

SOUTH PACIFIC COMMISSION

SEMINAR ON ICHTHYOSARCOTOXISM

(Rangiroa, French Polynesia, 16th - 22nd August, 1968)

TOXINS FOUND IN CIQUATOXIC FISHES IN THE RYUKYU ISLANDS

by Yoshiro HASHIMOTO*

Succeeding to the field investigation in the Ryukyu and Anami Islands, toxicity of fishes reported to cause ciguatera was examined, and the manifoldness of toxins involved has been clearly demonstrated. Four different types of toxin at least have been thus far found and a few others are postulated to be present.

In this paper are summarized chemical and pharmacological properties of toxins now under study in our laboratory.

Screening of toxic fishes

Some of specimens were at first screened by feeding the cooked flesh or liver to cats, and occurrence of two types of toxin was recognized. The one induced in cats paralysis, hypersalivation, inconsistent vomiting, and death in coma, and the other only transient vomiting. The both toxins were also different to each other in solubility, the former being fat-soluble and the latter water-soluble. To distinguish these toxins more sharply, a method for separating them prior to bioassay was devised as shown in Fig.1.

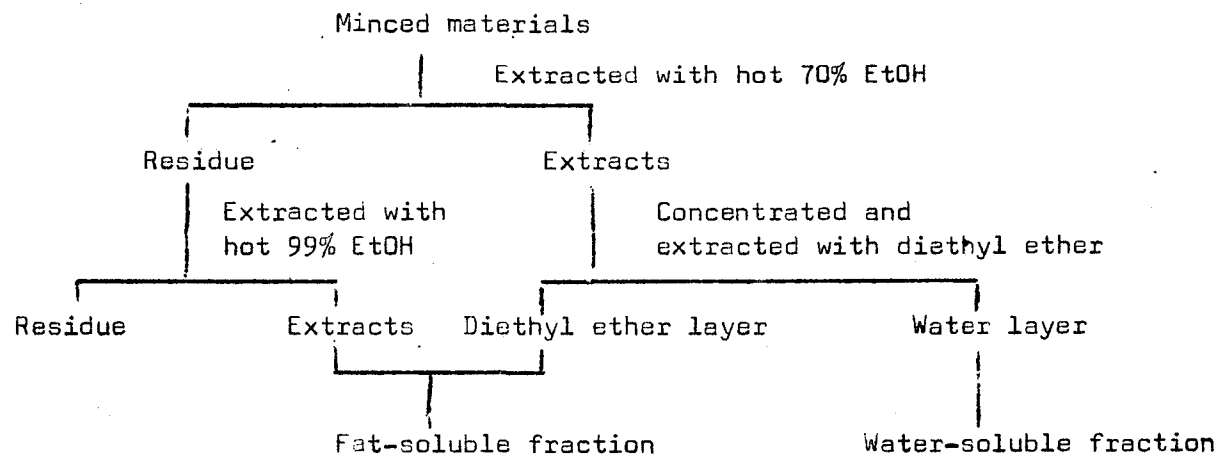


Fig. 1 Fractionation of the fat-soluble and water-soluble fractions

Occurrence of a fat-soluble toxin probably identical with ciguatoxin

By feeding the above-mentioned fat-soluble fraction to cats, all the specimens were examined, and 7 out of 22 specimens of Lutjanus monostigma were found to be toxic. A strongly toxic specimen and four slightly toxic ones were detected in a total of 34 specimens of L. bohar.

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Identity of toxins in a strongly toxic specimen of L. bohar from the Ryukyu Islands and in those from the South Pacific, supplied by Dr Banner, was established as follows. In all specimens, the fat-soluble fraction obtained by our extraction method caused quite identical symptoms in cats. This was also true for the diethyl ether extract from methanol layer prepared by the method which has been recently developed by Dr Scheuer for ciguatoxin in the muscle of Gymnothorax (P.J. Scheuer et al. : Science, 155, 1267, 1967). In addition, the toxic substance from each sample behaved quite similarly in thin layer chromatography and on the silicic acid column from which it was eluted as ciguatoxin with a mixture of methanol and chloroform (5:95).

It may be added here that a toxic amberjack (Seriola aureovittata) was also found to contain a fat-soluble toxin in the muscle. A toxin extracted from the muscle of fish which caused a poisoning case on May 10, 1967 at Katsuura near Tokyo behaved quite similarly to ciguatoxin in column and thin layer chromatography and developed similar symptoms in both mice and cats (Y. Hashimoto et al. : Bull. Jap. Soc. Sci. Fish., in press).

Distribution of ciguaterin and its chemical properties

The water-soluble fraction from toxic samples caused in cats violent vomiting and loss of activity, but the animals recovered without any ill effect within 48 hours. This toxin was named ciguaterin for convenience. It was only detectable with cats, but not with mice, rats, frogs, and chickens. Distribution of ciguaterin was therefore examined by the subcutaneous injection of the water-soluble fraction into cats.

The muscle was found to be weakly toxic rarely in both L. bohar and L. monostigma and rather frequently in G. flavimarginatus, Epinephelus fuscocuttatus and Variola louti. On the other hand, the liver of all specimens of these five species was almost always strongly or moderately toxic. Ciguaterin was detected in the gonad of L. bohar, too. A few species of fish common in Japan did not show any detectable amount of ciguaterin in their muscle and liver, suggesting that it may be peculiar to ciguatoxic fishes. It is interesting that some specimens of both L. monostigma and L. bohar revealed the co-existence of ciguaterin and a fat-soluble toxin in a body.

Ciguaterin extracted from the pooled liver of L. bohar was found to be considerably thermostable at pH 3 and 10, and not extractable from aqueous solution with ordinary organic solvents. It was not steam-distilled and easily dialyzed through a cellophane membrane. It was purified column chromatographically by using Dowex 1-X8 both in free and acetate forms and cellulose plate, and the final product showed the minimum dose lower than 0.4 Y/g to evoke vomiting in cats. The further purification is now under progress.

An ichthyotoxic and hemolytic substance in the skin of a sea bass

A sea bass (Pogonoperca punctata) showed some peculiarities in a screening test. When the water-soluble fraction was prepared from the muscle with skin by the routine method, a resinous substance giving a bitter taste and soluble in diluted acetic acid appeared as copious precipitates. The substance was then confirmed to originate from the skin and kill cats with ciguatera-like syndromes when force-fed or injected subcutaneously. It also killed mice and killifish and hemolyzed the rabbit blood cell. These activities were all useful for bioassay.

By using the hemolytic activity as index, chemical properties of the toxin were examined. It was extracted from the skin with dilute acetic acid but not with water and easily transferred into n-butanol or amyl alcohol from the aqueous layer, but not into diethyl ether from both acidic and alkaline media. It was heat-labile at pH 12. In dialysis

against distilled water with a cellophane membrane, about 35% of toxin were found in the outer layer. At a half saturation of ammonium sulfate or sodium chloride, the toxin was precipitated as complex quantitatively. The complex with sodium chloride was easily soluble in water and about 80% of hemolytic activity were recovered from the precipitates. The complex with ammonium sulfate, on the other hand, was hardly soluble in water and about a half of activity was found to be recovered from the complex by extraction with n-butanol in the water-soluble form. The toxin was effectively purified by extraction with n-butanol and gel-filtration on Sephadex G-25, but has not yet been obtained in the crystalline form.

These results suggest a close similarity of the toxin to an ichthyotoxin of soapfish (A. Maretzki *et al.* : *Toxicon*, 4, 245, 1967) rather than pahutoxin of boxfish (P.J. Scheuer *et al.* : *Science*, 155, 52, 1967). Our toxin was extractable directly from the skin of dead fish, whereas they were reported not to be obtainable directly from the skin. Recently, we have found that another sea bass (*Grammistes sexlineatus sexlineatus*) secretes an ichthyotoxic and hemolytic substance when placed in distilled water. The secreted toxin was almost identical with that obtained from *P. punctata* in solubility, precipitation with salts and thin layer chromatography.

L.P. Schultz has proposed the subfamily Grammistinae in the family Serranidae on the basis of morphological characteristics and included in this family four genera : *Rypticus*, *Pogonoperca*, *Grammistes* and *Grammistops* (L.P. Schultz : U.S. Naval Museum Bulletin, 202, 384, 1953). It is interesting that a soapfish (*Rypticus saponaceus*) found in Puerto Rico and our two species all belong to this subfamily.

A water-soluble toxin in a filefish

When the toxicity was examined on the various parts of a filefish (*Aluterus scriptus*) by injecting the hot 70% ethanolic extracts into mice, the liver, viscera and ingested materials were found to be toxic more or less. We obtained a specimen showing a considerably strong toxicity among 5 specimens thus far examined, and some chemical properties of the toxin were studied by using it. The viscera and digestive tract contents were fatal to mice at a dose equivalent to 0.05 g of raw material and 0.02 g, respectively.

Symptoms in mice were hypersalivation, paralysis of hind limbs, dyspnea, and death occurring in 10 minutes to 9 hours. Neither ichthyotoxic nor hemolytic activity was observed. There was no appreciable difference in symptoms of mice and chemical properties of the toxin described below between the viscera and ingested materials.

The toxin was soluble in methanol and insoluble in most fat solvents. It was extractable with n-butanol from aqueous layer, but not with diethyl ether from acidic or alkaline solutions. It was heat-labile at pH 2.0 and hardly dialyzable through a cellophane membrane. The preparations purified in parallel with Sephadex G-25 showed a minimum lethal dose, 1.2 Y/g in the case of digestive tract contents and 4.5 Y/g in the case of viscera. It was positive for Dragendorff reagent, and absorbed by Amberlite IRC-50 (H+) and eluted with diluted ammonia. The most purified toxin (0.45 Y/g) was obtained from the ingested materials by a series of procedures consisting of dialysis, extraction with n-butanol and gel-filtration with Sephadex G-25. These chemical properties may suggest that the toxic principle is a new marine toxin.

The ingested materials, mainly consisted of broken reef corals, which were tentatively identified as *Goniopora* sp. by Dr Yamazato, Ryukyu University. This may strongly suggest the presence of toxic reef corals which serve as a primary source of toxin in a filefish and presumably many other coral feeders.

Discussion

It was thought previously that ciguatoxic fishes in the Ryukyu Islands are characterized by the presence of ciguaterin (Y. Hashimoto et al. : Bull. Jap. Soc. Sci. Fish., 31, 452, 1965), but this conception was found inadequate, since some of them revealed presence of a fat-soluble toxin presumably identical with ciguatoxin. Judging from the symptoms of patients and test animals, we rather lean to the idea that ciguaterin is a less important factor than the fat-soluble toxin for development of ciguatera and that ciguatoxin is a principal toxin in ciguatoxic fishes in the Pacific as Dr Banner suggested (A.H. Banner et al. : Ann. N.Y. Acad. Sci., 90, 770, 1960).

The toxin in the skin of a sea bass is interesting from the biochemical point of view, but it may be of little importance in ciguatera, since its bitter taste may prevent a person from taking the skin excessively to cause symptoms more than vomiting.

A water-soluble toxin found in a filefish might be identical with the toxin detected by Dr Halstead mainly in the viscera of various kinds of reef fish (B.W. Halstead et al. : Pacific Sci., 5, 103, 1956), though he gave no detailed information on the chemical nature. The human illness from the ingestion of these coral feeders has been far less reported than those induced by ciguatoxin, but the toxin may be of not a small importance because of its possible wide distribution.

The complicated pattern of toxins in the phenomenon vaguely grasped as ciguatera has become evident, and any approach to control of toxic fish or search for the cause making fish toxic should be done inevitably in keeping this fact in view.

Original Text : English

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ABSTRACT

TOXINS FOUND IN CIGUATOXIC FISHES IN THE RYUKYU ISLANDS

by Yoshiro HASHIMOTO *

Evidence for multiplicity of toxin involved in ciguatera has been increasingly accumulated. At least four different toxins have been demonstrated in ciguatoxic fishes in the Ryukyu Islands.

The fat-soluble toxin probably identical with ciguatoxin was found in the muscle of Lutjanus bohar and L. monostigma. This toxin may be a principal one in ciguatera, as Dr Banner suggested.

A water-soluble toxin causing transient vomiting in cats was found and designated as ciguaterin. It was confirmed to distribute widely in the liver and occasionally in the muscle of representative ciguatoxic fishes. This toxin, however, may be a less important factor than ciguatoxin for development of ciguatera.

The skin of a sea bass (Pogonoperca punctata) was found to contain a toxin inducing in cats ciguatera-like syndromes when force-fed or injected. Besides, it revealed both ichthyotoxic and hemolytic activities and a close similarity to the ichthyotoxin found in soapfish rather than pahutoxin in boxfish.

Finally, a water-soluble substance toxic to mice was detected in the viscera and digestive tract contents of a filefish (aluteres scriptus). From the chemical properties it is supposed to be a new marine toxin. The toxin in the viscera was indistinguishable in chemical and pharmacological properties from that in the ingested materials which were mainly consisted of broken reef corals tentatively identified as Goniopora sp. This may strongly suggest the presence of toxic reef corals which serve as a primary source of toxin in a filefish and presumably many other coral feeders.

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