clear that the metabolism of 6-MP can be modified by the activity of the drug metabolizing enzyme in mouse liver. In many instances an increased rate of drug metabolism leads to decreased pharmacological action; however, in instances where the metabolite of a drug is more active than the parent compound, enzyme induction can lead to an increase in pharmacological activity of the drug. Since the increased levels in enzyme activity led to decreased toxicity, the present studies also confirmed the result reported by Yoshimura[4] that 6-MP can be catabolized in vitro by hepatic microsomal enzyme from rats.

The major pathway for the catabolism of 6-MP involves oxidation to thiouric acid by xanthine oxidase. An attempt to modify the catabolism of 6-MP by xanthine oxidase led to the development of allopurinol. Elion[9] reported that a reduction in doses of 6-MP was necessary when allopurinol was given concurrently, because allopurinol interferes with the enzymatic oxidation of 6-MP. However, there are some disagreements about the mechanism of the interaction between these drugs.

It has been shown that administration of allopurinol to healthy volunteers impairs the metabolism of antipyrine and coumarine, and further that this effect is caused by a reduction in hepatic microsomal cytochrome P-450 content[10]. In man, the proportion of inorganic sulfate generated is much higher following an oral administration of 6-MP, and lower following administration of allopurinol[9]. Yoshimura[4] also reported that the catabolism of 6-MP by hepatic microsomal enzyme was caused by desulfuration. These results also suggest that detoxification of 6-MP by hepatic microsomal enzyme may be more responsible for the catabolism of 6-MP.

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ATPase activity in mercury intoxicated eels

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Summary. Eels intoxicated by lethal doses of $HgCl_2$ accumulate mercury in their gills. Mercury inhibits the ouabain-sensitive Na^+K^+ATPase activity of gills involving the rupture of the fish NaCl balance.

We have previously shown that the lethal effect of mercury ($HgCl_2$) added to seawater on the seawater-adapted eel Anguilla anguilla could be attributed to a disruption of the NaCl balance of the animal[2]. Indeed, when considering fishes, the gill appears to be the most likely site of attack by heavy metals and, in our case, no inhibition of the respiratory processes occurs[3]. Moreover the effects of mercury are similar whether isolated gills or whole animals are intoxicated[3]. It appears then that mercury acts in the gills on the osmoregulatory processes, either active or passive ionic movements. Since the work of Skou[4], there is agreement on the fact that the ouabain-sensitive Na^+K^+ATPase has a fundamental role in the active transport[5,6]. In this paper, we have tried to see whether the disruption of NaCl balance could be attributed to an inhibition of the gill ATPase activity.

Material and methods. Fresh water eels are adapted for at least 10 days to natural seawater. Each control fish is then placed in a polyethylene bag containing 10 l of continuously oxygenated seawater; the fishes to be intoxicated are trapped in an identical bag containing 10 l of oxygenated seawater with 1 ppm of $HgCl_2$. Both series of eels are kept under these conditions for 30 h. They are then killed and the blood is collected after section of the ventral aorta. The left gills are cut off for the ATPase activity measurement and the right ones for the determination of mercury.

Determination of mercury in the gills. The digestion of the sample is performed following the method described by Sandell[7] and modified by Mayer[8], in order to avoid the loss of mercury associated with the use of the concentrated nitric acid. The analysis of the solution is made by the dithizone technique[7].

Determination of Na, K and Cl in the plasma. The blood is centrifuged at 1085 × g for 10 min (Sorvall S centrifuge). The ions are determined in the supernatant. The analysis of sodium and potassium is performed using an Eppendorf flame photometer. Chloride is determined with the Marius Chlor-o-Counter.

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Table 1. Reversibility of the effect of $HgCl_2$ on the Na^+K^+ATPase activity of gills by addition of cysteine to the homogenate

Experimental conditions	Percentage of Na^+K^+ATPase activity in the gill related to total ATPase activity
Optimal conditions	31%
Optimal conditions $+HgCl_2$ 10^{-5} M	10%
Optimal conditions $+HgCl_2$ 10^{-5} M $+$cysteine 10^{-3} M	29%
Optimal conditions $+$cysteine 10^{-3} M	32%

Table 2. Effect of $HgCl_2$ intoxication on Na and Cl concentrations in plasma of seawater adapted eels (mEq/l)

	Na	Cl
Control eels	144.0 ± 6.2	131.8 ± 4.5
Intoxicated eels	208.0 ± 18.0	175.3 ± 17.1

Determination of ATPase activity. Gill filaments are ground in a 0.5 M sucrose solution containing 5 mM EDTA (pH adjusted to 7.4 with Tris-HCl buffer) in an homogenizer with a Teflon pestle. The solution is centrifuged stepwise at 121 × g for 10 min and at 12,100 × g for 30 min. The precipitate is suspended in a 0.5 M sucrose solution containing 5 mM EDTA (pH 7.4) and is used immediately for testing its ATPase activity.

Assay of ATPase activity. The reaction mixture is adjusted to a final volume of 2 ml and contains 4 mM ATP, 100 mM NaCl, 25 mM KCl, 5 mM $MgCl_2$ and 0.2 ml of the enzyme solution[6]. After incubation for 30 min, the reaction is stopped by addition of 0.2 ml of 50% trichloroacetic acid. Inorganic phosphate is determined by the method of Fiske and Subbarow, and proteins are analyzed according to the method of Folin. Ouabain-sensitive Na^+K^+ATPase activity is obtained by substracting the ouabain-non sensitive ATPase activity from the whole ATPase activity (the concentration of ouabain used is 0.2 mM).

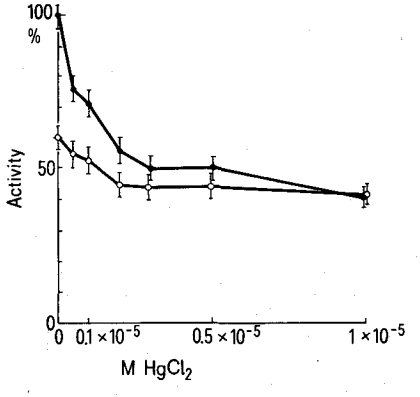

Fig. 1. Effect of $HgCl_2$ on ATPase activity in eel gills. ●, Total activity; ○, ouabain-non sensitive ATPase activity; I, 2 × SE.

Results and discussion. The total MgNaKATPase activity is 0.68 μM Pi/mg protein h (SE = 0.03; n = 6). The in vitro effect of $HgCl_2$ on the enzyme is shown in figure 1. In absence of mercury, the ouabain-sensitive ATPase activity is about 40% of the total activity. For 3×10^{-6} M $HgCl_2$ concentration, the ouabain-sensitive ATPase activity is completely inhibited. Moreover there appear to be 2 distinct fractions in the ouabain-non sensitive ATPase: one $HgCl_2$-sensitive (representing 15% of the total activity) and one $HgCl_2$-non sensitive (at concentrations $< 10^{-5}$ M) representing 45% of the total activity.

The effect of CH_3HgCl is shown in figure 2. The shape of the curves is similar to what is observed during inhibition by $HgCl_2$, but CH_3HgCl seems somewhat less toxic: the ouabain-sensitive ATPase is totally inhibited when the CH_3HgCl concentration reaches 8×10^{-6} M. As the inactivation is faster with $HgCl_2$, we have used this salt in the intoxication experiments described later in this paper.

Regarding the cause of the observed inhibition of the ouabain-sensitive ATPase, it is probably due to the great affinity of Hg^{++} for –SH compounds. Indeed, cysteine 10^{-3} M added to the preparation suppresses the inhibitory effect of mercury as shown in table 1.

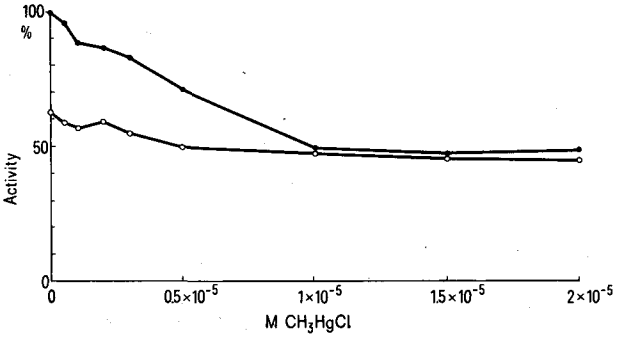

Fig. 2. Effect of CH_3HgCl on ATPase activity in eel gills. ●, Total activity; ○, ouabain-non sensitive ATPase activity.

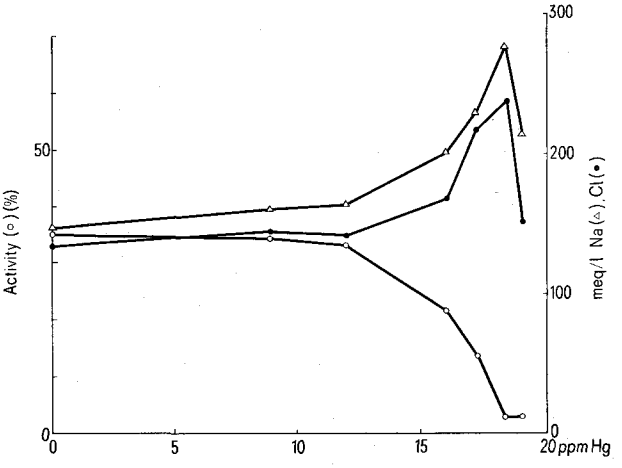

Fig. 3. Relation between Hg concentrations and ATPase activity in the gills and NaCl concentrations in the plasma. △, Na concentration; ●, Cl concentration; ○, percentage of ouabain-sensitive Na^+K^+ ATPase activity.

If mercury is able in vitro to react with the gill ouabain-sensitive ATPase, it remains to be shown that it does so in vivo. We have assayed the percent ouabain-sensitive ATPase activity in gill homogenates of intoxicated eels. Those values are compared with the percent activity in gill homogenates of control eels.

The percentage of ouabain-sensitive ATPase activity is only 10.2 ± 5.7% in the 6 intoxicated eels, whereas it represents 34.8 ± 4.0% of the total activity in the 6 control eels. The difference between those averages is significant when applying the t-test ($p < 0.05$).

Owing to the fact that gills contained 13.7 ± 1.5 ppm Hg and considering the different dilutions during the ATPase assay, leading to a final concentration of 10^{-6} M in the homogenate, figure 1 shows that we should only expect a 20% inhibition of the Na^+K^+ATPase activity instead of the 48% observed. It is concluded that the observed inhibition occurs in vivo, although a secondary effect caused by homogeneization cannot be totally excluded. The effect of intoxication on the NaCl concentrations in the plasma is shown in table 2. Each datum is the average of 6 determinations ± SE. The increases of Na and Cl concentrations are very significant (Na: $p < 0.01$; Cl: $p < 0.05$).

Considering the data corresponding to intoxicated eels, both ATPase activity assays and ion determinations, the SE appear to be rather high and reflect important individual variations. Individual variations are very important, but the relationship between the different data is clearly seen in figure 3. When the Hg concentration increases, the ATPase activity decreases in the gills and the Na and Cl concentrations increase in the plasma. The rupture of osmotic equilibrium appears only when the mercury concentration in the gill reaches about 10 ppm.

To conclude, the lethal effect of mercury previously related to a rupture of the NaCl balance[2] can be attributed to an inhibition of the Na^+K^+ATPase activity of the gills and consequently to an inhibition of the NaCl transport. It is otherwise interesting to note that no decrease of the K content both of plasma or gills occurs in such intoxicated eels[2,3], which shows to be debatable, as quoted by other authors[9,10], the theory of MAETZ[5] of a NaK coupled active transport in gills of seawater teleosts.

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Induction of the in vitro p-hydroxylation of ^{14}C-amphetamine stereoisomers in phenobarbital-treated rats

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Summary. The rate of p-hydroxylation of ^{14}C-(−)-amphetamine by liver microsomes was higher than that of (+)-isomer in phenobarbital-treated male rats. The apparent K_m values for (−)- and (+)-amphetamine hydroxylation were 4.54×10^{-5} M and 2.27×10^{-5} M respectively, in both treated and control animals.

The activity of the (+)-amphetamine on the central nervous system is generally higher than of the (−)-isomer. The differences[2-4] of pharmacological activity between the stereoisomers of amphetamine may be partly explained by their stereospecific metabolism. Dring et al.[5] showed in several animal species the main metabolite, p-hydroxyamphetamine, to be excreted in urine in greater amounts after administration of the (−)-amphetamine than from the (+)-isomer. The product of the metabolism of amphetamine in vitro is disputed. Daly[6] and Fuller[7] have only obtained oxidative deamination products, while Jonsson[8] and Rommelspacher[9] using rat liver microsomes have identified the p-hydroxyamphetamine as the major metabolite.

While Groppetti et al.[10] measuring the disappearance rate of d-amphetamine in homogenates of the whole rat found no induction effect after a phenobarbital treatment, the present paper relates the study of the microsomal enzyme induction to that of the amphetamine stereoselective metabolism. For this purpose, we have studied the aromatic hydroxylation of the ^{14}C-(−)-amphetamine and ^{14}C-(+)-amphetamine with liver microsomal suspensions from control and phenobarbital-treated rats.

Experimental conditions. The specific activity of the 7-^{14}C-(+)-amphetamine was 27 mCi/mmole and that of the 7-^{14}C-(−)-amphetamine was 12.4 mCi/mmole, with a radiochemical purity of 99%. These compounds were prepared by the techniques described by Lintermans et al.[11].

Male rats weighing about 220 g received phenobarbital 0.1% w/v (4.3×10^{-3} M) in their drinking water for 3 days,

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