Short Paper

Accumulation of Tetrodotoxin
by Marine Gastropods*¹

Recently there occurred a food poisoning by one of marine gastropods, the trumpet shell *Charonia sauliae*, and tetrodotoxin (TTX) was identified as the causative toxin.¹⁾ TTX was also detected in the ivory shell *Babylonia japonica*.^{2,3)} However, it was thought that TTX was not originally present in the gastropods but incidentally came from other marine organisms such as the starfish *Astropecten polyacanthus*⁴⁾ and puffer fishes.⁵⁾ The present work was conducted to confirm whether marine gastropods can actually take up and accumulate TTX from their baits.

Since the mid-gut glands of 6 specimens of *C. sauliae* caught at Kominato, Chiba Prefecture, in May and October 1981 were non-toxic (<5 MU/g), this species was judged not to become toxic there. The commercially obtainable ivory shells were also regarded as non-toxic. Therefore, 6 specimens of *C. sauliae* collected at Kominato from March to August 1982 and 22 specimens of the ivory shell (not identified) purchased at Tokyo Central Wholesale Market in December 1982 were used in this study. These specimens were transported alive to our laboratory. Then the *C. sauliae* specimens were transferred one by one into each aquarium (29×60×36 cm) containing 60 l of aerated sea water. In the case of the ivory shell, specimens were divided into two groups (14 and 8 specimens) and each group was also transferred into an aquarium. After kept in the aquarium for several days, each specimen or each group was reared using the muscle (toxicity 111–363 MU/g) obtained from 5 specimens of the puffer fish *Lagocephalus lunaris* as a feed. The puffer fish muscle was cut into small pieces and fed once a day to each specimen or each group. The weight of feed given daily depended on the appetite of specimens, varying from 2 to 8 g. Throughout the rearing, the puffer fish muscle did not cause any ill effects in the gastropods. At the end of rearing the muscle and mid-gut gland were removed from each specimen or each group and their toxicity was determined by the standard mouse assay.⁶⁾ One mouse unit (MU) was defined as the amount of toxin required to kill a 20 g mouse in 30 min.

As shown in Table 1, marine gastropods were found to accumulate TTX from their baits. The mid-gut gland exclusively became toxic while the muscle did not. Although the toxicity of mid-gut gland roughly paralleled the total amount of toxin given, the rates of

toxin accumulation remarkably differed from specimen (or group) to specimen. It is worth mentioning that the rates of toxin accumulation obtained in this study should be accepted as minimum values because a part of the toxin contained in the puffer fish muscle must have leaked into the rearing sea water during feeding. The toxin accumulated in the mid-gut gland of *C. sauliae* (No. 6 in Table 1) and ivory shell (No. 2 in Table 1) was purified according to the method of YASUMOTO *et al.*³⁾ and identified as TTX by thin layer chromatography and electrophoresis.

Preliminarily, the excretion of TTX from *C. sauliae* was also examined. When a specimen of *C. sauliae* was reared with the puffer fish muscle (total 5196 MU) for 3 weeks and thereafter with no feed for 10 days, the total toxicity in its mid-gut gland was 1910 MU. On the other hand, when another specimen, which had taken up TTX equivalent to 4687 MU, was reared with no feed for 4 weeks, its mid-gut gland showed no toxicity. These results suggest that TTX accumulated in *C. sauliae* can be excreted during rearing of more than 4 weeks without feed. In order to consider a counterplan when marine gastropods accumulate TTX in their bodies, further detailed studies on the excretion of TTX from them are necessary.

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Table 1. Accumulation of TTX by the trumpet shell and ivory shell

Species	No.	Rearing period (weeks)	Toxicity (MU/g)* ¹		Total MU		Rate of toxin accumulation (B/A × 100, %)
			Mid-gut gland	Muscle	Given (A)	Accumulated (B)* ²	
Trumpet shell <i>C. sauliae</i>	1	1	137	<5	5214	1918	36.8
	2	1	138	<5	6746	3643	54.0
	3	3	184	<5	6164	3478	56.4
	4	3	131	<5	7742	2319	30.0
	5	5	255	<5	13202	10965	83.1
	6	15	735	<5	46365	22932	49.5
Ivory shell	1	2	130	<5	9800	3484	35.6
	2	4	66	<5	6360	1366	21.5

*¹ Determined after rearing.

*² Calculated with mid-gut gland.

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