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AN EPIZOOTIC OF WATERFOWL ASSOCIATED WITH A RED TIDE EPISODE IN FLORIDA[1]

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Abstract: During February and March, 1974, an epizootic involving lesser scaup (*Aythya affinis*) occurred in the Tampa Bay area on the west coast of Florida. Several thousand ducks are estimated to have died. Concurrent with this epizootic was a red tide caused by heavy blooms of the toxic dinoflagellate *Gymnodinium breve* which caused severe wide-spread fish kills. Clinical signs consistent with *G. breve* intoxication were evident in some of the lesser scaup. A controlled experimental feeding of *G. breve* toxic material to White Pekin ducklings produced illness and death with signs comparable to some of those seen in the scaup.

INTRODUCTION

Every three to five years, blooms of the toxic dinoflagellate *Gymnodinium breve* occur on the west coast of Florida with resultant massive fish kills.[5,11] Such an event occurred from October, 1973, to May, 1974, and initially involved mainly tomtate fish (*Haemulon aurolineatum*) offshore and striped mullet (*Mugil cephalus*) inshore.[12,13] In February, the red tide and a severe fish kill occurred in Boca Ciega Bay and Tampa Bay where large numbers of waterfowl were over-wintering. During the last week in February, double-crested cormorants (*Phalacrocorax auritus*), red-breasted mergansers (*Mergus merganser*), and lesser scaup (*Aythya affinis*) were found moribund or dead in red tide areas. Deaths among cormorants and mergansers soon ceased, but lesser scaup mortality increased during March. Over the eight week period in which mortality occurred several thousand lesser scaup died.[16]

Details of clinical signs have been published elsewhere[11] and included weakness, reluctance to fly, slumping of the head, clear nasal discharge, viscous oral discharge, oil gland dysfunction, excessive lacrimation, chalky yellow diarrhea, dyspnea, tachypnea, tachycardia, decreased blood pressure, depressed body temperature, diminished reflexes and dehydration.

This report deals with studies performed to determine the cause of this epizootic.

MATERIALS AND METHODS

General Necropsy

Necropsies were performed on 12 moribund lesser scaup. Samples of heart blood were taken for hematocrit determinations, thin blood film preparation and for serum. Specimens of liver, spleen, heart, lungs, pancreas, proventriculus, gizzard, duodenum, caecae and

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kidneys were obtained from seven of the ducks, fixed in 10% buffered formalin solution, embedded in paraffin, sectioned at 7 μm thickness and stained with hematoxylin and eosin. Specimens of heart blood, liver, spleen, lung and large intestine were collected aseptically from nine birds for microbiologic studies. Swabs of the nasal sinus of one duck were taken for bacteriologic study. Samples of brain, liver, muscle and fat were obtained from two birds and wrapped individually in aluminum foil for pesticide residue analyses. Contents of the proventriculus and gizzard were collected and food items were preserved in 70% ethyl alcohol for later identification. Some gastrointestinal contents were saved for toxicologic studies. The remainder of the carcass of each duck was retained for parasitologic studies.

Bacteriologic Examination

Specimens of liver from nine of the ducks were cultured individually in cooked meat medium and on 5% sheep blood agar plates. Contents of the large intestine of each bird were cultured on Salmonella-Shigella (SS) and Eosin Methylene Blue (EMB) media and in selenite enrichment medium.[5] Cultures were incubated at 37 C.

Transmission Experiments

Four one-day-old White Rock chicks each was inoculated intraperitoneally with 0.5 ml of whole blood. Two chicks received blood from one scaup and two chicks received blood from another scaup. Two 2-day-old Muscovy ducklings were inoculated intramuscularly with 0.25 ml of whole blood. Each duckling received blood from a different scaup.

Ten percent suspensions of spleen and of a liver-lung composite from a moribund scaup were prepared in McCoy's 5A medium[6] containing 10% fetal calf serum, 100 units of penicillin, and 100 μg of streptomycin per ml. Four one-day-old White Pekin ducklings were inoculated intramuscularly with 0.2 ml of these preparations: 2 ducklings receiving the splenic material and 2 receiving the liver-lung material. Spleen, liver and lung material from two other moribund scaup was also inoculated into four two-day-old and two 16-day-old Muscovy ducklings in the same manner.

All chicks and ducklings were observed for signs of illness over the following two weeks.

Parasitologic Examination

Five scaup were examined for parasites utilizing the techniques described by Forrester *et al.*[4]

Pesticide Residue Studies

Tissue samples from two ducks were tested for the presence of chlorinated hydrocarbons and polychlorinated biphenyls following the methods described by Thompson *et al.*[15]

Toxicologic Studies

Individual serum specimens from two affected ducks and a pooled specimen from two additional affected ducks were inoculated into mice in an attempt to demonstrate clostridial or other toxins. One portion of each specimen was held at 100 C in a water bath for 20 min. Pairs of weanling white mice were inoculated intraperitoneally with 0.5 ml of each heated and unheated specimen after passage through 0.45 μm filters.[7] Mice were observed for one week after inoculation.

Contents of the small intestines of the affected ducks were tested also for clostridial or other toxins. The contents were flushed with distilled water and centrifuged at 1000 g for 20 min. in a refrigerated centrifuge at 5 C. Pairs of weanling mice were inoculated intraperitoneally with 0.5 ml of heated and

[5] Difco Laboratories, Detroit, Michigan, USA.
[6] Gibco Laboratories, Long Island, New York, USA.
[7] Millipore Corporation, Bedford, Maryland, USA.

unheated filtered (0.45 μm) washings. Mice were observed for one week after inoculation.

Four one-day-old White Rock chicks each was given orally 1 ml of a tap water suspension of pooled contents from the small intestine of two ducks. Two of the chicks received material from another scaup. The chicks were observed closely for 14 days for signs of intoxication.

Three two-day-old Muscovy ducklings each was given orally 0.5 ml of a tap water suspension of the contents of the proventriculus of a scaup on each of 3 successive days. Each duckling received material from a different scaup. Ducklings were observed for 14 days for signs of illness.

Twenty-five one-day-old White Pekin ducklings were divided at random into five groups of five ducklings each, housed in a poultry brooder and fed 22% protein poultry starter mash *ad lib*. To stimulate the salt glands of the ducklings to function, groups 1, 2 and 3 were acclimated to gradually increasing concentrations of sea water over a 10-day-period until they were tolerating sea water matching the salinity of Tampa Bay (sp.g. = 1.334) at the time of the epizootic. Groups 4 and 5 were maintained on fresh water. On day 12, exposures to toxic red tide material began. Group 1 was force-fed toxic clam tissue (*Mercenaria campechiensis*), which had been assayed at 48 mouse units per 100 g tissue.[3,10] Each duckling received 7 grams at each of two feedings per day. This group of ducklings was exposed also to sea water collected from a concentrated patch of red tide and determined to contain 22 million dinoflagellate cells per liter. Group 2 received toxin-free clams (purchased from a commercial source) in similar amounts plus the same toxic sea water as group 1. Group 3 received clams and sea water both of which were free of red tide toxins. Group 4 received toxin-free clams and fresh water and group 5 received fresh water and no clams. Groups 1, 2, 3 and 4 received only clam material for the first day of the toxin exposures and thereafter received both poultry mash *ad lib*., and the force-fed clams (14 grams/duckling/day). Exposure to toxic clams and toxic sea water was continued for an eight-day period (until day 20) when all remaining ducks were given fresh water and poultry mash *ad lib*., until the experiment was terminated on day 25.

Every two or three days each bird was weighed and blood samples were taken for blood film preparations and for hematocrit determinations. Blood films were stained with Giemsa and cells were differentially counted for thrombocyte and leucocyte proportions (%) utilizing the identification procedures of Lucas and Jamroz.[8] Ducks which died during the course of the experiment and all ducks remaining at the end of the experiment were examined at necropsy. Samples of heart, liver, pancreas, spleen, kidney, lung, proventriculus, gizzard, small intestine, large intestine, bursa, skin, muscle, brain, salt gland and bone marrow were obtained from representative survivors in each group and from each duck that died during the experiment. These samples were fixed in Davidson's fixative, embedded in paraffin, section at 6 μm and stained with hematoxylin and eosin for histopathologic study.

RESULTS

Pathology

All scaup examined had substantial subcutaneous fat and normal breast muscles indicating that the disease was acute rather than chronic. Most of the ducks had fed recently, as indicated by the presence of several species of turritellid, pyramidellid and opisthobranch gastropods and clams (*Gemma gemma*) in their proventriculi and ventriculi. No gross lesions were seen. Histopathologic findings were not consistent and included parasitic enteritis, renal trematodiasis, inhalation of foreign material, sepsis and, in one animal, superficial inflammation of the ventriculus. Hematocrits varied from 50 to 70% which is considered

above normal for this species.[7] Blood viscosity was increased in some scaup and in one the blood was blue-purple in color. Additional details on pathologic and hematologic observations are presented elsewhere.[11]

Microbiology

No aerobic or anaerobic bacteria were isolated from the livers of the affected ducks and no enteric pathogens were recovered from the contents of the small and large intestines.

None of the chicks or ducklings that were inoculated with blood, spleen or liver-lung preparations showed signs of illness during the observation period. Gross examination at necropsy showed body organs to be normal.

Parasitology

Haemoproteus nettionis was found in one scaup. Several species of nematodes (*Capillaria, Amidostomum, Tetrameres* and *Streptocara*), trematodes (*Zygocotyle* and several species of microphallids), and cestodes (hymenolepids) were encountered in the digestive tracts, but were present in small numbers. No *Sarcocystis* or coccidian infections were detected.

Pesticide Residues

Tissues from both of the two ducks tested contained small amounts of DDT in the fat (2.1 to 4.0 ppm) and trace amounts of DDT in brain, muscle and liver samples. Trace amounts of dieldrin occurred in samples of liver, muscle and brain of one duck and in muscle and fat of the other duck. Fat samples also contained 7.7 and 15 ppm of polychlorinated biphenyls (Aroclor 1254). The other tissues contained trace amounts of Aroclor 1248, 1242 and 1254.

Toxicology

No evidence of clostridial toxins or other acutely toxic material was indicated by mouse inoculation tests with serum specimens or washings of the intestinal tracts of typically affected ducks.

None of the mice, chicks or ducklings given stomach or intestinal contents showed signs of illness during the period of observation. At necropsy, all appeared grossly normal.

White Pekin ducklings exposed to red tide toxins showed signs of toxicity the second day after exposure began. The ducklings appeared lethargic and unthrifty. On the third day, two ducks in group 1 (toxic clams plus toxic sea water) showed ataxia, spastic movements of the head and a tendency to droop the head to one side. Both ducks rested with legs extended to the rear. These signs were most severe shortly after the force-feeding of the clams, and some recovery was evident within 2 h. Late on the third day, one duck in group 1 died. Over the next 3 days the remaining four ducks of group 1 died (Table 1). Two ducks in group 2 (toxic clams only) died, one on the fifth day and one on the ninth day. The remaining three ducks in group 2 were severely affected and possibly would have died if exposure to toxic sea water had been continued. Hematocrits for ducks in group 1 were slightly elevated (37 to 41% compared to controls which varied from 33 to 39%). Weight gains of ducks in groups 1 and 2 stabilized when toxic feed and water were administered (Fig. 1). The three moribund ducks in group 2 showed rapid recovery after the exposure to toxic sea water was terminated and fresh water was made available once again. Thereafter growth rates returned to normal. None of the ducks in control groups (groups 3, 4, and 5) showed signs of illness during the course of the experiment. Analysis of blood films showed definite hematologic changes in intoxicated birds. There was a sharp drop in the number of lymphocytes of ducks in groups 1 and 2. Thrombocytes decreased severely in all ducks receiving sea water (groups 1, 2, and 3) and especially in group 1 ducklings. Examination of ducklings that died during the experiment or were killed at the end of the experiment showed no gross or histopathologic changes attributable to the experimental procedure.

TABLE 1. Results of exposure of White Pekin ducklings to sea water and clams containing red tide toxins.

Group No.	Exposure regime	No. ducks exposed	No. deaths	Day of death (post exposure)
1	Toxic clams, toxic sea water	5	5	3-6
2	Normal clams, toxic sea water	5	2	5,9
3	Normal clams, normal sea water	5	0	—
4	Normal clams, fresh water	5	0	—
5	No clams, fresh water	5	0	—

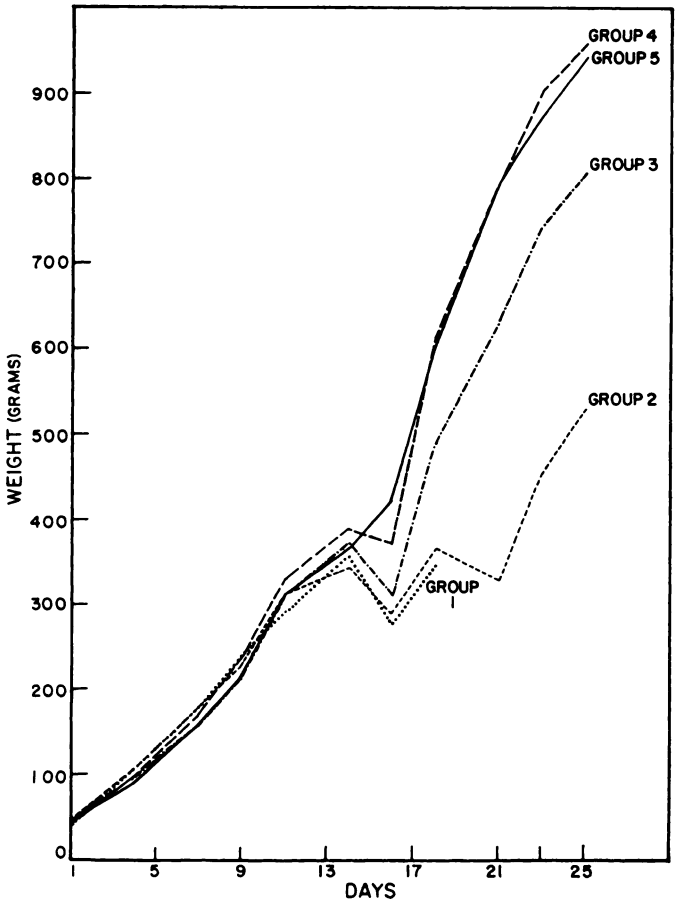

FIGURE 1. Weight gains of White Pekin ducklings exposed to red tide toxins.

DISCUSSION

Much of the evidence associating the lesser scaup mortality with the red tide is circumstantial, and can be summarized as follows:

1. Moribund and dead scaup began appearing two to three weeks after the red tide initially entered Boca Ciega Bay and Tampa Bay, where large numbers of scaup were over-wintering. This time span would allow ample exposure to toxic materials for development of a neuro-intoxicative syndrome. Throughout this period most of the scaup were located in the Hillsborough Bay region of Tampa Bay where *G. breve* concentrations remained very low or negative during the epizootic. The majority of scaup deaths were reported from Hillsborough Bay. However, no data are available on local movements and habitat utilization of the areas in question for feeding, etc. Possibly the scaup could have fed on filter-feeding mollusks (which concentrate the toxin) in areas of high *G. breve* densities and then moved to other areas such as Hillsborough Bay where subsequent morbidity and mortality occurred.

2. Many signs, both neurologic and hematologic, were common to the scaup and to fishes undergoing red tide intoxication. The behavioral responses of the moribund scaup were comparable to the behavioral sign complex of fishes undergoing chronic hemolytic intoxication,[12] although the hyperactivity noted during terminal phases of neurointoxication in fishes was never seen in the scaup. Quick and Henderson[11] noted some similarities between the hematologic signs observed in fish afflicted by red tide toxins and those signs that occurred in the lesser scaup, although they were much less severe in the birds. They listed cyanosis, normoblastosis, increased hematocrits and increased whole blood clotting times as being consistently present. In various combinations with these conditions they sometimes observed plasma hemoglobin, serum debris, hemal hyperviscosity, thrombocytopenia and leucocytopenia. Some of the signs observed in the moribund scaup were seen also in White Pekin ducklings exposed to red tide toxins in the experiment described in the present paper. These consisted of lethargy, ataxia and droopiness of the head. Other than elevated hematocrits however, there was little correlation of hematologic signs between the scaup and Pekin ducklings. Neurologic signs such as loss of equilibrium have been reported also in experimental studies on the effects of *G. breve* toxin on White Leghorn chicks.[14]

3. Initial recovery of the scaup and Pekin ducklings was rapid, being achieved, often dramatically, within 1 to 6 h. after toxin was removed from the diet in the case of the ducklings, or after fresh water intake and a period of body temperature stabilization in the case of the scaup. Seven to 14 days were required for oil gland function and flight capability to return to normal in scaup that recovered.

4. During the course of the epizootic, observations on 6,606 living scaup in the area revealed that 66% were females and 34% were males, whereas of 837 dead scaup, 31% were females and 69% were males.[16] Thus, mortality was much higher in males than in females. This type of differential sex susceptibility has been reported in other intoxication problems, such as aflatoxicosis,[1,19] and adds credence to the idea that the cause of the epizootic was a toxin and not an infectious agent.

5. Tests conducted to detect infectious agents were negative. Parasitism was minimal and considered of little consequence. The lack of consistent pathologic findings contributed to the conclusion that a microbial or parasitic cause of disease was unlikely. Pesticide studies showed subclinical levels of chlorinated hydrocarbons. Similar findings were reported by other laboratories in which some scaup from this epizootic were studied.[8] [9]

[8] Jasman, A. M. and E. D. Stoddard. Personal Communication. 1974. Florida Dept. Agric. and Consumer Services, Bur. Diag. Lab., Kissimmee, Florida, USA.

[9] Kocan, R. M. Personal Communication. 1974. Fish and Wildlife Service, Eastern Fish Disease Laboratory, Kearneysville, West Virginia, USA.

The feeding experiment demonstrated that material collected during the red tide occurrence was toxic for Pekin ducklings, although toxin concentrations utilized were above that which was representative of the toxicity of Tampa Bay waters. However, since other toxins may have been present as a result of industrial pollution in Tampa Bay, it is not possible to make a definitive statement that the red tide was responsible for the die-off of scaup. Nevertheless, much circumstantial and experimental evidence is suggestive of that association and that it was at least partially responsible.

Two questions arise concerning the epizootiology of this disease outbreak. First, one could ask why other species of waterfowl or shore-birds were not affected. Small numbers of double-crested cormorants and red-breasted mergansers were found moribund and dead in the red tide area during the first weeks of the epizootic. However, as previously indicated, mortalities of these species soon ceased, whereas mortalities of scaup continued, increased and spread. In addition, moribund specimens of all three species were treated at a local bird clinic and a high percentage of the cormorants and mergansers responded well to antibiotic therapy, but the scaup did not.[10] Thus the deaths among cormorants and mergansers may have been due to a different and possibly unrelated disease syndrome, although observations in England suggest that the shag (*Phalacrocorax aristotelis*) and the cormorant (*Phalacrocorax carbo*) are susceptible to the toxins produced by the dinoflagellate *Gonyaulax tamarensis*.[2]

Scaup may be more susceptible to red tide toxins than other species, but more likely the food habits of the scaup would exert an important influence in a red tide induced epizootic. Scaup are diving ducks and are known to utilize mollusks as food items to a significant degree.[6,15] Filter-feeding mollusks are known to concentrate large amounts of red tide toxin[17] and scaup would be prime targets for a red tide elicited epizootic because of their feeding habits. In the present study, some of the scaup examined at necropsy contained filter-feeding mollusks in their stomachs. Other birds which utilize different food sources would be less prone to red tide intoxication.

A second question is: Why have there been no previous reports of lesser scaup epizootics due to red tide intoxication, since scaup have overwintered in Tampa Bay in large numbers for many years and red tide outbreaks have been known to occur for some time on the west coast of Florida? The answer to this question might be as follows:

1. Red tides in the Tampa Bay area usually occur in spring and summer since blooms of dinoflagellates require warm waters.[5] The 1974 red tide occurred because of unusually warm winter water temperatures. Therefore, when most red tides occur, the scaup would not be present, having migrated north to their breeding grounds.

2. Red tides usually do not enter Tampa Bay due to the low salinity of the water. Most red tides occur offshore and enter inshore and bay waters only when winds, temperatures and salinities are favorable. This occurs usually during mild, dry, winter weather such as during the epizootic reported herein. Although there are records of seabird deaths due to toxins produced by *Gonyaulax catenella* on the coast of Washington[9] and *G. tamarensis* on the coast of England,[2] this is the first such report of extensive mortality associated with a bloom of *G. breve*.

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[10] Heath, R. T., Jr. and H. A. Albers. Personal Communication. 1974. Suncoast Seabird Sanctuary, St. Petersburg, Florida, USA.

LITERATURE CITED

1. ALPERT, M. E. and C. S. DAVIDSON. 1969. Mycotoxins. A possible cause of primary carcinoma of the liver. Am. J. Med. 46: 325-328.
2. COULSON, J. C., G. R. POTTS, I. R. DEANS and S. M. FRASER. 1968. Exceptional mortality of shags and other seabirds caused by paralytic shellfish poison. British Birds. 61: 381-404.
3. CUMMINS, J. M. and A. A. STEVENS. 1970. Investigations on *Gymnodinium breve* toxins in shellfish. Pub. Health Serv. Bull. U.S. Dept. of Health, Educ. and Welfare. Washington, D.C.
4. FORRESTER, D. J., A. O. BUSH, L. E. WILLIAMS, JR. and D. J. WEINER. 1974. Parasites of greater sandhill cranes (*Grus canadensis tabida*) on their wintering grounds in Florida. Proc. Helm. Soc. Wash. 41: 55-59.
5. GUNTER, G., R. H. WILLIAMS, C. C. DAVIS and F. G. W. SMITH. 1948. Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida, November 1946 to August 1947. Ecol. Mon. 18: 310-324.
6. HARMON, B. G. 1962. Mollusks as food of lesser scaup along the Louisiana coast. Trans. N. Amer. Wildl. Nat. Res. Conf. 27: 132-137.
7. KOCAN, R. M. 1972. Some physiologic blood values of wild diving ducks. J. Wildl. Dis. 8: 115-118.
8. LUCAS, A. M. and C. JAMROZ. 1961. Atlas of avian hematology. United States Dept. Agric. Mono. No. 25: 1-271.
9. McKERNAN, D. L. and V. B. SCHEFFER. 1942. Unusual numbers of dead birds on the Washington Coast. Condor. 44: 264-266.
10. MORTON, R. A. and M. A. BURKLEW. 1969. Florida shellfish toxicity following blooms of the dinoflagellate, *Gymnodium breve*. Fl. Dept. Nat. Res. Mar. Res. Lab. Tech. Ser. 60: 1-26.
11. QUICK, J. A., JR. and G. E. HENDERSON. 1975. Effects of *Gymnodinium breve* red tide on fishes and birds: a preliminary report on behavior, anatomy, hematology and histopathology. Proc. Gulf Coast Reg. Symp. Dis. Aquatic Animals: 85-115.
12. ———— and ————. 1975. Evidences of new ichthyointoxicative phenomena in *Gymnodinium breve* red tide. Proc. Ist Int. Conf. Tox. Dinoflagellate Blooms: 413-422.
13. ———— and ————. 1975. Behavioral, hematological and histological evidences of new ichthyointoxicative mechanisms in *Gymnodinium breve* red tides. Proc. Fla. Red Tide Conf. Fla. Marine Res. Publ. No. 8: 8-9.
14. RAY, S. M. and D. V. ALDRICH. 1965. *Gymnodinium breve*: induction of shellfish poisoning in chicks. Science. 148: 1748-1749.
15. ROGERS, J. P. and L. J. KORSCHGEN. 1966. Foods of lesser scaups on breeding, migration and wintering areas. J. Wildl. Manage. 30: 258-264.
16. SCHREIBER, R. W., F. M. DUNSTAN and J. J. DINSMORE. 1975. Lesser scaup mortality in Tampa Bay, Florida, 1974. Fl. Field Nat. 3: 13-15.
17. STEIDINGER, K. A., M. BURKLEW and R. M. INGLE. 1973. The effects of *Gymnodinium breve* toxin on estuarine animals. pp. 179-202. In: *Marine Pharmacognosy: Action of Marine Biotoxins at the Cellular Level*. Academic Press, New York.
18. THOMPSON, N. P., P. W. RANKIN and D. W. JOHNSTON. 1974. Polychlorinated biphenyls and p,p'-DDE in green turtle eggs from Ascension Island, South Atlantic Ocean. Bull. Environ. Contam. Tox. 11: 399-406.
19. WOGAN, G. N. 1966. Chemical nature and biological effects of the aflatoxins. Bact. Rev. 30: 460-470.

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