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.

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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. *** $p < 0.001$.

Propranolol (10 mg/kg i.p.) or atropine (10 mg/kg i.p.) administered 10 min prior to physostigmine, successfully blocked the physostigmine-induced stimulation of brain oxygen uptake (table 1). Under these conditions, neither propranolol nor atropine alone altered the rate of brain oxygen uptake in control animals (table 1). Unlike the low doses of physostigmine, higher (sub-lethal) doses inhibited oxygen consumption in the rat brain cerebral cortex. The significant decrease of oxygen uptake (about 18%) was found after i.v. injection of 0.4 mg/kg of physostigmine (table 2). This effect was antagonized by atropine (10 mg/kg 10 min prior to physostigmine). The injection of neostigmine, which was tested at a wide dose range (0.025–0.2 mg/kg), was without effect upon oxygen uptake in rat cerebral cortex (table 2).

*Discussion.* The results obtained show that physostigmine injected i.v. in a dose of 0.1 mg/kg expressly stimulates oxygen uptake in rat cerebral cortex tissue. This increase of oxygen uptake in rat cerebral tissue is certainly due to the central action of physostigmine since neostigmine, a cholinesterase inhibitor, which does not readily cross the blood-brain barrier, is unable to produce a similar effect. The stimulant effect of physostigmine may be considered as a result of adrenergic activation in the central nervous system. The antagonism by propranolol supports this hypothesis. Our earlier results have also shown that propranolol successfully blocked the stimulant effect of adrenergic substances on the oxygen uptake in rat cerebral cortical slices[8].

On the other hand, the fact that atropine also antagonized the stimulant effect of physostigmine on the brain tissue respiration suggests the possibility that the action of physostigmine is primarily cholinergic and that the adrenergic effect is a secondary phenomenon.

The injection of a higher dose of physostigmine did not produce a further increase in the stimulation of brain respiration, but a pronounced depression. This result is in agreement with other reports[9,10] which indicate that anticholinesterase substances in massive systemic doses depress brain oxygen uptake. The exact mechanism whereby anticholinesterase compounds depress cellular respiration remains to be determined. However, it is interesting to note that some sympathomimetic drugs in high concentrations also cause a reduction in cerebral metabolism[11].

Table 2. The effect of neostigmine and physostigmine on the oxygen uptake in rat cerebral cortex slices

| Treatment* (mg/kg) | $QO_2$ (μM $O_2$/g/h)** | Change of controls (%) |
|---|---|---|
| Saline | 71.6±4.3 | - |
| Neostigmine | | |
| (0.025) | 69.4±3.9 | −3.0 |
| (0.1) | 70.4±4.2 | −1.6 |
| Physostigmine | | |
| (0.3) | 65.7±3.8 | −8.3 |
| (0.4) | 58.9±2.1 | −17.7*** |
| Physostigmine (0.4)+ | | |
| Atropine (10.0) | 66.2±4.2 | −7.7 |

* See results. ** The figures represent the mean value (of 10–15 experiments)±SE. *** $p<0.01$.

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## Effect of dichloromethane on the sciatic motor conduction velocity of rats

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*Summary.* There is a correlation between dichloromethane dosis (X) of 1–6 mmole/kg administered i.p. to rats and the sciatic motor conduction velocity (Y): $Y=57.1-1.091\,X$. The correlation coefficient 'r' is 0.437 ($p<0.01$). Presumably, the decrease of nerve conduction velocity is caused by the endogenous carbon monoxide production due to dichloromethane biotransformation.

There are 2 aspects which prompted us to study the effect of dichloromethane ($CH_2Cl_2$) on motor nerve conduction velocity of rats: (a) Carbon monoxide (CO) induces a decrease of conduction velocity[1-3] and (b) an increase of endogenous CO production and in blood carboxyhemoglobin (COHb) concentration was observed when humans or laboratory animals were exposed to $CH_2Cl_2$[4-21].

*Methods.* Male albino rats of our colony-bred strain weighing between 160 and 200 g (age: 60 days) were used. They were maintained on standard laboratory diet and tap water ad libitum. $CH_2Cl_2$ was administered by i.p. injection. The rats were anaesthetized by hexobarbital and the sciatic motor conduction velocity (SMCV) determined according to the method of Glatzel et al.[22] at room temperature (24±1 °C). COHb concentration in blood was determined using the equation

$$\%\ \mathrm{COHb}=\frac{75.1\ (\mathrm{ml\ CO/100\ ml\ blood})}{\mathrm{g\ Hb/100\ ml\ blood}}$$

with the CO capacity of 1.331 ml/g Hb[23]. Hemoglobin (Hb) concentration was determined as cyanmethemoglobin, CO