Toxicity of the Common Puffer Fish in Hong Kong

It is universally accepted that the puffer is a poisonous fish which accounts for numerous cases of puffer poisoning, or tetrodotoxism[1,2]. A toxin, known as tetrodotoxin, has been isolated from the ovary of *Spheroides rubripes* by TSUDA and KAWAMURA[3]. To-date, the origin of the toxin is still obscure[4], as similar extraction on different tissues of the fish has not been reported. Apart from the possible occurrence of seasonal variations, it has recently been reported that in *Spheroides* sp., the following order of toxicity in various tissues is found: ovary > spleen > liver > blood > eye > gill > skin > testis[5]. Little is known of this sort of comparative study using other puffers which comprise over 40 species[1]. This study presents some preliminary findings on the possible origin and nature of the toxin in *Lagocephalus lunaris* (Block and Schneider) and *Fugu xanthopterus* (Temminck and Schlegel) which are the common species of puffer in Hong Kong and in Southeast Asia[6].

Mature specimens of *Lagocephalus* (3 male and 1 female) measuring 18–22 cm and weighing 130–230 g and of *Fugu* (3 male and 2 female) measuring 9–13 cm and weighing 41–95 g were caught in August, 1970 in the coastal waters of Hong Kong. They were killed. The liver, testis or ovary, and muscle of each sex, after having been cleared as much as possible of the connective tissue, were weighed and pooled. The tissues were homogenized with an equal weight of distilled water, centrifuged at 224 *g* for at least 10 min. The supernatant watery layer (extract) was pipetted out, and stored in a freezer at −25 °C.

To test the toxicity of the various tissues, the method of HALSTEAD and BUNKER[7,8] was used. The prepared extract, upon defrosting, was injected i.p. into groups of 2 or 3 animals (1 ml each) which were 5 months old, male mice of WHT/HT strain (to avoid any complications in response due to differences in sex, age and strain of mice). Groups of control mice were given either 1 ml of distilled water or muscle extract (similarly prepared as that of the puffer) of another fish, a Morey eel, *Gymnothorax*. The duration of time after injection when death occurred was recorded.

In the course of the experiment, it was found desirable to compare further the degree of toxicity of the muscle and liver in the male, using diluted solutions of the original extracts (Figure 1). An attempt was then made to correlate the intervals of time during which death of the experimental mice occurred and the extent of dilution.

An attempt was also made to detoxicate the various tissue extract by heating the original extracts for 10 and 60 min at various temperatures (Figure 2).

The results obtained are presented in the Table and in Figures 1 and 2. In the male puffer of both species, death resulted in all tested mice within the first minute of injection of the original extract, except in the case of muscle extract of *Fugu* (see Table). Indeed, death was almost instantaneous with testis extract injection. The first visible symptoms after treatment in these mice were stretching of the neck and restless probing of the head. The eye became fixed and protruding upon death. The limbs seemingly failed to support the body which swayed from side to side. After a few kicks in the air with the forelimbs, the mice toppled over. Panting was obvious, after which the mice died. This last observation seems to agree with the earlier suggestion that death was probably due to asphysia. Similar extracts from the female species indicated a time lag of over 10 min before death occurred in *Fugu*, and the mice injected with ovary extract of *Lagocephalus* suffered none of the symptoms described above (see Table), nor did the controls. Our results therefore suggest that firstly, the origin of the toxin was from the gonad; secondly, the order of toxicity of the following tissues was: Gonad (testis/ovary) > liver > muscle; thirdly, differences in the degree of toxicity between sexes exist: tissues of the male being more toxic than the counterparts in the female; and lastly, the musculature in puffer is not usually free of the toxin[1].

With diluted solutions of the original extracts of the liver and muscle, death was delayed (Figure 1). Mice survived throughout the period of observation of 36 h with $^1/_{16}$th of original concentration of *Lagocephalus* and *Fugu* muscle extract, and with $^1/_{128}$th that of *Lagocephalus* liver extract (but not in *Fugu*). This indicates that the liver is more toxic (?) or contains at least 8 times more toxic substance compared with body musculature.

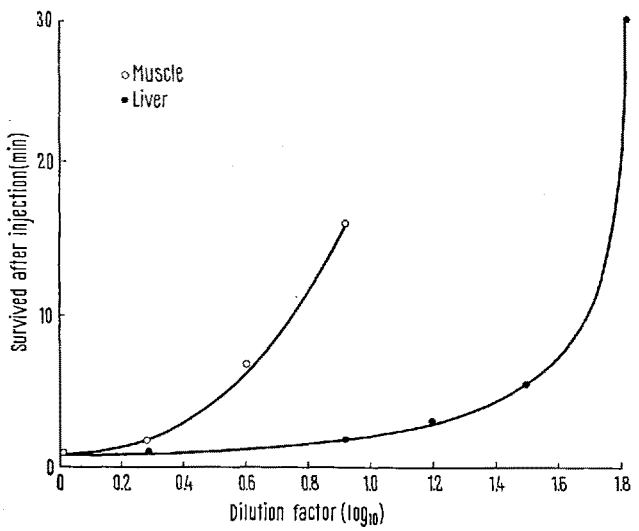

Fig. 1. Relation of time survived by mice and concentration of the original extract administered.

Time in min survived by mice after an injection of 1 ml original extract of the various tissues of *Lagocephalus* and *Fugu*

Species	*Lagocephalus lunaris*		*Fugu xanthopterus*	
Tissue	Male	Female	Male	Female
Liver	1	[a]	1	21
Muscle	1	[a]	2.5	18
Gonad	<1	No effect	<1	11.5

[a] No data were obtained.

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In *Lagocephalus*, heating the extract as high as 170 °C for 10 min did not detoxicate the toxic substance in the original extract of the liver, muscle and testis (Figure 2). There were indications, however, that the toxic effect, with subsequent death, would be considerably delayed. As expected, those animals injected with ovary extract showed no apparent changes. When the heating period was prolonged to 60 min instead of 10, the mice receiving muscle extract (of *Lagocephalus* and *Fugu*) injections showed no obvious changes and survived as long as 36 h, suggesting that detoxication was probably successful (although those receiving liver extract died). The fact that the muscle can be detoxicated at 120 °C while no apparent effect was observed with the liver extract provides circumstantial support to the contention that the toxin is less concentrated in the muscle than in the liver. It further suggests that it would take longer (8 times?) to detoxicate the liver when the same temperature was used. This finding on the relation of temperature and toxicity in the present study is in agreement with that in *Spheroides*[5].

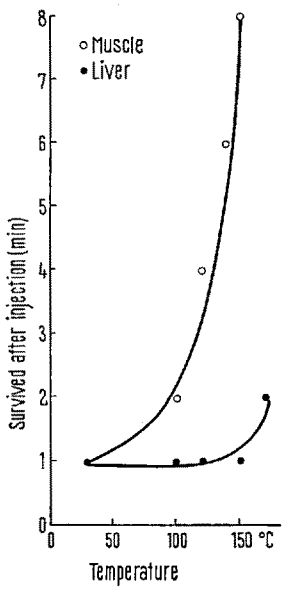

Fig. 2. Relation of temperature and time survived by mice after injection.

It has been shown that the OH^- group at C_4 and O- between C_5 and C_{10} of tetrodotoxin are probably important functional groups so that changes in these eliminate toxicity[4,9]. It is tempting to suggest that prolonged heating might dispose of the OH^- through dehydration and/or break up the oxygen link with C_5 and C_{10}. Further studies along this line are being planned.

Résumé. L'origine et la nature probable de la toxicité chez deux espèces de poissons (*Fugu* et *Lagocephalus*) a été étudiée. Les tissus énumérés dans l'ordre de toxicité décroissant sont les gonades, le foie et les muscles, avec une différence entre les deux sexes, les tissus mâle étant plus toxiques que les tissus femelles. On a éliminé la toxicité par un traitement thermique prolongé et maintenu à 120 °C.

L. L. Yip and K. W. Chiu

Department of Biology,
The Chinese University of Hong Kong,
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Sulfhydryl Group Reagents: Effect on Intestinal Smooth Muscle

Previous work in this laboratory has been aimed at obtaining more information on the area surrounding the anionic sub-site of the muscarinic receptor which might be involved in binding antagonists[1]. Our attention was drawn to the work of Karlin which indicated that a disulfide linkage and a sulfhydryl group were present on the nicotinic receptor of eel electroplax[2-4]. Karlin reported that treatment of electroplax with the reducing agent dithiothreitol (DTT) reduced sensitivity to carbachol, but mild oxidation reversed this effect by reformation of disulfide bonds. Brief exposure of the reduced preparation to N-ethylmaleimide (NEM) prevented reoxidation but had no effect on the unreduced electroplax[2]. The vastly enhanced alkylating ability of 4-(maleimido)phenyltrimethylammonium compared to NEM was considered evidence for the close proximity of the disulfide linkage to the anionic sub-site of the acetylcholine (Ach) receptor[3]. Also, bromoacetylcholine (BAC), normally a reversible agonist, became an irreversible agonist after exposure of the electroplax to DTT had freed a sulfhydryl group for reaction[4]. Karlin and Bartels[2] had also proposed that another sulfhydryl group was involved in depolarization of the electroplax, since they found that *p*-chloromercuribenzoate (PCMB) acted as an irreversible inhibitor even without prior DTT treatment. However, Župančič[5] has challenged the idea that the PCMB is active at the nicotinic receptor. We have investigated the action of some sulfhydryl-group reagents on the rat jejunum in order to determine the presence or absence of sulfhydryl or disulfide groups on the muscarinic receptor.

Methods. Jejunum from Wistar rats was suspended in 10 ml muscle baths containing Tyrode's solution at pH 7.4. Baths were aerated with 95% oxygen and 5% carbon dioxide except during incubation with DTT. Control responses to agonists were obtained and muscles were washed before exposure to DTT for 10 min. NEM or PCMB were added for an additional 10 min period in some studies. Muscles were then washed repeatedly and responses elicited. Agonists included Ach, potassium ion and BAC. Receptor protection experiments consisted

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