Evidence of absorption of bromsulphthalein from the biliary system of the rat

Many drugs and their metabolites are excreted in the bile (Smith, 1966), but it remains unclear which factors are involved in the bile elimination of these compounds. It has been suggested that the bile during its passage through the biliary tract may be altered to some extent by reabsorption and secretion of water and solutes (Andrews, 1955; Brauer, 1959; Sperber, 1959; Goldfarb, Singer & Popper, 1963; Wheeler, 1968). Furthermore a reabsorption and secretion of some organic compounds in the biliary tract has also been suggested (Clark, Hirom & others, 1971). These authors have investigated the biliary absorption from the biliary tree in the rat given different organic compounds by retrograde biliary injection. According to their results, compounds with low molecular weights (179–285) were poorly excreted by bile after intravenous infusion and retrograde injection whereas compounds with higher molecular weights (335–752) were excreted in high amounts under these conditions. Furthermore, the compounds of the latter group showed a slowly increasing biliary excretion after retrograde injection. The authors therefore supposed that some absorption from the biliary system followed by excretion may occur in the group with higher molecular weight.

To test this hypothesis we have investigated the biliary excretion of bromsulphthalein (BSP) in the rat giving this compound by intravenous and by retrograde biliary administration. The results of these experiments are shown in Table 1.

BSP is excreted about 85% after intravenous and about 81% after retrograde biliary administration. In the latter instance the biliary excretion slowly increases indicating that this effect is possibly preceded by some absorption of BSP within the biliary tree.

By thin-layer chromatography it was possible to show that after retrograde administration a metabolic transformation of BSP took place. Qualitatively there was no difference in the number of BSP metabolites found in the bile after intravenous infusion or retrograde administration. To exclude the possible transformation of BSP by bile we incubated comparable doses of BSP with bile. No BSP metabolite could be detected by these *in vitro* experiments which could be interpreted in the sense that BSP must enter the hepatocytes before the metabolic transformation could occur.

Table 1. *Recovery of BSP from the bile of rats after either intravenous or retrograde biliary administration.* BSP (5–6 μmol/kg) was administered to biliary cannulated male rats either intravenously or by retrograde biliary administration using a Hamilton micrometer syringe joined to a short polythene cannula inserted into common bile duct. In the latter case the dose (0·02 ml was washed in with 0·9 NaCl solution the syringe being held in place for 1 min after the injection of the saline before bile collection was commenced. In the Table the means (N = 3) and their standard deviations are given.

	% Dose excreted in bile in			
	5′	15′	30′	60′
BSP intravenous administration	14·4 ± 4·4	61·8 ± 16·7	78·8 ± 11·0	85·3 ± 4·7
BSP retrograde administration	17·9 ± 13·0	42·6 ± 17·6	67·9 ± 11·0	80·8 ± 7·2

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