A Note on the Toxicity of a Chinaman Fish^{*1}

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An outbreak of food poisoning with 25 patients occurred from the ingestion of a chinaman fish *Glabrilutjanus nematophorus* in Okinawa Island in 1974. The symptoms recorded, such as diarrhea, chill, fatigue, joint-ache and dry-ice sensation, were very close to those of ciguatera. The toxicity of the flesh recovered from the fish dealer was tested on cats and mice, in which the effects were very close to those induced by ciguatoxin.

The toxic principle was partially purified by column chromatography using silicic acid and DEAE-cellulose. The toxicity was checked by a mouse test. The toxin was eluted with chloroform-methanol (9: 1) from a silicic acid column. By subsequent DEAE-cellulose column chromatography, two toxic components were obtained; the one was eluted with chloroform and the other with chloroform-methanol (1: 1). The major toxin eluted with chloroform-methanol (1: 1) was not distinguishable from ciguatoxin by either thin layer chromatography or effects in mice, while the minor toxin eluted with chloroform was clearly different from ciguatoxin.

An outbreak of food poisoning occurred from the ingestion of a chinaman fish *Glabrilutjanus nematophorus* at Gushikawa in Okinawa Island on May 10, 1974. According to the survey made by the Ishikawa Health Center, the fish was consumed by a total of 50 persons as "Sashimi" and soup. Twenty-five persons of 11 families were affected and developed symptoms very close to those of ciguatera, consisting of diarrhea, nausea, chill, fatigue, langour, joint-ache, numbness of extremities, and dry-ice sensation. It took more than a week to recover completely and even a month in severe cases. There was no death.

Fortunately we obtained a fillet and head of the toxic fish recovered from the fish dealer, and could demonstrate the presence of ciguatoxin and an unidentified fat soluble toxin in the flesh. The present paper deals with the results of toxicity test and some chemical properties of the toxin.

Materials and Methods

A fillet and head of the causative fish recovered by the Ishikawa Health Center were used as specimens. The fish weighed about 15 kg and was landed at fish market in Naha on May 9, 1974. The fishing ground was unknown. The fish was identified as "Itofukifuedai" *Glabrilutjanus nematophorus* by Mr. S. Inoha, Okinawa Prefectural Fisheries Ex-

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perimental Station, who examined the head of the fish. It is locally called "Inubâ" in the Ryukyus. The specimens were stored in a freezer at -20°C until used.

The toxicity was tested by using cats and mice as test animals. In a cat assay, each 50 g of raw flesh was fed to cats weighing 1 and 1.5 kg at first. The water soluble and fat soluble fractions were then prepared from the flesh by our routine method for ciguateric fishes,¹¹ mixed well with a small portion of canned mackerel, and fed to cats each weighing 0.5 kg at a level of 10% body weight on the basis of raw flesh.

In a mouse test, the fat soluble fraction was injected intraperitoneally into mice weighing about 20 g after being emulsified with 1% Tween 60 in physiological saline. The serial dilutions of the sample were assayed and the minimum dose that killed mice within 48 hr was sought. Two mice were used for each dilution. The toxicity was expressed in μg per g of mouse.

By using mice as test animals, the toxic principle was partially purified for characterization as follows. The fat soluble fraction prepared from 100 g of the muscle was fractionated on a column (30 g, 2×21 cm) of silicic acid (Mallinckrodt, 100 mesh) with chloroform, chloroform-methanol (9:1), chloroform-methanol (1:1) and methanol, in order. The toxic eluate was then applied to a column (2×8 cm) of DEAE-cellulose (Brown, acetate form) and eluted in sequence with chloroform, chloroform-methanol (1:1) and methanol.

In thin layer chromatography, silica gel H (Merck) combined with chloroform-methanol-acetic acid-water (90:9.5:0.2:0.3) was used. A portion of toxin was applied to a layer (0.5 mm thick, 20×20 cm) and developed to a height of 10 cm. The plates were then divided into several bands by monitoring with fluorescence under ultraviolet light. Each band was scraped off and extracted with chloroform containing 20% methanol. After removal of solvent, the extract was emulsified with Tween 60 in physiological saline and injected into mice.

Results

Signs of laboratory animals A cat weighing 1.5 kg fed 50 g of raw flesh revealed paralysis in hind limbs 17 hr after and in four limbs 30 hr after administration. The cat fell into a coma that continued for 2 weeks. Thereafter, the animal recovered gradually and started to walk within 3 weeks. Neither vomiting nor diarrhea was recognized in the observation period. A cat weighing 1 kg fed the same amount of raw flesh showed paralysis of limbs, prostration, coma and death after 2 weeks. The fat soluble fraction prepared from 50 g of the flesh caused paralysis, coma and death 7 days after administration in a cat weighing 0.5 kg. While the water soluble fraction induced no signs in another cat of the same weight.

In mice the fat soluble fraction caused loss of activity, severe diarrhea, dyspnea and death, but lacrymation and salivation were not recognized. The death time ranged from

6 to 20 hr depending on dose.

Partial purification and chemical properties of the toxins The fat soluble fraction (solids 640 mg, toxicity 840 $\mu\text{g/g}$) from the muscle was fractionated by silicic acid column chromatography. The eluate (67 mg) with chloroform-methanol (9:1) was toxic at a level of 250 $\mu\text{g/g}$, and the other eluates were nontoxic at a level of 1,000 $\mu\text{g/g}$. By subsequent DEAE-cellulose column chromatography, two toxic components were obtained; the one was eluted with chloroform (28 mg, 350 $\mu\text{g/g}$) and the other with chloroform-methanol (1:1) (24 mg, 160 $\mu\text{g/g}$). In thin layer chromatography, the component appearing in the chloroform fraction gave the toxic band at R_f 0.4–0.7 on silica gel H plates. The signs in mice were labored breathing, abnormality in walking, paralysis in hind limbs and death, differing from those induced by ciguatoxin. The component found in the chloroform-methanol (1:1) fraction gave the toxic band at 0.1–0.4 in thin layer chromatography under the same conditions. The signs in mice were similar to those induced by ciguatoxin.

Discussion

A chinaman fish is reputed to become poisonous occasionally. Several outbreaks of food poisoning due to this species were recorded in Australia,²⁾ but none in the Ryukyus.³⁾ We confirmed the presence of ciguatoxin in the toxic flesh which caused human illness in the Ryukyus. Signs in cats and mice were very close to those induced by ciguatoxin. These results indicate that this fish is classified as ciguateric.

In addition to ciguatoxin, we detected an unknown fat soluble toxin in the flesh, that evoked paralysis in hind limbs and dyspnea in mice. The toxin, however, seemed to be far less important than ciguatoxin in this fish. Further study was impossible due to the limited availability of the specimen. In the previous paper,⁴⁾ we reported that a surgeonfish *Ctenochaetus striatus* from Tahiti bears a secondary toxin in addition to ciguatoxin. It is of interest that a chinaman fish from the Ryukyus also has the secondary toxin in the flesh.

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