CIGUATERA II. CIGUATERA FISH POISON STUDIES IN THE CARIBBEAN

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INTRODUCTION

Ciguatera fish poison appears to have been a problem in tropical and subtropical waters since before recorded history. It is the most important marine toxicity problem in the world today judged by its impact upon the health of many people and its inhibition of the development of tropical and sub-tropical reef fisheries. Ciguatoxic fish have been reported in the Red Sea, Indian Ocean, Pacific Ocean, Caribbean Sea and Gulf of Mexico. It has been assumed, although not proven, that ciguatera is caused by similar or identical toxins wherever it is observed.

Halstead (1967) lists over 400 species of ciguatoxic fish which include both herbivores and carnivores as well as cartilaginous and bony species. These species include some of our more prized food fish such as groupers, jacks, eels, flounder, snappers, croakers and barracuda. Without exception fish of ciguatoxic species are non-toxic in some waters although toxic in others. In some cases only a few miles separate ciguatoxic and safe fish of a given species. Each of the ciguatoxic species is a bottom feeder or includes bottom feeding species in its diet. This has led many to conclude that these toxins or their precursors originate in the benthic flora of a reef (Banner, 1974).

Although fish seem to tolerate the ciguatera toxins, humans who eat ciguatoxic fish become very ill. Toxic symptoms may include abdominal pain, nausea, vomiting, diarrhea, numbness and a tingling sensation about the mouth, headache, numbness in the extremities, metallic taste, muscular weakness, muscle aches, reversal of the sensation of hot and cold, and itching. Death from ciguatera poisoning sometimes results especially when it occurs in the Western Pacific. Recovery is often slow from the neurological symptoms and may require weeks or even months.

Scheuer (1967), following several years of careful research, isolated a purified toxin from extracts of moray eels, *Gymnothorax javanicus*

(Bleeker), collected in the waters of Johnston Island in the Pacific. This toxin, which he named ciguatoxin, has an apparent empirical formula of $(C_{35}H_{65}NO_8)_3$, is basic, yields glycerol and a mixture of long chain fatty acids upon hydrolysis, and has a molecular weight of 1500-1800. Ciguatoxin is a fat soluble toxin. It has been identified in several species of ciguatoxic fish by Scheuer, Hashimoto and their colleagues in the University of Hawaii and University of Tokyo respectively.

Hashimoto(1969) discovered a water soluble toxin, which he named ciguaterin, in addition to ciguatoxin in some ciguatoxic fish. Ciguaterin was subsequently purified and shown to be a peptide derivative (Kamiya, 1973) and hence quite different chemically from ciguatoxin.

Many reports of ciguatera fish poisoning in the Caribbean and lower Gulf of Mexico have been documented by Halstead (1967) and Brody (1972). Some 100 species of fish have been implicated. Only a few preliminary research investigations, however, have been made in this region. An investigation of ciguatera fish poisoning in the Carbbean was begun in our laboratory in August 1973 (Cheng, 1975 and Granade, 1975).

CIGUATOXIC FISH FROM ST. THOMAS

Portions of the flesh of six ciguatoxic fish were furnished us by Dr. Ed Towle, director, Island Resources Foundation, St. Thomas, U.S. Virgin Islands. Four specimens were remains of fish recovered by the Caribbean Research Institute from families which had been poisoned. Two were specimens which were found toxic in the mongoose assay (Banner, 1960) by the Islands Resources Foundation. These specimens included an amberjack *(Seriola dumerili)*, an almico jack *(Seriola rivoliana)*, a horse-eye jack *(Caranx latus)*, a misty grouper *(Epinephelus mystacinus)*, a snapper and an unidentified fish. The specimens were shipped, packed on dry ice. to our laboratory.

These specimens were extracted by a procedure similar to that of Yasumoto and Scheuer (1969), figure I. Fractions B and D were found to contain nearly all the toxin(s). Samples of these two fractions were injected intraperitoneally into mice as oil in water emulsions stabilized with Tween 60 and as corn oil solutions (Banner, 1961). The following observations were made:

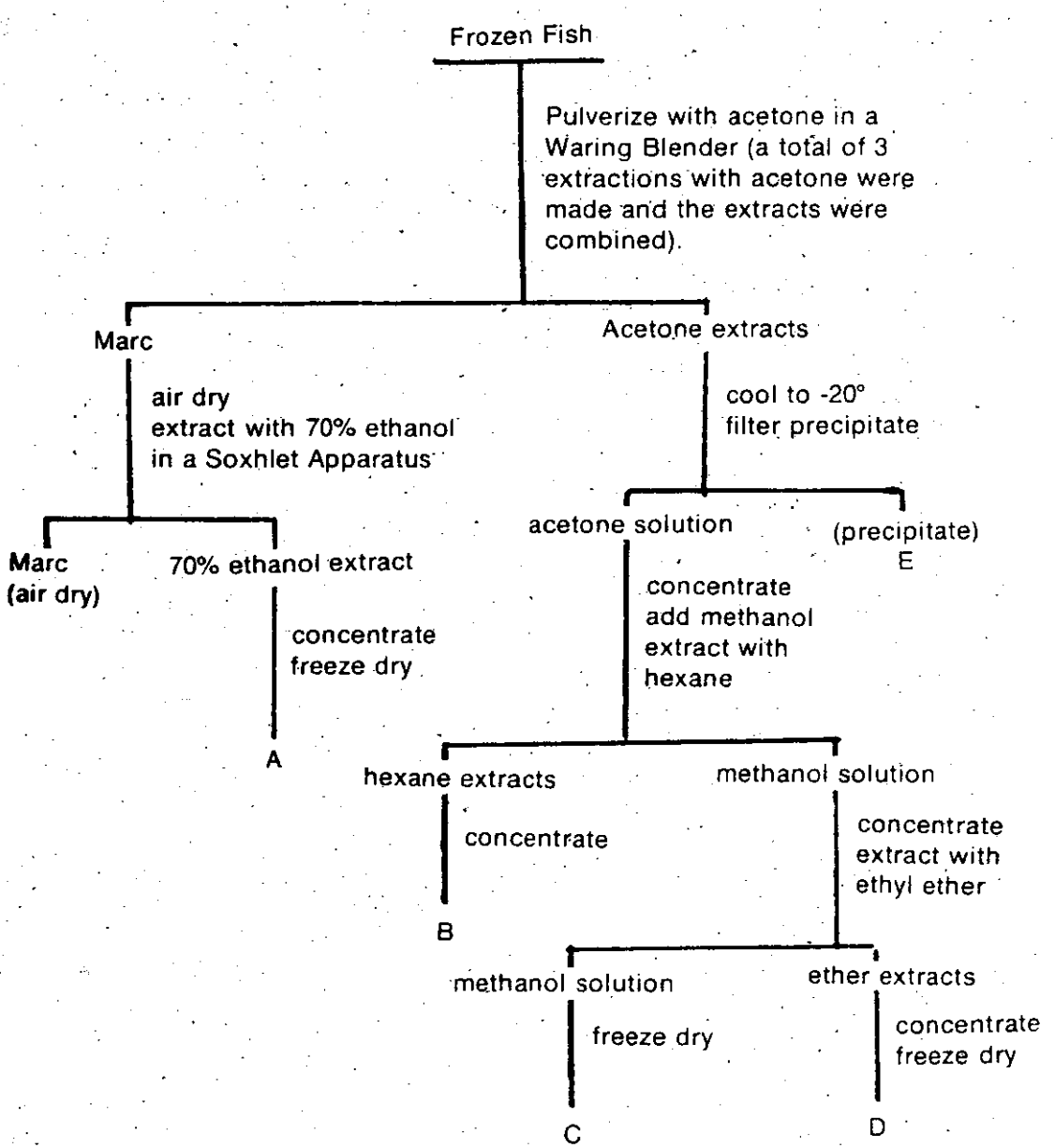
(1) The D fraction was always more toxic than the corresponding B fraction. This is consistent with the observations of Yasumoto and Scheuer (1969).

(2) Each fraction produced abdominal irritation after injection as judged by the humped appearance of the mice and their sensitivity to being handled in the abdominal region. Injection of the vehicles alone did not produce this effect.

(3) A mixture of autonomic effects, e.g., mydriasis, piloerection. diarrhea and vasodilation, were observed suggesting that more than one bioactive substance may have been present in these extracts.

Figure I

Extraction and Fractionation Procedure



(4) In most instances body temperature was lowered 2-7° even in those mice which survived.

(5) A disassociation from the environment was noted in which there was little or no response to usual anxiety producing situations.

(6) Some extracts produced a curious "springing" behavior in which a mouse would leap from one part of his cage to another.

(7) Labored breathing always preceded death.

(8) The limited quantities of material which we had prohibited us from determining LD_{99} or LD_{50} values for these fractions. They were less toxic, however, than the most toxic fractions reported by Yasumoto and Scheuer (1969).

Some of these observations are not reported in the literature. This may mean that these specimens did not contain the same toxin as being studied in the Pacific because (1) the toxin decomposed during storage of the fish prior to our receiving them, (2) the ciguatera toxins of the Caribbean and Pacific are different or (3) these fish were toxic due to a toxin other than that of ciguatera. It may also mean that we have examined the mice in greater detail for pharmacological effects or that these fish specimens contain toxins in addition to ciguatoxin.

NEW BIOASSAY PROCEDURES FOR CIGUATERA

At the present time bioassay procedures are needed to detect ciguatera toxins since there is no suitable chemical assay. The most frequently used bioassays include feeding suspect fish to a mongoose (Banner, 1960) or a cat (Halstead, 1967) and the intraperitoneal injection of certain extracts into mice (Banner, 1961). Many other animal species have been used experimentally but were not adopted for bioassay purposes because of lack of sensitivity to ciguatera, large size or cost.

There is an urgent need for simpler and more sensitive tests for ciguatera toxins in order to facilitate chemical and biological research, to aid in tracking down the source(s) of ciguatera in the food chain, and to aid in identifying non-toxic fish and safe fishing waters in the ciguatera areas of the world.

A number of potential new bioassays were explored in our laboratory utilizing extracts of the ciguatoxic fish obtained from St. Thomas. Three potentially useful new bioassay procedures were discovered (Granade, 1975). One involved the intraperitoneal injection of a toxic extract into frog tadpoles. The tadpoles were killed at doses much lower than those required by mice. A second promising bioassay involved the addition of a small sample of a toxic extract to a culture of a photobacterium (species unknown) which we have in our laboratory. Light output was markedly reduced with small amounts of toxin but unaffected by similar extracts of non-toxic fish.

The third bioassay discovered involves the use of brine shrimp, *Artemia salina*, larvae. We were prompted to examine brine shrimp because of Rayner's report (1972) that ciguatoxin made certain cell membranes more porous toward passive diffusion of sodium ions. *Artemia salina*, because of its efficient sodium pump, is one of the few living species capable of adapting to the high salt concentrations of Great Salt Lake. It was reasoned that since this sodium pump is so efficient, it may, as a result of evolutionary processes, now perform important functions in some vital physiological processes. If so, the bring shrimp might be very sensitive to a substance, such as ciguatoxin, which facilitates the passive transport of sodium ions. Although our reasoning may not be correct, the brine shrimp proved to be very sensitive to extracts of the ciguatoxic fish from St. Thomas.

Fractions B and D of the extracts of the six fish specimens obtained from St. Thomas were highly toxic to brine shrimp larvae. Similar fractions of extracts of non-toxic fish were relatively non-toxic to the brine shrimp. The twelve fractions (B and D) from the St. Thomas fish and fraction D from a non-poisonous mackerel were ranked from one to thirteen based upon toxicity to mice. Precisely the same ranking was obtained when these samples were ranked based upon toxicity to brine shrimp (Granade, 1975). This gave us confidence that we were detecting the same toxin(s) in the mouse and brine shrimp assays. The brine shrimp assay has the advantage of being much more sensitive than the mouse assay.

The assay procedure consists of adding 0.5 ml. of a solution of a known quantity of extract in one percent Tween 60 to 0.5 ml. of artificial sea water containing about 100 brine shrimp. Newly hatched brine shrimp larvae are more sensitive than those which are several days old. The percent brine shrimp which were dead at 20 minutes, 1, 2, 10, and 24 hours were recorded. The quantity of extract required to produce toxic effects under these conditions was 10-500 μg. with the St. Thomas extracts. The quantity of extract required could be reduced approximately tenfold by reducing the total volumes for the assay to 0.1 ml. and utilizing 10-20 brine shrimp.

Studies are now in progress with the objective of modifying the brine shrimp assay to make it suitable for use as a field assay. These studies are directed toward developing a simple procedure for isolating the toxin from liver, viscera or flesh utilizing solvents, equipment and procedures suitable for field situations.

Fraction D of the St. Thomas misty grouper (*Epinephelus mystacinus*) was chromatographed on silica gel plates using each of five chromatographic systems described by Hashimoto (1968). We failed to detect a spot positive for an alkaloid with Dragendorff's reagent which corresponded with the ciguatoxin spot reported by Hashimoto in any of his systems. We were able to locate four Dragendorff positive spots in the misty grouper extract when 5 percent methanol in chloroform was used as the developing solvent. The Rf values were 0.15, 0.25, 0.60 and 0.90 for these spots. It was found that the Rf 0.90 spot was highly toxic to brine shrimp

since rapid death occurred with concentrations of less than 1 μg./ml. Rf 0.15 and 0.25 spots were also very toxic. The Rf 0.60 spot was found to be non-toxic.

CIGUATERA STUDIES ON CAYMAN BRAC

Vital to ciguatera research is the availability of a supply of ciguatoxic fish and facilities for making observations, collections and conducting experiments in waters which contain ciguatoxic fish. The facilities of the Mosquito Research and Control Unit on Cayman Brac were made available to us during the summer of 1974 through the kind cooperation of Dr. M.E.C. Giglioli, Director of Mosquito Research and Control and Natural Resources Study and the Honorable Warren W. Conolly, Office of the Governor, Cayman Islands.

Many fish specimens were collected, freeze dried, and brought back to our laboratories for study. A variety of observations were made and information collected which may ultimately be significant. We would like to share some of this information at this time.

Toxic fish are more frequently encountered in the vicinity of Grand Cayman in West Bay, off Northwest Point and in one of the outer fish banks west of the Cayman Islands. Each of these areas contains many ship wrecks. There are a few ship wrecks in other areas among these islands where toxic fish are less frequently encountered. The horse-eye jack *(Caranx latus)*, which is known locally as the "Crevalle" jack, is considered very dangerous and seldom eaten. It has been a frequent cause of poisoning. The true crevalle jack *(Caranx hippos)* is also found in the waters of the Cayman Islands and is apparently toxic less often. Almost everyone over 30 interviewed had been poisoned at least once, usually with a small (2-5 pounds) barracuda. Large barracuda are considered dangerous and consequently are eaten much less often.

Some of the symptoms of poisoning reported to us differed from the literature. For example men frequently described disorders of the urinary tract along with the other more usual symptoms. These disorders included pain, burning on urination, inability to urinate and in some cases blood in the urine. Symptoms of yellow jack, *(Caranx bartholomaei)* poisoning are often restricted to severe dermatitis with itching for periods of two days to several weeks.

A tea made from "Bissie" (pronounced busy) which consists of the ground kernels of the dried fruit of *Cola acuminata* and an infusion made from the leaves of "Bitter Sage" *(Pluchea odorata* Cass.) are used for the treatment of ciguatera poisoning. Local people make great claims for each of these treatments.

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