August 3, 1973

MEMO TO: Dr. John Bardach
Director, H.I.M.B.

FROM: Robert M. Nakamura
Associate Animal Scientist

SUBJECT: TB and Salmonellosis in Green Sea Turtles (C. mydas)

This is a belated report on our disease studies on the turtles (C. mydas) while these were being housed at the Henke Annex on the U.H. Campus. We have, thus far, examined 32 turtles in all. Twenty-two of these died prior to January 1973 and for the most part were frozen specimens. We do not have complete data on the circumstances or dates of these deaths. Many of these were underweight and exact cause of death could not be established on all of these turtles. Between January 29 and March 13, 1973, 10 dead turtles were examined. On March 26, the turtles were moved to Coconut Island, and we have not examined animals since that date.

1. Tuberculosis

Four turtles from the latter group of 10 were found to have multiple, focal granulomatous lesions in the lung, liver, and kidneys. Acid fast bacilli were found in smears from these lesions. Histological changes were consistent with a diagnosis of tuberculosis. Tissue specimens were sent to the State Animal Diagnostic Laboratory, and forwarded to the National Animal Disease Laboratory in Ames, Iowa. The bacilli isolated were found to belong to the Mycobacterium Avium complex. This is, as far as we know, the first instance in which avian TB has been identified in turtles. Four out of 10 turtles in a two-month period constitutes a high attack rate, in my opinion.

2. Salmonellosis

We have isolated Salmonella from the turtles on 9 occasions in March 1973. We were isolating this bacteria almost at will from turtles that were housed in the Henke Annex. These isolates were sent to the Public Health Laboratory for sero-typing and were all found to be Salmonella weltevreden. As discussed in our telephone conversation, this serotype is one of the more common ones in Hawaii, causes a severe type of infection, and the exact source of this serotype in human infection is largely unknown. In turtles, Salmonellosis is a mild or non-clinical disease, but long-term carrier states have been described.

3. Compacted Turtles

In 9 cases (from the original 22 and the latter group of 10), the problem was found to be a compaction (constipation) in the lower intestines. This condition was thought to be nutritionally induced.

4. Dermatitis

Many of the turtles were afflicted with a skin condition which we felt was caused by a bacteria. Histologic sections showed numerous bacteria in the deeper portions of the lesions. Various treatment regimes were not completed and definitive conclusions were not formed.

5. Miscellaneous

Other turtles were afflicted with miscellaneous disorders such as gas (3) and congestion (4) in the intestines probably not severe enough to cause death. Fourteen were found to be underweight. In a large number, no changes could be found to indicate the cause of death.

Proposed Studies of Salmonellosis and Tuberculosis in Turtles

1. Salmonellosis

We are, at present, studying and evaluating a method for treating turtles with potassium permanganate, acriflavine, and malachite green for the elimination of the carrier state of Salmonellosis. In preliminary studies, we were very surprised to find that cures were effected in coin turtles.

We would like to continue our studies and perhaps extend these studies to the turtles on Coconut Island; and if this treatment method proves to be effective, to attempt to completely eradicate Salmonellosis from the turtles. In this effort, we would need to: (1) assess the Salmonella situation in your turtles at the present time; (2) select a few positive-culture turtles and subject them to the treatment; (3) place them in a clean and isolated environment; (4) monitor the feces for Salmonella; (5) stress (by dehydration) and retest for Salmonella; and (6) if negative results are obtained in step 5, other turtles may be similarly treated.

2. Tuberculosis

There are several steps in studies on TB that we would like to undertake:

(a) We would like to tuberculin test 3-4 turtles using avian TB antigen. If the results are negative, we would produce a positive subject by injecting the avian TB antigen into these turtles. After an appropriate time interval, these turtles would be retested to determine if turtles are capable of responding to a skin test. As far as I know, turtles have never been skin-tested for TB.

The antigen, avian tuberculin, can probably be used in the skin test. If not, an antigen will have to be prepared from our isolate. I have asked Dr. C. Thoen of the National Animal Disease Lab concerning this and have not, as yet, received an answer.

If any turtles turn out to be positive on the skin-test, I feel the animal should be killed and examined or at least quarantined to prevent spread to other turtles. We may even consider chemotherapy in the control (prevention) of TB in the turtles.

(b) If the skin test is found to be feasible for detecting TB, the source of the TB organisms in these turtles can be ascertained by TB testing of turtles and birds in the natural habitat (the Leeward Islands). If facilities for holding turtles and birds for a period of 72 hours is available, this is the period of time needed to inject the tuberculin and read the skin test.

These are the results of our studies on the turtles and proposals for continued studies on TB and Salmonellosis. As I said in our telephone conversation, I feel that these two diseases should preclude any considerations of release of these turtles. The proposed studies could still result in effective means for control of these diseases and safe release of the turtles. If turtles are being considered for aquaculture, then studies on diseases of turtles should commence with the proposed studies on TB and Salmonellosis and continue into studies on control of the dermatitis.

Sorry for such a long memo. I did want to get my thoughts on paper. If you have any questions, please feel free to call me (948-8334).

RMN:esm

cc: Dr. Allen Miyahara
Mr. George Balazs
Mr. Jim Brock

August 7, 1973

Dr. H. Robert Bustard
Australian National University
Canberra, A. C. T.
Australia

Dear Dr. Bustard:

A large portion of my research here in Hawaii deals with the nutritional aspects of captive reared marine turtles. In addition, I have recently completed a seven-week field study on the green turtle breeding colony at French Frigate Shoals. As you are probably aware, this remote area hosts the largest number of nesting turtles throughout the Hawaiian Archipelago.

Our present group of 100 green turtles were obtained from French Frigate Shoals at one-day of age. They are now 11 months old, healthy and range from 1.0 to 2.5 kg in weight. However, at approximately four months of age we experienced a 30 percent mortality over a four to five week period. As there were no external signs other than an accompanying weight loss, we turned the specimens over to a pathologist for examination. Ten of the animals were found to have granulomatous lesions in the lungs. In addition several had similar lesions in the liver and kidneys. Acid fact bacilli were found in smears from these lesions. Two of these samples were subsequently sent to the National Animal Disease Laboratory in Ames, Iowa. The bacilli ~~a~~isolated were recently found to belong to the Mycobacterium avium complex. To my knowledge, this is the first identification of avian Mycobacterium in turtles. In my opinion, the most logical route of infection would have been through the numerous sea birds (terns, shearwaters, albatross, etc.), which utilize the same islands as the turtles. Since much of your work has taken place on similar islands where birds nest, I thought it might be possible for you to have knowledge of this condition. If you have observed similar lesions or have information on this subject from your own personal experiences, I would greatly appreciate hearing from you at your earliest convenience.

Sincerely,

George H. Balazs
Jr. Marine Biologist

mk

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HRB:jjo

20 August 1973

Dr. G. H. Balazs
Marine Biologist
University of Hawaii
Institute of Marine Biology
P.O. Box 1346
Coconut Island KANEOHE. HAWAII 96744

Dear Dr. Balazs,

Thank you for your letter of 7 August 1973. I would certainly appreciate you keeping in touch with me in view of your interests in the nutritional aspects of captive reared marine turtles. Might I suggest that I would be most grateful for any reprints you may have and I would be happy to send you any of mine that you may request. I have had no experience with the lesions to which you refer. Most of the Islands where we are involved do not have nesting sea bird colonies and in view of the situation you describe, the occurrence of Mycobacterium avium is not surprising.

Do please continue to keep in touch.

Yours sincerely,

(H. R. Bustard)

April 29, 1974

Mr. Palmer Sekora
Refuge Manager
Hawaiian Islands National Wildlife Refuge
337 Uluniu St.
Kailua, Oahu 96734

Dear Mr. Sekora:

For the past 5 years we have been studying the immune response and the characteristics of the immunoglobulins (antibodies) in the sea turtle. This study was initiated as a result of a casual conversation with Dr. John Hendrickson, former Professor of Zoology, who told me that it has not been possible to trace the migration of these turtles. I suggested that an "internal marker" might be possible; that is, to immunize with an antigen (vaccine) which the animal would not come in contact with in nature, and then bleed sometime later and assay for the production of specific antibody synthesized to this antigen. This is a common procedure in the field of immunology. The idea was reasonable, thus, we initiated a study on the characteristics of the immune response and the antibodies. As a result, we have made some fundamental discoveries in regards to "immunity" in turtles. As far as I know, we are the only group studying this aspect of sea turtles. In addition, we have found that turtles, given a single injection of the antigen, still synthesize detectable antibodies to this antigen 5 years after injection. The animals at the Waikiki aquarium are being used for this work. Thus, we know that if we inject an adult animal (over 60 lbs) they can be recognized by this assay method 5 years later. We had received 3-month sea turtles some time ago from Dr. Hendrickson, and we have followed the ontogeny of the immune response in this animal--several very important basic finding were made in this regard. Now, we are in a position to ask the questions: (1) Will a newly hatched turtle injected with the antigen be recognized 1, 2, 3,.... years later?/ (2) What are the characteristics of the antibodies and "immunity" of very young turtles? In order to answer these questions I should like to request permission to obtain about 20 newly hatched turtles and perhaps a few eggs. I have talked with George Balazs about this project and he has agreed to help in any way that he can.

As part of our continuing interest in the immune response of lower vetebrates, we also are interested in studying in turtles a response known as "cell-mediated immunity". One manifestation of this reaction is the

"tuberculin reaction" (delayed-type hypersensitivty). This has not been studied in turtles. George also is interested in this response, because a positive tuberculin reaction is an indicator of exposure to the bacterium which causes tuberculosis (Mycobacterium sp.). George and I propose to do a fundamental study on the conditions required to elicit the tuberculin reaction. Perhaps this study would be of interest to you since development of this method, as well as other immunologic procedure, for detection of disease in turtles obviously will be related to their preservation.

Thank you for any help that you can give us.

Sincerely,

Albert A. Benedict
Professor

AAB:msm

cc: George Balazs, HIMB

UNIVERSITY OF HAWAII
Hawaii Institute of Marine Biology

MEMORANDUM
December 26, 1974

TO: DR. M. KREMRIN
DR. M. BALK
Clinical Investigation Service
Tripler Army Hospital

FROM: GEORGE H. BALAZS
Hawaii Institute of Marine Biology

CONCERNING: MARINE TURTLE STUDIES

In reviewing my records I find that over the past 10 weeks a total of three separate samplings for Salmonella organisms have been made of both green and hawksbill fecal matter and the sea water associated with the turtle facility. In addition, on one of these occassions samples were also taken of various feedstuffs as well as the intestinal contents of fish inhabiting the Coconut Island lagoon. It is my understanding that the only positive results obtained from these tests came from fecal matter isolated from a single pen (#2) which contained three animals.

Based on these results and the results of post-mortem examinations conducted on four greens and one hawksbill, I wonder if there is presently any reason why those animals not contained in pen #2 cannot be released from captivity into their natural environment. As you are aware, I have been planning the release of most of these turtles for some time, contingent of course on your clinical findings. If we can now be reasonably satisfied that it is safe to do so, I would like to proceed with this action.

cc
Dr. J. Bardach, Director HIMB
Dr. N. Palumbo, Comparative Medicine
Dr. A. Benedict, Microbiology

Clinical Investigation Svc
Box 251, Tripler Army Medical Center
APO San Francisco 96438

21 January 1975

Mr. George Balazs
Hawaii Institute of Marine Biology
P. O. Box 1346
Kaneohe, Hawaii 96744

Dear George:

It is our professional opinion that all of the green sea turtles presently housed at Coconut Island, except those in cage #2, could be released into their natural environment. With the exception of cage #2, Salmonella has not been cultured from any of these turtles during the multiple attempts to isolate this organism.

The public health hazard associated with the release of the turtles in cage #2 will probably be debated at length. We feel that the release of these animals in the open ocean does not represent a significant public health hazard when compared to open sea dumping of minimally treated sewage.

Sincerely,

Martin H Crumrine
MARTIN H. CRUMRINE, Ph.D.
Research Microbiologist

Melvin W Balk
MELVIN W. BALK, D.V.M., M.S.
Laboratory Animal Veterinarian

United States Department of the Interior

FISH AND WILDLIFE SERVICE

Hawaiian Islands and Pacific Islands
National Wildlife Refuges
337 Uluniu Street
Kailua, Hawaii 96734

February 4, 1975

Mr. George H. Balazs
Hawaii Institute of Marine Biology
University of Hawaii
P. O. Box 1346
Kaneohe, HI 96744

Dear George:

In reply to your letter of January 22, 1975, we have no objection to the release of those turtles obtained as hatchlings at French Frigate Shoals, Hawaiian Islands National Wildlife Refuge, during August 1972, under a U. S. Fish and Wildlife Service permit. However, we have been informed by the Hawaii Division of Fish and Game you will require a permit from them to release in Kaneohe Bay.

Prior to the release of any turtles, they should be certified free of any parasites and/or diseases which would be detrimental to other turtles and marine life. We will require that you return approximately ten tagged turtles to French Frigate Shoals for release there. This may provide further insights into their movements especially any possible return as nesters or breeders in ensuing years. Shipment of these turtles could be by Coast Guard Buoytender during their spring resupply mission to French Frigate Shoals. We will coordinate this activity. We suggest also that some be released in Kailua Bay where from time to time small turtles are reported.

This has been discussed with the Endangered Species Coordinator Eugene Kridler and he concurs.

Please inform us when the turtles are released on Oahu so that we may observe this event.

Thank you for the publications sent and we will look forward to receiving other papers when written.

Sincerely yours,

Palmer C. Sekora

Palmer C. Sekora
Refuge Manager

CONSERVE AMERICA'S ENERGY

Save Energy and You Serve America!

cc: Mr. M. Dillon, Mr. E. Kridler, Mr. M. Takata

J. HILLIS MILLER HEALTH CENTER • UNIVERSITY OF FLORIDA • GAINESVILLE, FLORIDA 32610
HEALTH CENTER ANIMAL RESOURCES DEPARTMENT Ph. 904/392-2977
P. O. BOX J-6

March 19, 1981

Dr. James A. Brock
Aquaculture Disease Specialist
Division of Fish and Game
Dept. of Land and Natural Resources
1151 Punchbowl Street
Honolulu, Hawaii 96813

Dear Dr. Brock:

Thank you for allowing me to review the material on the green turtles.

The periocular lesions described by both you and Dr. Balazs are commonly seen in aquaculture reared marine turtles. I have seen them both in Ridley's at Galveston, Texas, and in green turtles at Cayman Farm, Grand Cayman, B.W.I. I am more familiar with the syndrome in the Caymans.

As far as I know, grey patch disease has only been conclusively demonstrated in green turtles at Cayman Turtle Farm. This does not mean it is found nowhere else, but it has only been from turtles at Cayman Turtle Farm that the virus has been isolated and demonstrated in tissue section. Over the last few years I have seen hundreds of cases. Morbidity and mortality vary from year to year with each new group of hatchlings. It is generally not seen within the first two months following hatchling, nor after one year of age. Lesions can be seen over the entire body surface (plastron and carapace included) and start out as small papules that eventually coalesce into patches (in this month's issue of "The Compendium on Continuing Education for the Practicing Veterinarian" there is a gross photo of grey-patch in an article I have contributed). Very rarely is there involvement at only one site such as the periocular tissue. Generally it will become diffuse across the cervical skin and forelimbs. Secondary bacterial and mycotic infection add to the problem. Enclosed you will find a slide of grey-patch. Note the characteristic hyperplasia and hypertrophy of epithelial cells with lightly basophilic (somewhat glassy appearance) intranuclear inclusions.

The tissues that you sent represented necrotizing ulcerative epidermal lesions with dermal necrosis and inflammation in most areas. The surface was covered with necrotic debris containing myriads of bacteria. By Gram stain the predominant organisms were gram-negatives. GMS stained sections failed to reveal any fungi. The lesion, in the material I reviewed, was necrotizing compared to a more proliferative response for Gray-Patch. No inclusions were seen in your material.

In my experience most green turtles raised in aquaculture are kept under crowded conditions. Palpebral lesions commonly begin as abrasions from turtles rubbing against one another. Further damage, or initial damage, may be from rubbing against the walls of the holding tanks. Since these animals are constantly being bathed in a sea of microorganisms, traumatic lesions easily become infected. I would be curious in knowing the type of filtration system that is being used. Have the eyelid lesions been cultured, and have you tried any type of antibiotic therapy? Dr. George Leong at Galveston, and the people at Cayman Turtle Farm have had some success in controlling these lesions with antibiotic soaks.

Thus, in summary, I do not believe that you have a herpes virus infection. It very well may be that this is a husbandry problem that may be controlled by improved husbandry techniques. If you could supply me with information regarding the rearing techniques maybe I could be of further help.

I look forward to hearing from you and if you have any other material that you would like me to evaluate please send it along.

With best regards,

Elliott Jacobson, D.V.M., Ph.D.

EJ:ck

TURTLES THAT DIED AT THE WAIKIKI AQUARIUM OBTAINED FROM FFS ~ Sept 84 (most died)

ADP Case #85-132 (5/15/85)
Species: Bodianus sp., Gymnothorax sp. and Chelonia mydas. Small flipper tag attached
Culturette swabs from skin and shell lesions submitted for general aerobic bacterial isolation/identification.

Bodianus - Isolated bacteria resembling Moraxella - like bacteria Gp. M-6.

Gymnothorax - Isolated bacteria resembling Vibrio alginolyticus, Enterobacter agglomerans and Micrococcus sp.

Chelonia mydas - Isolated organisms resembling Vibrio alginolyticus, Vibrio fischeri and Vibrio sp.

ADP Case #85-137A (5/20/85)
Species: Chelonia mydas
Turtle shell biopsy specimen for microscopy and fungal culture.

GROSS FINDINGS: Large focal to confluent and spreading ulcerative shell, skin and flipper lesions. Affected tissues appeared necrotic. Wet-mount preparations revealed abundant branching, septate fungal hyphae. Bacteria were also present in the superficial tissues.

HISTOPATHOLOGY FINDINGS: Ulcerative, necrotic shell cuticle heavily infected with branching, septate fungal hyphae. Fungal spores were not observed. Bacteria present in the tissues.

MICROBIOLOGY FINDINGS: Isolated a fungi resembling Fusarium sp., possibly F. solani.

DIAGNOSIS AND COMMENTS: Ulcerative, necrotic shell disease probably caused by Fusarium sp..

ADP Case #85-137B (5/20/85)
Species: Pricanthus cruentatus.
Gross examination of ulcerative skin lesions, "holes", on the head and a biopsy specimen of thickened skin from the caudal edge of the operculum.
Skin biopsy specimen from operculum was decalcified prior to blocking/staining.

HISTOPATHOLOGY FINDINGS: Epidermis and dermis appeared to be thickened. Focally, in the basal epidermal layer, cellular necrosis was observed. An etiologic agent was not apparent in the examined tissue section.

DIAGNOSIS AND COMMENTS: Focal epidermal basal cell degeneration and necrosis. The cause of the focal cellular necrosis was not apparent in tissue biopsy specimen.

Since the "holes" on the head region has been recognized from a variety of species of marine fish at the aquarium, it is probably worthwhile to try to obtain several specimens that can be sacrificed so a more complete study of this syndrome can be undertaken.

ADP Case #85-146 (5/25/85)
Species: Chelonia mydas
Juvenile green sea turtle found dead in A.M. Postmortem examination performed. Note: this is the same animal for tissue biopsy specimen 85-137A.

GROSS FINDINGS: Turtle emaciated, with shrunken eyes and taught skin suggestive of dehydration. Severe ulcerative shell, flipper and skin lesions. However, necrotic process appeared to be limited to the outer 50% of the shell and was not found to obviously penetrate into the underlying tissues. Approximately 5 ml or red colored fluid was present within the abdominal cavity. Nodular lesions or apparent granulomata were not noted within internal organs. The small bowel was empty for a small amount of yellow, mucoid fluid. Lower intestine was partially distended with a frothy, malodorous greenish-brown fluid. Intestinal metazoan parasites were not grossly observed. Lungs atelectic, but floated in water; bladder empty. Swab cultures were collected of the necrotic shell tissue (fungal culture), abdominal fluid (aerobic bacteriology) and lower bowel intestinal contents (Salmonella culture).

MICROBIOLOGY FINDINGS: Abdominal fluid - Isolated bacteria resembling Corynebacterium sp., Streptococcus sp., Vibrio alginolyticus and Proteus morganii. Fecal culture - no Salmonella isolated. Necrotic shell - isolated a fungus in the Genus Fusarium, probably F. solani.

HISTOPATHOLOGY FINDINGS: Abundant fungal mycelia within the epidermis and outer layers of the dermis. Little to no host inflammatory response to the invading fungal mycelia was found. In some areas of the epidermis, individual epidermal cells contain slightly basophilic, circular cytoplasmic inclusion bodies, which frequently were found to displace the nuclei of the cell. Internal tissues were found to be moderatley to severely autolytic. Large colonies of bacteria were commonly observed in the liver and within areas of the intestinal tract. The liver sinusoids appeared to be congested with blood. Foci of acute inflammatory cell infiltration in several areas of the ventricular myocardium, and degeneration of renal tubular epithelium were found.

DIAGNOSIS AND COMMENTS: Severe mycotic shell disease caused by Fusarium sp. was the primary reason for this turtle's death. The apparent dehydration of the turtle probably resulted because the animal could no longer osmoregulate due to the extensive damage to the shell and skin. The focal

colonies of bacteria were probably either opportunistic pathogens invading a debilitated host, or represent a postmortem event. However, the focal, acute inflammatory lesions in the heart muscle suggest that bacterial invasion probably occurred prior to death of the animal.

The apparent cytoplasmic inclusions within epidermal cells of the skin may indicate infection by a viral agent or be a type of degenerative process which these cells were undergoing. Additional histological work and possibly T.E.M. study may be required to clarify this. These possible inclusion bodies are not consistent with herpesvirus infection known from green sea turtles reared in mariculture farms in the Cayman Islands.

Fungal shell disease has been reported from a number of turtle species including C. mydas. I am unaware of reports of Fusarium being a cause of shell disease of turtles, but it has been implicated as the cause of necrotic skin lesions of snakes.

ADP Case 85-162 (6/13/85)
Species: Chelonia mydas
Necrotic shell tissue for fungal culture

MICROBIOLOGY FINDINGS: Isolated Fusarium sp. probably F. solani from the necrotic shell tissue. A subculture of this fungus was submitted to the National Veterinary Services Laboratory, Ames, Iowa for confirmation of the identification. The NVSL reported the isolate to be Fusarium sp.

COMMENTS: The same fungus has now been isolated from two green sea turtles with the necrotic shell lesions. This strengthens the argument that Fusarium sp. is the cause of the necrotic shell lesions of these turtles.

Griseofulvin while being an effective therapeutant for the dermatophyte fungi which are theprimary cause of fungal skin lesions of people, is not supposed to be effective as a treatment for other types of fungi. It is therefore unlikely that the Fusarium sp. associated with the shell lesions of the turtles is susceptible to griseofulvin therapy. Thus, the current griseofulvin treatment of the turtle with the shell lesions should be discontinued.

It may be of some significance that the shell lesions of one, and possibly two turtles have appeared to respond to daily 1 hr. bath treatment with formalin at 1 ml/gal. (250 ppm). Formalin is not generally recognized as a good fungicide, not at least in my experience, and if it is in fact effective then it may be that the repeated exposure (daily bath treatment) has been of significance as to why it has seemed to help. The observations on the effectiveness of the use of formalin bath for treatment of fungal shell disease of turtles may be of practical importance to others who work with juvenile green sea turtles.

THE SYMPOSIA OF THE NATIONAL ZOOLOGICAL PARK

MYCOBACTERIAL INFECTIONS

# of Zoo Animals

RICHARD J. MONTALI, *Editor*

Proceedings of a Symposium Held at the
Conservation and Research Center
National Zoological Park
Smithsonian Institution
October 6–8, 1976

Smithsonian Institution Press
Washington, D.C.
1978

DAVID G. BROWNSTEIN, D.V.M.
Department of Pathology
Johns Hopkins University Hospital
Baltimore, Maryland 21205

Reptilian Mycobacteriosis

ABSTRACT

Reptilian tuberculosis is a sporadic disease with an annual incidence of 0.1 to 0.5 percent in well-managed colonies. Organisms reported from cases with cultural confirmation have all been within Runyon groups I and IV. *M. marinum*, an ubiquitous saprophyte of warm aquatic environments, is most commonly isolated. Less commonly isolated are *M. chelonei* [*borstelense, runyoni, abscessus*], also of wide environmental distribution, and *M. thamnopheos*. The latter, originally isolated in 1929 from garter snakes at the Philadelphia Zoo, has unusual links with *Nocardia*.

Disease production is a complex interaction of organism, host, and environment with stress and debility playing an important role. Snakes ingesting birds infected with *M. avium* may harbor the organism for many months. Inoculation with *M. avium* has been shown to induce disseminated disease in snakes. Inoculation of lizards with *M. ulcerans*, a slow growing, nonchromogen, has also produced disease.

Reptilian tuberculosis may be localized or disseminated. Infections usually originate in skin and respiratory or alimentary tracts. Chelonia most frequently have cutaneous or pulmonary primary infections, while other orders frequently have primary infections of the digestive tract. Primary lesions often contain abundant caseation with minimal histiocytic response and therefore resemble the inflamatory response that is typical to a wide variety of pathogens in the reptile. Secondary foci of mycobacterial infection are usually more typical of the tubercles noted in other species. Acid-fast organisms are abundant in caseous areas as well as within macrophages. The reptilian organisms are larger than mammalian or avian types and usually have a beaded appearance.

Mycobacteriosis of reptiles is a sporadic disease with an annual incidence of 0.1 to 0.5 percent in well-managed collections reporting the disease (4, 9). The infections may be local or disseminated and originate in skin, and respiratory or alimentary tracts (2, 14, 20). Primary foci in the skin or lungs are reported to be most frequent in chelonians while alimentary origins are common in crocodilians and Squamata (16).

Gross autopsy findings are generally characteristic when the disease is disseminated and consist of firm, noncalcified nodules up to 3 mm in diameter with a miliary pattern in many tissues, including liver, spleen, and kidneys (Figure 1). Such lesions are indistinguishable from systemic mycoses and have been observed on occasion in visceral gout and subacute bacterial sepsis. This lack of specificity correlates with the more primitive type of inflammatory response in reptiles where a wider range of agents are capable of eliciting a granulomatous reaction than in higher vertebrates.

Primary (local) lesions may assume a variety of forms from caseous consolidation of an organ or tissue (Figure 2) to ulcers or sarcomatous proliferations (12). They may therefore mimic a number of other pathological processes.

Histopathologic examination of disseminated lesions generally reveals typical tubercles; i.e., well-circumscribed accumulations of epithelioid cells, some with caseous centers. These tubercles differ from mammalian tubercles by the rarity of Langhans' giant cells, the absence of calcification, and a less prominent zone of lymphocytes at the periphery of the tubercle (Figure 3).

Histopathologic examination of primary (local) lesions often shows abundant caseation with a minimal histiocytic response (Figure 4). This type of reaction may be impossible to distinguish morphologically from the nonspecific caseation frequently associated with acute inflammatory lesions from non-mycobacterial causes. This caseation is thought to result from the lack of liquifying lysosomal enzymes of reptilian leucocytes.

Acid-fast organisms are present in large numbers in most mycobacterial lesions both intra- and extracellularly. They are large (up to 7 μm in length) and often have a beaded appearance (Figure 5).

Organisms isolated from spontaneous reptilian mycobacterioses have been either photochromogens or rapid growers and therefore belong to Runyon groups I and IV, respectively (21). In cases reported from the London Zoological Park from 1924 to 1933, Griffith isolated *Mycobacterium marinum* from 71 percent of the cases (7, 9). This is an ubiq-

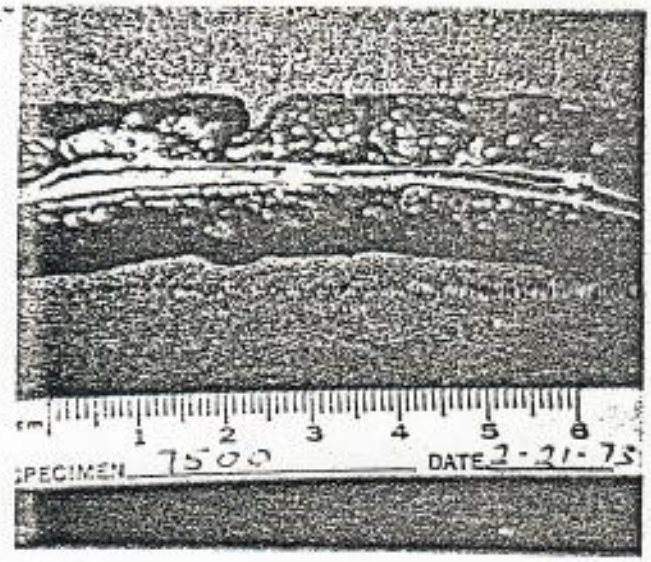

Figure 1. Disseminated mycobacteriosis in the liver of a snake. Miliary pattern of nodules occurs throughout the organ.

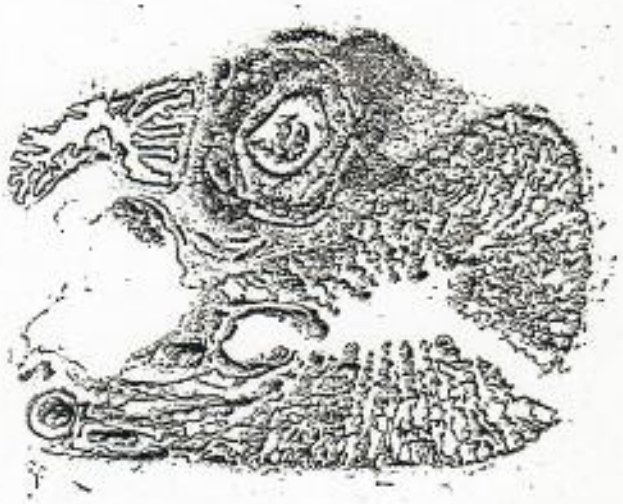

Figure 2. Lung section from a snake with a caseous mycobacterial lesion on the pleura. H&E, X 40.

uitous saprophytic photochromogen of warm aquatic environments which is infective for a wide variety of poikilotherms and occasionally has been reported to infect mammals (7, 8, 22). Less commonly isolated from poikilotherms are *M. chelonei* (*borstelense, runyoni, abscessus*) (9), a rapid grower of wide environmental distribution, and *M. thamnopheos* (1). The latter, originally isolated by Aronson from diseased garter snakes at the Philadelphia zoo, has unusual links with Nocardia. Lechavalier, comparing the carbon skeleton of mycolic acids in the bacterial cell wall with that of Nocardia, proposed that *M. thamnopheos* should be more properly classified as Nocardia due to the smaller carbon skeleton (11).

Older literature also reports *M. tropidonatus* (8)

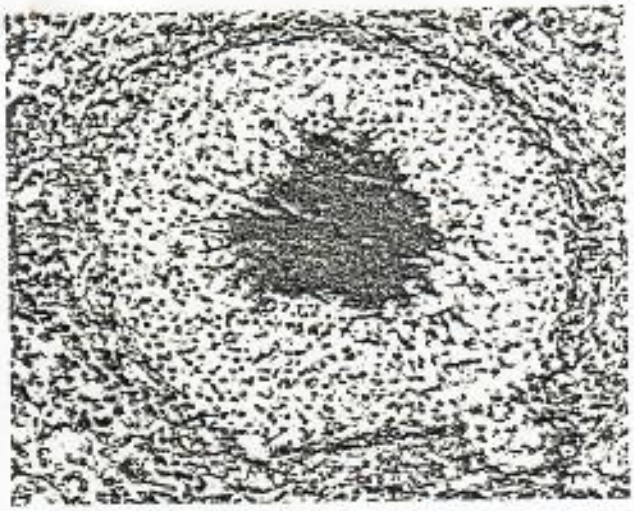

Figure 3. Section of liver from a snake with a tubercle typical of that seen in disseminated mycobacteriosis in reptiles. The caseous center is surrounded by activated macrophages. Note the absence of Langhans' giant cells and calcification and the paucity of the lymphocytes at the periperphy of the lesion.

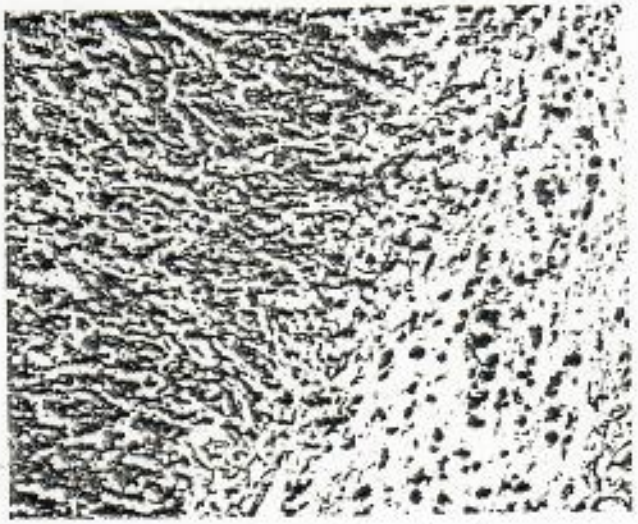

Figure 4. Section through the periphery of a primary mycobacterial lesion from a snake. It consists mainly of caseous necrotic tissue and heterophiles. H&E, X 500.

and *M. schlangen* (15) as being isolated from spontaneous disease in snakes, but Bergey's manual no longer lists these species (5).

Experimentally, reptiles have been infected with mycobacteria that are nonchromogens, *M. avium* (10) for example, and a wide variety of mycobacteria classified as rapid growers (13). Experimentally, disseminated disease has been produced in reptiles with *M. tuberculosis* when environmental temperatures were elevated (3, 19). Early accounts of spontaneous mycobacterial disease in snakes, without cultural confirmation, have been correlated with the feeding of tuberculous birds suggesting that *M.*

Figure 5. Section of a caseous mycobacterial lesion from a snake with numerous acid-fast bacilli. The organisms are 5–7 μm in length and many have a beaded appearance. Ziehl-Neelsen, X 1200.

avium is the causative agent (6, 18).

The epidemiology of mycobacteriosis of reptiles is undoubtedly different from tuberculosis of birds and mammals. It is unlikely that any mycobacteria are primary pathogens of reptiles, although the status of *M. thamnopheos* in this regard is unclear. Most of these organisms are saprophytic or marginally pathogenic and produce disease either when there is immunologic deficiency or when very large numbers of organisms are introduced into the host. Therefore, the incidence of mycobacteriosis rises in poorly managed reptile collections in which specimens are often stressed and malnourished. Poor sanitation allows large numbers of saprophytic mycobacteria to proliferate in moist environments. Compounding the problem are defects in exposed epithelial surfaces such as skin ulcers or respiratory infections due to other infectious agents which allow secondary colonization by acid-fast organisms. Ingestion of a mycobacterial-infected poikilotherm by another may also be a route of infection particularly when the lesions primarily involve the alimentary tract. *M. marinum* and *M. chelonei* are the most common mycobacteria isolated from infected reptiles, probably because they are ubiquitous in aqueous environments where most reptiles are exhibited (17).

The clinical diagnosis of reptilian mycobacteriosis, when there are no external lesions, is often difficult. However, antemortem diagnosis is not as imperative to colony management as the comparable situation in mammalian and avian tuberculosis since contagion is apparently not a feature. Post-mortem diagnosis is important for purposes of identifying contaminated

quarters. The source of mycobacteria can then be identified, utilizing the known natural history of the species isolated. These are often in aqueous habitats that are rich in organic material (slime) and metalic ions (water faucets) (17).

There are currently no reports on the clinical management of mycobacteriosis of reptiles.

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