Figure B indicates that the attacking fish displayed intervals (modes: 9 and 15 msec = 111 and 67 Hz) shorter than the shortest interval observed during control.

The remaining seven *G. petersii* displayed a similar pattern of 'high frequency' burst activity during aggressive behaviour: the burst frequency (75 to 140 Hz) always surpassed the highest value of intantaneous frequency observed in the resting fish (50 to 66 Hz).

These 'high frequency' bursts appeared only when *Gnathonemus peterssi* attacked the intruder. If a parallel or anti-parallel lateral display followed this attack, bursting of discharges continued and the bursts, now, were of longer duration. Bursting ended when *G. petersii* retreated.

In the literature the only 'high frequency' bursts (130 Hz) in mormyrids were indicated by LISSMANN[1] for a mechanically stimulated *Gnathonemus senegalensis*. In contrast, in our experiments continuous 'high frequency' bursts (up to 140 Hz) of fighting *G. petersii* were recorded during long periods of attack, whereas in 14 h of control the same resting fish never emitted instantaneous frequencies higher than 71 Hz. Also, swimming *G. petersii* never discharged frequencies exceeding 30 Hz[4].

The significance of this particular discharge pattern, described for the first time for *G. petersii*, will be the subject of further observations.

Zusammenfassung. Der elektrische Fisch *Gnathonemus petersii* sendet beim Angriff auf einen anderen Mormyriden, *Mormyrus rume*, und während des Breitseitimponierens «burst»-artige elektrische Entladungen von Frequenzen bis zu 140 Hz aus.

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A Method for Distinguishing Tetrodotoxin from Saxitoxin, by Comparing Their Relative Stabilities when Heated in Acid Solution

Tetrodotoxin (TTX) occurs naturally in puffer fish of the order Tetraodontidea and in newts within the genus *Taricha*. Saxitoxin (STX) is produced by marine dinoflagellates of the genus *Gonyaulax*, and is also found in many species of bivalve molluscs after they have been feeding on these dinoflagellates[1-3]. The newts and puffer fish are unaffected by TTX; bivalves are not poisoned by TTX or STX. Most vertebrates, however, are paralysed by either toxin in doses of 5–20 μg/kg i.v. or i.p. Since these toxins have virtually the same mode of action they are very difficult to distinguish by means of their pharmacological effects[2,3]. As no specific chemical tests have been discovered for either poison, identification of an unknown poison having these pharmacological effects is not easy[4]. The nerves of *Taricha* newts resist TTX but are blocked by STX[5]. TTX is not found in European newts and their nerves are not resistant[6,7].

Several remarks in the literature imply that STX is stable in strong acids, whereas TTX is most stable near pH 4 to 5[2,8-14]. The poisons were therefore tested for stability at various pH values, diluting solutions of pure TTX and STX to concentrations equal to 8 mouse units/ml. The mouse LD_{50} (1 mouse unit, MU) for TTX was found to be 0.24 μg/20 g body weight, so the TTX solution was made up to 1.92 μg/ml. The corresponding figures for STX were 0.206 μg/20 g and 1.65 μg/ml. The diluent was either HCl or 0.025 *M* sodium acetate/HCl buffer, depending upon the desired pH. The ability of the poisons to withstand heating to 100°C, for various times between 2.5 and 60 min, was tested at pH 0.64, 1.0, 1.28, 2.0, 3.0, 4.0 and 5.0. Portions of 4 to 5 ml were pipetted into conical centrifuge tubes. One tube was left unheated as a control and the others were placed in a boiling water bath for 2.5, 5, 7.5, 10, 20, 40 or 60 min, then removed and quickly cooled. The samples were brought to pH 2.5–3.5 with HCl or NaOH before bio-assay on mice, and loss of water by evaporation was corrected.

The amount of poison remaining in each tube after heating was estimated by a mouse death time technique similar to that described by SCHANTZ, MCFARREN and their colleagues[15-18]. The samples were assayed on groups of 3–14 mice. Almost all the points shown in the Figure were estimated using groups of 12–14 mice. The volume of solution injected i.p. was 0.5 ml/20 g body weight. Therefore, if no destruction of poison had occurred in the sample, each mouse received 4 MU of poison. This amount of TTX would be expected to kill mice after a median time to death of 4 min; 4 MU of STX would produce a median time of about 3 min[17]. The unheated controls gave median times to death that did not differ significantly from these figures, when corrected for the presence of sodium

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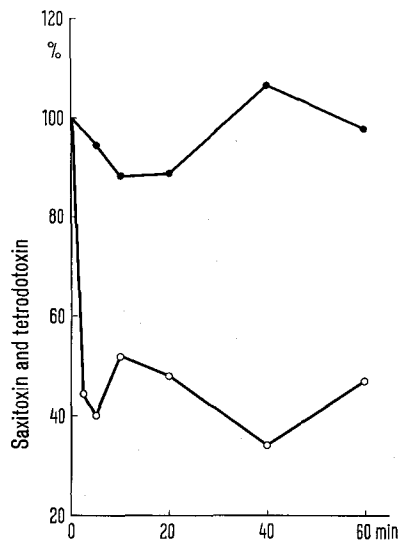
chloride formed when the samples were brought to pH 2.5–3.5. Salt tends to prolong the death times in this assay[15,18,19]. Smaller doses of poison result in longer death times, so the toxicity remaining after heating could be estimated from published tables[17] or from graphs of the dose/death time previously determined by us. Doses of less than 1 MU cannot be assayed by this technique, therefore the percentage of poison that remained after heating could be measured only if it was not less than 25% of the initial concentration.

Virtually every sample of STX showed better stability than the corresponding sample of TTX, heated at the same pH for the same length of time. In strongly acid solutions TTX rapidly lost toxicity at 100°C, while STX remained stable, but above pH 2 there was less difference. The Figure shows the results of heating the poisons at pH 1.0 for times up to 60 min. There was no significant change in the toxicity of the STX even after 60 min at 100°C, but the TTX lost more than half its toxicity within the first few minutes. At pH 0.64 TTX lost toxicity even more rapidly, while STX was as stable at pH 0.64 as at 1.0.



Amounts of saxitoxin (●) and tetrodotoxin (○) found remaining in solutions which had been heated to 100°C for 2.5–60 min at pH 1.0, expressed as a percentage of the amount of poison initially present.

STX is not indefinitely stable when heated with concentrated HCL[20,21], but in 3N acid there is only a slight loss of toxicity after 24 h at 100°C[10]. One would not expect significant loss of toxicity after heating for 1 h or less at pH 1.0 and the results reported here have confirmed this. On the other hand, they show that TTX loses activity rapidly at pH 1.0 and 100°C, and can easily be distinguished from STX by this property. In more strongly acid solutions the destruction of TTX is even greater, but difficulties might arise if weakly toxic solutions were tested below pH 1.0 because of the larger amounts of salt that would be formed when the more concentrated acid was neutralized before bioassay. The effect that salt has in prolonging survival times in this assay makes the estimation of near-threshold amounts of poison unreliable or even impossible in concentrations of NaCl greater than about 0.1 *M*[15,18,19].

It is therefore suggested that bio-assay of suitable dilutions, before and after heating to 100°C at pH 1.0 for 20–30 min, is a simple and reliable way for distinguishing between solutions of TTX and STX.

Resumen. La tetrodotoxina pierde toxicidad rapidamente cuando se calienta en solución acida por debajo de pH 2, mientras que la saxitoxina es estable. Se puede distinguir facilmente entre estos venenos calentándolos a 100°C en solution pH 1.0 durante 20–30 min.

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Evidence for a Different Site of Action of Phenoxybenzamine and Desmethylimipramine on the Catecholamine Uptake System

It is well known that both phenoxybenzamine and desmethylimipramine behave as very potent blockers of the catecholamine uptake system (AXELROD et al.[1]; SIGGS et al.[2]; FURCHGOTT and KIRPEKAR[3]; TITUS et al.[4]; IVERSEN and LANGER[5]). The blocking effect induced by these agents could be considered essentially irreversible.

The purpose of the present experiments was to determine whether the site of action of phenoxybenzamine and desmethylimipramine, on adrenergic nerve terminals, is or is not the same. The changes in sensitivity of an atrium to norepinephrine was used as indication of the catecholamine uptake blockade induced by either phenoxybenzamine or desmethylimipramine in the presence or in the absence of a protecting agent (FURCHGOTT[6].) Provided that norepinephrine and tyramine are taken up by a common uptake system (COMMARATO et al.[7]; FURCHGOTT et al.[8]; SÁNCHEZ GARCIA et al.[9]), tyramine was used in these experiments in order to protect the uptake system for catecholamines, during exposure of atria to the blocking agents.

Methods. Guinea-pigs weighing from 300–500 g were used. The animals were killed by a blow on the head. The hearts were removed and the left atria were prepared for mounting as previously described by FURCHGOTT and SÁNCHEZ GARCIA[10]. In each experiment one half of the atrium served as a control. The bathing solution was