Gill lesions in Cichlid fishes after intoxication with the insecticide Fenthion[1]

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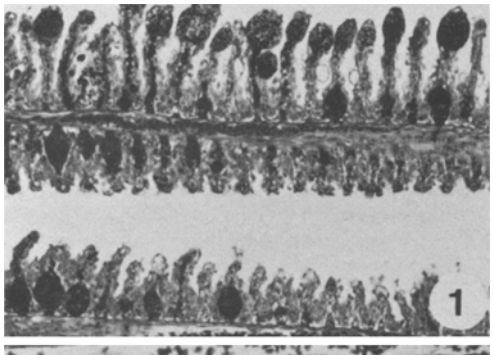
Summary. The toxic effects of Fenthion on the gills of Cichlid fishes were investigated. In Herotilapia multispinosa hyperplasia and separation of the respiratory epithelium, lamellar telangiectasis and thrombosis occurred. The lamellar epithelium of surviving fish regenerated in all cases. In Tilapia leucosticta no telangiectasis was found, but all the secondary lamellae were fused due to the severe hyperplasia of the gill epithelium.

The organophosphorous insecticide Fenthion (Lebaycid®, Baytex®, Baycid®) is a highly effective larvicide and used for Mosquito and Simulium control in many tropical countries[2-5]. As fishes are essential in those countries as a protein source in human nutrition[2,6], and moreover are the only natural enemies of the insect larvae[7], it is important to discover the toxic effects of Fenthion on fishes[2,4,5,8]. The aim of this investigation was to study the effects of Fenthion on fish gills in relation to the insecticide concentration and incubation period, as well as the ability of the fishes to regenerate from their induced injuries.

Materials and methods. 40 Cichlid fish of the species Herotilapia multispinosa with an overall length of 10 ± 1 cm were exposed to 4 Fenthion concentrations from 1.1 mg/l up to 2.8 mg/l (in dilutions of Lebaycid® 50EC) for 96 h, to concentrations of 4.4 mg/l, 5.0 mg/l and 5.5 mg/l for 48 h and of 7.2 mg/l for 24 h. In a 2nd series, 40 specimens were exposed to 2.8 mg/l for 6, 12, 24, 48 and 96 h and to 5.5 mg/l for 6, 12, 24 and 48 h; 45 specimens of Tilapia leucosticta of the same size were exposed to Fenthion concentrations of 2.8 mg/l, 5.5 mg/l, 7.2 mg/l and 8.3 mg/l for periods of 24 and 96 h. In each experiment, 3 or 5 fish were put in an aerated glass aquarium with a capacity of 10 l. After the experiments, the fish were killed and the complete gills and histological sections of them (thickness 5 μ, Azan- and H.E.-staining) were examined.

Results and discussion. In Herotilapia, Fenthion caused hyperplasia and separation of the respiratory epithelium. Lamellar telangiectasis and thrombosis occurred (figure 1) and could be recognized even in the gills of intoxicated fish in situ. At 1.1 mg/l, no damage was found. For the first time slight hyperplasia, telangiectasis and thrombosis occurred with a concentration of 1.7 mg/l. Up to 2.2 mg/l, no increase of the damage was seen and no fish died with these concentrations. With 2.8 mg/l, the lesions suddenly became more serious and 19% of the fish died. Under these conditions, the first slight hyperplasia and separation of the epithelium were seen after an incubation time of 12 h. At this time, 1.3% of the secondary lamellae were thrombosed. The degree of thrombosis increased with the incubation time (3.0% after 24 h, 8.0% after 48 h). After 96 h, 16.4% of the secondary lamellae were thrombosed and most of them showed epithelial hyperplasia. It was surprising to see how the patterns of intoxication changed at Fenthion concentrations of more than 4.4 mg/l. Whilst all other damage increased with increasing concentrations, the amount of telangiectasis and thrombosis began to decrease. A concentration of 5.5 mg/l thus caused a further progress in



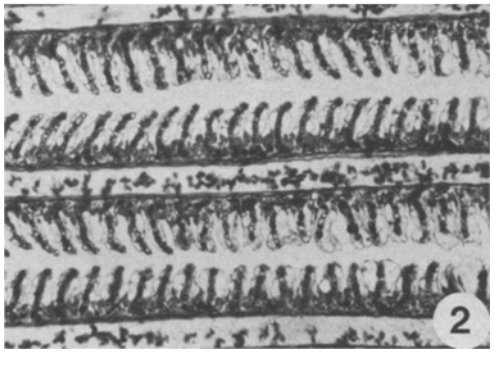
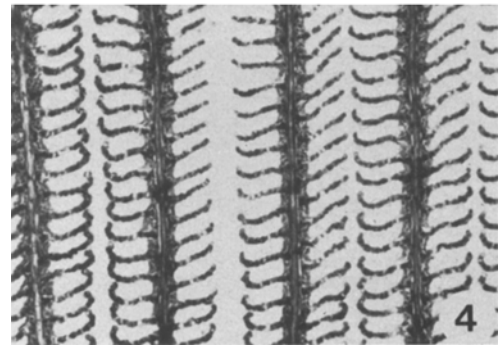


Fig. 1. Gill of Herotilapia treated with 2.8 mg/l for 96 h. Note epithelial hyperplasia in the secondary lamellae, sporadic lamellar epithelial separation and multiple telangiectasis. Most of the enlarged capillaries are thrombosed, some of them contain normal blood. H.&E.-staining, × 120. Fig. 2. Severe lamellar epithelial separation after treatment with 7.2 mg/l for 24 h. Azan-staining, × 120. Fig. 3. Gill of Tilapia after treatment with 8.3 mg/l for 96 h. The secondary lamellae are nearly completely fused due to the severe hyperplasia of the respiratory epithelium. Azan-staining, × 120. Fig. 4. Normal gill of control Tilapia. Azan-staining, × 120. The gills of Herotilapia have exactly the same structure.



epithelial separation. The 1st gill damages already occurred after an incubation period of 6 h; and after 48 h, in some cases the epithelium was completely destroyed, but only 1.1% of the secondary lamellae showed thrombosis. With 7.2 mg/l finally, scarcely any thrombs were to be found (figure 2).

Surviving fish recovered in fresh water within several days. Thrombosis almost completely disappeared after 2 days. Lamellar epithelium regenerated in all cases; after 28 days, the intoxicated gills barely differed from the control gills (figure 4).

Tilapia turned out to be much less sensitive to Fenthion than Herotilapia. At a concentration of 5.5 mg/l and an incubation period of 96 h, 5% of the fish died. The histopathological patterns in Tilapia differed from those in Herotilapia. Epithelial separation and telangiectasis did not occur with any concentration at any incubation time. In concentrations of less than 5.5 mg/l, no gill lesions were found. From 5.5 mg/l up to 8.3 mg/l at an incubation time of 24 h, only a slight swelling of the lamellar epithelium was found. After 96 h, with 5.5 mg/l, the lesions did not increase significantly, but with 8.3 mg/l all the secondary lamellae were fused due to the severe hyperplasia of the gill epithelium (figures 3 and 4).

The gill lesions observed in the 2 Cichlid genera point to the fact that the histopathological patterns of insecticide poisoning are nonspecific[9-11] and may differ very much even in closely related species. From the gill damages found here, one can conclude that the respiratory O_2-supply is rather complicated, especially in tropical waters of low O_2-contents. Even if the intoxication is not lethal, the O_2-deficiency in the tissues may cause further organic injuries, for example in nerve cells[12,13]. Such fishes may easily fall prey to their enemies. The results show that even in an insecticide with a high factor of safety[4,5], the dispersion must be carried out with extreme care in rivers and lakes with dense fish populations. High concentrations of the active substance at the application area could induce serious injuries within a short incubation period.

1 This work was undertaken with the aid of Prof. Dr. E. Kulzer.
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The effects of physostigmine on the oxygen uptake in rat brain tissue[1]

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Summary. Physostigmine in a dose of 0.1 mg/kg i.v. expressly stimulated the oxygen uptake in the rat cerebral cortex. This effect was blocked by propranolol and seems be mediated by catecholamines. Since atropine also antagonized the stimulant effect of physostigmine, it appears that the action of physostigmine is primarily cholinergic and that the adrenergic effect is a secondary phenomenon. The higher dose of physostigmine (0.4 mg/kg i.v.) caused a depression of rat brain oxygen uptake.

Some experimental studies have provided evidence for a possible relationship between central cholinergic and adrenergic systems[3,4]. In order to explore this relationship further, we considered it pertinent to study the effects of 2 cholinergic drugs, physostigmine and neostigmine, on the oxygen uptake in rat brain tissue. It is known that physostigmine activates the central cholinergic and adrenergic systems of rat, whereas neostigmine is unable to cross the blood-brain barrier[5,6].

Materials and methods. The animals used in this study were Wistar rats of either sex, weighing 100–120 g. Oxygen uptake in cerebral cortex slices (thickness of slices was about 0.5 mm and the weight, 15–20 mg) was determined by the direct manometric method in a Warburg apparatus[7]. The suspending fluid was Krebs-Ringer phosphate solution with 13 mM glucose and the gas phase was oxygen. Oxygen consumption was measured at 37 °C and expressed as μmoles of oxygen consumed/g of fresh tissue. This value is expressed as QO_2.

Physostigmine salicylate (Sandoz) and neostigmine (Prostigmin Hoffmann-La Roche) were injected i.v. at different dose levels. The rats were sacrificed by decapitation 30 min after the injection of the anticholinesterases. Propranolol hydrochloride (Inderal-ICI) was used as beta adrenergic antagonist and atropine sulfate (Lek), as cholinergic antagonist.

Results. Physostigmine salicylate, administered i.v. at doses from 0.075 mg/kg to 0.125 mg/kg increased oxygen consumption in rat cerebral cortex. The maximum effect (23% raise) was found 30 min after administration of 0.1 mg/kg of physostigmine, and then gradually disappeared over a period of 45 min.

Table 1. The effect of physostigmine (0.1 mg/kg) on the oxygen uptake in rat cerebral cortex slices

Treatment*	QO_2 (μM O_2/g/h)** Controls	Physostigmine	Change of controls (%)
Saline	75.0±2.9	92.3±0.7	+23***
Propranolol	76.3±1.9	72.7±2.6	−4.3
Atropine	77.2±1.3	75.5±1.8	−2.2

* See results. ** The figures represent the mean value (of 10–15 experiments) ± SE. *** $p < 0.001$.