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Author: ALBERT H. BANNER, Professor of Zoology, University of Hawaii

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13 ABSTRACT

As this final report covers not only the contract listed above but also the previous contract of about 8 years duration, it deals in summary form only with the advances made on the study of ciguatera and other marine toxins during the period. On the problem of ciguatera, a disease that results from the ingestion of certain fishes associated with coral reefs, we have developed techniques for purification and isolation of the causative toxin, and we have developed a crystalline product; we have not yet determined the structural formula. In pharmacology, we have shown that the major effect of the toxin is to upset the ionic balance of excitable membranes particularly the balance of the Na⁺ ion. In biology, we have shown that the toxin apparently arises in the benthic flora of coral reefs as it is found in the gut contents of reef herbivores, and that it can be passed from reef herbivores to reef carnivores; we have also shown that in some of the reef carnivores the toxin may be stored for long periods. We have also found a number of other toxic algae, invertebrates and fish, some of which have been remarked upon in preliminary notes but others we have not yet reported.

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EXPLORATION OF TOXIC MARINE ANIMALS IN THE TROPICAL PACIFIC

FINAL REPORT

INTRODUCTION

When the present study was initiated in 1955, little was known of the toxins found in marine animals in the tropical Pacific. The Japanese were working upon the isolation and identification of tetrodotoxin and exploring the cause of scombrotoxin; reports of toxic invertebrates were diffuse and unconfirmed. Ciguatera, the most important intoxication from marine animals in the central Pacific, was most imperfectly known through the work of Hiyama (in Japanese, in translation by Van Campen) and faunal surveys by Halstead and his group. In 1956 Hashimoto, working on a toxic barracuda, showed that the toxin in its flesh was soluble in polar organic solvents, yet survey tests of several authors still continued to use extractions of the flesh with aqueous solutions. There was considerable question as to whether the toxins in the various fishes found in the various regions of the tropical Pacific were the same; Halstead, for example, had separated intoxication by moray eels from what he considered true ciguatera.

In the 16 years of investigation our attention was concentrated primarily on the cause of the disease ciguatera. To prevent confusion of results by multiple toxins, we early decided to limit ourselves to the toxins from one species of fish from one locality, and to develop at least working definitions of the toxin through chemical and pharmacological studies before spreading our investigations to other fish or to an intensive investigation of the biological origin of the toxin.

The work of our interdisciplinary team was supported by many national and a few foreign or international agencies which included, in addition to the Office of Naval Research, the U. S. Public Health Service under various branches,

the National Science Foundation, the Atomic Energy Commission, the Hawaii State Department of Health, and at times the governments of Micronesia, Fiji and of Polynesia Francais, the South Pacific Commission, etc. The support by the Office of Naval Research was under two consecutive contracts which ran from 1958 through January 1971; in most years the ONR contract was primarily for field studies, for exploration of new toxins and for logistic support, although during some years it supported phases of the laboratory work. In 1969 funds were placed in the contract for the study of the "Crown-of-Thorns" starfish.

In the report below the results of all phases of the investigation at the University of Hawaii for the entire period are summarized and differentiation is not made between the sources of funding of the interdigitating studies. As almost all results herein summarized have appeared, or are appearing, in publication, details are not given; as most conclusions listed were built up over a series of separate publications, individual citations are not offered, but a complete bibliography is given as an appendix. It should be noted that a comprehensive review of the ciguatera problem has been written by the principal investigator and submitted for publication in a two volume work on coral reefs edited by O. A. Jones and R. Endean (to be published in 1971 or 1972).

STUDIES ON CIGUATERA

CHEMISTRY:

We have reported that:

1. The toxin that we consider to be the principal toxin is insoluble in water, soluble in polar organic solvents, heat stable up to the temperature of boiling water, stable under refrigeration (below 0°C.) and in dried flesh for months.

2. This toxin is unstable, however, in the semipurified or purified condition unless extracted, purified and stored under inert atmospheres and low

temperatures.

3. The toxin can be purified by solvent-solvent extraction, and further purified by column and thin-layer chromatography, with final purification with Sephadex (last step yet unpublished).

4. The yield of the toxin is 5-10 mg/kg of highly toxic flesh with a toxicity of 0.025 mg/kg when injected intraperitoneally into mice (final results unpublished).

5. Combustion data upon the non-crystalline product gave an empirical formula of $(C_{35}F_{65}NO_9)_n$, and the molecule has indications of a quaternary nitrogen atom, one or more hydroxyl groups and a carbonyl function. It is not a phospholipid. The compound has been named "ciguatoxin" by Scheuer *et al.* The same toxin occurs in both the flesh and the liver of moray eels and red snappers.

6. Ciguatoxin, either in an identical or a closely related form, occurs in numerous species of carnivorous fish associated with coral reefs, and in at least one herbivore.

7. No simple colorimetric or other chemical tests for the toxin could be developed empirically.

We have now obtained a crystalline product, unreported as yet, either ciguatoxin or some close derivative, and that we hope to be able to derive its structural formula within the next two years under a current grant from the Public Health Service.

PHARMACOLOGY:

We have reported that:

1. We tried 37 different species of animals for a suitable screening bioassay and found the most satisfactory to be the mongoose, but that we could obtain nonquantifiable responses from the cat, the common aquarium turtle and the crayfish.

2. If the toxin were extracted and repurified at least to a second or third step, a quantitative bioassay for ciguatera toxin could be obtained through IP or IV injections into a mouse or a baby chick; miosis in the rabbit eye can also be used.

3. We experimented at some length with the injection of crude extracts into the crayfish; while this technique had considerable promise as a screening test for Johnston Island eels, it was found to be unreliable with other species from other areas, presumably because of multiple toxins carried by them (unpublished).

4. We surveyed symptoms in humans and in test animals; most include the loss of reflexes, progressive paralysis, and eventual death by respiratory failure; in test animals with life prolonged by artificial respiration, death occurred through cardiac failure. Early symptoms may also include gastrointestinal disorders.

5. An epidemiological survey of the tropical Pacific based on questionnaires and partly on interviews was made; however, in the years since the publication was issued in 1964, the distribution of the disease in the islands has changed.

6. Early work showed the toxin to behave primarily as a neurotoxin.

7. In several papers it was stated that the toxin behaved as an anticholinesterase *in vitro* and it was suggested that this was the primary effect *in vivo*; however, subsequent work proved that its effects within a living system was not that of an anticholinesterase.

8. We now believe the primary effect of ciguatera toxin on excitable membranes to be that of increased permeability of Na^+ ions, thus upsetting the ionic balance of the membrane.

9. No specific therapy could be endorsed upon the basis of the reaction of intoxicated animals under laboratory conditions.

10. No pathological changes could be detected in the nervous system of an

animal maintained at a stage of sublethal intoxication for a month.

Current experiments, presently being carried out under the funds from the Public Health Service, are investigating further the effects of ciguatoxin on the membrane ionic potential; there are indications that other cations may mitigate the action of ciguatoxin and may even lead to a rationally based therapy.

BIOLOGY:

We have reported that:

1. By more accurate tests of chemistry and pharmacology than previously used, most carnivores apparently carry the same toxin and that this toxin is the one we have described from red snappers (*Lutjanus bohar*) and moray eels (*Gymnothorax javanicus*) (full confirmation of the identity of the toxin cannot be achieved until the structure of the toxic molecule is determined).
2. True ciguateric fishes appear to be only those fishes tied directly to the flora and fauna of coral reefs, either by direct grazing on the bottoms or by feeding upon animals that so graze.
3. The distribution of ciguateric fishes in space and time varies, with some islands showing a rapid, almost catastrophic, rise in incidence and a slow decline; on other islands ciguatera may remain at a high level for long periods. Ciguatera appears to be confined to the smaller islands of the central and western Pacific and does not occur along the continental masses, with the exception of the Great Barrier Reef of Australia, or the great islands of the western Pacific (Philippines, Indonesia, etc.).
4. Within fish the toxin is carried without detectable differences in concentration in all parts of the musculature, but may be 50 to 100 times as concentrated in the visceral organs, especially the liver.
5. In our studies of the biology of the transmission of the toxin, we have shown that:

- a. Normally nontoxic omnivores will become toxic when fed toxic flesh in small amounts daily;
 - b. Toxic specimens of *Lutjanus bohar* when maintained on a nontoxic diet will retain the toxin within their bodies for up to 30 months;
 - c. A detrital feeding acanthurid from Tahiti (*Otenochaetus striatus*) has demonstrable ciguatoxin in the flesh, in the visceral organs and in the ingested food in its crop.
 - d. No demonstrable increase in ciguatera in cichlid fishes could be found after natural disasters which exposed "new surfaces" as from floods from Fiji and Hawaii, or from predation by *Acanthaster*; similarly, channel blasting in the Cook and Gilbert Islands caused no increase in the disease.
 - e. Other changes in marine conditions, as the contamination of Wake Island by wax esters, have been found to have no effect on ciguatera.
6. In our studies of the origin of the toxin, we have found numerous toxic species of algae, yeasts and molds, but none as yet producing toxins similar to ciguatoxin (studies suspended because of lack of funds).

OTHER TOXINS:

Fish toxins:

1. We have shown preliminary evidence that fish labelled as ciguatoxic may have toxins differing from ciguatoxin in their chemistry and pharmacology; this may account for the differences reported in human symptoms.
2. Working with our Japanese colleagues (but primarily their work) we have shown that some fish bear toxins other than ciguatoxin, tetrodotoxin and the other previously reported toxins; these toxins include ciguaterin and aluterin as described by Washimoto and colleagues.
3. We have described the symptoms of hallucinatory mullet poisoning in

Hawaii and poisoning by toxic sardines in Fiji but we have done no laboratory work upon these toxins.

4. Our students have investigated box-fish toxins and puffer toxin in Hawaii.

Invertebrate toxins:

1. We made the original discovery (after the Hawaiians) of the highly toxic zoanthid subsequently studied in detail by the DOD.

2. We made preliminary investigations of the toxins found in toxic *Tridacna* in Bora Bora.

3. One of our students made preliminary chemical and detailed pharmacological studies of the toxin found in the salivary glands of ophistobranch molluscs.

4. With our Japanese colleagues (and again the studies were made mostly by the Japanese) we studied and reported upon toxic reef crabs and toxic turban shells.

Toxic microbial forms:

As indicated above, we have found many forms of fine marine algae, yeasts and molds that produce toxins, but we have been financially unable to follow these leads.

ACANTHASTER:

Not as part of our marine toxins program, our contract was used as a vehicle to help support the University of Hawaii's participation in the *Acanthaster* survey of 1969. University of Hawaii personnel, under the principal investigator, visited Guam for the planning sessions, the Palaus and the central Marianas; we surveyed the Marshall Islands, Johnston and Hawaii. We found large but seemingly harmless concentrations of the starfish in the three areas, particularly on the island of Molokai. The studies were continued under funding from Sea Grant.

APPENDIX

All publications issued by the Marine Toxins Program, University of Hawaii:

- 1953a Banner, A. H. and H. Borroughs. Observations on toxins of poisonous fishes. *Proc. Soc. Exptl. Biol. and Med.* 98:776-778.
- 1958b Martin, W. and A. H. Banner. The effect of poisonous fish upon intestinal parasites. *Trans. Amer. Micro. Soc.* 77(3):304-306.
- 1959 Banner, A. H. A dermatitis-producing alga in Hawaii. *Hawaii Med. J.* 19:35-36.
- 1960a Helfrich, P. A study of the possible relationship between radioactivity and toxicity in fishes of the central Pacific. U. S. Atomic Energy Comm. Tech. Information Ser. Publ. TID-5748. 16 pp.
- 1960b Helfrich, P. and A. H. Banner. Hallucinatory mullet poisoning. *J. Trop. Med. and Hyg.* 63(4):36-89.
- 1961a Banner, A. H., P. Scheuer, S. Sasaki, P. Helfrich, and C. Alender. Observations on ciguatera-type toxins in fish. *Annals N. Y. Acad. Sci.* 90(3):770-787.
- 1961b Banner, A. H., S. Sasaki, P. Helfrich, C. Alender, and P. Scheuer. Bioassay of ciguatera toxin. *Nature* 189(4760):229-230.
- 1961c Banner, A. H. Fish poisoning in the tropical Pacific. *South Pac. Bull.* 11(4):18-21, 65.
- 1961d Helfrich, P. Fish poisoning in the tropical Pacific. 16 pp. [Also issued in French by South Pacific Commission].
- 1963a Helfrich, P. and A. H. Banner. Experimental induction of ciguatera toxicity in fish through diet. *Nature* 197(4871):1025-1026.
- 1963b Helfrich, P. Fish poisoning in Hawaii. *Hawaii Med. J.* 22:361-372.
- 1963c Glossaries of island names [Preliminary working copies, issued in mimeographed form by A. H. Banner and F. C. C. Goo. August, 1963].
- A preliminary compilation of animal and plant names of the Caroline Islands, 49 pp.
 - A preliminary compilation of Samoan animal and plant names, 123 pp.
 - A preliminary compilation of Gilbertese animal and plant names, 70 pp.
 - A preliminary compilation of animal and plant names of the Mariana Islands, 43 pp.
 - A preliminary compilation of Tahitian animal and plant names, 57 pp.
 - A preliminary compilation of Marshallese animal and plant names, 43 pp.
 - A preliminary compilation of Tuamotuan animal and plant names, 52 pp.
 - A preliminary compilation of Fijian animal names, 45 pp.
 - A preliminary compilation of Tongan animal names, 52 pp.

- 1964a Banner, A. H., S. Shaw, C. Alender, and P. Helfrich. Fish intoxication: Notes on ciguatera, its mode of action and a suggested therapy. *South Pac. Comm. Tech. Paper No. 141*. 17 pp.
- 1964b Banner, A. H., P. Helfrich, P. Scheuer, and T. Yoshida. Research on ciguatera in the tropical Pacific. *Proc. Gulf and Carib. Fish. Inst. 16th annual, 1963:87-98*.
- 1964c Banner, A. H. and P. Helfrich. The distribution of ciguatera in the tropical Pacific. Hawaii Mar. Lab. Tech. Rept. No. 3, pp. iii + 48 [Mimeo].
- 1964d Cooper, M. J. Ciguatera and other marine poisoning in the Gilbert Islands. *Pacific Science* 18(4):411-440.
- 1964e Scheuer, P. J. The chemistry of toxins isolated from some marine organisms. *Fortschr. Chem. Org. Naturstoffe* 22:265-278.
- 1965a Li, K. M. Ciguatera fish poison: A cholinesterase inhibitor. *Science* 147(3665):1580-1531.
- 1965b Banner, A. H. Ciguatera in the Pacific. *Hawaii Med. J.* 24:353-354.
- 1965c Li, K. M. A note on ciguatera fish poison and action of its proposed antidotes. *Op. Cit.* 24:358-361.
- [Two publications listed immediately above issued with: Okihiro, M. M., J. P. Keenan, and A. C. Ivy, Jr. Ciguatera fish poisoning with cholinesterase inhibition. *Op. Cit.* 24:354-357.
In reprint "Ciguatera fish poisoning: A symposium" with separate pagination.]
- 1965d Li, K. M. Fish poisoning in the Far East. *Far East Med. J.* 1(1):29-33.
- 1966a Cooper, M. J. Destruction of marine flora and fauna in Fiji caused by the hurricane of February, 1965. *Pacific Science* 20(1):29-33.
- 1966b Banner, A. H., P. Helfrich, and T. Piyakarnchana. Retention of ciguatera toxin by the red snapper, *Lutjanus bohar*. *Copeia* (2):297-301.
- 1966c Banner, A. H., B. J. Stephens. A note on the toxicity of the horseshoe crab in the Gulf of Thailand. *Nat. Hist. Bull. Siam Soc.* 21(3-4):197-201.
- 1967a Banner, A. H. Marine toxins from the Pacific, 1-Advances in the investigation of fish toxins. *Animal Toxins*, pp. 157-165.
- 1967b Scheuer, P. J., W. Takahashi, J. Tsutsumi, and T. Yoshida. Ciguatoxin: Isolation and chemical nature. *Science* 155(3767):1267-1268.
- 1967c Banner, A. H. Poisonous marine animals, a synopsis. *J. Forensic Sci.* 12(2):190-192.
- 1967d Kosaki, T. and J. Stephens. Pupillary miosis to ciguatoxin(s) (from *Gymnothorax javanicus*). *Fed. Proc.* 26(2):322.

- 1968a Helfrich, P., T. Piyakarnchana, and P. S. Miles. Ciguatera fish poisoning I. The ecology of ciguateric reef fishes in the Line Islands. *Bishop Museum Occasional Papers* 23(14):305-369.
- 1968b Helfrich, P., and A. H. Banner. Ciguatera fish poisoning. II. General patterns of development in the Pacific. *Bishop Museum Occasional Papers* 23(14):371-382.
- 1968c Rayner, Martin D., T. I. Kosaki, and E. L. Fellmeth. [Marine toxins from the Pacific. V.] Ciguatoxin: More than an anticholinesterase. *Science* 160(3823):70-71.
- 1968d Kosaki, T. I., B. J. Stephens, and H. H. Anderson. Marine toxins from the Pacific. III-Comparative bioassay of ciguatoxin(s) in the mouse and chicken. *Proc. West. Pharm. Soc.* 11:126.
- 1968e Kosaki, T. I., and H. H. Anderson. Marine toxins from the Pacific. IV-Pharmacology of ciguatoxin(s). *Toxicon* 6(1):55-53.
- 1969a Banner, A. H., J. C. Nevenzel, and W. R. Hudgins. Marine toxins from the Pacific. II-The contamination of Wake Island Lagoon. *Atoll Research Bulletin* (122):1-12.
- 1969b Rayner, M. D., M. Baslow, and T. I. Kosaki. Marine toxins from the Pacific, VI: Ciguatoxin: not an *in vivo* anticholinesterase. *J. Fish Res. Bd. Can.* 26(8):2208-2210.
- 1969c Banner, A. H. Marine Toxins from the Pacific, II: A freshwater kill on the coral reefs of Hawaii. *Hawaii Inst. Marine Biol. Tech. Rept.* No. 15, 29 pp.
- 1969d Yasumoto, T. and P. J. Scheuer. Marine Toxins of the Pacific--VIII. Ciguatoxin from Moray Eels livers. *Toxicon*, 7(4):273-276.
- 1970a Rayner, M. D. Ciguatoxin: Muscle membrane actions of a possible ecological precursor. *The Pharmacologist* 12, abstr. no. 546.
- 1970b Rayner, M. D. In "Proceedings of Food/Drugs from the Sea Conference, 1969" ed. H. W. Youngken. pp. 345-350. Marine Technology Society, Washington, D. C. [Marine Toxins from the Pacific VII: Recent advances in the pharmacology of ciguatoxin]
- 1970c Rayner, M. D. and T. I. Kosaki. Ciguatoxin: Effects on Na fluxes in frog muscle. *Federation Proc.* 29:Abstract no. 1639.
- Setliff, J. A., M. D. Rayner, and S. K. Hong. Effect of ciguatoxin on sodium transport across isolated frog skin. *Toxicol. Appl. Pharmacol.* [In press 1971]
- Yasumoto, T., Y. Washimoto, R. Bagnis, J. E. Randall, and A. H. Banner. The toxicity of the surgeonfishes, *Ctenochaetus striatus* and *Acanthurus lineatus*. [Submitted to *Bull. Japan. Soc. Sci. Fisheries*]