Although this compound is extracted only to a minor extent from hepatocytes into the bile. It was possible to show histologically by freeze-drying techniques that BSP entered the hepatocyte after retrograde injection. The cytoplasm and the nuclear membranes showed a dark-blue colour after being made alkaline giving evidence of BSP within the hepatocytes. On the other hand we were not able to detect any destructive alterations of the liver cells which might have been caused by the retrograde biliary injection of a total of 0·07 ml.

These results provide clear evidence that an absorption from the biliary system into the hepatocytes is possible and support the suggestion given in this respect by Clark & others (1971).

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Interaction of fenfluramine analogues with the *in vivo* metabolism of (+)-amphetamine in the rat

Jonsson & Gunne (1972) have shown that pretreatment of rats with fenfluramine before administration of amphetamine increased plasma and brain concentration of amphetamine with a concomitant prolongation of the amphetamine-induced excitatory behaviour. We wished to establish whether fenfluramine and related derivatives interfere with the metabolism of amphetamine through inhibition of aromatic hydroxylation as do several other compounds such as tricyclic antidepressants and phenothiazines (Consolo, Dolfini & others, 1967; Lewander, 1969). (+)-[7-^{14}C]Amphetamine sulphate CEA, France (5 mg/kg, i.p., 7·2 μCi; purity >96% by ion-exchange chromatography, Lewander, 1971) was injected to male Sprague-Dawley rats, 180–220 g. Urine was collected from rats, housed individually in metabolic cages, for 24 h after the injection. The rats had free access to food and water. Amphetamine metabolites were separated by paper chromatography (Ellison, Gutzait & Van Loon, 1966) and the radioactivity, eluted from the paper strips, was measured by a liquid scintillation spectrometer. The drugs (Table 1) to be tested for inhibition of *p*-hydroxylation of amphetamine, were administered subcutaneously in equimolar concentrations before injection of amphetamine. The urinary pH in the drug-treated groups was not significantly different from that in the control group (pH = 7·1 $\pm$ 0·44; mean $\pm$ s,d,),

All the fenfluramine analogues reduced the aromatic hydroxylation of amphetamine as indicated by the change in the pattern of distribution of amphetamine metabolites from *p*-hydroxylated metabolites towards unchanged amphetamine (Table 1). Fenfluramine (10 mg/kg, s.c. 1 h before amphetamine) was the most potent inhibitor

Table 1. *Influence of* m-(*trifluormethyl*)- *and* p-*chloramphetamine derivatives on the pattern of urinary metabolites of* (+)-[^{14}C]*amphetamine* (7·2 μCi, 5 mg/kg) *in male rats.* The metabolites are expressed as the percentages (mean ± s.e.) of the total radioactivity of the urine collected during 24 h after injection of the amphetamine. Figures in brackets refer to number of observations.

Treatment	Dose (mg/kg, s.c.)	Injection of drug (h before amphet.)	Excretion (% of injected [^{14}C] amphet.)	Amphet. unchanged	*p*-Hydroxy-amphet. free + conjugated	Hippuric acid	Unidentified
Saline (11)			61·8 ± 3·41	22·6 ± 2·48	68·4 ± 2·34	4·1 ± 0·48	4·8 ± 0·22
Fenfluramine (6)	10 × 5a	2	79·6 ± 2·25**	81·6 ± 1·22***	8·2 ± 0·79***	8·8 ± 0·76***	1·4 ± 0·23***
Fenfluramine (3)	10	2	69·0 ± 7·14	66·7 ± 1·88***	24·2 ± 1·96***	6·8 ± 0·76*	2·2 ± 0·08***
Fenfluramine (3)	10	1	69·2 ± 9·72	75·7 ± 0·55***	15·2 ± 0·65***	7·6 ± 0·93**	1·4 ± 0·12***
Norfenfluramine (3)	8·9	1	69·5 ± 5·86	42·0 ± 2·05**	47·9 ± 2·02***	5·5 ± 0·91	4·6 ± 0·82
780 SE (3)	14·5	1	68·6 ± 2·31	43·0 ± 3·67**	48·9 ± 3·18**	4·2 ± 0·72	3·9 ± 0·35
780 SE (3)	14·5 × 5a	1	71·9 ± 5·99	68·5 ± 4·62***	19·6 ± 3·43***	8·4 ± 1·02**	3·4 ± 0·42
p-Chloramphetamine (3)	7·7	1	68·4 ± 5·86	26·8 ± 1·74	64·2 ± 2·22	3·3 ± 0·11	5·7 ± 0·66
p-Chlormethamphetamine (3)	9·9	1	61·1 ± 4·12	25·3 ± 1·45	66·0 ± 1·36	4·2 ± 0·14	4·3 ± 0·35

Difference from saline control: ***P <0·001; **P <0·01 ;*P <0·05.
(a) The rats were given the drug twice daily.

in this series and reduced the excretion of *p*-hydroxylated products from 68 to less than 20%. The total amount of *p*-hydroxyamphetamine and its conjugate was further reduced when fenfluramine or *N*-(2-benzoyloxyethyl)norfenfluramine (780 SE) was given repeatedly to the rats. When the time interval between the injection of fenfluramine and amphetamine was lengthened from 1 to 2 h, a diminished efficiency in reducing the *p*-hydroxylation was obtained. This finding may be explained by a decrease in the concentration of fenfluramine at the metabolic site with time. Norfenfluramine, the dealkylated metabolite of fenfluramine, like 780 SE had less pronounced effects, when given as a single injection. The amount of the unidentified metabolite decreased after pretreatment with fenfluramine which suggests that this substance is a hydroxylated metabolite of amphetamine.

The percentage of hippuric acid, the final metabolite of the minor deamination pathway of amphetamine metabolism in the rat (Axelrod, 1954), was significantly higher after pretreatment with fenfluramine or 780 SE. Thus, side-chain metabolism seemed to be increased when the availability of the aromatic pathway was reduced.

It has been suggested that the microsomal oxidative systems are located inside a lipid membrane penetrable only by lipid soluble substances (Gillette, 1963). This could explain why norfenfluramine was less effective than fenfluramine as an inhibitor of amphetamine hydroxylation, since norfenfluramine is the less lipid soluble (Brookes, 1968). Other explanations, such as different rates of metabolism and excretion or degree of protein binding of the fenfluramine derivatives (Gillette, 1963), resulting in different concentrations at the site of amphetamine *p*-hydroxylation, can not be excluded.

The effect of *p*-chloramphetamine and *p*-chlormethamphetamine was investigated to see if lipid-soluble amphetamine derivatives with a *p*-substituent influence the metabolism of amphetamine. These compounds are probably not *p*-hydroxylated (Fuller & Hines, 1967). However, neither derivatives, reduced aromatic hydroxylation or oxidative deamination of amphetamine, nor did they increase the latter pathway of metabolism. Thus, an unoccupied *p*-position may be of importance for the action of fenfluramine as an inhibitor of the aromatic hydroxylation of amphetamine.

From the present results it can be concluded that the *m*-trifluoromethylamphetamine derivatives tested inhibited the parahydroxylation of (+)-amphetamine.

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A new method for the assay of orally active hypolipaemic agents

In 1943, Hahn reported that heparin was able to clarify hyperlipaemic sera while Anfinsen, Boyle & Brown (1952) showed that the plasma of animals previously treated with heparin contained a clearing factor which is responsible for the diminution of absorbance of lipaemic sera mixed with such plasma. Several aspects of the mechanism have been discussed by Robinson & French (1960). Although the mechanism of the clarifying activity of heparin has not been completely elucidated, Wolff & Brignon (1959) suggested that it releases cellular enzymes, whose coenzymes are lipoproteins. Other substances having an anti-lipaemic effect have been sought, as heparin is effective only parenterally and its anticoagulant activity is undesired (Oliver, 1967).

Several substances called heparinoids were proposed as orally active hypolipaemic agents by Bianchini & Osima (1959) and Szabó, Larrocha & Sandor (1968). These were obtained either from natural sources or by chemical synthesis. No simple method is known for a laboratory assay of their hypolipaemic activity using the oral route. We propose the following method for this purpose.

The method is based on the inhibition of the hyperlipaemic effect of ACTH (Meakin & Nelson, 1960; Robinson & French, 1960; Hollenberg, Raben & Astwood, 1961; Lebowitz, Breyant & Frohman, 1965; Oliver, 1967). We observed that several natural heparinoid preparations administered by stomach tube antagonized the ACTH-induced increase of free fatty acids in rabbits. Albino rabbits of 2·5–3 kg and of either sex, were used. The animals were allowed food freely. They were then placed in individual cages and blood was extracted from the marginal ear vein 30 min before the injection of ACTH.

Blood FFA were assayed by Dole's method (1956). Every rabbit received subcutaneously 60 units of ACTH. After 2 and 4 h, respectively, blood was extracted and assayed for FFA. Initial values of blood FFA varies between 0·30 and 1·51 μ equiv/ml with a mean value 0·74. Two h after giving 60 units of ACTH the values were between 3·70 and 8·90 μ equiv/ml, with a mean value 6·52. After 4 h there was a diminution of these values (between 0·70 and 7·40, mean value 3·98), without return to the original levels. An activity coefficient, R, was calculated from the following formula: