Research on Ciguatera in the Tropical Pacific[1]

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Abstract

Ciguatera, a disease caused by eating toxic fish, is widespread among the islands of the central Pacific; it is sometimes restricted, however, to narrow areas of the reefs of certain islands. Over a period of years the toxicity of the reef fish fauna may wax and then wane.

Laboratory studies, using mice and mongooses for bioassay, have lead to the isolation of the toxin from the red snapper, *Lutjanus bohar*, but its chemical nature has not yet been determined. Parallel studies indicate that some of the other euryphagous reef carnivores, such as certain species of *Gymnothorax, Epinephelus, Caranx*, and *Sphryaena* probably bear the same toxin, but that found in *Ctenochaetus striatus* appears to be different. Pharmacological tests show that the toxin causes a blockage in the neuromuscular junction; this has led to the development of a therapy using anticurarial drugs.

Field and pond studies show that fishes may accumulate the toxin through their diet, and that *L. bohar*, one of the most consistently toxic fish in the Pacific, stores the toxin for a long period of time when maintained on a non-toxic diet. The diet of the toxic carnivores, moreover, includes toxic acanthurids which are obligate herbivores. The ultimate source of the toxin in the environment has not been discovered.

INTRODUCTION

WHILE THE DISEASE, ciguatera or tropical fish poisoning, was first recorded from the Caribbean, and while the name itself is of Spanish Caribbean origin, ciguatera has been known to Europeans in the Pacific since de Quiros was poisoned by fish eaten in the New Hebrides in 1606. Moreover, in the tropical Pacific it is widespread and of considerable importance to the health and economy of the people dwelling on the Pacific islands.

In the Pacific there are at least three types of fish poisoning in addition to ciguatera: puffer fish or tetraodon poisoning, scrombroid poisoning, and hallucinatory mullet poisoning; there are likely to be other types, as from certain clupeoid fishes and from shark livers, but too little is known about them to classify them at this time. Other authors (as Halstead, 1959) have suggested that the eels and sharks also carry a toxin that differs from that producing ciguatera, but the evidence on this point is questionable. Moreover, it is not known that all of the various species of fish which cause the symptoms known as ciguatera from archipelago to archipelago actually carry the same toxin; this is especially true when the Pacific is compared to the Caribbean.

Consequently, to eliminate possible confusion, all of our initial work at the Hawaii Marine Laboratory on the toxin causing ciguatera has been carried out on a single species of fish, the red snapper, *Lutjanus bohar* Forskål, from a single archipelago, the Line Islands (an archipelago of small islands

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near the equator about 1,000 miles south of Hawaii). In the last several years the studies have been extended to other fish and other archipelagoes to give a broader base to our studies.

Our studies, extending back eight years, have had the formal support of various national and international agencies. Our primary objectives have been three: the determination of the biological origin and mode of transmission of the toxin, the chemical isolation and identification of the toxin, and the pharmacology of the toxin. Collateral and minor studies have included the epidemiology of ciguatera in the Pacific, preliminary investigations on other types of toxins to determine their identity or non-identity with the toxin we are studying, the efficacy of certain native remedies, and an investigation of the possible relationship of radioactivity from the nuclear-testing programs to the toxicity of fish in the Marshall and Line Islands (Helfrich, 1960). Our investigations were severely delayed by a fire in December, 1961, which destroyed the building at the Hawaii Marine Laboratory that housed our investigation; this loss of our records is reflected in some of the data given below.

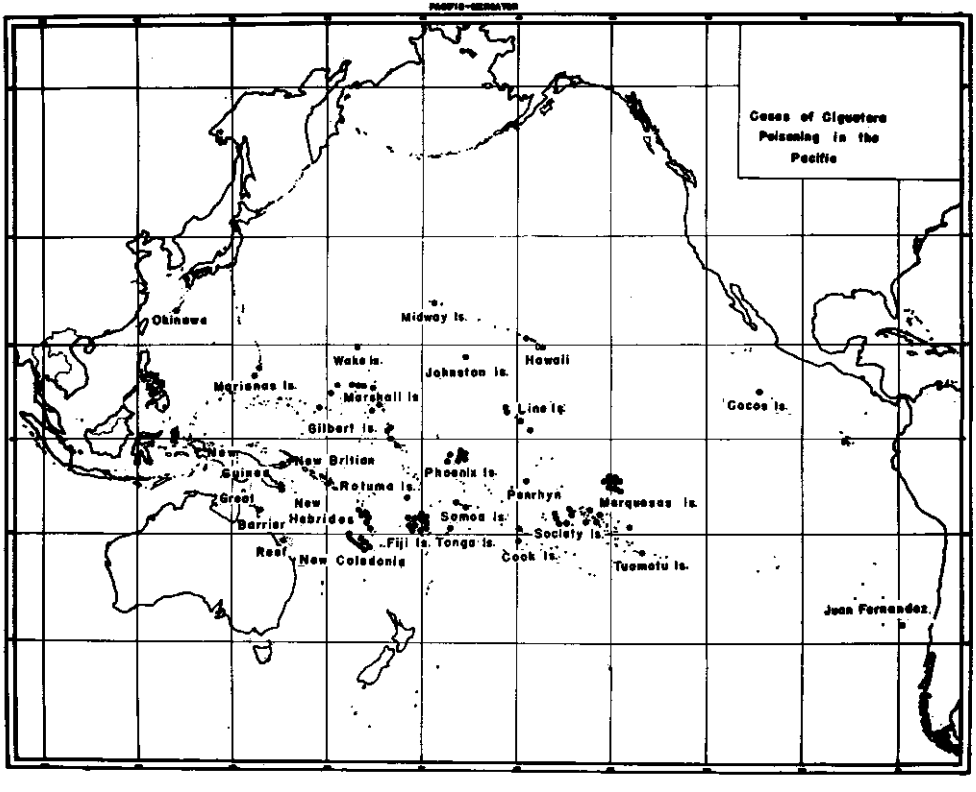

FIG. 1. Map of the Pacific showing extent of ciguatera. The archipelagoes from which ciguatera has been reported are named; the individual islands where cases have been reported are indicated by the larger circles.

EPIDEMIOLOGY

Under the sponsorship of the N.I.H. and with the cooperation of the South Pacific Commission, we have distributed questionnaires on fish poisoning to medical personnel throughout the South Pacific. To date, only incomplete returns have been received so that no final or complete picture can be drawn of the extent of ciguatera. However, from these returns, from personal letters and interviews, and in some cases from secondary sources, the following distributional map may be drawn (Fig. 1).

It is noteworthy that we have not been able to find any reports of the disease either on the continental coasts or from the large islands of the western Pacific except for the offshore areas of the Great Barrier Reef of Australia. On the smaller islands of the central Pacific, there is no readily apparent correlation between toxicity and any geographic, oceanographic, or meterologic

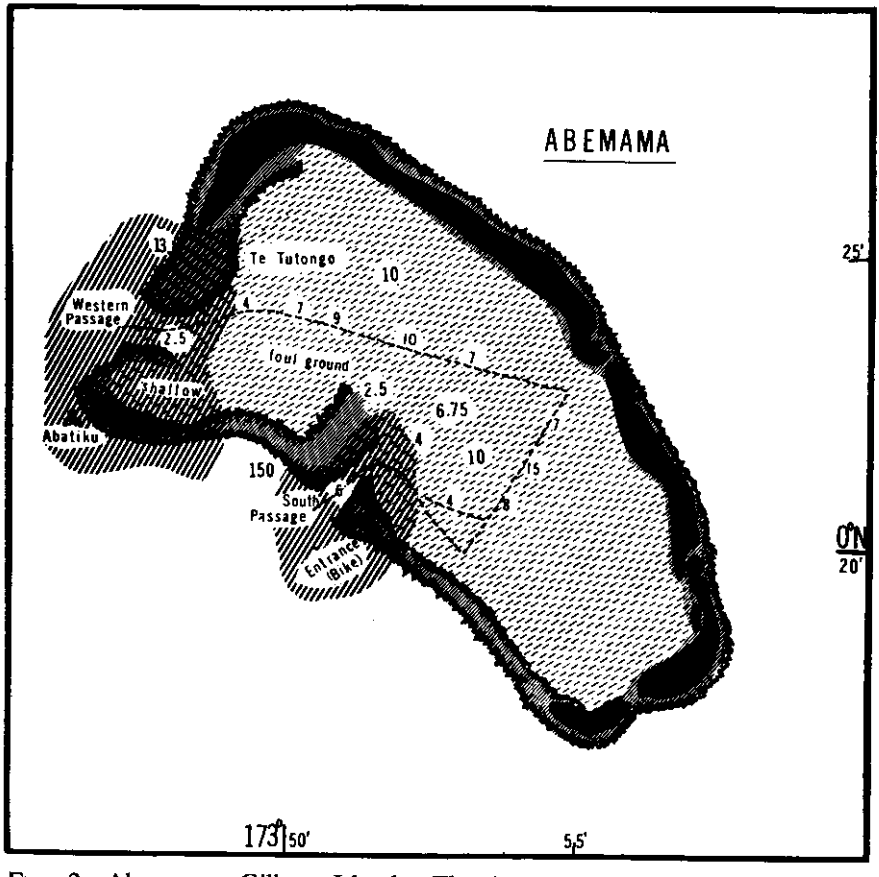

FIG. 2. Abemama, Gilbert Islands. The heavily hatched areas are those reported by the Gilbertese as locations where toxic species occur; the same species of fish from other areas are safe to eat (map from M. J. Cooper, in press). (Numbers indicate soundings in fathoms.)

conditions, save that the toxicity is confined to warmer seas and often to the lee shores of an atoll.

An interesting phenomenon, reported before but now more precisely delineated, is the narrow regionality on a single island of the fish that cause ciguatera. An associate of our program, Mrs. Margaret Jane Cooper, has prepared a report on her seven years' study of the toxicity in the Gilbert Islands (in press). She was able to obtain her information from Gilbertese fishermen in their own language; the Gilbertese proved to be an excellent source of information, for the chief source of protein food on the impoverished atolls is the sea and the inhabitants deliberately risk ciguatera in their desire for fish. Of the sixteen atolls in the Gilberts, ten have had a serious problem of fish poisoning during the years since World War II. Yet in no case was the entire reef around the atoll, or much of the lagoon, toxic. In all cases the toxic fish were restricted to a narrow area, always on the lee side of the atoll, and usually near the channels of the lagoon; seldom were the toxic fish found within the lagoon. A map of Abemama in the central Gilberts will illustrate the general pattern (Fig. 2).

Previous studies have indicated that the toxicity of the fish on a coral reef will increase, and then decrease, over the years. Thus, increases in toxicity in the last decade have been reported from certain of the Marshall Islands and from Midway in the Hawaiian chain (Bartsch and McFarren, 1962, Banner *et al.*, 1960). More recently and previously unreported are the studies of Dr. Hubert Voison of the Service de Santé of French Polynesia, which have shown a marked increase in fish toxicity in the Marquesas (personal communication). Dr. Voison was stationed in the Marquesas from 1954 to 1956, and again from 1960 to 1962. For the eight year interval he reports that an original toxic area on the eastern tip of Hiva Oa has extended westward at two to four miles a year, until in 1962 almost all of the bottom fish on the southern coast and about two-thirds of the northern coast are toxic; similarly, he reports, an increase of toxicity in all of the islands of the archipelago. On the other hand, Mrs. Cooper's study of toxicity in the Gilberts

TABLE I

PER CENT OF *Lutjanus Bohar* OVER 5 LBS. CAUSING PARALYSIS OR DEATH IN MONGOOSES (10% BODY-WEIGHT EQUIVALENT FEEDINGS)*

CHRISTMAS ISLAND (Cochrane reef area)

	1959	1960	1961	1962	1963
Percent toxic	—	—	15.0%	8.7%	5.4%
Sample size			113	57	55

PALMYRA ISLAND (South & Southwest reefs)

	1959	1960	1961	1962	1963
Per cent toxic	55.0%	41.1%	—	26.8%	—
Sample size	60	51		465	

*Those categories of +3 to +5 as established by Banner *et al.* (1960:773)

shows that on all ten toxic atolls the number and extent of toxic fish has been decreasing since a post-war peak.

These records, however, are not based on a systematic sampling program and laboratory testing but rather upon individual recollections (and in some cases, hospital records) of chance eating of the toxic fish. From our laboratory studies we have been able to document the decrease in toxicity in two of the Line Islands, Palmyra and Christmas, from 1959 to 1963, working consistently with the same species and from exactly the same portion of the reefs.

ASSAY METHODS

In our previous reports (Banner *et al.*, 1960, 1961) we have summarized our bioassay techniques. These involve the use of mongooses, fed the sample to be tested at 10% body weight equivalent, for the preliminary screening of the raw fish, and the use of intraperitoneal injections of the extract in various stages of purification into closed-strain white mice, using Tween 40 as an emulsifier. In an attempt to develop a simplified chemical assay method, one of our Japanese associates, Dr. M. Asano, working in our laboratory, explored 40 known colorimetric tests for the steroids and other naturally occurring compounds and functional groups; while he found some correlation between toxicity and positive color reactions in the Liebermann-Burchard and Salkowski tests for sterols, the results were not consistently reliable (unpublished). We have also found that a pharmacological test, using the impedence of nerve transmission across the nerve-muscle junction *(vide infra)* may offer a rapid and reliable test for the semi-purified and purified toxin. This remains to be investigated more thoroughly.

ORIGIN AND TRANSMISSION OF THE TOXIN

In 1958 Dr. John E. Randall published a coherent hypothesis on the origin of the toxin and its transmission through the food chain; this paper was based in part upon his studies in the Society Islands. He suggested that the toxin originated in benthic organisms, probably a fine blue-green alga; and, as the alga was consumed by various herbivores, it was accumulated in their flesh and viscera. The principal carriers were probably the abundant acanthurids or surgeon fish. These in turn were eaten by large carnivores like the snappers, groupers, and barracudas, which in turn would accumulate the toxin.

Several pieces of evidence obtained in our work tend to confirm this hypothesis. First, we have carried out a series of experiments in which we have induced toxicity into normally non-toxic species (Helfrich and Banner, 1963). *Acanthurus xanthopterus* Cuvier & Valenciennes from Kaneohe Bay, Hawaii, a common food fish that has never been known to cause ciguatera in Hawaii, was fed the flesh of *Lutjanus bohar* of known and high toxicity. The experimental fish were shown by bioassay to have developed varying toxicity, with two of the four fish capable of causing death in test mongooses. Unfortunately the quantitative data from this experiment were also destroyed in the fire, so the experiment is being repeated on a quantitative basis, using several species of non-toxic Hawaiian fish.

In Randall's hypothesis it would be logical to assume that the toxin was not rapidly metabolized or excreted in the fish that remain toxic for long periods after the other fish on the reef lose their toxicity. In the Gilberts these include the snappers, groupers, and eels (Cooper, in press). To establish whether

toxic fish do lose their toxicity or not when maintained on a non-toxic diet, a number of fish from Christmas Island were caught and maintained alive in the live wells of the U.S. Fish and Wildlife Service research vessel, CHARLES H. GILBERT. These were delivered to the tidal ponds of the Hawaii Marine Laboratory where they are being maintained on a diet of commercial Hawaiian skipjack tuna and herring from the Puget Sound. The results are presented in Table 2.

TABLE 2
TOXICITY OF *Lutjanus bohar* MAINTAINED ON A NON-TOXIC DIET*
Mongoose Reaction

Date Sampled	Non Toxic	+1	+2	+3	+4	+5	Total
Oct. 1961	33	5	9	1	1	3	52
Jan. 1962				1			1
Feb. 1962	1	1		2			4
March 1962			1			1	2
Dec. 1962		1					1
Jan. 1963	1	1					2
Feb. 1963	2	1					3
May 1963		1		2			3
Sept. 1963	3						3
Total Sampled	7	5	1	5	—	1	19
Fish Remaining							20

*The fish used to establish the toxicity were captured between 7 and 11 October, 1961, on Cochrane Reef, Christmas Island. The pond samples were captured on the same reef on 28-29 November, 1961, transported to Hawaii, and maintained on a non-toxic diet in the laboratory tidal pond. All fish were over 2.2 kg in weight and tested by standard mongoose bioassay. (Data for toxicity of sample of 28-29 November, to be used as the base line, were lost in the fire.)

With the low toxicity of the original sample and the small number of fish tested, we cannot as yet show reliably in this unfinished experiment if there is or is not a slow decline in toxicity. However, the three moderately toxic fish in the May, 1963, sampling show there is not a marked and rapid loss of the toxin in 18 months.

The only finding so far that does not follow the Randallian hypothesis is that one of the postulated transmitters of the toxin, the toxic *Ctenochaetus striatus* (Quoy & Gaimard), may not carry the same toxin as does *Lutjanus bohar;* this will be discussed below.

Randall also suggested that the organism producing ciguatera "may be one of the first growing on new or denuded surfaces . . ." On this point our studies have produced no consistent evidence, either for or against; if this part of his hypothesis be true, then the toxin must long remain in the ecosystem, for the fish at Palmyra continue to be toxic and the last extensive dredging and reef modification was in the war years 1942-1945 (Dawson, 1959).

BIOLOGY OF CIGUATERIC FISH IN THE LINE ISLANDS

In our attempt to understand fully the toxicity of one species of fish from one area, we have studied the biology of *Lutjanus bohar* from the Line Islands, hoping that this study would give us clues on the transmission of the toxin (all data as yet unpublished).

The habitat of *L. bohar* is in more open water around atolls and islands—primarily off the seaward reefs and in passes into the lagoon in depths of 15 to 100 feet or more. They seem to be particularly prevalent around certain bottom discontinuities such as ledges, ridges, and overhangs. This species appears to be a fairly active fish observed singly or in loose aggregations up to several hundred fish swimming off the bottom to mid-depths. They will occasionally rise to the surface to take a moving lure, but the usual method of capture is with hook and line, using cut bait near the bottom to mid-water.

This species is one of the most abundant fishes off the seaward reefs in the Line Islands; it is less common around some of the high islands. Randall & Brock (1960) report it rare around the high islands of the Societies, but more common around the atolls of the Tuamotus. Voison (personal correspondence) reports it uncommon around the main Marquesas Islands (high volcanic islands) but common on some of the off-shore banks, such as Clark Bank. In the Philippines, the fishery for this species is on an off-shore bank, and in New Caledonia it is more common on the barrier reef off the Isle of Pines (30 miles southeast of the main island of New Caledonia) than around the main high island.

A positive correlation between size and toxicity occurs in these fish. In a random sample of 437 fish from the Line Islands taken in 1959 and 1960,

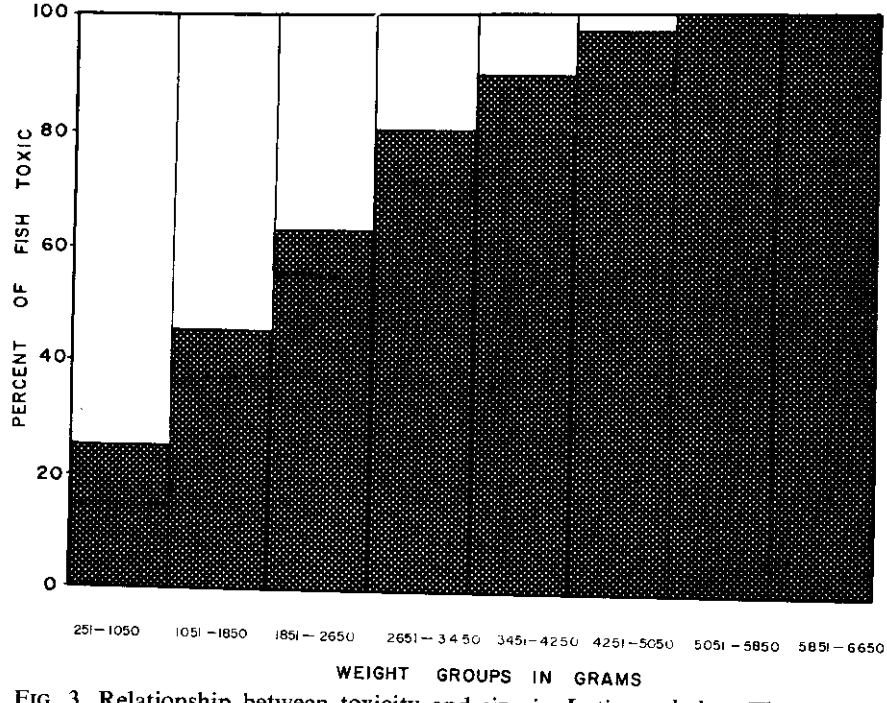

FIG. 3. Relationship between toxicity and size in *Lutjanus bohar*. The sample on which this graph is based is of 437 specimens collected in the Line Islands in 1959; the toxicity was established by standard mongoose feedings, and all levels of toxic response are included.

the toxicity in all degrees increases from about 25% in those below 1 kg in size to 100% in those over 5 kg (Fig. 3). This corresponds well to the sample of 190 specimens reported by Hessel & Halstead (1960) from the same area. Moreover, the degree of toxicity of the groups also increased with the size of the fish, with those in the smallest group being at most weakly toxic (+1, +2 reactions, as reported in Banner, *et al.* 1960), while those in the largest size category were moderately (+3) to strongly toxic (+4, +5).

The feeding activity of *L. bohar* is irregular. Analysis of catch data showed no significant differences between morning and afternoon catches. Fishing throughout the night yielded about equal catches at all hours from scattered locations over the entire Cochrane Reef area of Christmas Island, and at various locations near the main channel at Majuro, Marshall Islands. On the contrary, successful diurnal fishing depended upon location of concentrations of fish, which appeared to be "resting aggregations." These groups of fish ranging in size from about 300 to 700 mm in fork length were seen on a number of occasions near prominent ledges at depths of 60 to 100 feet off Palmyra and Christmas Islands (Fig. 4). In aggregations, *L. bohar* were observed swimming about slowly in irregular patterns or holding their position

FIG. 4. A typical aggregation of *Lutjanus bohar* near Cochrane Reef, Christmas Island, in about 15 feet of water. Note that in upper center and upper left are two *Monotaxis grandoculis* with pale bars on the dorsal surface, while on the lower center and left are four unidentified acanthurids with lunate caudal fins.

in the current, but never feeding or engaging in other purposeful activity that could be detected. Catches from these aggregations were highly variable ranging from about one fish per minute per boat (3 lines fishing) for a period of two hours to no fish for a comparable effort. Attempts to correlate feeding activity were inconclusive, although both field data and observations on *L. bohar* kept in captivity suggests a positive correlation between temperature and feeding activity. Other factors, such as the abundance of natural food during the previous normal feeding periods (believed to be at night), undoubtedly affected feeding activity of individuals and aggregations.

In a study on the diet of *L. bohar*, based upon food items found in 1790 stomachs examined during the period 1959-62 from Palmyra and Christmas Islands in the Line Islands, the following major food categories were found:

Fish

The main diet of *L. bohar* consists of various species of reef fish (17 families from Palmyra and 13 families from Christmas). The volumes and the frequency of fish are lower in the samples taken from Palmyra than the Christmas. Among 17 families of fish from Palmyra only three categories (Acanthuridae, Balistidae, and unidentified fish remains) occurred in more than 1% of stomachs examined. However, of those from Christmas, seven categories (unidentified eels, Serranidae, Acanthuridae, Labridae, Scaridae, Balistidae, and unidentified fish remains) occurred in more than 1% of the stomachs. Among the recognizable fish in the stomachs, acanthurids are the major component in both Palmyra and Christmas fish.

Crustacea

On both Palmyra and Christmas Islands the frequency and the volume of Crustacea in the diet give roughly comparable figures, but these indices differ between the two islands. The main category was that of larvae, which averaged about 42% on Christmas and 17% on Palmyra. These larvae were mostly the megalopa of crabs which were found more commonly in the six months from November through May than in the months from June through October. This undoubtedly coincides with the spawning period of some common reef crabs; it should be noted that Reintjes and King (1953) reported concentrations of megalops near the Line Islands in May, 1950 and May and June, 1951.

Mollusca

Among the Mollusca, the animals in the class Gastropoda occurred more frequently than the other classes and the percentage of frequency from these two areas are very much the same (4.23% from Palmyra and 4.07% from Christmas Island). The animals in the class Cephalopoda are of lesser importance.

Miscellaneous Invertebrates

The planktonic tunicates in the family Salpidae are the most common among the miscellaneous invertebrate food from the Palmyra samples. However, this is not true for the Christmas samples. *Pyrosoma* is the other planktonic tunicate which was found frequently in the Palmyra samples. It is interesting to note that the frequency of *Pyrosoma* decreases during November to May and increases in June to October.

Non-Food Categories

Between 1.0% and 1.5% by volume of the stomach contents of *L. bohar* was found to be non-food items such as random scraps of marine algae, feathers, pieces of coral, etc.

This study indicates that the acanthurids could be the chief dietary source of the toxin as hypothesized by Randall; this is further emphasized by the fact that there is a positive correlation between the size of the predator and the size of the prey. Thus, only the larger snappers can eat the high-bodied herbivores, which may in part account for the observations that only the larger fish are strongly toxic.

Reproduction in *L. bohar* was not observed. From examination of egg diameters and from relative gonad weight determinations, there is an indication that spawning occurs throughout the year but with a peak in November to January, which coincides with the coolest surface water temperatures in that area. There was no correlation between possible spawning times and toxicity.

A preliminary study of the food habits of the acanthurids, especially *Acanthurus triostegous* (Linnaeus) and *Ctenochaetus striatus,* gave no clues as to the ultimate source of the toxin. The fish were found to eat almost all algae found on the reef, each in small quantities. *Plectonema terebrans* (Borinet and Flahautt) (identified by Dr. Francis Drouet of the Philadelphia Academy of Science), a blue-green suspected as being associated with fish poisoning in some locations in the Gilberts (Cooper, in press), was found to grow in abundance ephiphytically on most of the algae eaten by the common acanthurids at Christmas Island. (We are indebted to Dr. William J. Gilbert of Albion College, Michigan, for his aid in our initial studies of acanthurid stomachs from Christmas Island.)

CHEMISTRY OF THE TOXIN

Since the last published report on the extraction and purification techniques being used in the study of the chemistry of the toxin, many advances have been made. The procedure leading towards the isolation of the toxin, with the concentration factors and the toxicity of the product, that is now used is as shown in Figs. 5 and 6.

From our most highly toxic chromatographic fraction we have been able to remove by crystallization a low-melting compound of long-chain fatty acid character. The remaining toxin has not been crystallized, but it appears to be homogeneous by thin-layer chromatography, by starch block electrophoresis and by countercurrent distribution. Further work is in progress.

While the positive identification of the toxin found in other species with that of *Lutjanus bohar* must wait until the toxin from *L. bohar* itself is known chemically, solubility tests can give an indication of the nature of the toxin. By extracting the flesh of other species in a manner parallel to that used in the purification of *L. bohar* toxin, we have found that the toxin of species of *Epinephelus, Gymnothorax, Caranx,* and *Sphyraena* can be extracted in exactly the same way.

On the other hand, when toxic *Ctenochaetus striatus* are subjected to this extraction procedure, the toxin goes into an alcoholic medium, but instead of being removed by solvent-solvent extraction with diethyl ether, the second

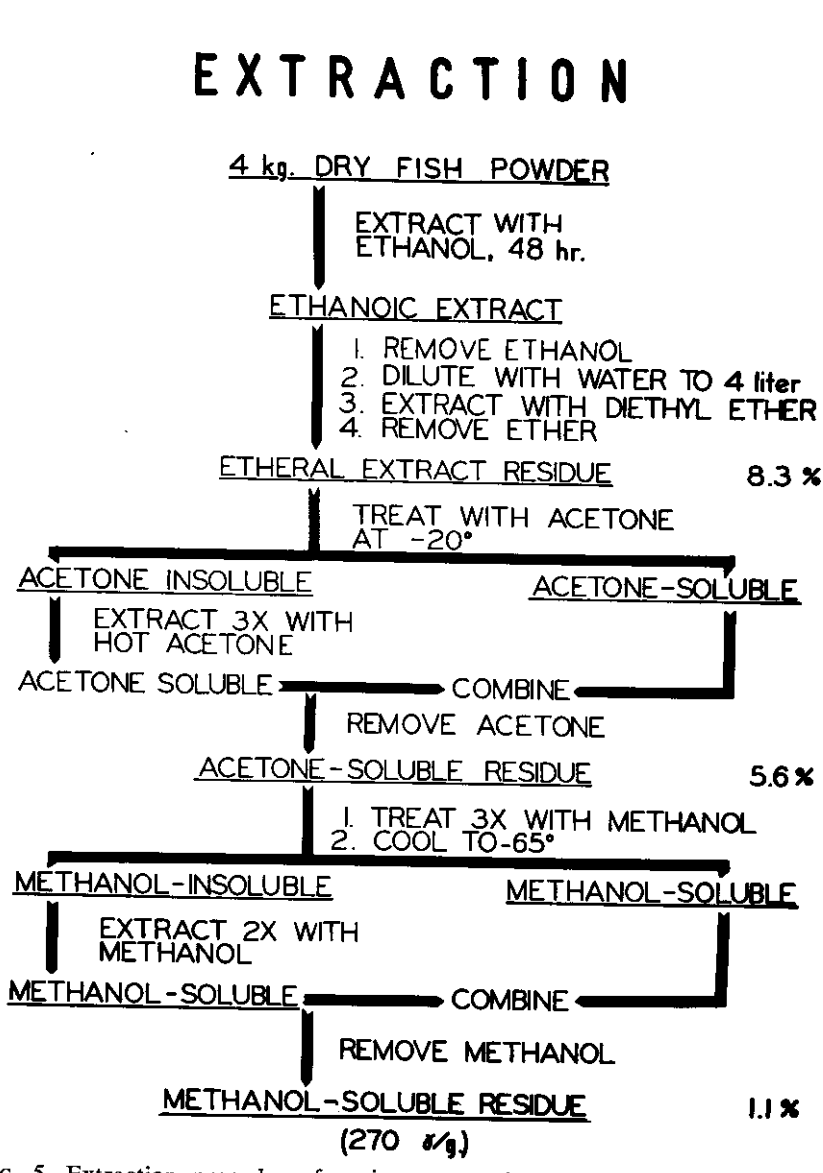

FIG. 5. Extraction procedure for ciguatera toxin from *Lutjanus bohar*. The original 4 kg of dried flesh, with skin and bones but without viscera, is equivalent to approximately 16 kg of whole fresh fish. The yields are expressed on the right as percentages of the original dry weight.

step in the extraction of the toxin of the carnivores, it remains in the aqueous alcohol. Whether this means that this acanthurid carries an entirely different toxin or a precursor of the carnivore toxin with slightly different solubility characteristics is not known.

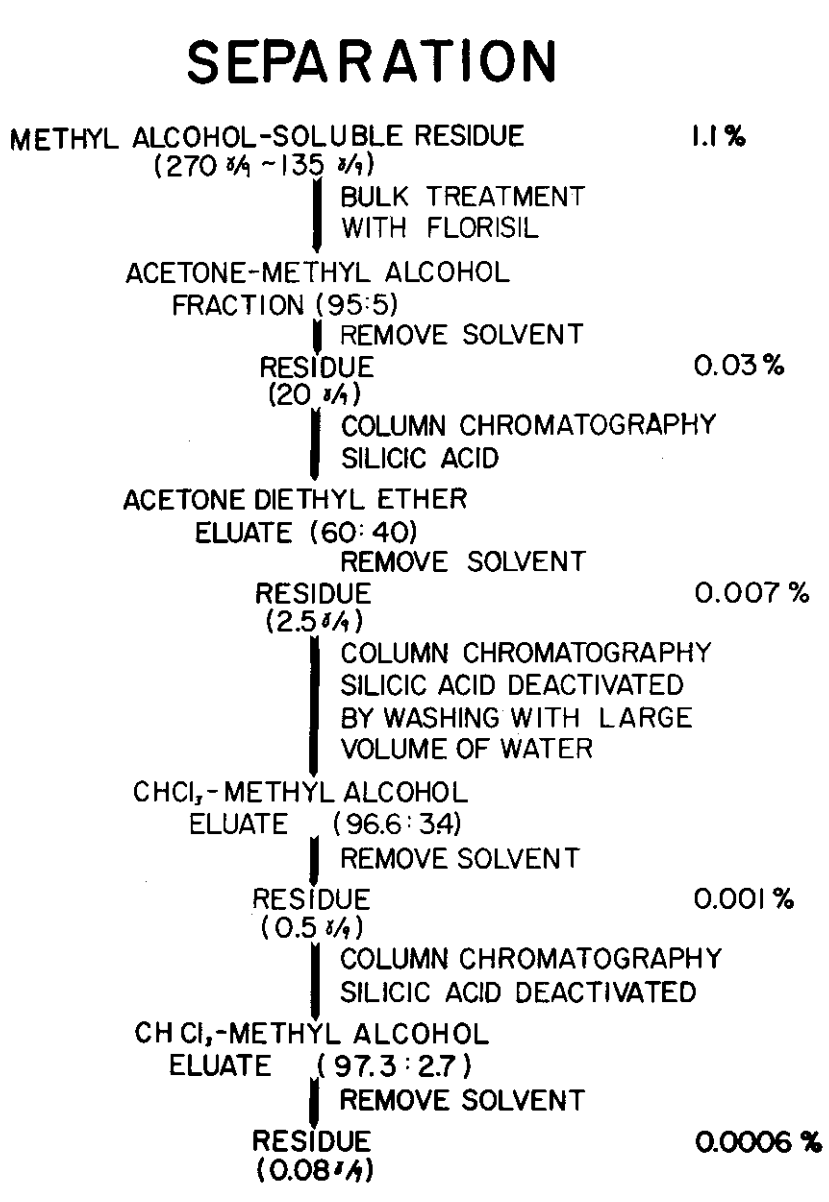

FIG. 6. Separation procedure for ciguatera toxin, continuing from Fig. 5. The toxicity indicated in parenthesis is that amount necessary per gram to kill a uniform strain of white mice by intraperitoneal injection. The percentage yield is on the right. The residue is apparently a pure compound but has not yet been crystallized.

PHARMACOLOGY OF THE TOXIN

In preliminary studies of the whole animal response to intraperitoneal and intravenous injections of the semipurified toxin, changes were observed in respiratory rate and blood pressure, but the electrocardiographic traces remained unchanged. In addition, preparations were made of the guinea pig phrenic nerve-diaphragm and toad sciatic nerve-satorius muscle (Banner, *et al.*, 1963). In both, after bathing the preparation with saline solution containing small amounts of toxin, there was an immediate impedence of stimulation of the muscle through the nerve fiber, but the contractility of the muscle on direct stimulation was not impaired.

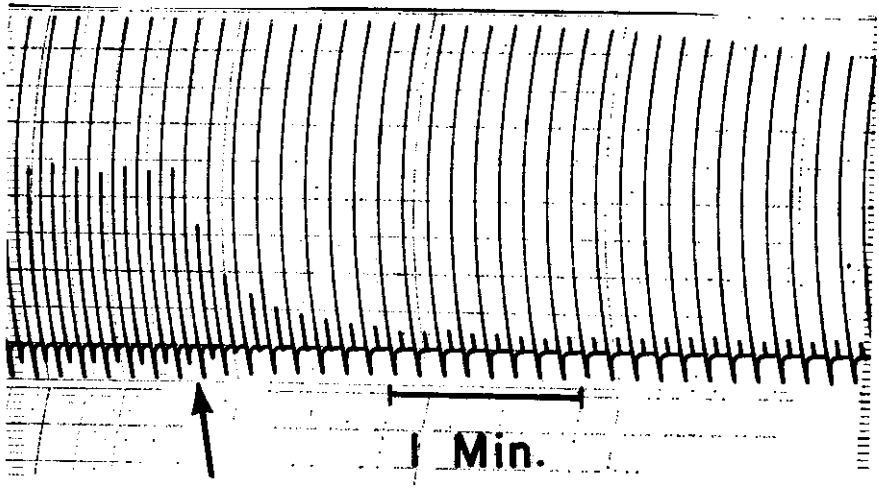

FIG. 7. Action of semi-purified toxin upon a toad sciatic nerve-gastrocnemius muscle preparation. The recording of the direct stimulation of the muscle is the higher contraction, and the lower contraction is that elicited by indirect stimulation through the nerve. The point of introduction of 20 μg of toxin to the bathing Frog Ringer's solution is indicated by the arrow. Within one minute the indirectly elicited response fell almost to zero; within ten minutes it had disappeared and would not reappear even after multiple washings with non-toxic saline solution.

This pharmacological action could be similar to the end-plate blocking action of d-tubocurarine, the depolarizing block induced by decamethonium, or the inhibition of the release of actylcholine from the nerve endings as caused by botulism toxin.

THERAPY FOR CIGUATERA

In 1961, when only preliminary studies on the pharmacology of the toxin had been made, a physician on Guam, Dr. Spencer W. Shaw, communicated with our laboratory, seeking advice on a patient who was apparently dying from ciguatera (Banner, *et al.*, 1963). The patient, a 19 year old girl, had eaten a portion of a 57 lb. barracuda some six days before. Originally she had been admitted to the hospital complaining of the usual nausea, numbness and

tingling of the skin, weakness of the limbs that characterize early symtoms of ciguatera. She was treated with calcium gluconate and released from the hospital in two days. However, on the evening of the third day she was re-admitted to the hospital in relapse, showing acute loss of control of her muscles. From the third to sixth day she was treated with hydrocortisone, chloropromazine, atrophine sulfate, edrophonium bromide *(Tensilon)* and the usual intravenous fluids. None of these drugs brought more than temporary relief, and the patient would lapse into deeper and deeper comas.

When consulted by Dr. Shaw, Dr. Helfrich of our group stated that the preliminary work indicated that the toxin caused a blockage of the neuromuscular junction, the exact nature of which had not been determined. This suggested to Dr. Shaw that perhaps an anticurarial drug might be indicated, so he tried neostigmine methylsulfate, coupled with a continuation of the hydrocortisone therapy. The next morning the patient was "awake and alert, showing no facial weakness and had good strength bilaterally . . ." Continuation of the treatment caused rapid improvement and the patient was released seven days later.

Subsequently the therapy was tried on 27 other patients, one in Hawaii and 26 on Ponape, Caroline Islands; in all but 5 cases a marked improvement in the symptoms was noted in as little as 30 minutes, and one group of 17 patients were reported as "completely cured" in six hours. Similarly, in an outbreak of ciguatera in pets in Hawaii caused by imported fish, several cats were treated successfully with physostigmine.

SUMMARY

Recent advances in the study of the toxin causing ciguatera in the tropical Pacific are discussed, including studies on the epidemiology, the biology, the chemistry and the pharmacology of the toxin, and an indication of a possible therapy for ciguatera is given.

ACKNOWLEDGMENTS

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