He did say they would welcome an unsolicited application for work on the papilloma problem. He said it might end up either in the N.C.I. or with the environmental division of N.I.H. In either case it would have to compete for general funds, not predesignated funds.

That is where I am sitting right now. I am very interested in helping work on the problem in any way that I can. I must struggle through the goal setting we are working on in the department before I know whether I can pursue this project (and my other turtle projects) or whether we are all going to be working on a "theme" in our department

In the mean time I would support Elliot's request for any funding you can possibly find for him. There is plenty of work for us all to do on this problem and the more people working on it the sooner it will be solved.

You mentioned earlier that others have expressed an interest in applying for money to work on the problem. Perhaps they are better located to work with you or Lew in collecting and sampling. I would like to continue to work on the problem until the cause is identified and preventive measures can be taken. If you feel that I can be a help here in Athens as we are now doing then I will continue to do what I can. I should know by early June if it will be appropriate for me to work on a papilloma grant proposal from my department. I can see that in any case my travel time will have to be limited.

How ever that comes out I would appreciate it if you could continue to send me samples if it is not too much trouble. I would like to continue to examine the papillomas and to help search for a solution to the problem.

Sincerely,

Fred

J. HILLIS MILLER HEALTH CENTER
COLLEGE OF VETERINARY MEDICINE
Department of Special Clinical Sciences
and
HEALTH CENTER ANIMAL RESOURCES
Box J-6
Gainesville, FL 32610-0006

Laboratory Animals
Zoo Animals
Wildlife
Birds
Reptiles
Mammals
Ph. (904) 392-2977

June 11, 1986

George H Balazs
NMFS
Southwest Fisheries Center
Honolulu Laboratory
2570 Dole St
Honolulu, Hawaii 96822-2396

Dear George:

Sorry to here there is no possibility of funding but I will continue to work at this end to get a few bucks to pursue these preliminary studies. Please collect as many samples from lesions at different stages of growth and fix them in the em fixative I recommended. Please make sure you collect detailed information on where the lesion was located, how large (measure), whether it was ulcerated, how many lesions per animal, where were other lesions located, and overall clinical appearance of the turtle. The more information the better.

I look forward receiving samples as soon as possible and hope to really start to unravel the etiologic mystery behind these lesions.

With best regards,

Elliott Jacobson, DVM,PhD

National Museum of Natural History · Smithsonian Institution

WASHINGTON, D.C. 20560 · TEL. 202-

17 July 1986

Dr. George H. Balazs
Zoologist
Honolulu Laboratory
Southwest Fisheries Center
National Marine Fisheries Service, NOAA
U.S. Department of Commerce
P.O. Box 3830
Honolulu, Hawaii 96812

Dear George:

Enclosed are accession forms, microslides, and evaluations for two green sea turtle cases, i.e., RTLA 3572 and RTLA 3615. Both are fibropapilloma and both contain a lot of trematode eggs. In contrast, a recent case from a Florida turtle sent by Dr. R. Erik Martin (RTLA 3581), had no trematode eggs, but was heavily infested by leeches and crustacea (barnacles ?). Maybe several different irritants can initiate the response.

Thank you for these good cases!

Sincerely,

John

John C. Harshbarger, Ph.D.
Director
Registry of Tumors in Lowwer Animals

Enclosures: Accession forms and evalulations
6 microslides - RTLA 3572
4 microslides - RTLA 3615

Accession Sheet

RTLA NO. 3572 Date Rec'd 04/01/86 Date Acc 05/23/86

Contrib. No.
CL-73.5

Common name Green sea turtle

Phylum Chordata
Class Reptilia
Order Testudines
Family

Genus Chelonildae
Species Chelonia
Strain mydas

Contributor BALAZS, G.H.

Material Submitted
Multiple, small, papilloma-like growths from the eyes, neck, and flippers, similar growth from inside the mouth, blockin g glottis

Origin of animal: M

Locality: Hawaii, Haleiwa: Island of Oahu

Date Coll 3/26/86 -

How Killed Died

Sex

Age

Stage Juvenile

Gross description:

Multiple, small, papilloma-like growths from the eyes, neck, and flippers, similar growth from inside the mouth, blocking glottis. Contributor's: "Small papillomas on eyes, neck and flippers. Similar (?) growth found in the mouth, blocking glottis. Turtle found in lethargic state floating at the surface. It died a few hours later in captivity."

Comments:

RTLA 3572 EVALUATION

Lesions consist of moderately cellular, collagenous, monotonous, dermal fibrous tissue with a verrucose surface of keratizing epidermis. Numerous epidermoid cysts occur near the surface. Trematode eggs are abundant in the connective tissue. Some are enveloped by foreign body giant cells. There are a few small unidentified metazoans infesting the surface, none of which are leeches. Two resemble some type of crustacean.

<u>DIAGNOSIS</u>:

Fibropapilloma

John C. Harshbarger: JC Harshbarger

Date: July 17 1986

Accession Sheet

RTLA NO. 3615 Date Rec'd 05/13/86 Date Acc 05/27/86

Contrib. No.
SCL-668

Common name Green sea turtle

Phylum Chordata
Class Reptilia
Order Testudines
Family

Genus Cheloniidae
Species Chelonia
Strain mydas

Contributor BALAZS, G.H.

Material Submitted
Six cauliflower-like growths from the neck and front flipper s; a growth (three pieces of tissue) from the tongue and glo ttis; a spherical growth from the external upper jaw

Origin of animal: M

Locality: Hawaii: Live turtle stranded at Haleiwa, Oahu

Date Coll 05/07/86 - How Killed Live--biopsied

Sex Age Stage Subadult

Gross description:

Six cauliflower-like growths (ca 0.8 cm x 0.4 cm to 1.0 cm in diameter) ranging from white to dark gray, from the neck and front flippers; a growth (three pieces of tissue: ca 1.7 cm x 1. 3 cm; 2.7 cm x 1.1 cm; 2.9 cm x 1.6 cm, respectively) from the tongue and glottis; a spherical growth (ca 2.5 cm in diameter) from the external upper jaw

Comments:

RTLA 3615 EVALUATION

Lesions consist of moderately cellular, collagenous, monotonous, dermal fibrous tissue with a verrucous surface of keratizing epidermis. Numberous epidermoid cysts occur near the surface. Trematode eggs are abundant in the connective tissue. Some are enveloped by foreign body giant cells. There are no metazoans infesting the surface.

DIAGNOSIS:

Fibropapilloma

John C. Harshbarger: JC Harshbarger

Date: July 17 1986

553 Ilimano St.
July 24, 1986

George H. Balazs
NOAA, NMFS
P.O. Box 3830
Honolulu, Hawaii 96812

Subject: SIGHTING OF A TURTLE WITH A TUMOR

On 20 July 86 at ~1:00pm, while diving in Kailua Bay approximately 500 meters northeast of a small rock island (locally referred to as "bird shit rock") a Green Sea Turtle, Chelonia mydas was observed resting on the bottom in 40 feet of water. The shell length was estimated as about 24 inches. It was aware of our presence but made no attempt to move. A single large tumor of about 6 by 4 inches was observed attached to the turtle's left hind limb.

When I approached the turtle it remained still and allowed me to lift it off the bottom. Then it bolted from my grip and settled down on the bottom about 20 feet from the place where it broke free. I repeated my attempt and the turtle bolted again and found an overhang under which to shelter. I then left the turtle, assuming that it was not disabled enough to warrant a rescue.

Sincerely,

Frank A. Parrish

Frank A. Parrish

553 Ilimano, St
July 27, 1986

George H. Balazs
NOAA, NMFS
P.O. Box 3830
Honolulu, Hawaii 96812

Subject: INCIDENT OF ENTANGLED TURTLE, WITH TUMORS

The Green Sea Turtle, Chelonia mydas was first observed approximately 8:30 am on 27 July 86 at the mouth of Kawainui Mokapu Canal at Kailua Bay. Originally seen by some children who were playing on the canal bank, its presence was brought to the attention of a local resident. The resident observed the head of a turtle breaking the surface repeatedly within a foot of a buoy which is moored in the middle of the canal. The greatest observed time interval between surfacings was twenty minutes. The concerned resident then called me.

When I arrived (approx 10:30 am), I saw the turtle surface close to the buoy. I swam out to the buoy, looked about the line, and found the turtle at the bottom of the line resting on the canal bottom. Entangled in 1/8 polypropylene line, the turtle had enough scope to migrate to the surface to breath and then return to the bottom to rest.

The water was murky and the turtle very active, so it was impractical to handle it in place. I tied a line from shore to the line in which it was entangled and then cut the turtle free of the mooring. With aid from shore, I was able to guide the turtle to the beach with minumum struggle.

Once ashore, it was apparent that a 4-inch tumor located on the turtle's right fore flipper had become tangled in the frayed buoy line. The line was securely fouled around the peduncle of the tumor. This may have occured while the turtle was feeding on algae growing on the line. At this time by quick count, 12 conspicous tumors of sizes between 2 and 6 inches were found distributed throughout all flippers, tail and head. The turtle was then rolled on its back to immobilize it while freeing it of the line and taking some photos (enclosed). A yard stick was included in the photos for scale Total length was ~38 inches, shell length ~30 (S-76.8cm) inches. A photo of the entangled tumor is included. No rupture of the tumor or injury to any other part of the turtle was observed. I looked for tags or marks on the shell and found none.

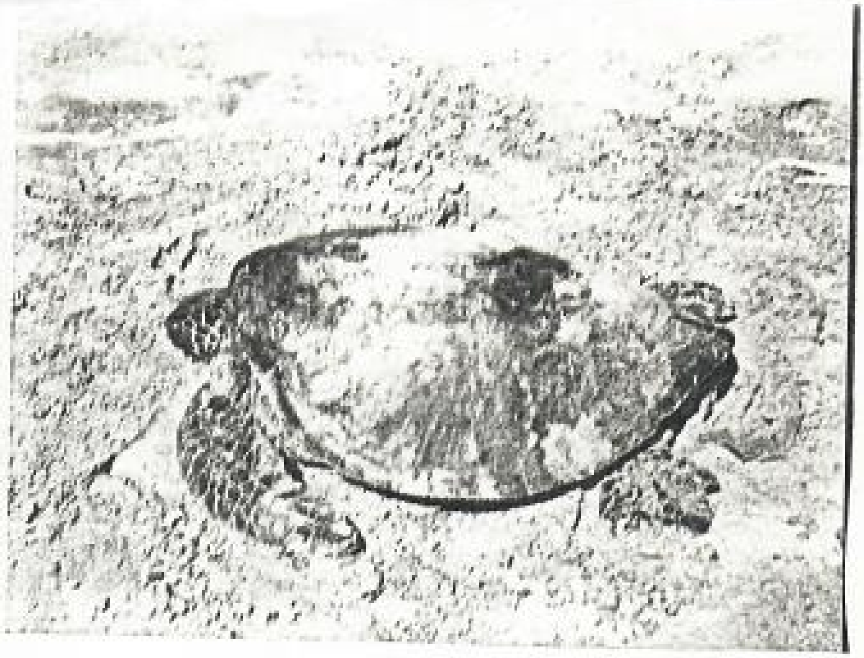
The turtle was then placed back in the water where it fled quickly. The total time it was exposed on the beach was kept to under ten minutes because local residents supplied equipment and aid upon request. I returned to the buoy and cleared the frayed line.

Deborah Goebert of 684 Kaimalino St. Kailua HI 96734 was responsible for calling me to the scene. Her neighbors, the Adams family, of 678 Kaimalino St. supplied the film and camera for the pictures.

Sincerely,

Frank A. Parrish

Frank A. Parrish

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Southwest Fisheries Center Honolulu Laboratory
2570 Dole St. • Honolulu, Hawaii 96822-2396

June 17, 1988 F/SWC2

Dr. John Harshbarger
Registry of Tumors in Lower Animals
Room W216
Smithsonian Institution
Washington, D.C. 20560

Dear John,

The enclosed report by Dr. Elliott Jacobson is the result of a small contract issued by our agency to learn more about the fibropapilloma problem in green turtles. The funds for this work originated from a branch of the National Marine Fisheries Service based in Florida. Consequently, I had very little opportunity to have turtles of Hawaiian origin included in the work. In fact even though the report is dated June 1987, I was only recently able to obtain a copy.

I am sending the report to you because of your longstanding involvement in green turtle fibropapillomas. Dr. Jacobson still seems to strongly believe that a virus is the responsible agent. However, as you will read, he has yet to demonstrate the presence of a virus. When your time permits, I would very much appreciate hearing your expert review comments on the work and conclusions of the report. Some sense of direction is needed here in Hawaii on this disease problem, and I am not at all certain our money will be best spent searching further for an elusive virus.

In closing, I should mention that during 1987, 41% of the 112 stranded turtles we dealt with here in Hawaii involved fibropapillomas. Both the numbers and percentage we are seeing continue to increase with each passing year.

Sincerely,

George H. Balazs
Zoologist

Enclosure

National Museum of Natural History · Smithsonian Institution
WASHINGTON, D.C. 20560 · TEL. 202- 357-2647

14 December 1987

VIA AIR MAIL

Dr. Hiroyuki Tachikawa
Research Staff
Ogasawara Marine Center
Byobundani, Chichijima
Ogasawara-mura, Tokyo 100-21
Japan

Dear Dr. Tachikawa:

Thank you for your letter of 25 November.

I would be quite interested in receiving well fixed tissues from the green sea turtle fibropapilloma-like lesions from your area. The kidney of a turtle with cutaneous fibropapillomas recently autopsied in Florida had half of the kidney replaced by a similar lesion. Therefore should you obtain a very fresh specimen you should also examine internal organs for lesions and send along fixed examples for diagnosis. Since the green sea turtle (Chelonia mydas) is an endangered species, legally you are supposed to get a CITES (CITES = Convention on International Trade in Endangered Species) export permit from your Government. While you do that I will get an import permit from my Government. Please let me know when you are ready to ship the tissues and I will send you my documents (or number) to attach to the shipment. We will supply you with a diagnosis and a set of microslides.

I am sorry this is such an effort, but if you request an open-ended export permit then any future shipments should be simple.

Sincerely,

John Harshbarger

John C. Harshbarger, Ph.D.
Director
Registry of Tumors in Lower Animals

cc: G. H. Balazs

HAWAII CLIPPING SERVICE
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Honolulu, Hawaii 96816
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DEC. 26 1990

workshop on mysterious green sea turtle tumors

For the first time, scientists had the opportunity to share their research on fibropapilloma, a life-threatening tumor disease in green sea turtles. The Marine Turtle Fibropapilloma Disease Workshop brought ten scientists from across the United States to Honolulu during December 4-6, said George W. Boehlert, Director of the Honolulu Laboratory, Southwest Fisheries Science Center, which organized the workshop.

The green sea turtle, which occurs worldwide in tropical and subtropical seas, is a protected species under the U.S. Endangered Species Act.

"The scientists not only shared what they know about the disease based on current research, but they also devised a comprehensive cooperative research plan to determine the cause of the disease," said Boehlert.

Although the disease was first observed in the late 1920s in the Florida Keys and in 1958 in Kaneohe Bay, it has spread simultaneously and reached epidemic proportions at certain sites in Hawaii and Florida during the past few years. Until recently, the disease was believed to be exceedingly rare in other areas. However, in the past few months, a number of cases have been reported in Australia and the Caribbean Islands.

The workshop also gave scientists an opportunity to visit one of the locations off Kaneohe Bay where reports of the disease have increased dramatically in the past few years. During the trip, hosted by the Hawaii Institute of Marine Biology at Coconut Island, visiting scientists observed Honolulu Laboratory researchers hand-capturing turtles for tagging. Of the seven turtles captured that day, six were afflicted with tumors.

"The Kaneohe trip really brought the gravity of this situation to life for those scientists who have analyzed tissue from the tumors but had never seen tumor-inflicted turtles themselves," said Balazs.

The scientists also visited Sea Life Park's sea turtle facilities, which have had no cases of the disease.

Turtles afflicted with this disease have disabling tumors that can grow up to 12 inches in diameter and may eventually lead to death. The tumors occur at a number of locations on turtles: In their mouths, throats and nasal passages, hindering breathing and making eating difficult or impossible. On their eyes, severely impairing vision and increasing their vulnerability to predators. On their jaws, necks, tails and flippers, impairing movement and swimming ability.

Scientists so far do not know what causes the disease, how it is spread, nor what the disease's impact will be on the recovery of the Hawaiian green turtle. Causes discussed at the workshop included a virus, parasites, and environmental pollution.

The tumor disease was designated the number one research priority by the draft Hawaiian Sea Turtle Recovery Plan.

The Sunday Star-Bulletin & Advertiser Honolulu, January 20, 1991 E3

Tumor growths are threatening green sea turtles

JAN TENBRUGGENCATE
Advertiser Environment Writer

Scientists remain baffled by the cause of severe tumor growths on green sea turtles.

The situation grows increasingly serious as the disease spreads among turtle populations in Hawaii and around the world.

As an example of how quickly the disease spreads, there had been no sightings of green sea turtles with the tumors known as fibropapilloma before 1985 off the south coast of Molokai at Palaau. That year there was one. In 1987, a second infected turtle was spotted.

By 1988, when scientists inspected 125 turtles there, they found nearly 5 percent had tumors. The next year it was nearly 10 percent.

In a survey in April last year, 17 percent had the disease, and in July, researchers found 26 percent were infected. In cases where the same turtle was caught a second time, scientists noted tumors got progressively worse, and none appeared to have gotten smaller.

In Kaneohe Bay, where the first infected turtle was spotted in 1958, the rate of infliction is much higher. Studies in 1989 and 1990 showed 67 percent of turtles inspected at Twin Reef infected, 92 percent at Deep Reef, 54 percent at Mark Reef, and 49 percent at Ahu-O-Laka.

The highest rates of infection were in turtles with shells more than 20 inches long. These rates ranged from 70 to 100 percent, depending on shell length.

George Balazs, a zoologist with the National Marine Fisheries Service, said he is still looking for old-time fishermen who may have seen the disease before 1958, but so far has heard of none.

The tumor disease has grown in Hawaii, and around the world. It is a serious problem in Florida waters, in the Caribbean islands and

See Turtles, Page E3

Turtles: Threat of tumors

FROM PAGE 1

around Australia.

The tumors are bulbous lumps of pink flesh. They are most common on the eyes and around the mouth. But they are also common on the flippers, in the throat, and on the internal organs. They tend to be tumors of skin tissue, but can be found elsewhere as well, Balazs said.

As the epidemic spreads, scientists grow more worried.

"Things just keep getting worse. Something is getting out of hand," he said.

The tumor disease was unknown to science until the 1920s, when it was reported on green sea turtles in Florida.

Ninety-nine percent of the cases across the globe are on green sea turtles, an endangered species. There are now two reports of similar tumors on loggerhead turtles off Cape Canaveral, Florida, and there are rare reports of tumors on olive ridley turtles off the Pacific coast of Mexico.

The tumors often grow around the eyes of turtles until they are blind and cannot feed themselves. They can also grow around the mouth and in the throat, until the animals cannot eat or breathe.

The number of turtles that strand themselves on Hawaii shores has grown to record levels, and most of the stranded turtles suffer from severe tumor disease.

There are still a few pockets of turtle populations without the disease, but it is not clear how long they will remain so. Balazs said there have been no diseased turtles found in samplings at Kiholo Bay on Hawaii, but at Punaluu Bay on Hawaii, which previously was disease-free, there have now been two cases of tumor disease.

Photo courtesy of George H. B

A Hawaiian green turtle, *Chelonia mydas*, with fibropapilloma disease.

Scientists from around the country met last month in Honolulu at a workshop to discuss what they know and how to proceed.

The most accepted theories about the cause of Marine Turtle Fibropapilloma Disease are these:

■ A very elusive virus causes the tumors, and science simply hasn't been able to locate the virus. There are similar tumors, which are known to be caused by viruses, in cows, deer, mice and other animals.

■ A virus in combination with some kind of chemical pollution, which suppresses the turtles' immune system and allows the virus to cause the tumors, the way AIDS suppresses the immune system and allows pneumonia, cancers and other diseases to proliferate.

■ A parasite of some kind, which directly causes the tumors.

■ A parasite that carries a virus, which in turn causes the tumors.

Balazs said he jokingly suggested to the workshop that the hole in the ozone layer might be responsible. Nobody laughed. Some kind of skin cancer promoted by increa solar radiation is at least a sideration, he said.

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Turtles: Threat of tumors

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Photo courtesy of George H. Balazs

A Hawaiian green turtle, *Chelonia mydas*, with fibropapilloma disease.

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It actually makes a little sense, since the tumors often appear first and are most serious around the eyes and mouth, which are exposed to the sun every time the turtle comes up to take a breath.

There is a human concern that if some kind of chemical pollution in the ocean is causing health problems with turtles, it could end up being a warning flag for potential human health dangers.

But for now, it appears the disease is limited to the turtles, the graceful flying saucers of the deep.

Vet Pathol 31:335–339 (1994)

Ocular Fibropapillomas of Green Turtles (*Chelonia mydas*)

D. E. Brooks, P. E. Ginn, T. R. Miller, L. Bramson, and E. R. Jacobson

Departments of Large and Small Animal Clinical Sciences—Ophthalmology Service (DB, TM),
Small Animal Clinical Sciences—Wildlife Service (EJ), and Comparative and Experimental Pathology (PG),
College of Veterinary Medicine, University of Florida, Gainesville, FL; and
The Turtle Hospital, Marathon, FL (LB)

Abstract. Histologic evaluation of four eyes from three stranded juvenile green turtles (*Chelonia mydas*) from Florida, USA revealed ocular fibropapillomas composed of an overlying hyperplastic epithelium, various amounts of a thickened, well vascularized, collagenous stroma, and a moderate-to-dense population of reactive fibroblasts. The histologic morphology of the ocular fibropapillomas varied depending on whether the eyelid, conjunctiva, limbus, or cornea was the primary site of tumor origin. Fibropapillomas arising from the limbus, conjunctiva, or eyelid tended to be polyploid or pedunculated with a high degree of arborization. They often filled the conjunctival fornices and extended externally to be ulcerated on the distal aspects. Corneal fibropapillomas were more sessile and multinodular with less arborization. Some corneal tumors consisted primarily of a broad fibrovascular stroma and mild epithelial hyperplasia, whereas others had a markedly hyperplastic epithelium supported by stalks of fibrovascular stromal tissue. In green turtles ocular fibropapillomas may be locally invasive and associated with severe blindness and systemic debilitation.

Key words: Fibropapilloma; green turtles; ocular neoplasia.

Green turtle fibropapilloma (GTF) is commonly reported in free-ranging green turtles (*Chelonia mydas*) in many parts of the world.[1–4] A dramatic increase in the prevalence of GTF in stranded green turtles has occurred since 1983. Up to 85% of individuals are affected in some regions.[2–3]

Fibropapillomas are thought to be of infectious origin in green turtles, as epizootics of papillomatosis have been reported.[2–3] In two cases of cutaneous GTF, degenerative superficial epithelial cells contained eosinophilic intranuclear inclusions that were found to consist of viral particles. Based on size, location, and morphogenesis, these particles were considered to be compatible with members of the family Herpetoviridae.[2] It may be, however, that these viruses represent only secondary infections, merely present in a growing tumor, and not the etiologic agent of GTF.[2] Determining the specific etiology of GTF is complicated by the common occurrence of spirorchid trematode eggs within dermal capillaries of the tumors.[2–4] The role of papillomaviruses in the pathogenesis of GTF has also not been fully elucidated.[2]

Severe cutaneous and multisystemic visceral involvement is common in GTF.[3] GTF of the skin, eyes, lung, heart, kidney, and gastrointestinal tract has been reported.[3] Ocular GTF has been previously noticed in captured juvenile and adult green turtles in the cornea, eyelids, periocular skin, and conjunctiva.[2,4] Surgical removal of periorbital and corneal GTF has been attempted.[4]

Although the actual percentage of green turtles afflicted with ocular GTF is not known, the eye is reported to be frequently and severely affected in stranded turtles.[3,4] In spite of these many observations, no histologic description of ocular GTF exists. The purpose of this paper is to describe the histology of ocular GTF in order to make comparisons to the cutaneous form of the tumor.

Materials and Methods

Four eyes from three different stranded juvenile green turtles (*Chelonia mydas*) from the Florida Keys with ocular, cutaneous, and systemic signs of GTF were available for histologic examination. The size of the lesions suggested that severe visual deficits were present in each of these turtles. Specimens were obtained at the time of anesthesia for surgical removal of the lesion or following death of the animal. Representative lesions were fixed in 10% neutral-buffered formalin, decalcified in formic acid-sodium citrate solution, embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin and periodic acid-Schiff stains.

Results

Gross examination revealed each eye to have single to multiple, darkly pigmented, verrucous papillomatous lesions varying from 2 to 4 cm in diameter. The lesions arose from the cornea, the limbus, the conjunctiva, or the mucocutaneous junction of the eyelids (Figs. 1, 2). Nonpigmented, whitish-tan areas of each mass were also present. In two globes, the lesion incorporated the entire cornea and limbus obscuring a

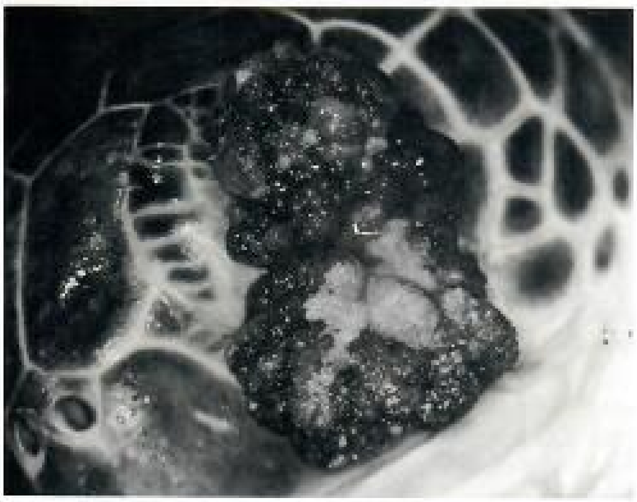

Fig 1. Eye; green turtle. Palpebral fissure is obliterated by large papillary fronds of this variably pigmented, protruding, verrucous fibropapilloma.

Fig 3. Eye; green turtle. An arborizing, pedunculated tumor at the eyelid mucocutaneous junction and a smaller conjunctival fibropapilloma (open triangle) are present. HE. Bar = 3.6 mm.

focal site of origin. A distinct, isolated conjunctival mass was also present in one of these globes. A third eye had a single lesion arising from the limbus, whereas the fourth eye had one mass arising from the eyelid mucocutaneous junction and a second mass arising from the conjunctiva of the fornix.

Histologically, the masses arising from the conjunctiva, limbus, or eyelid mucocutaneous junction tended to be polypoid or pedunculated with a high degree of arborization (Fig. 3). These masses usually enlarged to obliterate the conjunctival fornices and extended externally to become ulcerated on their peripheral surfaces. The surfaces tended to become more verrucous as the lesions increased in size. The lesions associated primarily with the cornea were more sessile and multinodular with less arborization (Fig. 4).

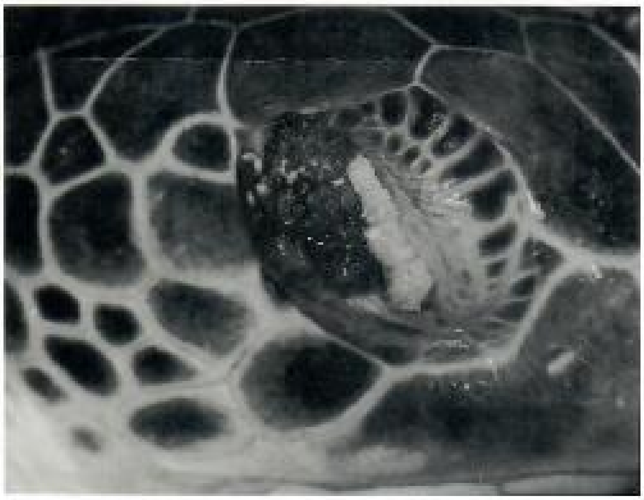

Fig 2. Eye; green turtle. The corneal surface is replaced by this sessile fibropapilloma.

The lesions were composed of a hyperplastic epithelium overlying varying amounts of a thickened, well vascularized, collagenous stroma containing a moderate-to-dense population of reactive fibroblasts (Figs. 5, 6). Depending on the anatomic location, three predominate epithelial types consisting of noncornified stratified squamous, cornified stratified squamous, and epithelia consisting of large goblet cells were noted.

The epithelium of the limbal mass was up to 30 cells thick and was either a superficial noncornified stratified squamous epithelium or a cornified stratified layer with goblet cell differentiation at its base. Masses arising from the conjunctiva or mucocutaneous junction of the eyelids demonstrated a similar degree of hyperplasia. They were lined in the basal regions of the mass by goblet cells and on the more superficial aspects by cornified stratified squamous epithelium characterized by moderate orthokeratotic hyperkeratosis, cleftlike invaginated accumulations of excess keratin, and accumulated mucin (Fig. 5).

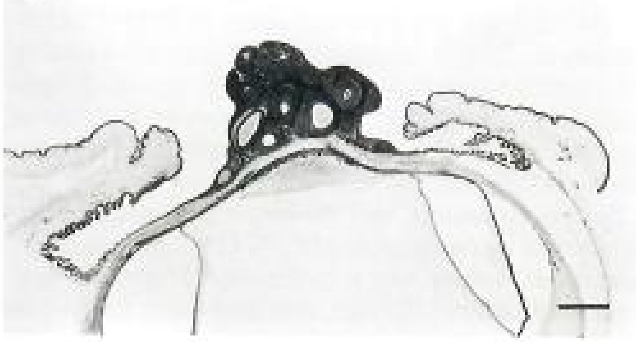

Fig 4. Cornea; green turtle. Endophytic nodular masses with cavitated centers in this corneal fibropapilloma. HE. Bar = 2.3 mm.

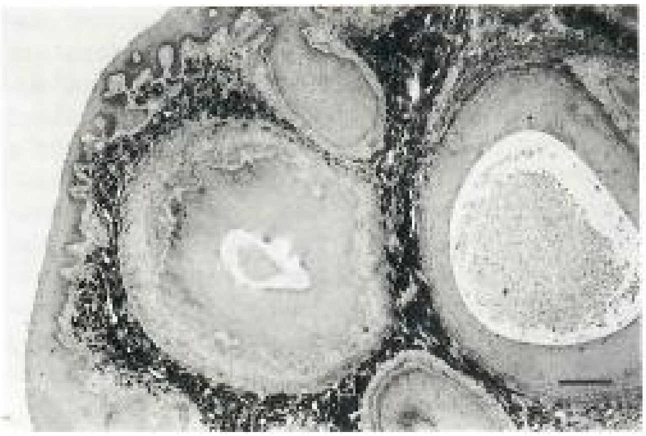

Fig 5. Cornea; green turtle. An endophytic nodular mass consists mainly of thickened, dysplastic epithelium with a cavitated center of sloughed epithelial cell remnants. Note dermal pigmentation. HE. Bar = 300 μm.

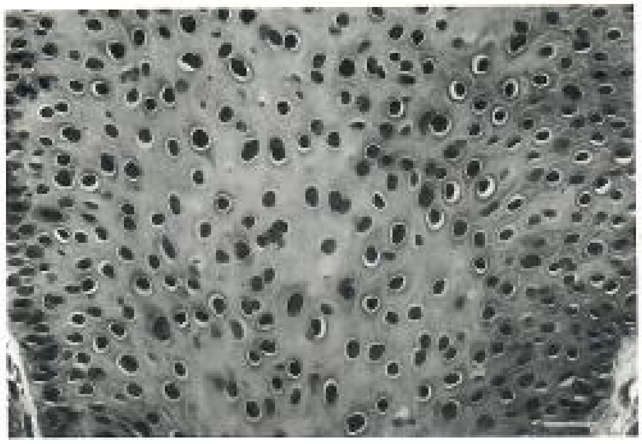

Fig 7. Cornea; green turtle. Clear areas surround homogeneous, amphophilic epithelial nuclei. HE. Bar = 50 μm.

Two morphologic variations appeared in the corneal lesions. One corneal lesion was composed primarily of a broad fibrovascular stroma with only mild hyperplasia (up to 15 cells thick) of the overlying noncornified stratified squamous epithelium. The epithelium formed deep stromal invaginations and had multifocal areas of goblet cell differentiation (Figs. 3, 6). No normal corneal epithelium remained, and the stroma of the cornea was confluent with the mass of fibroblastic tissue. The lesion also incorporated and disrupted the scleral ossicles (Fig. 3). The other more sessile corneal lesion had a markedly hyperplastic epithelial component (up to 40 cells thick) that formed numerous endophytic nodular masses and anastomosing cords of cells supported by scant intervening fibrous tissue (Figs. 4, 5). The cells of the noncornified stratified squamous layer of this lesion retained a fairly organized pattern of growth with respect to the basement membrane, with only scattered foci of gentle swirling of some layers. The endophytic nodules often had cavitated centers filled with ghostlike remnants of sloughed epithelial cells (Fig. 5). This lesion was also invasive of the cornea and sclera, causing obliteration of the corneal stroma and Descemet's membrane. Pronounced edema of the epithelium was noticed in some of the corneal sections. The size of individual epithelial cells in areas of epithelial hyperplasia varied from small cells with hyperchromatic nuclei to cells that were three times the normal size. These enlarged epithelial cells had large, homogeneous, amphophilic nuclei, often surrounded by a clear zone (Fig. 7). Epithelial necrosis with occasional ballooning degeneration of the cells of the wing cell layer occurred in some lesions.

Fig 6. Conjunctiva; green turtle. Conjunctival tumor obliterates the conjunctival sac (space around tumor). HE. Bar = 300 μm.

Fibroblasts within the stromal component of the masses were haphazardly arranged, but appeared to be more dense near the basement membrane of the epithelium. They had poorly defined borders, plump, oval-to-elongated nuclei with basophilic-to-vesicular chromatin, and prominent single to occasionally multiple nucleoli. Many capillaries lined by prominent endothelial cells were present throughout the stroma. A few capillaries in some lesions contained single trematode eggs with a morphology consistent with that of the spirorchid trematode, *Laeredius laeredii.* The stroma was often moderately to heavily pigmented due to the presence of numerous melanin-containing melanophores. Pigmentation of epithelial layers was absent. Moderate numbers of lymphocytes and plasma cells were found subconjunctivally in some sections. Inflammatory cells were not conspicuous near the epithelial cell layers.

Discussion

The term fibropapilloma comes from the papillary pattern (proliferating epithelial cells on thin fibrovascular stalks) of the early lesions and the prominence of the fibrous dermal connective tissue.[4] Cutaneous fibropapillomas have been reported in reptiles, birds, and a wide variety of mammals.[1-8] Although papillomavirus is a common cause of cutaneous fibropapillomas in mammals and has been reported in three species of birds,[9] members of this group of viruses have not been identified in green turtles with fibropapillomatosis.[2-4] Evidence of herpesviruses have been reported in cutaneous fibropapillomas of such diverse species as African elephants,[5] European green lizards,[10] and recently in two cases of GTF,[2] but their role in the pathogenesis of cutaneous or ocular GTF is not clear. In green turtles with cutaneous fibropapillomas infected with herpesvirus, it may be that the virus has simply colonized a preexisting tumor.

Intranuclear inclusions in hyperplastic and hypertrophied epithelial cells of proliferative cutaneous papillomas in African elephants were similar morphologically to those seen with herpesviruses; attempts to demonstrate papillomavirus involvement gave negative results.[5] Although predominantly localized to the elephant's trunks, eyelid papillomas were noticed.[5]

In deer, fibromas found around the eyes may interfere with vision.[6] Local tissue infiltration or metastasis is infrequently found with these fibromas, although spreading to visceral organs has been reported and has been interpreted as representing multiple sites of attack by the causative agent rather than true metastases.[6] Viruslike particles have been observed in ephithelial cells from deer fibromas. The mode of natural transmission is not known in deer, but papillomatosis in cattle may represent a deficiency of cell mediated immunity.[6] Similar to deer fibroma, GTF may commonly involve the periorbital tissues and the globe itself. As in deer, ocular GTF may interfere with vision and thus limit the animal's ability to feed. In contrast to deer, it is not uncommon to find visceral nodules in turtles with cutaneous or ocular GTF. GTF may be multicentric in development, rather than a metastasis from a primary site. There is no evidence of vascular invasion or a high mitotic index in cutaneous GTF.[3]

The histologic appearance of cutaneous GTF varies with what has been interpreted as early lesions displaying a more proliferative, papillary, arborizing epithelial pattern, to those with a less verrucous surface and a more prominent fibrovascular stromal component.[4] These differences may be due to the time of onset, stage of tumor regression, host immune status, or presence of coinfection, or could be related to the anatomical site of origin of the tumor.

The variation in histologic appearance noted in the four turtle eyes between the sessile corneal lesions and the pedunculated, arborizing conjunctival fibropapillomas may simply indicate normal progression in GTF maturation, or could be related to differences in ocular microenvironments provided for tumor or viral replication. Similar regional variation in histologic pattern is found in humans with ocular papillomas. Conjunctival papillomas in humans tend to be pedunculated when they arise at the lid margin, but sessile with a broad base at the corneoscleral junction.[8] The proliferative, limbal epithelial stem cell population, biochemical differences in the epithelial extracellular ground substances, variations in ocular immune response, differences in corneal and conjunctival vascularity, and ultrastructural variations may also play a role in the development of specific forms of the tumors.

Herpesvirus-like, eosinophilic intranuclear inclusions have been noted by light microscopy in epidermal cells of cutaneous fibropapillomas of green turtles, and the presence of herpesvirus has been confirmed by transmission electron microscopy (TEM).[2] Such eosinophilic intranuclear inclusions were not found by light microscopy in ocular GTF.

Adult spirorchid trematodes are commonly found in the chambers of the heart and within the great vessels of green turtles. Their eggs have been reported in dermal blood vessels of GTF and were seen in one of the eyes we studied, but may only represent incidental findings.[2,3]

Ocular GTF presents a severe epizootiologic and therapeutic problem for marine turtle biologists and veterinarians working with these threatened animals. This is a debilitating disease that seriously diminishes this turtle species' survivability. Obstruction of vision is known to deleteriously affect the ability to swim and feed.[2-4] Because the tumors are fast growing, prompt surgical removal is recommended in cases with minimal systemic involvement.[4] The deep posterior infiltration of the cornea by the tumors makes keratectomies very difficult to accomplish successfully. Healing of periorbital GTF is reported to be satisfactory.[4] Cryotherapy, radiation therapy, and laser photoablation are other therapeutic options that might be beneficial for ocular GTF.

Acknowledgement

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Request reprints from Dr. D. E. Brooks, PO Box 100126, University of Florida, Gainesville, FL 32610-0126 (USA).

TEAR OUT ACCESSION SHEET

PLEASE SUBMIT TO:

Dr. John C. Harshbarger
Registry of Tumors in Lower Animals
National Museum of Natural History
Room W216-A
Smithsonian Institution
Washington, D.C., 20560, USA

DATE RECD. ______________________

CONTRIBUTOR'S NO. ______________________

RTLA NO. ______________________

USNM NO. ______________________

NIH HISTOPATH NO. ______________________

COMMON NAME ______________________ FAMILY ______________________

PHYLUM ______________________ GENUS ______________________

CLASS ______________________ SPECIES ______________________

ORDER ______________________

CATEGORY: NEOPLASM ____ INFLAMMATION ____ INFECTION ____ PARASITIC ____ TOXIC ____
DEVELOPMENTAL ____ TRAUMATIC ____ NORMAL ____ OTHER ____

CONTRIBUTOR (NAME & ADDRESS): COLLECTOR (NAME & ADDRESS):

ITEMS SUBMITTED (QUANTITY):

GROSS MATERIAL ______________________ PHOTOGRAPHS ______________________

SLIDES ______________________ REPRINTS ______________________

BLOCKS ______________________ OTHER ______________________

ORIGIN OF ANIMAL: MARINE ____ FRESHWATER ____ ESTAURINE ____ TERRESTRIAL ____

WHERE COLLECTED ______________________

DATE COLLECTED ________ SEX ___ AGE ________ STAGE OF LIFE CYCLE ________

HOW KILLED ______________________ FIXATION ______________________

GROSS DESCRIPTION ______________________

MICROSCOPE DX ______________________

COMMENTS (e.g., possible exposure to chemical pollutants or infectious agents, incidence and duration of this condition in the population, etc.)

(Please use reverse side, if more space is needed)

FIG. 1. The distribution of *Ozobranchus margoi* on the posterior flippers, on the external cloaca and in the folds of soft tissue between the carapace and plastron on *Caretta caretta*.

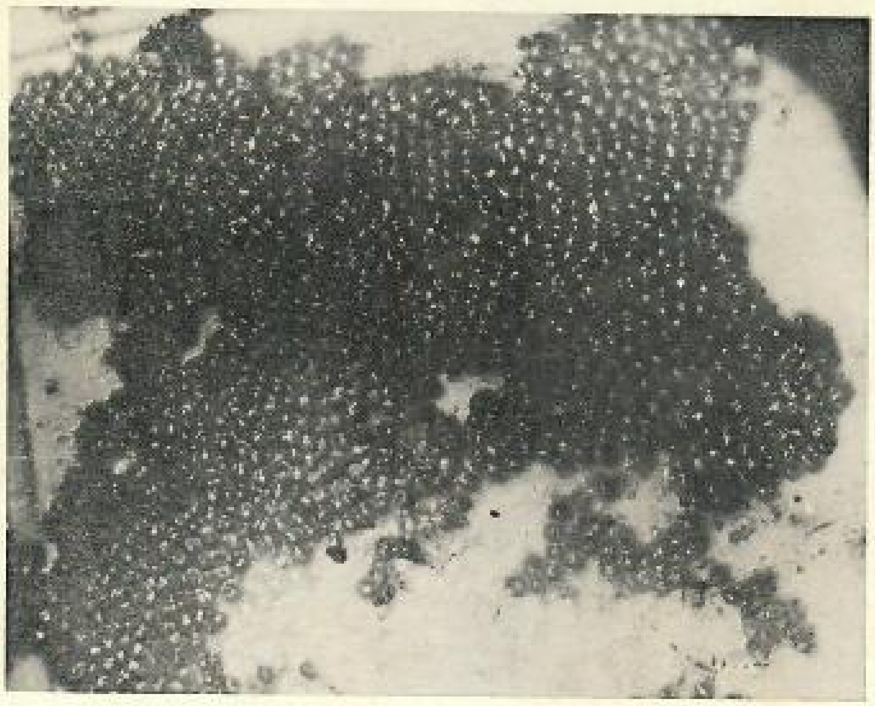

FIG. 2. Egg clusters of *Ozobranchus margoi* on the plastron of *Caretta caretta*.

First Record from North America of the Piscicolid Leech, *Ozobranchus margoi*, a Parasite of Marine Turtles

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Wometco Miami Seaquarium, Miami, Fla. USA

DAVIES, R. W., AND C. G. CHAPMAN. 1974. First record from North America of the piscicolid leech, *Ozobranchus margoi*, a parasite of marine turtles. J. Fish. Res. Board Can. 31: 104–106.

Ozobranchus margoi is recorded from North America for the first time, as an ectoparasite of the marine turtles *Chelonia mydas mydas*, *Caretta caretta caretta*, *Eretomochelys imbricata imbricata*, and *Lepidochelys kempii*. The pathology and treatment of the leech infestation are described and the presumed distribution of the species compared with that of *Ozobranchus branchiatus*, the only species of the genus previously recorded from North America.

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Les auteurs signalent pour la première fois en Amérique du Nord *Ozobranchus margoi*, ectoparasite des tortues marines *Chelonia mydas mydas*, *Caretta caretta caretta*, *Eretomochelys imbricata imbricata* et *Lepidochelys kempii*. Ils décrivent les manifestations pathologiques et le traitement de l'infestation par cette sangsue, et comparent ce qu'ils croient être la répartition de l'espèce avec celle d'*Ozobranchus branchiatus*, la seule espèce du genre déjà signalée en Amérique du Nord.

Received July 20, 1973

IN April 1973 an infestation of leeches identified as *Ozobranchus margoi* was discovered on the marine turtles in a pool at the Wometco Miami Seaquarium (Florida, USA); the infestation was first noted when the turtles continually attempted to haul out. Of the 25 specimens of *O. margoi* examined in detail, all appeared to be sexually mature, with the united genital pores visible. They ranged in length from 18 to 25 mm in comparison with published records of 15–30 mm (Apathy 1890) and 17–26 mm (Cordero 1929). *Ozobranchus branchiatus* is much smaller, ranging in length from 5 to 11 mm (Ghosh et al. 1963).

The pool containing the infected turtles was circular (15 m in diameter), encircling an island, and filled with filtered bay water pumped in at a high turnover rate. There appeared to be no preference by *O. margoi* as to host species, all 85 turtles in the pool being infected. The host species were mostly Atlantic green turtles (*Chelonia mydas mydas*) and Atlantic loggerhead turtles (*Caretta caretta caretta*), the others being several Atlantic hawksbill turtles (*Eretomochelys imbricata imbricata*) and Atlanti Ridley turtles (*Lepidochelys kempii*). The large loggerhead turtles were the most heavily infectec However, *O. margoi* was not found on variou species of local terrestrial turtles on the island or o various numbers of small lemon sharks, nurse sharks tarpons, southern stingrays, assorted Atlantic ree fishes (parrotfish, black durgons, trunkfish), an spiny lobsters. An adjacent pool also containin the same species of turtles and on the same wate supply, except that the direction of water flow wa towards the affected pool, has not harbored *O margoi*.

On all species of turtles, *Ozobranchus margo* was always found on the ventral surface of th flippers, the neck, the external cloaca, and th corners of the mouth, and in the folds of the soft tissue between the carapace and the plastron (Fig. 1). No leeches were found on the carapace. The numbers found on individual turtles varied greatly but in many cases 800–900 were estimated. Large clumps of eggs covered by a chitinous membrane were also commonly observed attached to the plastron (Fig. 2).

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FIG. 3. Lesions at the sites of attachment of *Ozobranchus margoi* on the plastron of *caretta*.

The only pathology observed was the excavation and destruction of the plastron at the sites of attachment. (Fig. 3). There were no signs of the fibro-epithelial tumors observed by Nigrelli and Smith (1943) and Hendrickson (1958) at the sites of attachment of *O. branchiatus* on *Chelonia mydas*. All the specimens of *O. margoi* examined had blood in their guts; samples of this blood and blood taken directly from turtles were examined unsuccessfully for the presence of blood parasites.

Treatment was attempted in two ways. Firstly, 10% formalin was applied directly to the leeches and egg masses of beached turtles, which were subsequently kept out of the water for at least 3 hr. This treatment was not highly effective. Secondly, copper sulphate was added to the pool through the inlet pipe and a 0.3 ppm concentration maintained in the pool (monitored with a spectrophotometric method) for 6 hr. This procedure was repeated twice, 7 days apart, and both the leeches and their egg clumps were eliminated from the turtles. Three months after treatment, *O. margoi* was again found in low numbers on most turtles.

Of the 86 infected turtles three died: one large loggerhead, although no internal lesions were discovered; and two Ridleys, the day after the completion of the copper sulphate treatment, whether as a result of the infestation or of the treatment being unknown.

The genus *Ozobranchus* de Quatrefages, 1852, is represented by six species, all of which are primarily ectoparasites of chelonids. The only species previously recorded from North America, *Ozobranchus branchiatus* (Menzies 1791), was collected from *Chelonia mydas* (Linn.) from Key West Florida (MacCallum and MacCallum 1918) and from the west coast of Florida (Nigrelli and Smith 1943). *Ozobranchisus branchiatus* has also been recorded from the Pacific Ocean between the tropics (Menzies 1791), Australia (Baird 1869), Japan (Oka 1910), Sumatra (Hendrickson 1958), India (Ghosh et al. 1963), and the Malay Peninsula and Sarawak (de Silva and Fernando 1965).

Ozobranchus margoi (Apathy 1890) was originally described from specimens collected from *Thalassochelys corticata* caught in the Bay of Naples (Mediterranean), the location from which Selensky (1915) described the species. Oka (1927) recorded it as an ectoparasite of both a dolphin (*Delphinus longirostris*) and the marine turtle *Caretta olivacea* (= *Lepidochelys olivacea* Escholtz) off the coast of Kyushu (Japan), Cordero (1929) recorded *O. margoi* as an ectoparasite of *Thallassochelys caretta* (= *Caretta caretta caretta* Linn.) off the coast of Montevideo (Uruguay), and Richardson (1969) recorded it on *Chelonia mydas* from Heron Island, Australia.

Though *O. margoi* and *O. branchiatus* are now clearly distinguished on the basis of five and seven pairs, respectively, of digitiform branching branchiae, some confusion has occurred in the past. Menzies (1791), in describing *Hirudo branchiata* (=*Ozobranchus branchiatus*) as having "a row of soft pellucid branchy bristles on each side, opposite to one another, making in all seven pairs," showed only five pairs of branchiae in his illustration of the species. Oka, in 1910, described *O. branchiatus* in Japan as having either five or seven pairs of branchiae, and in 1912 he recorded only *O. branchiatus* in Japan while clearly distinguishing between *O. branchiatus*, *O. margoi*, and *O. jantseanus* Oka 1912; yet, in 1927, he recorded *O. margoi* for the first and only time from Japan. The paucity of distributional data combined with this possible confusion between *O. branchiatus* and *O. margoi* makes speculation on the distributional patterns for both species tenuous. Since many of the green turtles in the Wometco Seaquarium were captured on Ascension Island in 1970 while all the other species were caught locally, it is not possible to know whether *O. margoi* occurs naturally in Florida waters or is a recent introduction. Evidence from the distribution of *O. branchiatus* and the more limited distributional evidence for *O. margoi* would indicate that *O. margoi* might be expected in all warm water areas in the distribution of the host species.

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Cutaneous Fibropapillomas of Green Turtles (*Chelonia mydas*)

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Summary

Six juvenile green turtles (*Chelonia mydas*) from the Indian River Lagoon System, Florida, U.S.A., with multiple cutaneous fibropapillomas, were kept in isolation and examined over a 6-month period. Histologically, the fibropapillomas consisted of a slightly to moderately hyperplastic epidermis overlying a thickened hypercellular dermis. In the earliest lesions, ballooning degeneration was present predominantly in the stratum basale where rete ridges extended into the dermis; aggregates of mixed inflammatory cells were present around dermal vessels. As the lesions matured, they developed an arborizing, papillary pattern. More mature lesions had a less verrucous, often ulcerated surface, with the dermis composed primarily of large collagenous fascicles and relatively few fibroblasts. While numerous trematode eggs were present within dermal capillaries of a histologically similar biopsy specimen from an Hawaiian green turtle, no trematode eggs were observed in any of 28 biopsies examined from the six Florida green turtles in this study. Low stringency Southern blot hybridization and a reverse Southern blot failed to demonstrate papillomavirus DNA in any of the samples extracted. Ultrastructural evaluation of the earliest lesions demonstrated membrane-bound intracytoplasmic vacuoles within epidermal cells in the stratum basale. Similar vacuoles were also observed in the epidermal intercellular spaces and within the dermis. Occasionally, particles with electron-dense centres and measuring 155 to 190 nm were observed in these vacuoles.

Introduction

Papillomas, fibromas, and fibropapillomas were first described in captured adult green turtles (*Chelonia mydas*) 50 years ago (Lucke, 1938; Smith and Coates, 1938). Papillomas were distributed over the dorsal cervical region, axillary regions of the hindlegs, eyelids and conjunctivae. These lesions ranged from papillary projections of hyperplastic keratinized epithelium supported by a fibrous core to smooth round fibrous tumours composed of dense connective tissue covered by thickened epithelium. In 1980, papillomatosis was studied by one of us (ERJ) in a breeding group of green turtles raised in mariculture at Cayman Turtle Farm, Grand Cayman, British West Indies. The history suggested an infectious aetiology. The papillomas were first noted in adults

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caught in the wild and, over a period of several years, developed in farm-reared turtles previously free of gross lesions.

Recently, an epizootic of papillomatosis was identified by one of us (LME) in juvenile green turtles from the Indian River Lagoon System of east central coastal Florida, U.S.A. Before 1982, although hundreds of green turtles were captured in this area, no papillomatous turtles were observed. A single papillomatous green turtle was captured in 1982, and from November 12 1985 to June 11 1986, 30 of 53 turtles (57 per cent) captured in this study area were affected with the disease (Ehrhart, unpublished data).

Similarly, an increasing number of green turtles with papillomas have been reported from the Hawaiian Islands (Balazs, pers. comm.). In 1985 and 1986, tumours were present in 35 per cent of all stranded turtles recovered.

The purpose of this study was to define the pathological features of these tumours and attempt to identify and characterize an aetiological agent.

Materials and Methods

Green Turtles

Six juvenile green turtles, weighing 5·2 to 9·2 kg and with multiple cutaneous and conjunctival papillomas, were collected in the Indian River Lagoon System, Indian River County, Florida, U.S.A. and transported to Sea World of Florida, Orlando, Florida for the duration of the 6-month study. The turtles were housed in an outdoor cement pool measuring 35′ × 12′ × 41″ deep and containing approximately 13 500 gallons of sea water. Water temperature ranged between 19°C and 26°C. All turtles were fed a mixture of fish and marine invertebrates including smelt, herring, bluefish, krill, squid and clam throughout the study period.

Microscopic Evaluations

Following arrival, each turtle was sedated with an intramuscular injection of ketamine hydrochloride (25 mg per kg body weight) and four full skin-thickness biopsies were collected from four different lesions per turtle; two biopsies of normal appearing skin were also collected from each turtle. Each biopsy specimen of a fibropapilloma was divided into two pieces; one was fixed in neutral buffered 10 per cent formalin for light microscopic evaluation and the other was fixed in a modified 4 per cent formalin and 1 per cent glutaraldehyde (FG) mixture for electron microscopic evaluation. One biopsy specimen of the smallest recognized lesion was fixed in 3 per cent glutaraldehyde for electron microscopic evaluation.

Two months after arrival, several large fibropapillomas were surgically removed from the right conjunctiva, periocular skin and right inguinal soft tissue area between the right marginal bridge and right hindleg of one green turtle. A second turtle had lesions removed from the intermandibular area, the right foreleg axillary soft tissue adjacent to the costal scutes, and the right hindleg inguinal soft tissue adjacent to the plastron. Sections of tissues from all lesions were fixed in neutral buffered formalin for light microscopy and in FG for electron microscopy.

Sections of an FG-fixed fibropapilloma from a stranded green turtle in Hawaii were received and evaluated by both light and electron microscopy.

For light microscopy, fixed lesions and normal green turtle skin were embedded in paraffin wax, sectioned 6-μm thick and stained with haematoxylin and eosin (HE). Paraffin-embedded sections 6-μm thick were also examined for papillomavirus group-specific structural antigens, by the peroxidase-antiperoxidase technique (Sundberg, Junge and Lancaster, 1984). Cutaneous fibromas from two mule deer were used as papillomavirus-positive controls. Normal rabbit serum was substituted for the primary rabbit antiserum as a negative control. A formalin-fixed biopsy specimen of the smallest recognized lesion was embedded in 7·5 per cent gelatin, sectioned 4-μm thick in a cryostat and stained with periodic acid-Schiff (PAS) and oil red 0.

For electron microscopy, lesions fixed in either FG or 3 per cent glutaraldehyde were post-fixed in 1 per cent osmium tetroxide and embedded in an epon/araldite mixture. Thick (1 μm) sections were stained with toluidine blue and examined with a light microscope. Ultrathin sections were placed on copper grids, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope.

Tests for Episomal Papillomavirus DNA

Several portions of lesions from each of six green turtles with fibropapillomas were minced finely and homogenized in 300 μl of neutral buffered saline solution containing 10 mM EDTA. Sodium dodecyl sulphate was added to make a final concentration of 0·7 per cent. The homogenate was digested with 1 μg of protease per ml for 3 h at 56°C. Undigested material was removed by low-speed centrifugation ($10\,000 \times g$). Supernates were extracted twice with equal volumes of phenol–chloroform (1 to 1) and once with chloroform alone. The DNA was precipitated with 2·5 volumes of ethanol in the presence of 0·3 M sodium acetate at −20°C for 8 h. The precipitate was recovered by centrifugation at $12\,000 \times g$ for 20 min, dried, and dissolved in 10 mM Tris–HCl (pH 8·0) and 1 mM EDTA buffer containing 20 μg of ribonuclease A per ml. After 1 h at 37°C, the DNA was reprecipitated with ethanol.

Ten μg of each DNA sample were electrophoresed on 1 per cent agarose gel along with a bovine papillomavirus type 2 control. The gel was transilluminated with ultraviolet light after staining with ethidium bromide. Subsequently, the electrophoresed DNA samples were transferred to a Genescreen membrane by the manufacturer's modification of the method described by Southern (1975). A control consisting of bovine papillomavirus type 2 DNA was electrophoresed on the same filter. The filter was prehybridized overnight at 42°C in a solution consisting of 20 per cent deionized formamide, 1 M NaCl, 10 mM Tris, 10 mM EDTA, Denhardt's solution (0·1 per cent ficoll, 0·1 per cent polyvinylpyrrolidone and 0·1 per cent bovine serum albumin), with sonicated and denatured salmon sperm DNA (100 μg per ml). On the following day, 7×10^6 counts per min of a ^{32}P nick-translated bovine papillomavirus type 2 virion DNA probe (specific activity $= 5 \times 10^7$ cpm per μg) was added and allowed to hybridize for 70 h at 42°C. The filter was washed in 3 × 0·45 M NaCl and 0·045 M sodium citrate and 0·1 per cent sodium dodecyl sulphate at 52°C, air-dried and exposed to X-ray film for 7 days.

Next, a reverse Southern blot was done in which 0·2 μg of the total DNA of samples from green turtles 2 and 3 was radiolabelled and allowed to hybridize under conditions of low stringency (25 per cent formamide) with blots that contained 25 different cloned papillomaviruses digested from their respective vectors (O'Banion, Sundberg, Reszka, and Reichmann, 1987). The cloned viral genomes included all six of the known bovine papillomaviruses, seven of the human papillomaviruses, and dog, rabbit, coyote, mouse, rat and parrot papillomavirus DNA.

Results

Morphological Appearance

Fibropapillomas ranged from 0·5 cm in diameter up to a size of 11 × 8·5 × 6·5 cm. Fibropapillomas involved all soft integumentary tissue, but were particularly numerous in the axillary and inguinal soft tissue adjacent to both forelegs and hindlegs (Fig. 1). Fibropapillomas also commonly involved the conjunctivae. Occasionally, fibropapillomas grew from the suture lines between adjacent scutes.

The smallest recognizable lesions were slightly raised, light-brown in colour, oblong in shape and had a rough surface. As lesions increased in size, the surface became verrucose. Advanced, more mature lesions were rounded with a less verrucous, often ulcerated surface. Fibropapillomas projecting from the conjunctivae were often darkly pigmented. In two cases, fibropapillomas had completely obscured vision in the affected eye (Fig. 2).

Four months after arrival, all turtles had gained weight (range: 5·9 to 10 kg) and increased in size (plastronal length 30·7 to 38·5 cm). While some fibropapillomas in most turtles had decreased, many had slowly increased in size. In one turtle, a conjunctival fibropapilloma had increased to a size where normal swimming and feeding were affected. This turtle was anaesthetized and the periorbital fibropapilloma was removed. Three other sea turtles were anaesthetized and selected fibropapillomas were removed.

Two months after surgery, surgical sites either showed good epithelization and scar formation or had become infected and were ulcerated. Three months after surgery, all surgical sites had partially or completely healed.

One turtle became extremely debilitated and was killed 5 months after arrival. No postmortem examination was performed.

Light Microscopic and Electron Microscopic Findings

Histologically, normal green turtle skin from the foreleg axillary soft tissue had a relatively thin epidermis composed of stratified squamous epithelial cells, ranging from four to seven cells in thickness. Epithelial cells were flattened at the surface, their nuclei became pyknotic and were lost and the cells were covered by a layer of "keratin-like" material which was approximately one-half to two-thirds the thickness of the cellular layer (Fig. 3). In several sections the surface had a "spiked" pattern, although in most sections examined, the surface was relatively smooth.

In normal skin, the epidermis was separated from the dermis by a basement membrane, adjacent to which the dermis was organized into a thin layer corresponding to the papillary layer of mammals (Fig. 3). For the most part, the junction between the basement membrane and dermal papillary layer was smooth, although occasional papillary projections into the epidermis were seen. The papillary layer consisted of thin collagenous bundles, small mononuclear cells, small blood vessels and chromatophores. The chromatophores extended into the subjacent reticular layer. Below the papillary layer, the reticular layer consisted of large collagenous bundles and fibroblasts, with blood vessels scattered throughout.

The cutaneous lesions of affected turtles were diagnosed as fibropapillomas because of the papillary proliferation of the epidermis on broad fibrovascular stalks (Fig. 4). In what was interpreted as the earliest lesion, the epidermis was

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Fig. 1. Two large verrucous fibropapillomas in the soft tissue area adjacent to the right hindleg of a green turtle.

Fig. 2. Large verrucous fibropapillomas in the intermandibular area and covering the globe of the right eye of a green turtle.

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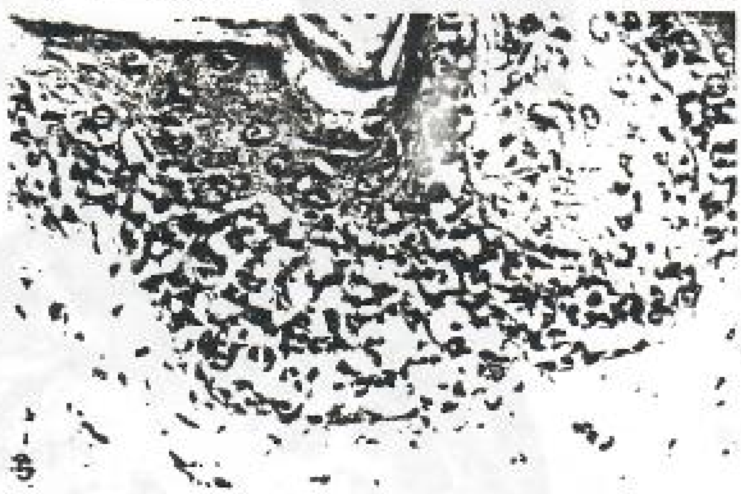


Fig. 3. The normal skin of a green turtle. The epidermis is approximately four to seven cells thick and is covered by a thin keratinized layer corresponding to the stratum corneum. Immediately below the papillary layer (p), is the reticular layer (r), which is composed of large collagenous bundles. HE × 160.

Fig. 4. A fibropapilloma showing an arborizing pattern. Dermal proliferation is seen as the major component of the tumour. HE × 62.

Fig. 5. The skin of a green turtle with multiple fibropapillomas. In what was interpreted as the "earliest" lesion, ballooning degenerative changes are seen in cells within the stratum basale. HE × 400.

hyperplastic, with rete ridges extending into the dermis. Multifocal areas of ballooning degeneration were seen in the stratum basale (Fig. 5). The papillary layer of the dermis consisted of numerous fibroblasts and compact bundles of collagen fibres; perivascular cuffs of lymphocytes and plasma cells were seen throughout the dermis.

While some fibropapillomas showed no substantial increase in the thickness of the stratum corneum, some were hyperkeratotic. In others, cleft formation was observed between epidermal and dermal layers. The epidermis was slightly to moderately hyperplastic, ranging from 7 to 15 cells thick. The conjunctival/palpebral fibropapillomas ranged from 15 to 20 cells thick. In some sections, epithelial cells within the stratum spinosum were hypertrophic with vacuolated nuclei. In these areas, amphophilic intranuclear inclusions were occasionally seen.

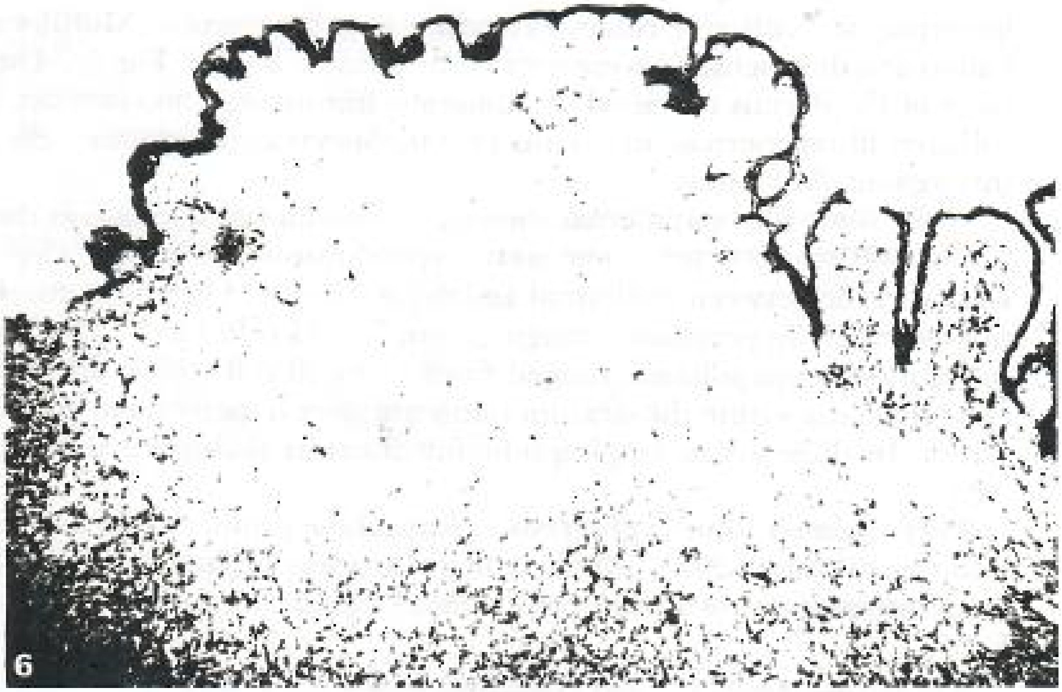
There seemed to be a progression from these proliferating, arborizing early tumours to those with a relatively less verrucous surface, having a minimal to moderate amount of folding and a more significant dermal component (Fig. 6).

Ultrastructurally, normal epidermis had a maturation pattern of basal cells differentiating into a single cell type. The stratum basale was a single cell layer and was composed of columnar cells with irregular cytoplasmic and nuclear outlines. The average size of cells in this layer was $4 \cdot 5 \times 9 \cdot 5$ µm; the nuclei measured $2 \cdot 5 \times 5$ µm. The cells were joined to each other and to the cells in the overlying layer by desmosomes and to the basal lamina by hemidesmosomes. The cytoplasm of cells in the stratum basale had pronounced bundles of tonofilaments. In the cytoplasm, lipid droplets (containing flocculent or granular material), scattered ribosomes, mitochondria and endoplasmic reticulum were seen.

Above the basal cells was a layer 1 to 2 cells wide which corresponded to the stratum spinosum of mammals. The average size of these cells was 5×11 µm; nuclei measured 4×7 µm. The more superficial cells in this layer were flattened, with their long axis oriented parallel to the epithelial surface. Compared with the stratum basale, there were increased numbers of mitochondria, endoplasmic reticulum, Golgi and ribosomes in the cytoplasm, but there were fewer tonofilaments. The outermost cells of this layer had cytoplasmic vesicular bodies and granules of various electron densities which measured approximately 150 to 170 nm (Fig. 7).

Above the stratum spinosum was a layer of cells which had lost their nuclei. These cells contained granules near the cell margins, or groups of granules coalescing and being discharged into the extracellular space. Above this layer, the cells of the stratum corneum were flat, had a thick cell membrane, were filled with electron-lucent material and had no subcellular organelles.

The epidermis of fibropapillomas was thrown into multiple folds associated with proliferation of the dermal component. Dermal papillae projected towards the surface of the epidermis. The epidermis was thicker than normal epidermis and consisted of four to seven nucleated cell layers, several layers containing nuclear remnants and a stratum corneum. Intercellular spaces were larger than those of normal epidermis. Compared with the normal stratum



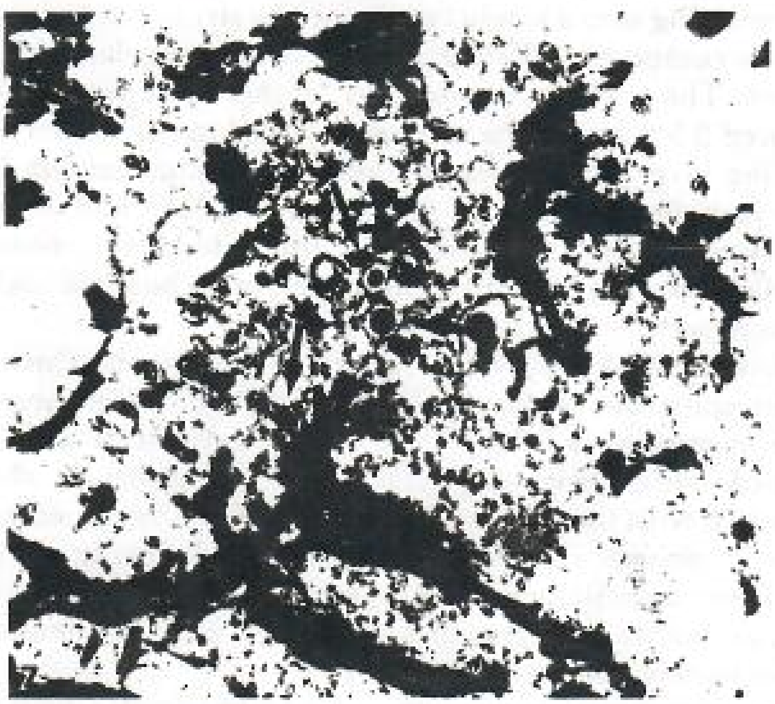


Fig. 6. A mature fibropapilloma from a green turtle. The surface shows minimal folding and the dermal mesenchymal connective tissue is the major component of this tumour. HE ×62.

Fig. 7. Transmission electron micrograph showing intracytoplasmic particles (arrows) of irregular shape, some having an electron-dense core, within the cytoplasm of a cell in the stratum spinosum of a normal turtle. ×20 200.

basale, there were increased numbers of mitochondria and endoplasmic reticulum. The stratum spinosum was three to six cells thick and the cells were pleomorphic. There was hypertrophy and hyperplasia of the cells in this layer. The subcellular organelles in these cells were more numerous than in the corresponding layer of normal epidermis. Mitochondria, vesicular bodies, hyperplastic, with rete ridges extending into the dermis. Multifocal areas of ballooning degeneration were seen in the stratum basale (Fig. 5). The papillary layer of the dermis consisted of numerous fibroblasts and compact bundles of collagen fibres; perivascular cuffs of lymphocytes and plasma cells were seen throughout the dermis.

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Above the stratum spinosum was a layer of cells which had lost their nuclei. These cells contained granules near the cell margins, or groups of granules coalescing and being discharged into the extracellular space. Above this layer, the cells of the stratum corneum were flat, had a thick cell membrane, were filled with electron-lucent material and had no subcellular organelles.

The epidermis of fibropapillomas was thrown into multiple folds associated with proliferation of the dermal component. Dermal papillae projected towards the surface of the epidermis. The epidermis was thicker than normal epidermis and consisted of four to seven nucleated cell layers, several layers containing nuclear remnants and a stratum corneum. Intercellular spaces were larger than those of normal epidermis. Compared with the normal stratum endoplasmic reticulum, Golgi bodies and ribosomes were abundant; nucleoli were more prominent in a greater proportion of cells than in normal epidermis. Granules with electron-dense cores were also present in the more superficial cells of this layer.

As in normal skin, a well-defined basement membrane separated the dermis from the epidermis. A narrow band of collagen fibrils and anchoring fibrils was present. However, the superficial dermis was not arranged into loosely spaced bundles as seen in the normal epidermis, but consisted of a thick matrix of fibroblasts and collagen fibrils, both randomly arranged. The collagen bundles were denser and more tightly packed and the fibroblasts were larger, more pleomorphic and more numerous than in normal dermis. In deeper areas of the dermis, there were clusters of cells around blood vessels; numerous lymphocytes, a few heterophils, plasma cells, and macrophages were observed.

In the earliest lesions, ballooning degeneration, as determined by light microscopy, was found by electron microscopy to result from development of membrane-bound vacuoles, which were located above and below the epidermal basement membrane and were filled with granular electron-dense material. In the basal cell layer, vacuoles were both intracytoplasmic and extracellular (Fig. 8); their size varied greatly and ranged from 1 to 11 μm. Occasionally, vacuoles were seen just above the basal cell layer. In frozen sections, vacuoles failed to stain with PAS or oil red 0.

In the dermis, vacuoles were also both intracytoplasmic and extracellular and occurred mainly in the papillary layer directly below the basement membrane (Fig. 9). Rarely, particles 155 to 190 nm in diameter with electron-dense centres were observed within vacuoles (Fig. 10). The specific cells affected could not be identified. Large vacuoles completely filled the cytoplasm and at times it was difficult to determine whether vacuoles were intra- or extracellular.

The biopsy specimen from an Hawaiian green turtle tumour was similar to those of the green turtles from Florida except for one notable difference: trematode eggs, having a distinct golden yellow capsule (with HE staining) and consistent with eggs of members of the family Spirorchidae, were commonly seen within dermal vessels of the fibropapilloma from the Hawaiian green turtle. There was often a minimal to moderate inflammatory response around these vessels consisting of small mononuclear cells and occasional giant cells. No trematode eggs were seen in any of the biopsy specimens from the Florida green turtles examined.

Histochemical and Molecular Findings

All fibropapillomas examined were negative for papillomavirus group-specific antigens by the peroxidase–antiperoxidase technique.

Papillomavirus DNA could not be demonstrated in any of the six fibropapillomas examined by ethidium bromide-stained gells or by low stringency Southern blot hybridization, with a bovine papillomavirus type-2 DNA probe. A reverse Southern blot against 25 different cloned papillomaviruses also failed to demonstrate papillomavirus DNA in both samples examined.

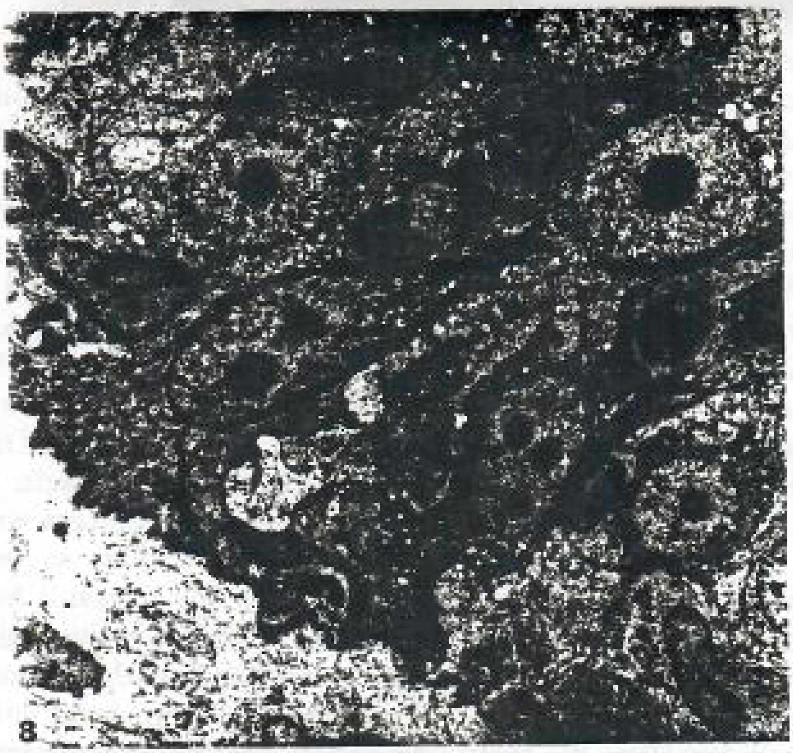

Fig. 8. Transmission electron micrograph of an "early" lesion. Membrane-bound intracytoplasmic (arrow heads) and extracellular (arrow) vacuoles within the stratum basale. × 2100.

Fig. 9. Transmission electron micrograph of the epidermal/dermal junction of an "early" lesion. Membrane-bound vacuoles are seen in the extracellular space in the stratum basale (arrow heads) and in the dermis (arrows), just below the basement membrane. Numerous iridophores (i) containing reflecting platelet profiles are also seen within the dermis. × 2100.

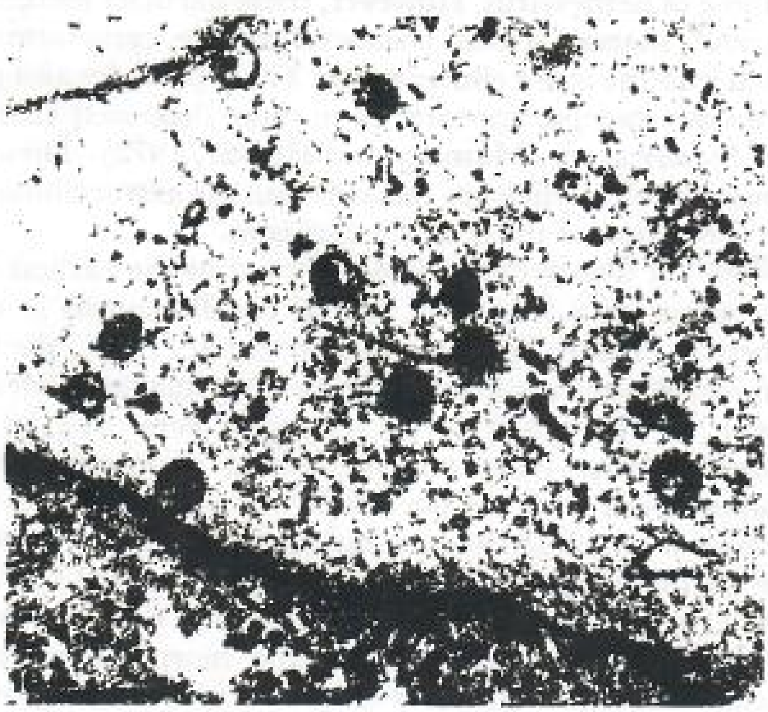

Fig. 10. Transmission electron micrograph of a membrane-bound vacuole within the dermis. Several particles with electron-dense centres are seen. × 32 000.

Discussion

The proliferative cutaneous lesions of the green turtles of the present report were consistent with previously reported fibropapillomas of green turtles from Florida (Lucke, 1938; Smith and Coates, 1938). Morphologically, the lesions in the turtles had some similarities to cutaneous fibromas of deer and fibropapillomas of cattle, i.e., epithelial hyperplasia overlying marked dermal proliferation. However, koilocytotic atypia, a cytopathological feature of productive papillomavirus infection in mammals (Koss and Durfee, 1956; Sundberg, 1987), was not present in the green turtle fibropapillomas.

By light microscopy, with HE staining, hypertrophic nuclei containing amphophilic intranuclear inclusions were occasionally observed. These inclusions superficially resembled intranuclear inclusions seen in grey patch disease of green turtles, a skin disease caused by a herpesvirus (Rebell, Rywlin and Haines, 1975). Herpesviruses have been incriminated in the pathogenesis of papillomas in other animals including such diverse species as the European green lizard, *Lacerta viridis* (Raynaud and Adrian, 1976) and African elephants, *Loxodonta africana* (Jacobson, Sundberg, Gaskin, Kollias, and O'Banion, 1986).

By electron microscopy, structures corresponding to the amphophilic intranuclear inclusions seen by light microscopy could not be identified. The composition of these structures remains unknown.

Intracytoplasmic particles containing electron-dense cores were commonly seen within the layer of cells between the stratum spinosum and stratum corneum of both normal and abnormal epidermis. Superficially they resembled those of herpesvirus. However, these particles merged with larger granules of various shapes, which coalesced at the cytoplasmic margins and were released into the intercellular space. These particles and granules are similar in electron microscopic appearance to those described for the normal skin of the turtle, *Pseudemys* sp. (Matoltsy and Huszar, 1972). These are considered to be mucous granules which are released into the extracellular space and contribute to the formation of the stratum corneum.

Ballooning degeneration was observed in the earliest lesions. Although this was reported as a feature of genital papillomatosis in sperm whale bulls by Lambertsen, Kohn, Sundberg and Buergelt (1987), the ballooning changes in sperm whales were confined to the stratum intermedium and stratum spinosum whereas, in the green turtle, changes were seen in the stratum basale. Ultrastructurally, these cells contained membrane-bound vacuoles which contained electron-dense flocculent material; vacuoles were also seen in the epidermal intercellular spaces and in the cytoplasm of cells within the dermis. These vacuoles failed to stain with PAS or oil red 0. Particles with electron-dense cores and measuring 155 to 190 nm in diameter were rarely seen within these vacuoles. Their regular size and morphology were suggestive of viral particles. Vacuoles were never seen in what were interpreted as older papillary-type lesions and their nature remains unknown.

Sarcoid, a common cutaneous neoplasm of horses believed to be caused by bovine papillomavirus types 1 or 2 (Amtmann, Muller and Sauer, 1980; Lancaster, 1981; Trenfield, Spradbrow and Vanselow, 1985) has several histopathological features in common with green turtle fibropapilloma. Like green turtle fibropapilloma, the epidermis is acanthotic, the bulk of the lesion consisting of mesenchymal proliferation (Ragland, Keown and Spencer, 1970). However, the picket-fence pattern of fibroblasts at the junction of epidermis and dermis characteristic of equine sarcoid was not seen in green turtle fibropapilloma.

In many instances, papillomavirus infections may not be productive (Pfister, 1984), and without specific probes, the viral genome may be impossible to identify. As an example, an integrated papillomavirus genome has been identified in equine sarcoids by a labelled probe of bovine papillomavirus type 1 (Amtmann *et al.* 1980; Lancaster, 1981; Trenfield *et al.* 1985). In the present report, the inability of the cloned DNA probes to demonstrate the presence of papillomavirus in green turtle fibropapilloma may be the result of phylogenetic divergence and lack of common DNA sequences between cloned mammalian papillomaviruses and (if it exists) a green turtle papillomavirus. Since a papillomavirus cloned from an African grey parrot hybridized to a minimal degree with mammalian papillomaviruses at low stringency (O'Banion *et al.* 1987), it would be expected that a turtle papillomavirus would also show limited (if any) hybridization. Thus, the involvement of papillomavirus in the development of green turtle fibropapilloma cannot be discounted.

Green turtles are commonly infected with the spirorchid trematode *Laeredius laeredei*. The adult parasites are located in the chambers of the heart and within the lumen of the great vessels (Griner, Forrester and Jacobson, 1980). Since trematode eggs have been seen within dermal vessels of fibropapillomas, their role in the pathogenesis of green turtle fibropapilloma has been questioned. It is the experience of one of us (ERJ) that eggs, released into the vascular compartment and a variety of extracardiac sites including lungs, viscera, gastrointestinal tract and skin, often result in vasculitis and perivasculitis, with vessels becoming obstructed. However, since Smith and Coates (1939) only observed these ova in approximately half of the 250 tumours evaluated, they believed that they were not of primary importance in the aetiology of the disease. Although, in the present study, trematode eggs were identified in biopsy specimens from an Hawaiian turtle, none were seen in multiple sections of 28 biopsies examined from six juvenile green turtles collected in the Indian River Lagoon System. Thus, trematode eggs are most likely to be an incidental finding.

While some fibropapillomas in most turtles decreased in size over the period of evaluation, many slowly increased in size. Ulceration, due to abrasion, was common in the largest lesions. Fibropapillomas which involved the conjunctiva and palpebra grew rapidly and obscured the vision of the affected turtles. This was similar to fibromas reported for white tailed deer (Sundberg and Nielsen, 1981). Based upon these experiences with captive turtles, it is expected that fibropapillomas ultimately can be life-threatening to free-ranging turtles either by obscuring vision and thus affecting the ability to feed or by becoming large enough to impair normal swimming.

In this study, several large fibropapillomas were surgically removed. Those that had a wide excisional margin did not show any signs of recurrence by 2 months after surgery and in many instances showed complete healing. For captive green turtles with fibropapillomas, surgical removal should be performed as soon as possible.

The occurrence of fibropapillomas in dysjunct populations of green turtles, and the increased numbers of affected turtles within the Indian River Lagoon System of east central Florida suggests an infectious aetiology. On a comparative basis, a viral agent seems most likely. As with many viral-associated neoplasms of mammals and birds, cell cultures of green turtle fibropapilloma may be necessary to determine whether a virus is involved in the pathogenesis of this disease. Transmission studies would also be necessary to demonstrate a causal relationship between an isolated agent and green turtle fibropapilloma.

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ANALYSIS OF A FIBROPAPILLOMA OUTBREAK IN CAPTIVITY

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In mid-September 1986 two facilities in the Florida Keys received hatchling green sea turtles (Chelonia mydas) from Hutchinson Island, Florida. One facility was a pen (approximately 4 by 2 by 1.5 meters deep) built under the dock at Lignum Vitae Key State Botanical Site specifically for headstart turtles. These were the only turtles housed at this site, and the pen design ensured constant flushing with very clean seawater. The second facility was Theater-of-the-Sea, a tourist attraction on Windley Key, approximately 7 km northeast of Lignum Vitae Key. Theater-of-the-Sea cooperates in the sea turtle stranding network, housing sick and injured turtles. The facility has an array of pools excavated in the fossil coral bedrock of the island. Sea water flows into the facility through an excavated trench from a nearby marina and through a culvert under a road. A pair of pumps lift 12 million gallons per day one to two meters into a higher trench. From there it flows by gravity throughout their pool system. The pool system includes a series of eight small pools used for displaying sea life, then a much larger pool housing several dolphins. The upstream display pool contains two female California sea lions. The second display pool, immediately downstream of the sea lion pool, houses a variety of fish, including several sharks, and at one time housed a large green sea turtle. This turtle developed fibropapillomas after at least several years in captivity, apparently without ever coming into direct contact with other green sea turtles. The third, fourth, fifth, and sixth housed fish and a few rehabilitating and permanently captive sea turtles. The fourth contained a fenced "hospital" enclosure in the mid-1980s that was used for rehabilitating injured and ill turtles, including turtles with fibropapilloma. The seventh houses rays, and the eighth, nurse sharks. The large dolphin pool is at the downstream end of the system. No surface outlet exists, and water returns to the surrounding ocean through the highly porous fossil coral. Water quality in the system is surprisingly high, but some nutrification may be occurring in the source marina.

The Lignum Vitae Key facility received 35 hatchling green sea turtles on 17 September 1986, and at about the same time, Theater of-the-Sea received 24. All 35 turtles at Lignum Vitae prospered, and in the course of a year, outgrew their pen. At Theater-of-the-Sea, the turtles were placed in a floating pen in the hospital enclosure in pool four. Survival was poor, but none developed visible papillomas. On 11 August 1987 the 35 turtles from Lignum Vitae were transferred to Theater-of-the-Sea for the second year of their life. After a brief period in the floating pen in the hospital enclosure, all the headstart turtles were transferred to a pen opening into the second pool, just downstream of the sea lions. A total of 30 of the turtles, including 24 from the Lignum Vitae batch, survived the second year of captivity. In June 1988, approximately ten months after the transfer from Lignum Vitae, small fibropapillomas were noticed on some of the turtles. By July all 30 surviving turtles had developed fibropapillomas. Blood samples were taken from all 30 turtles in July 1988 for sex determination. Four turtles were transferred to the House of Refuge for examination of the papillomas, and on 15 September 1988 were released into the Indian River Lagoon. The remaining 26 were released on 6 August 1988 at Sombrero beach, Marathon.

At the time of release, papillomas were growing around the eyes, on the flippers, and elsewhere on the skin. They were particularly prevalent on the perforations in the flippers caused by tagging. At the time of release most papillomas were less than 2 cm in diameter.

This incident provides some insight into the epidemiology and etiology of fibropapilloma in green sea turtles. If the turtles were all from the same clutch, embryonic transmission from the mother is a possibility to keep in mind. The most likely explanation for the outbreak, however, is that the as yet unidentified infectious agent was introduced to Theater-of-the-Sea by one or more infected turtles brought into the facility for treatment and rehabilitation. It is our understanding that infected turtles may have been in direct contact with the Theater-of-the-Sea headstart turtles in the first year, and briefly with the Lignum Vitae turtles in August 1987. Subsequently, all the headstart turtles were maintained in isolation, upstream from the "hospital." This location, however, is contiguous to the pool formerly occupied by the large green sea turtle that contacted fibropapilloma in relative isolation.

The infectious agent could possibly have been transmitted by serial contact with employees of the facility during feeding or other care, but no direct evidence for such transmission is available. The agent also could have persisted in the bottom of pool two, previously occupied by an infected turtle. The agent could have been dispersed through the water, or by direct contact through the pen walls during August 1987. If so, the infection would have remained latent for about nine months. The turtles initially housed at Theater-of-the-Sea could have become infected prior to August 1987, and subsequently infected the Lignum Vitae turtles, in turn. If this happened, the latency periods for the two groups must have been very different (or the turtles were infectious long before showing symptoms). Such different latency periods are unlikely unless turtles are not competent to develop fibropapillomas until a certain developmental stage (reached in this case at age 21 months).

Alternatively, marine leeches (Ozybranchus sp.) present in the facility could have acted as vectors. These leeches commonly are found on green sea turtles with fibropapilloma, and often attach directly to the papillomas. It is our personal opinion that transmission by leeches is the most likely mode of infection. We think transmission by leeches best explains the infection of the large turtle in pool two, although we cannot rule out the possibility of unrecorded direct contact. We are also unsure that these leeches are capable of travelling upstream from pool to pool, although such travel by creeping along the pool walls seems plausible. During a recent visit, the water flow between pools two and three was timed at one-third to one-half meter per second.

We believe that this incident demonstrates a need to develop protocols for the handling and housing of green sea turtles with fibropapilloma. We would suggest the following:

1) Until transmission is better understood infected turtles should be maintained at some level of quarantine from apparently uninfected turtles. At a minimum, turtles with fibropapilloma should be physically segregated from noninfected turtles, and facilities used to hold infected turtles should be disinfected, if possible, before re-use.

2) We also recommend controlling leech populations in holding facilities, and removing leeches from turtles recovered from the wild.

3) Housing infected turtles in separate sea-water systems from uninfected ones would be preferred, where possible. Where possible, treating papilloma turtles in separate facilities from other turtles is advisable.

4) Until the mode of transmission is better understood, the release of turtles with a history of papilloma into the wild may be unwise. Green turtles at the Cayman Islands turtle farm have survived fibropapilloma infections and presumably have become non-infectious. The same likely would be true of rehabilitated turtles, once tumor growth ceased, but this issue needs to be tested.

5) In any case, we strongly recommend that turtles with young, growing papillomas not be released in the future, and in particular, not be transported to be released away from the region where the infection occurred.

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Herpesvirus in cutaneous fibropapillomas of the green turtle *Chelonia mydas*

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ABSTRACT: Two juvenile green turtles *Chelonia mydas* with multiple cutaneous and ocular fibropapillomas were evaluated. Both turtles were anesthetized and fibropapillomas were surgically removed and examined by light microscopy. Turtle No. 1 died postsurgically and was necropsied. Turtle No. 2 recovered and was anesthetized 3 wk later to remove remaining fibropapillomas. Three weeks after the second surgery, Turtle No. 2 died and was necropsied. Histopathologic evaluation of hematoxylin and eosin stained sections of fibropapillomas of both turtles revealed areas of ballooning degeneration of epidermal cells associated with eosinophilic intranuclear inclusions. By electron microscopy, inclusions consisted of virus-like particles measuring 77 to 90 nm. Envelopment of these particles was observed at the nuclear membrane and mature enveloped particles measuring 110 to 120 nm were present in the cytoplasm. Based upon morphology, size, and location the particles were compatible with those of the family Herpetoviridae.

INTRODUCTION

While relatively few types of tumors have been reported in chelonians (Jacobson 1981), fibropapillomas are commonly encountered in free ranging green turtles *Chelonia mydas* (Jacobson et al. 1989). Green turtle fibropapilloma (GTF) was first described in green turtles from the Florida Keys (USA) in 1938 (Lucke 1938, Smith & Coates 1938) and almost 50 yr later from green turtles in Hawaii (Balazs 1986). Based upon anecdotal reports of fishermen, GTF was seen around 1900 in Florida (Ehrhart pers. comm.) and in 1958 in Hawaii (Balazs pers. comm.). One of us (E.R.J.) has been informed by sea turtle biologists that GTF has been seen in near-shore sites in: Puerto Rico, Cayman Islands, Virgin Islands, Barbados, Venezuela, Colombia, Panama, Belize, and Australia. Over the last 10 yr there has been an increased incidence of GTF in the Indian River Lagoon System of east central Florida (Jacobson et al. 1989) and Hawaii (Balazs pers. comm.).

The light- and electron-microscopic characteristics of GTF have recently been described (Jacobson et al. 1989). The earliest lesions exhibited ballooning degeneration of basal epidermal cells, with intracytoplasmic vacuoles occasionally containing particles with electron-dense centers; the nature of these particles could not be determined. Molecular studies using cloned mammalian papillomavirus probes failed to identify members of this group of viruses in GTF. While spirorchid trematode eggs are commonly observed within dermal capillaries (Smith & Coates 1939), tumors without eggs have also been observed and the role of these parasites in the development of GTF has been questioned (Jacobson et al. 1989). The etiology of GTF remains unknown.

This paper presents preliminary evidence of herpesvirus involvement in GTF.

METHODS AND MATERIALS

Two juvenile green turtles *Chelonia mydas*, found in a debilitated condition in the vicinity of Key West, Florida, USA, were transported by air to the Veterinary Medical Teaching Hospital, University of Florida, Gainesville, Florida, for clinical evaluation. Turtle No. 1 was estimated to be 3 to 4 yr old and weighed 8 kg. It was submitted with generalized cutaneous fibropapillomatosis involving multifocal areas of the soft integument, periocular tissues and corneas. The turtle was anesthetized with a tiletamine/zolazepam combination (Telazol, A. H. Robbins, Richmond, VA, USA), given intramuscularly at 15 mg kg^{-1} body weight.

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Following anesthesia, the turtle was intubated and manually ventilated at 6 breaths min^{-1}. Cryosurgery was performed on 2 large tumors on the cervical integument and tumors on the corneas were removed by keratectomy. Following surgery, ventilation was continued until the turtle started lifting its head and moving its limbs. However, at 10 h following injection of the anesthetic agent, the turtle became visibly depressed and apneic, and died 2 h later. Subsequently, it was submitted for necropsy. Three cutaneous fibropapillomas and multiple visceral tissues were fixed in neutral buffered 10 % formalin, embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin (H & E).

Turtle No. 2 was estimated to be 3 to 4 yr old and weighed 11 kg. It also had generalized cutaneous fibropapillomatosis involving both the soft and hard integument, periocular tissues, and corneas. The turtle was anesthetized with ketamine hydrochloride (Ketaset, Aveco, Fort Dodge, IA, USA), given intramuscularly at 15 kg kg^{-1} body weight. Once immobilized, it was intubated and administered an isoflurane (Forane, Anaquest, Madison, WI, USA) in oxygen mixture. Fourteen fibropapillomas were surgically removed and bisected. Half of each tumor was placed in neutral buffered 10 % formalin, embedded in paraffin, sectioned at 6 µm and stained with H & E. The corresponding half of each tumor was placed individually in a plastic bag and frozen in an ultra-freezer at −70 °C. Following surgery, the turtle recovered without any complications. Three weeks later the turtle was again anesthetized and, utilizing a laser technique, 2 broad-based fibropapillomas were removed from the pericloacal region. Although the turtle recovered from anesthesia, it became anorexic and died 3 wk later. A necropsy was performed and multiple tissues were fixed in neutral buffered 10 % formalin, embedded in paraffin, sectioned at 6 µm and stained with H & E.

A piece of formalin-fixed paraffin-embedded fibropapilloma from Turtle No. 1 was removed from the block, post-fixed in osmium tetroxide, and embedded in effapoxy resin (Ernest F. Fullam, Inc., Latham, NY, USA). Thin section were stained with toluidine blue and examined by light microscopy. Ultrathin sections (800 nm) were placed on copper grids, stained with uranyl acetate and lead citrate, and examined with an electron microscope.

RESULTS

Gross pathology

At necropsy, Turtle No. 1 had ca 500 ml of clear, dark red fluid in the coelomic cavity. The gastrointestinal serosa, the surfaces of the liver, spleen, kidney, pericardium, and the fasicles of the skeletal muscle were surrounded by edema fluid. Trematode eggs were observed on light microscopic examination of a fecal sample.

The coelomic cavity of Turtle No. 2 contained a small amount of clear fluid. The surface of the right kidney had a few small discrete white, firm foci, which extended into the inner cortex; the largest focus was 1 mm in diameter. The left kidney contained multiple, white, discrete masses (1 to 4 cm in diameter). These masses extended from the cortex into the medulla and altered the shape of the kidney. Both lungs contained small (1 to 2 cm in diameter) white, firm masses in the parenchyma.

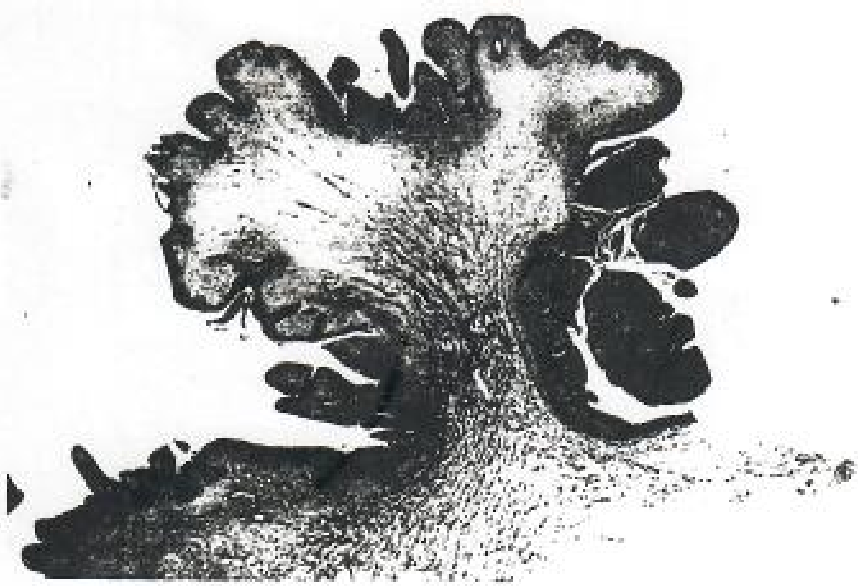

Fig. 1. *Chelonia mydas*. Histologic section of a cutaneous fibropapilloma of a green turtle. The fibropapilloma consists of a hyperplastic epidermis overlying a thickened dermis composed of proliferating fibroblasts and abundant loosely arranged collagen. H & E stain, × 7.5

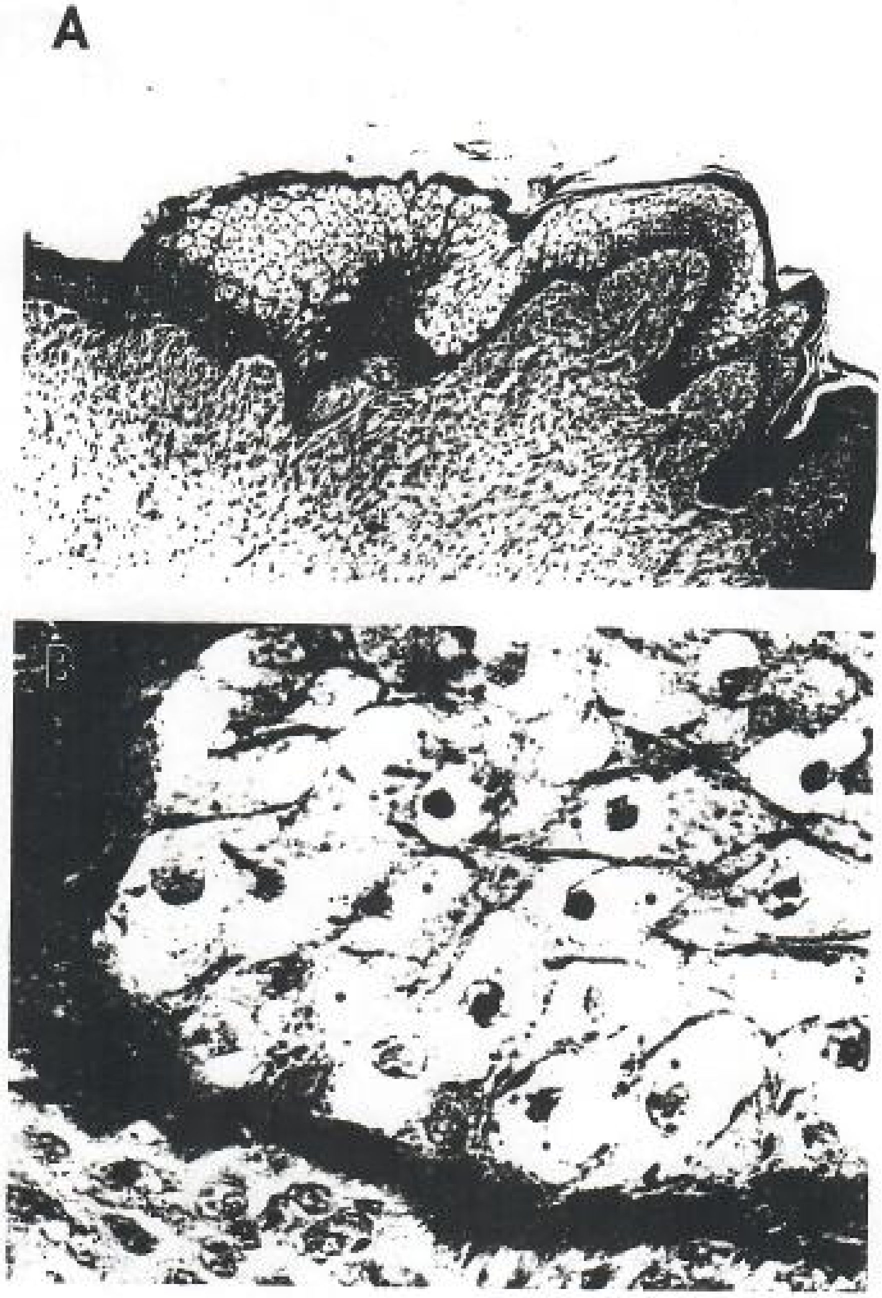

Fig. 2. *Chelonia mydas.* Green turtle fibropapilloma. (A) Focal area of ballooning degeneration. H&E stain, ×75. (B) At higher magnification, epidermal cells can be seen to contain intranuclear viral inclusions. H & E stain, × 750

Histopathology

Histologic examination of the liver, spleen, and lung of Turtle No. 1 revealed multiple granulomas surrounding trematode eggs. The 3 cutaneous fibropapillomas examined were characterized by marked hyperplasia of the epidermis with anastomosing rete ridges extending into the dermis. The epidermis also was thrown into papillary fronds (Fig. 1) and in several foci, there was marked cytoplasmic vacuolation and ballooning degeneration of superficial epidermal cells (Fig. 2A). Many of these spongiotic cells contained eosinophilic intranuclear inclusions (Fig. 2B). The underlying dermis consisted of proliferating fibroblasts, with occasional trematode eggs within capillaries.

Histologic examination of the lung of Turtle No. 2 revealed that most of the parenchyma was replaced by hyalinized fibrous tissue. Several trematode eggs, measuring 20 to 50 µm, were embedded in the fibrous tissue. Eggs were also present within the interstitium of

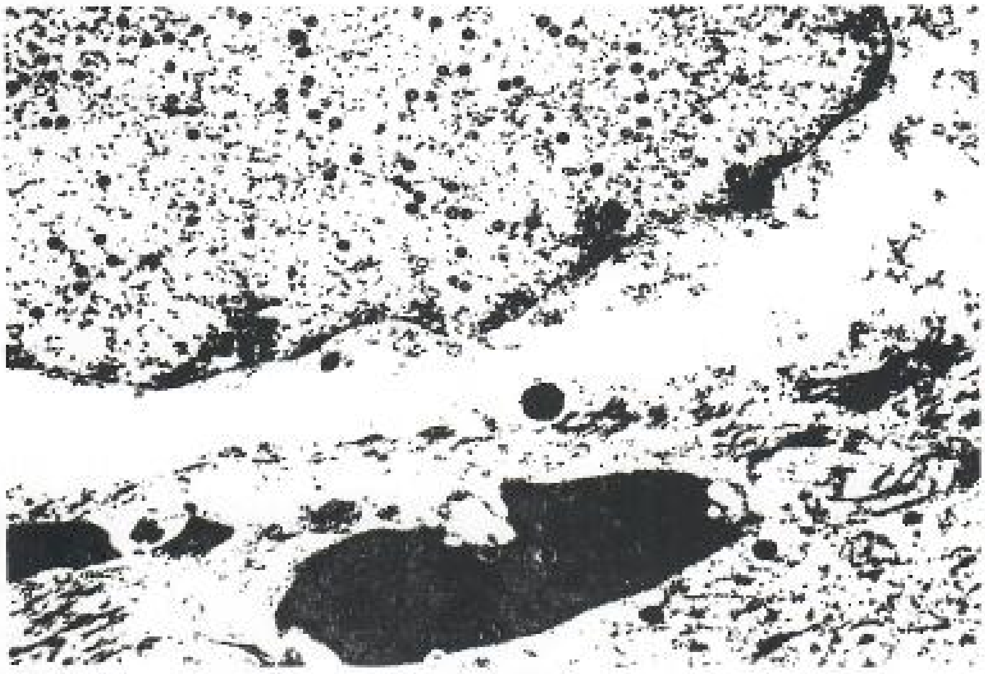

Fig. 3. *Chelonia mydas.* Low magnification electron micrograph showing intranuclear (I) and intracytoplasmic (C) viral particles within epidermal cells of a fibropapilloma. × 29 575

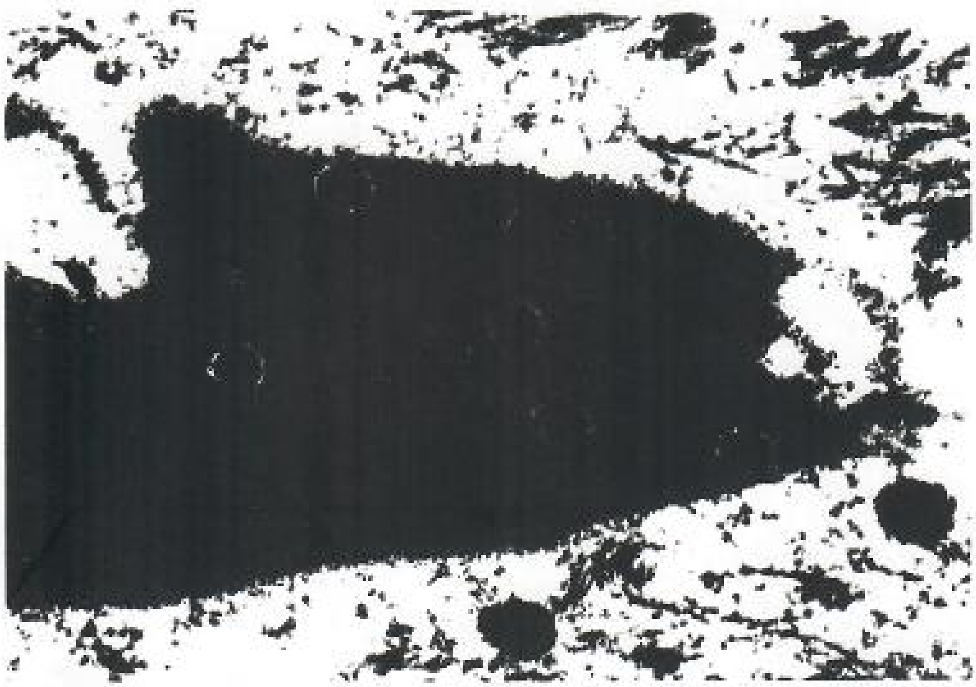

Fig. 4. *Chelonia mydas.* Higher magnification electron micrographs of mature particles within the cytoplasm with morphology typical of herpesvirus. × 91 975

uninvolved pulmonary parenchyma. There were similar areas of fibromatosis in the kidneys, with trematode eggs scattered within both affected and unaffected areas. Light microscopic examination of the 14 cutaneous fibropapillomas revealed that the surfaces were thrown into papillary projections with epidermal hyperplasia and proliferation of dermal fibroblasts. Trematode eggs were occasionally observed within dermal capillaries. In one small area of one of 14 tumors (7 %), there was ballooning degeneration of epidermal cells and eosinophilic intranuclear inclusions similar to those seen in Turtle No. 1.

Ultrastructural findings

By electron microscopy, intranuclear inclusions contained immature viral particles with electron-lucent and electron-dense centers; viral particles were also within adjacent areas of the cytoplasm (Fig. 3). Immature viral particles measured from 77 to 90 nm and obtained an envelope from the nuclear membrane. Mature, enveloped particles seen within adjacent areas of the cytoplasm measured from 110 to 120 nm (Fig. 4). Based upon size, location, and morphogenesis both types of particles were consistent with those of the family Herpetoviridae.

DISCUSSION

The cutaneous tumors observed in the green turtles had gross and light microscopic features typical of green turtle fibropapilloma (Jacobson et al. 1989). In addition, previously unobserved areas of epidermal ballooning degeneration, with eosinophilic intranuclear inclusions, were seen in several tumors. In Turtle No. 2, inclusions were confined to one small portion of one of 14 (7 %) tumors examined. This distribution indicated that for each turtle, many tumors need to be examined in order to determine the presence or absence of inclusions. The productive viral infection may only occur during very limited times in the pathogenesis of GTF.

By electron microscopy, intranuclear inclusions were found to consist of immature viral particles. In adjacent areas of the cytoplasm of infected cells, mature enveloped particles, compatible with those of herpesvirus, were observed. The only previously reported herpesviruses of green turtles are those responsible for grey patch disease (Rebell et al. 1975) and associated with a respiratory disease of green turtles at Cayman Turtle Farm, Grand Cayman, British West Indies (Jacobson et al. 1986a). The green turtles from Key West, Florida, represent the first green turtles outside of Cayman Turtle Farm to be observed with a herpesvirus infection.

Herpesviruses have been associated with and/or demonstrated to be the cause of neoplastic diseases of a variety of vertebrates including renal adenocarcinoma of the leopard frog (Lucké 1952), Marek's disease of poultry (Nanoyama 1982, Powell 1985) and Burkitt's lymphoma of humans (Werner & Gertrude 19[illegible]). Herpesviruses have also been incriminated as the causative agent of papillomas in the European green lizard *Lacerta viridis* (Raynaud & Adrian 1976) and African elephant *Loxodonta africana* (Jacobson et al. 1986b). However, the significance of herpesvirus as the primary pathogen versus a secondary infection can be debated. For example, while infectious bovine herpesvirus was isolated from bovine ocular squamous cell carcinomas (Sykes et al. 1961) and bovine herpesvirus-5 antigens were detected by indirect immunofluorescence in the cytoplasm of tumor cells derived from bovine ocular squamous cell carcinomas (Anson et al. 1982), a causal relationship between these bovine herpesviruses and bovine ocular tumors has not been firmly established. It is quite possible that these herpesviruses may only represent secondary infections, merely present in a growing tumor. Similarly, it is unknown whether or not the herpesvirus identified in the fibropapillomas of the green turtle of the present report is the etiologic agent of this disease. The identified virus needs to be isolated and Koch's postulates fulfilled in order to establish a causal relationship. Isolation attempts are in progress.

Trematodes of the family Spirorchidae are commonly encountered in the cardiovascular system of marine turtles and include members of the genera *Amphiorchis, Carettacola, Haemoxenicon, Hapalotrema, Laeredius, Monticellius, Neospirorchis* and *Squaroacetabulum* (Lauckner 1985). Pathological findings associated with eggs of these parasites have been described for the loggerhead sea turtle *Caretta caretta* (Wolke et al. 1982) and in both farmed (Greiner et al. 1980, Glazebrook & Campbell 1990a) and wild (Glazebrook & Campbell 1990b) green turtles.

Smith & Coates (1939) found trematode eggs in 50 % of fibro-epithelial growths on green turtles at the New York Aquarium and trematode eggs were occasionally seen within dermal capillaries of fibropapillomas of green turtles of the present report. Based upon the absence of trematode eggs in any of 28 biopsies of fibropapillomas examined from 6 green turtles, Jacobson et al. (1989) did not consider spirorchid eggs as the cause of GTF. In studies on diseases of farmed and wild marine turtles in Australia, although spirorchid trematode eggs were commonly associated with visceral granulomas in green turtles, fibropapillomas were not noted in any of the examined turtles (Glazebrook & Campbell 1990a, b). Still, transmission studies utilizing spirorchid eggs or egg components need to be conducted in order to conclusively determine if a causal relationship exists between spirorchid eggs and tissue fibromatosis. Regardless of the role of spirorchids in GTF, the visceral tissue reactions and alterations from eggs collecting at visceral sites probably contributed to the debility of the 2 green turtles in the present report.

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the resumption of commercial whaling, ostensibly to alleviate economic and cultural hardships suffered by their coastal communities. Their negative reaction to this opportunity to do so suggests that their stance may have been a sham. Realising that the Irish Initiative would be more restrictive than the *status quo*, they have lost interest in providing a few whales for their coastal communities, opting for larger whale kills on the open sea. A Greenpeace spokesman commented on the indifference to world opinion expressed by such reactions. Greenpeace is calling for a global whale sanctuary to cover coastal waters as well as the high seas, effectively ending Japanese and Norwegian whaling.

Despite this seeming impasse, the Irish Initiative will continue to be discussed until the IWC's next meeting in April.

SHIRLEY HENDERSON

Japanese Nuclear Waste Shipment Continues on its Way

The *Pacific Swan*, a British flagged ship reported as carrying a nuclear waste shipment to Japan (see *Mar. Pollut. Bull.* **35**) has passed through the Panama Canal and is heading across the Pacific Ocean despite protests from Panamanian environmentalists and Greenpeace activists.

According to Greenpeace the Pacific Swan is transporting 60 canisters of highly radioactive waste packed inside three transport casks containing 30,000,000 curies of radioactivity. The shipment is the largest of its kind and is part of a programme to ship some 3,000 canisters of nuclear waste from Britain and France to Japan. The environmental group also claims that the two countries also 'have clandestine plans to ship tens of tonnes of weapon-useable plutonium to Japan in the next decade'.

It is reported that countries and environmental groups throughout the Caribbean and Central America have protested about the current shipment and the Caribbean Community, the Organization of Eastern Caribbean States and the Latin American and Central American Parliaments have issued statements of opposition. In addition, US Representatives from Hawaii, American Samoa and Guam have written to President Clinton asking him to intervene against the transport.

While the route of the *Pacific Swan* remains a secret kept by the Japanese, French and British officials, it is

Human Herpes Responsible for Turtle Deaths?

It is feared that the tumour epidemic that is currently raging world wide (see *Mar. Pollut. Bull.* **34**, 989–990) is threatening the survival of endangered sea turtles species. For example, increasing numbers of loggerheads, olive ridley and green sea turtles are being found with the non-cancerous, but debilitating tumours that cover the eyes and noses of the victims. In some Hawaiian waters in excess of 90% of green turtles have been infected with tumours, with more than 50% recorded in the Indian river lagoon and Florida Bay. These tumours grow to large sizes affecting the eyes and nose, causing blindness and affixation, with internal tumours causing the constriction of organs which in many cases leads to death.

Biologists have noted an increased prevalence of tumour affected turtles in near shore areas and suggested possible links with terrestrial pollution. Experts have speculated that the turtle immune system is weakened by such pollution increasing their susceptibility to any viral pathogens.

An infectious viral agent has been identified in turtles which is genetically similar, but not identical to human herpes *Simplex* virus 1 and 2 and *Varicella zoster* virus, which causes chicken pox. Researchers believe that the turtle virus, which is similar to the human form of herpes, may be the cause of the tumours. Researchers at the University of Florida's College of Medicine, are unsure whether the virus is the cause of the disease or is just an associated. Other leading laboratories in the United States and Hawaii are having difficulties isolating and growing the virus in order to obtain conclusive results.

University of Florida researchers and the PathoGenesis Corp. and have developed a molecular diagnostic method to determine if the virus exists in individual tumours and normal tissues. Pathological studies over the past 6 years at the Florida Key turtle hospital have demonstrated that the tumour disease is caused by a viral agent, which has been consistently found to be associated with the tumours and not normal turtle tissues.

ADRIAN BUNKER

Nigerian Oil Spill

Due to a pipeline rupture at the Idoho platform off the coast of Nigeria, forty thousand barrels of crude oil were spilled into the sea on January 12. The platform, owned by Mobil Producing Nigeria Unlimited