Toxicity of a Turban-shell in the Pacific*

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The term 'ciguatera' is now used to represent the intoxication of a low mortality rate that follows the ingestion of various kinds of toxic reef and semi-pelagic fish in the tropics and subtropics. The term is reported to have originated from 'cigua', a local name of a marine snail, *Cittarium* (*Livona*) *pica*, in the Caribbean Sea, where it caused a mild food poisoning[1]. No detailed description, however, has been available on the toxicity of *C. pica* and the poisoning due to it.

In 1968 we obtained information from the Japan Meteorological Agency that several staff members of that agency had been intoxicated from the ingestion of a marine snail caught at Marcus Island. The information attracted our attention in connection with origin of the term 'ciguatera' and led us to make the epidemiological investigation. This was followed with toxicity tests of specimens collected at various islands in the Pacific. The suspected species was identified as a silver mouthed turban-shell, *Turbo* (*Marmarostoma*) *argyrostoma*, and found to contain both the water-soluble and fat-soluble toxins in the mid-gut gland and gut contents.

The present paper deals with the results of epidemiological investigation and toxicity test, together with a report on the purification and properties of toxins found in the turban-shell. The possible significance of this marine snail to the study of ciguatera is discussed.

Epidemiological Investigation

The investigation was made mainly by interviews with the patients under the assistance of the Japan Meteorological Agency. The poisoning occurred in both 1962 and 1968, and 6 and 2 persons were intoxicated, respectively. In the outbreak of 1968, 6 other persons were also poisoned at the same time but are not included in the present paper, as they ate not only turban-shells but also fishes caught around Marcus Island, where the fishes were suspected to be ciguatoxic. Symptoms reported on a total of 8 patients were fatigue (7 persons), dry-ice sensation (6), diarrhea (4), and itching (4). The first sign appeared after several hours in 2, one day in one, and 2–3 days in the remaining

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5 patients. For complete recovery a long period was required; in the first poisoning, 5 out of 6 patients complained of certain disorders even after 2 months and, in the second one, 2 patients were still suffering from some disorders when interviewed 2 weeks after having eaten the snails.

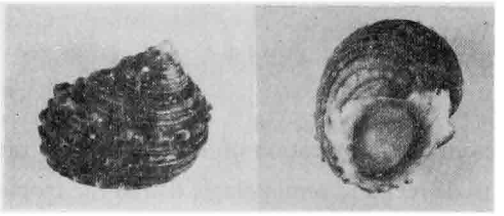

Fig. 1. The silver mouthed turban-shell, *Turbo* (*Marmarostoma*) *argyrostoma*.

The causative species was identified as a silver mouthed turban-shell, *Turbo* (*Marmarostoma*) *argyrostoma* (Fig. 1), by Dr. Tadashige HABE, Department of Zoology, The National Science Museum. It belongs to the family Turbinidae, while *C. pica* to the family Trochidae.

First poisoning

The turban-shells were caught on coral reefs at Sakamoto-zaki of Marcus Island on June 16, 1962. The muscle was cooked with or without the viscera in their own shells and ingested. Out of 9 persons who consumed each 2–7 individuals, 6 were affected.

Case A (male), who ingested the cooked muscle and viscera from 5–7 individuals of the turban-shell, was affected most seriously. Several hours after ingestion he had a severe diarrhea, which continued all the night, and subsequently languor in the legs. On the next morning when he immersed his hands into cold water, he felt stinging pains. The languor in the legs became more severe. Fatigue then appeared in the arms and prickling pains on the back. The languor in the legs and dry-ice sensation continued for more than 3 months.

Case B (male, aged 39) took the muscle of 2 individuals and the viscera of one of them after cooking. He did not feel unwell until he had diarrhea 2 days after ingestion. He felt then acute pain on wiping himself with a towel and fatigue in the legs. On the fourth day, he began to complain of both strong pain and itching when he rubbed his body. After about a week, severe itching occurred intermittently. The itching lasted for about 10 hours in the worst period. Even after 2 months he felt acute pain like electric shock on dipping his hands into cold water. It took about 3 months to recover completely.

Second poisoning

In May of 1968, 2 persons were intoxicated following the ingestion of the raw and cooked muscles of turban-shells which were caught on the north-west reefs of Marcus Island and kept below −5°C in a cold room for several days.

Case C (male, aged 41) ate the cooked muscle from 4–5 individuals and the raw muscle from one individual. After 2 days he had stinging sensation on drinking water and languor in the lower part of the body. He also developed an intermittent itching after a week. The dry-ice sensation and itching did not disappeared even after 2 weeks.

Symptoms of Laboratory Animals Administered with Turban-shell

As a preliminary experiment, responses of cats and mice were observed after administration of various test samples. The specimens were caught at Marcus Island in June, 1968 and transported frozen to our laboratory.

Cats

A part of the sliced muscle or the homogenized viscera each pooled from 10 specimens was fed to cats as shown in Table 1. A cat administered with the viscera at a level of 3.9% body weight had vomiting after 2.5 hours, lachrymation and diarrhea after about 5 hours, and subsequent loss of both agility and appetite. Two cats receiving the muscle at different levels and a cat receiving the viscera at a lower level did not develop any appreciable symptoms except vomiting and loss of activity.

Table 1. Responses of cats to test samples.

Test sample	Body wt. of cat, g	Dose, g (%)	Responses
Muscle:			
Raw	900	69 (7.7)*	Vomiting and loss of activity.
〃	500	23 (4.6)*	Vomiting and loss of activity.
Water-sol. fr.	500	2.1 (5.8)**	None
Fat-sol. fr.	500	0.3 (10.0)**	None
Viscera:			
Raw	750	29 (3.9)*	Vomiting, lachrymation, diarrhea, and loss of activity and appetite.
〃	375	1.2 (0.3)*	Vomiting.
Water-sol. fr.	500	1.3 (5.0)**	None
Fat-sol. fr.	400	0.5 (7.3)**	Vomiting.
〃	600	0.4 (4.0)**	Vomiting, nausea, diarrhea, paralysis or weakness of the limbs, and death.

* Percent body weight.
** Percent body weight on the basis of starting raw material.

The water-soluble and fat-soluble fractions prepared from the muscle and viscera were then fed to cats (Table 1), according to the routine method used in our laboratory for screening ciguatoxic fishes[2]. Only the fat-soluble fraction from the viscera induced symptoms, while the other fractions did not show any ill effect on the test animals. At 7.3% body weight on the basis of raw material, a cat began vigorous vomiting after 20 minutes. At a level of 4.0%, a cat revealed vomiting and nausea after 4 hours, vomiting and diarrhea after 3 days, and paralysis or weakness of the limbs after 4 days. She died after 8 days.

Mice

Test samples were prepared by dissolving the water-soluble fraction in water or by emulsifying the fat-soluble fraction with 0.8% Tween 60. A 0.5 ml portion of each sample was administered intraperitoneally into two mice weighing approximately 20 g (Table 2). As were observed with cats, the fat-soluble fraction of the viscera was fatal to mice at 0.5% level, while the other fractions were non-toxic at a level of 5% body weight on the basis of raw material. These results indicated that this bioassay method with mice can be used for toxicity test of the turban-shell.

Table 2. Responses of mice to test samples.

Test sample	Dose, mg (%)*	Responses	Approximate death time, hr
Muscle:			
Water-sol. fr.	72.8 (5.0)	None	—
Fat-sol. fr.	6.4 (5.0)	None	—
Viscera:			
Water-sol. fr.	71.6 (5.0)	None	—
Fat-sol. fr.	8.0 (2.5)	Loss of activity, anorexia, and death.	6 and 9
〃	4.0 (1.25)		23 and 30
〃	1.6 (0.5)		39 and 99
〃	0.8 (0.25)	None	—

* Percent body weight on the basis of starting raw material.

Toxicity of the Turban-shells from Various Islands in the Pacific

The source of specimens is given in Table 3. Specimens of *Turbo setosus* collected at Hao, Tuamotu Islands, were also examined. The turban-shells were frozen on collection at each island and brought to our laboratory, where they were kept at −20°C until used. Seventeen to fifty specimens from each place were dissected into the muscle, midgut gland, gut contents, and other viscera to prepare the water-soluble and fat-soluble fractions as mentioned above. Since the toxin involved in the water-soluble fraction was found to be non-dialyzable in a preliminary experiment, the fraction was dialyzed to remove inorganic salts in Visking cellulose tubing (20/32) for 5–6 hours against 50 times its volume of water, replacing the external solution 2 times. The inner solution thus obtained was condensed under reduced pressure to an appropriate volume. The subsequent bioassay method was essentially the same as described above; a 0.5 ml portion of serial dilutions of the both fractions was injected intraperitoneally into each of two mice. The mice were observed for a week and the approximate minimum lethal dose was sought. When the mice survived at a dose level corresponding to 2 g of raw material, the sample was regarded as non-toxic.

Results obtained are tabulated in Table 3 and may be summarized as follows. The muscle is non-toxic in both the water-soluble and fat-soluble fractions. The viscera without mid-gut gland are also non-toxic with the exception of a weakly poisonous water-soluble fraction of HA–1. On the other hand, the both fractions of mid-gut gland and the water-soluble fraction of gut contents are fatal to mice in most of the specimens, although their lethalities are diverse. The fat-soluble fraction of gut contents is also toxic in some specimens. It is noteworthy that the water-soluble fractions of the mid-gut gland and gut contents of HA–1 are remarkably high in toxicity.

Table 3. Toxicity of the turban-shells from various islands in the Pacific.

Code of sample	Place of collection	Date of collection or receipt	No. of specimens used	Fraction*	Toxicity**			
					Muscle	Mid-gut gland	Gut contents	Other viscera
MA–1	Marcus Is.	June, 1968	20	F	—†	—	0.5 (205)	—
				W	—	—	0.5 (1060)	—
MA–2		June, 1969	50	F	—	0.5 (895)	—	—
				W	—	0.5 (395)	0.5 (395)	—
OF–1	Ofoppsyaka Is., Palau	Jan., 1969	17	F	—	1.0 (1420)	—	—
				W	—	—	1.6 (1390)	—
IE–1	Ie Is., Ryukyus	May, 1969	30	F	—	1.0 (1090)	—	—
				W	—	0.5 (710)	0.5 (270)	—
IS–1	Ishigaki Is., Ryukyus	Apr., 1969	18	F	—	2.0 (2500)	—	—
				W	—	2.0 (2100)	—	—
HA–1††	Hao, Tuamotus	May, 1969	44	F	—	1.0 (650)	2.0 (320)	—
				W	—	0.16 (350)	0.06 (82)	1.0 (870)

* F: Fat-soluble fraction, W: Water-soluble fraction.

** The toxicity is expressed as the approximate minimum lethal dose (g) on the basis of raw material per mouse weighing about 20 g. Figures in parentheses indicate the minimum lethal dose (μg) of solid per g of body weight of mouse.

† Non-toxic.

†† *T. setosus*.

Symptoms of mice which received toxic samples were almost the same irrespective of their solubility and were loss of activity, anorexia, difficulty in respiration, and death. Some of mice developed inflammation of the eyelids after about 20 hours. The death time ranged from a few hours to 3 days depending upon the dosage.

The outer solution obtained in the dialysis was also examined for toxicity after being concentrated *in vacuo*, and some samples from the mid-gut gland, gut contents and other viscera were found to kill mice. However, they were not considered in the present study, since a large amount of inorganic salts was suspected to be responsible for the lethality.

Properties and Purification of Water-soluble Toxin

In preliminary studies, we found that the water-soluble toxin in the mid-gut gland and gut contents is non-dialyzable in Visking cellulose tubing, extractable with *n*-butanol, and precipitable with acetone. It was not extracted with diethyl ether from aqueous solution adjusted to pH 11 with 1 N sodium hydroxide. Taking advantage of these properties and using the gut contents of IE–1, purification of the toxin was carried out as follows (Fig. 2).

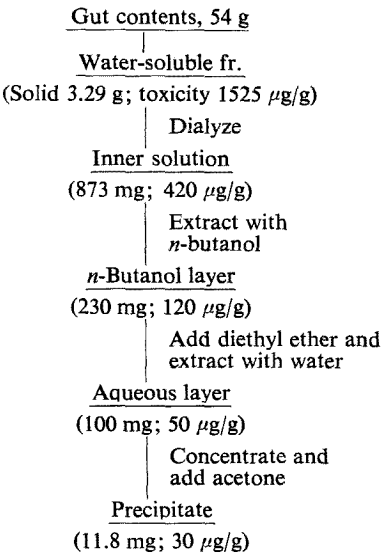

Fig. 2. Purification of the water-soluble toxin from the gut contents (IE–1).

The water-soluble fraction was dialyzed against water as described in the preceding section and the inner solution was extracted with *n*-butanol. The toxin was then recovered by extraction with water from the butanol layer, to which was added the same volume of diethyl ether. After the aqueous solution was condensed under reduced pressure, 6 times its volume of cold acetone was added to precipitate the toxin, which was collected by filtration and dried in a desiccator under reduced pressure. The toxicity at each step in the purification process is given in Fig. 2. The final preparation of the water-soluble toxin was a very hygroscopic and greenish powder having a lethality 30 μg/g. It was positive to the Dragendorff and ninhydrin reagents and showed a hemolytic activity corresponding to one-fifth of that of a standard saponin (Merck, B6, Darmstadt), when compared by measuring photometrically a 50% hemolysis of the rabbit blood cells after BOYLAN and SCHEUER[3].

From the gut contents and mid-gut gland of MA–2, the partially purified preparations were obtained in the same way; their toxicity was found to be 40 and 65 μg/g and hemolytic activity about 30 and 50% of that of the standard saponin, respectively.

An Attempt to Purify the Fat-soluble Toxin

According to the purification method adopted for ciguatoxin[4], the fat-soluble fraction of gut contents of MA–1 was chromatographed on a silicic acid column (Mallinckrodt, 1.8×34 cm). As shown in Fig. 3, the fraction eluted with a mixture of chloroform and methanol (1:1) was fatal to mice at a dose 20 μg/g, while the other fractions did not show any toxic effect on mice at higher dose levels. The further purification was not successful.

Fat-soluble fraction
(171 mg; toxicity 205 μg/g)
Silicic acid column chromatography

Solvent	$CHCl_3$	$CHCl_3$–MeOH (9:1)	$CHCl_3$–MeOH (2:1)	$CHCl_3$–MeOH (1:1)	MeOH
Yield (mg)	121	32	28	16	9
Toxicity (μg/g)	—	—	—	20	—

Fig. 3. Chromatography of the fat-soluble fraction of gut contents (MA-1).

Discussion

Outbreaks of food poisoning following the ingestion of silver mouthed turban-shell shave been recorded for the first time. The staff of the Japan Meteorological Agency confirmed in interviews that they had previously consumed the snail without any trouble very often at Marcus Island. A field investigation in the Ryukyus showed that inhabitants there usually ate the snail safely. Together with lack of the previous reports, this information may suggest that poisoning from *Turbo* occurs only rarely.

The toxicity test of specimens of *T. argyrostoma* from various places revealed that the mid-gut gland and gut contents were somewhat toxic in most of the specimens, whereas the muscle was almost non-toxic. This may support the report of patients that those who ingested the muscle with viscera were more seriously affected than those who ate only the muscle. It may be concluded from these results that the muscle is only rarely toxic, if any, but the viscera are always more or less toxic. This was also true for *T. setosus* from Hao, supporting the report of BAGNIS[5], who found a moderate toxicity only in the hepatopancreas in a cat assay on specimens from the same atoll. It was briefly referred to by him that the ingestion of *T. setosus* recently caused there a ciguatera-like poisoning of three cases who revealed the prominent neurological symptoms.

It is interesting that the snail contained both the water-soluble and fat-soluble toxins. The symptoms of patients resembled those in typical ciguatera, differing only in the delayed appearance of the first symptom, the development of serious itching, and absence of joint-ache[6]. The role of each toxin in developing symptoms is a problem to be solved in future.

The fat-soluble toxin from the gut contents was eluted from a silicic acid column with a mixture of chloroform and methanol (1:1), while ciguatoxin was reported to be recovered in a less polar solvent[7]. In addition, symptoms of mice suggested dissimilarity of the both toxins; ciguatoxin is described to cause diarrhea and excessive salivation[7] which were not observed on mice affected by the toxin of turban-shells. Inflammation of the eyelids induced by the latter may de another distinguishing criterion.

The water-soluble toxin from the turban-shell resembles aluterin, a toxic principle found in the ingested materials of a filefish, *Alutera scripta*[8]; the both toxins are extractable with *n*-butanol from aqueous solution and non-diffusible through a cellophane membrane. The former is, however, distinguishable from the latter in having the hemolytic activity.

It is noteworthy that the gut contents were fatal to mice in most of the specimens. On the basis of gut-contents analysis for food items. Drs. R. T. TSUDA and J. E. RANDALL (personal communication, 1969) described that the turban-shell appears to be a non-selective grazer on benthic algae. It is interesting that the ingested materials of a surgeonfish, *Ctenochaetus striatus*, caught at Tahiti, have been recently shown to reveal a similar pattern of toxicity and to contain a toxin quite similar to the water-soluble toxin of turban-shell[9]. The surgeonfish is a well-known grazer on benthic algae, and the marine snail like the fish may indicate an ecological pathway leading to toxic algae on coral reefs, which have been postulated as the primary source of toxins found in ciguatoxic fishes.[10]

Summary

Peculiar human intoxications following the ingestion of silver mouthed turban-shell, *Turbo* (*Marmarostoma*) *argyrostoma*, have been described. The poisonings have some resemblance to typical ciguatera, but are apparently distinguished from it. Toxicity tests using mice with the water-soluble and fat-soluble fractions from specimens collected at various islands in the Pacific revealed that the mid-gut gland and gut contents were more or less toxic, while the muscle was non-toxic. The water-soluble toxin was partially purified and found to be non-dialyzable, extractable with *n*-butanol, precipitable with acetone, and hemolytic. The fat-soluble toxin differed from ciguatoxin to some extent.

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