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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Effect of phenobarbital treatment on some biochemical parameters in male rat

Parameters	Control animals	Phenobarbital-treated animals
Liver weight/body weight (%)	3.8 ± 0.3	5.2 ± 0.4*
Microsomal proteins (mg/g liver)	31.7 ± 6.4	46.2 ± 6.3*
Cytochrome P-450 (μmoles/g liver)	28.6 ± 5.2	94.7 ± 26.2*
Aniline p-hydroxylation (μmoles p-aminophenol formed/g liver)	0.6 ± 0.1	1.0 ± 0.3*

Phenobarbital 0.1% in drinking water was given for 3 days. Mean values of 6 animals ± SD. * Values significantly different from control values: $p < 0.05$ using Student's t-test.

while control animals were given water only. 24 h after stopping the treatment, the animals were killed and exsanguinated and their liver microsomes were separated by differential centrifugation as described by La Du et al.[12]. The microsomal protein level was measured by Lowry's method[13] and the cytochrome P-450 level by Estabrook's method[14]. For the measurement of aniline p-hydroxylation the incubation medium contained: 0.8 mM NADP, 5 mM glucose-6-phosphate, 5 mM $MgCl_2$, 7 IU of glucose-6-phosphate dehydrogenase and 1 mM of aniline in 0.25 M Na^+/K^+-phosphate buffer pH 7.4. The final volume was 2 ml and contained microsomes equivalent to 125 mg liver wet weight. The incubation time was 30 min. Aniline p-hydroxylation was stopped by the addition of 1 ml TCA 20% and the p-aminophenol formed was measured by the method of Kato[15].

The same incubation medium was used for in vitro amphetamine metabolism, amphetamine at concentrations of 3, 6, 12, 25 and 50 μM substituted for the aniline. The incubation time was 10 min. Amphetamine metabolization was stopped by adding 1 ml $HClO_4$ 2 N[9] and the separation of the metabolites was carried out by paper and thin layer chromatography in the system: toluene-n-butanol-acetic acid-water 2/2/1/1 (v:v). The radioactive chromatography sectors corresponding to amphetamine and metabolites were localized with a scanner and quantified by liquid scintillation. The metabolites of amphetamine were identified by comparing their R_f values with those of the following reference compounds: p-hydroxyamphetamine[16], norephedrine[17], p-hydroxynorephedrine[17], and phenylacetone[18] in different chromatographic systems described by Glasson et al.[19]. The identity of p-hydroxyamphetamine was confirmed by UV-spectrometry, according to Noirfalise[20].

Results and discussion. To verify the induction state, we determined the liver weight expressed as a percentage of body weight, the amount of microsomal protein and of cytochrome P-450 and the activity of in vitro aniline p-hydroxylation in control and in phenobarbital-treated rats (table). The increase of all measured parameters in phenobarbital-treated rats compared to the control ones is significant ($p < 0.05$). Figure 1 shows the percentage of ^{14}C-(+)-amphetamine and ^{14}C-(−)-amphetamine metabolized in control and phenobarbital-treated animals. In this figure, no difference in the rate of the in vitro metabolism of the 2 amphetamine isomers is observed, while the stereo selective induction of this metabolism is already obvious. The increase in the metabolism of ^{14}C-amphetamine in phenobarbital-treated rats is greater when the (−)-isomer is used as substrate than with the (+)-isomer, where the difference are not significant (differences were considered significant when $p < 0.05$ using Student's t-test). Quantitatively, the most important metabolite of amphetamine is the p-hydroxy compound. The kinetic constants of the in vitro p-hydroxylation of (+)- and (−)-amphetamine in control and phenobarbital-treated rats, calculated using the Lineweaver-Burk plots, are shown in figure 2. The formation of p-hydroxyamphetamine is greater with ^{14}C-(−)-amphetamine used as substrate than with the (+)-isomer. Values of apparent

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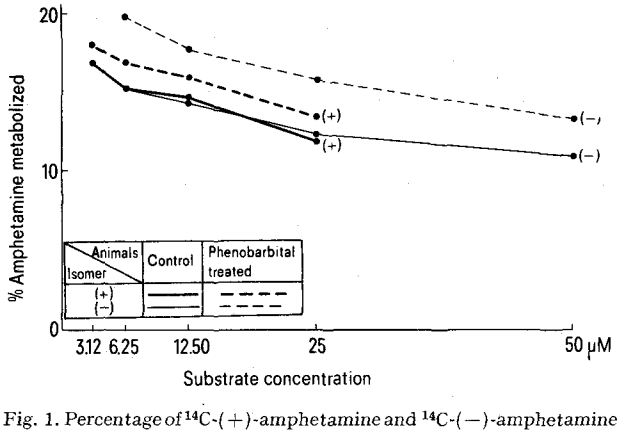

Fig. 1. Percentage of ^{14}C-(+)-amphetamine and ^{14}C-(−)-amphetamine metabolized in control and phenobarbital-treated rats according to the substrate concentration. Each point represents the mean value of 6 animals.

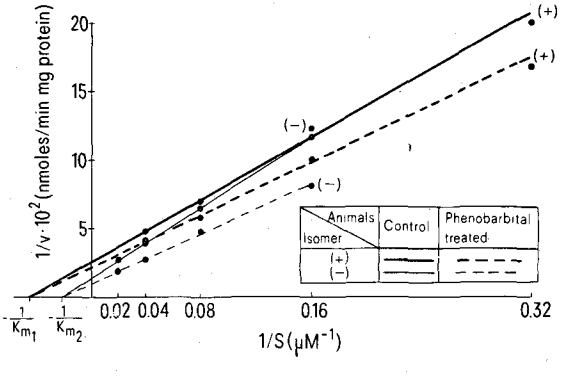

Fig. 2. Lineweaver-Burk plots of the in vitro p-hydroxylation of ^{14}C-(+)- and -(−)-amphetamine in control and phenobarbital-treated rats. Mean values of 6 animals.

K_m are 2.27×10^{-5} M for (+)-amphetamine and 4.54×10^{-5} M for (−)-amphetamine. Furthermore, the p-hydroxylation of ^{14}C-(−)-amphetamine is more sensitive to the inducing effect of the phenobarbital treatment than that of the (+)-isomer. In the phenobarbital-treated rats, we observed an increase of apparent V_{max} (from 41.7 to 47.6 and from 71.4 to 111.1 nmoles p-hydroxyamphetamine/min mg protein for (+)- and (−)-amphetamine respectively) without effect on apparent K_m, as it is generally the case for induction phenomena.

Our results are consistent with data obtained in vivo[5,21,22] and with some results previously obtained in vitro[8,9]. Further, we could obtain under our experimental conditions a stereospecific induction of the in vitro amphetamine metabolism, (−)-amphetamine p-hydroxylation rate being greater than that observed with the (+)-isomer. Although we are unable to explain the mechanism of stereospecific induction, such a phenomenon may be of significance with regard to the pharmacological activities and interactions of these compounds.

Studies on GABA accumulation induced by γ-glutamyl-hydrazide in regions of rat brain following treatment with a tyrosine hydroxylase inhibitor and 6-hydroxy-dopamine[1]

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Summary. Reduction of DA receptor activity via depletion of DA stores does not seem to influence GABA turnover in the forebrain and in the DA cell body rich region of the midbrain.

In recent papers[2–4] evidence has been obtained that changes in GABA accumulation in brain after GABA aminotransferase inhibition using γ-glutamyl-hydrazide (GAH)[5] probably reflect changes of GABA turnover in the neuronal pool, since it has been found to be nerve impulse dependent[4]. Using this model to study changes in GABA turnover, it was possible to show that the dopamine (DA) receptor agonist apomorphine increased GABA turnover in striatum, subcortical limbic regions (rich in DA nerve terminals) and in the DA cell body rich regions of the midbrain. This increase was blocked by pretreatment with the DA receptor blocking agent pimozide. However, pimozide by itself did not cause any change of GABA turnover[2,3]. On the other hand, in dorsal neocortex and in cerebellar cortex, lacking DA cell bodies and nerve terminals, apomorphine produces no effects on GABA turnover, or a trend for a reduction (cerebellar cortex)[6], supporting the view that the increases of GABA turnover observed are due to stimulation of specific DA receptors innervated by DA terminals.

In view of this, it is of interest further to evaluate the influence of a reduction of DA receptor activation on the turnover of GABA. Therefore, it has been studied whether combined treatment with the tyrosine hydroxylase inhibitor α-methyl-tyrosine methylester (H 44/68) and the neurotoxic compound 6-hydroxy-dopamine (6-OH-DA), causing a relatively selective degeneration of catecholamine (CA) neurons in the brain[7] without affecting e.g. GABA levels[8–10], can change the GABA turnover in the nuc. caudatus, subcortical limbic regions (tuberculum olfactorium, nuc. accumbens and tractus diagonalis area) and the DA cell body rich regions of the midbrain.

Material and methods. Male specific pathogen-free Sprague-free Sprague-Dawley rats were used. Unilateral lesion of

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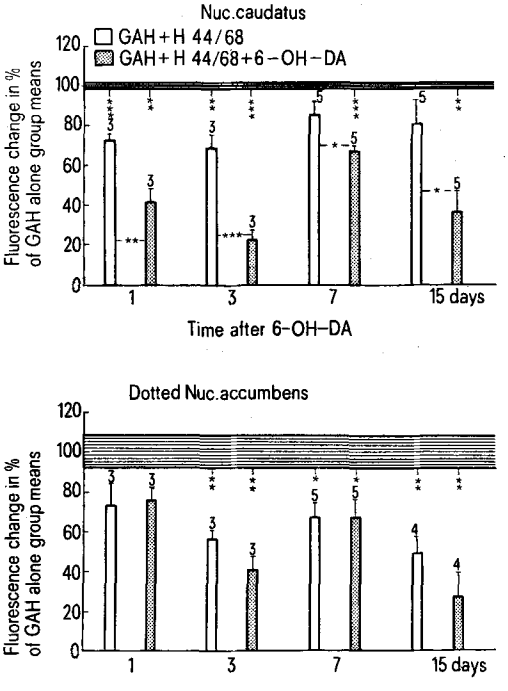

Fig. 1. Effect of 6-OH-DA and H 44/68 on DA fluorescence in the nuc. caudatus and dotted nuc. accumbens. On the x-axis days after 6-OH-DA treatment (see Materials and methods) are shown. H 44/68 (250 mg/kg) was injected i.p. 3 h 15 min and GAH (160 mg/kg) 3 h before killing. On the y-axis the DA fluorescence (means ± SEM) is shown in percent of the GAH alone group mean value. Absolute mean values for the GAH alone group expressed in arbitrary fluorescence units ± SEM were for nuc. caudatus 15.6 ± 0.2 (n = 4) and for the dotted nuc. accumbens 32 ± 2.8 (n = 4). Number of animals in parenthesis. Paired Student's t-test was used.
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.