differed significantly (266·0 ± 15·9 *vs* 242·0 ± 13·0 g, $P < 0.01$). The mean body weight before phenobarbitone treatment was 290·0 ± 5·7 g.

Phenobarbitone is an agent which enhances the microsomal enzyme activity and thus increases the rate of biotransformation of a large number of drugs. Since bilirubin is conjugated with glucuronic acid and sulphobromophthalein sodium with glutathione before their excretion in the bile, it might be assumed that the increased plasma disappearance of these substances after phenobarbitone treatment is due to the increased metabolism of these organic acids (Fujimoto, Eich & Nichols, 1965; Klaassen & Plaa, 1968). However, phenobarbitone treatment can also enhance the plasma disappearance of phenol 3,6-dibromophthalein disulphonate and indocyanine green, dyes which apparently are not biotransformed before their biliary excretion (Klaassen, 1970). This suggests that the increased metabolism of sulphobromophthalein sodium may not play a major role in enhancing the plasma disappearance of the dye. The results presented in this work show that the reabsorption of ^{35}S-BSP from the rat biliary tree was significantly reduced after phenobarbitone treatment. It is suggested that a decreased biliary reabsorption of sulphobromophthalein sodium may explain at least partly the enhanced biliary excretion of the dye after pretreatment with phenobarbitone.

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The effect of route of administration on the biliary excretion of phenolphthalein and its glucuronide

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Two compounds excreted unchanged in bile of the rat, phenolphthalein glucuronide (Millburn, Smith & Williams, 1967a) and succinylsulphathiazole (Millburn, Smith & Williams, 1967b), are unaffected in their biliary excretion by prior administration of the inhibitor of microsomal oxidation, SKF 525A (Levine, Millburn, & others, 1970). In contrast the dye, indocyanine green, also excreted extensively in the bile of the rat largely unchanged (Cherrick, Stein & others, 1960), has its excretion depressed by SKF 525A (Levine & others, 1970), an effect largely attributable to the depression of body temperature by SKF 525A which leads to reduced bile output (Levine, 1970). It was suggested that the dye might be excreted by a mechanism different from that involved in the biliary excretion of the other two compounds. However, in the work cited, the dye was injected intravenously whereas succinylsulphthiazole and phenolphthalein glucuronide were injected intraperitoneally. If absorption from the abdominal cavity were the limiting step in the excretion of the latter two compounds, then their excretion might be expected to be relatively insensitive to the effects of agents acting on later, non-rate limiting steps. To test this possibility, we have examined the effects of the route of administration two model compounds, phenolphthalein and its glucuronide.

Phenolphthalein (BDH), phenolphthalein β-D-glucosiduronic acid, sodium salt (Koch Light) and thiopentone sodium (M & B) were purchased.

Wistar albino rats (230 ± 15 g) of either sex were anaesthetized with thiopentone sodium (60 mg kg^{-1}; i.p.) and biliary fistulae were established as described by Abou-El-Makarem, Millburn & others (1967). The rectal temperature of the rats was maintained at 37° using heating lamps regulated by 'Thermistemp' control units (Yellow Springs Instrument Co.). Bile from each rat was collected into tared containers for two 5 min periods before dosing and subsequently at 5 min intervals for 1 h.

Phenolphthalein glucuronide, monosodium salt, was dissolved in 0·9% saline (2 mg ml^{-1}) and was injected either into the abdominal cavity or into a femoral vein at a dose of 8·1 mg kg^{-1}. Phenolphthalein (2 mg ml^{-1}) was dissolved in saline by addition of 2·2 equivalents of sodium carbonate; the final pH of the solution was 10·2. The phenolphthalein was administered as above

* Correspondence.

at 5 mg kg^{-1}, a dose equivalent to that of the glucuronide.

Biliary phenolphthalein glucuronide was determined as follows. Bile samples (approximately 100 μl), including pre-dose control samples, were incubated in the collection tubes with 1 ml of 0·2 M sodium acetate buffer, pH 4·5 containing molluscan β-glucuronidase (1 mg, 400 Fishman units) for 1 h at 37°. The mixtures were made alkaline with 9 ml of 0·3 M glycine buffer, pH 10·4 and the extinction of the solutions at 550 nm was measured. Recovery of added phenolphthalein glucuronide was greater than 95%. Free phenolphthalein was not detectable in bile. In some animals, urine samples were taken at the end of the collection period and treated as above. Urinary phenolphthalein was never greater than 3% of the administered dose.

Results of the experiments are shown in Table 1. The cumulative excretion of phenolphthalein as a percentage of the administered dose is shown after injection of either phenolphthalein (PP) or its glucuronide (PPG) by either the intravenous or intraperitoneal route.

Rates of excretion after injection of either compound via the intraperitoneal route do not differ significantly and are comparable with the percentage excretion rates quoted by Levine & others (1970) after intraperitoneal injection of phenolphthalein. Rates of excretion were significantly higher after intravenous injection of either compound. Measured over the first 10 min, the rate of excretion after intravenous injection of phenolphthalein is increased by a factor of seven and for the glucuronide the factor is approximately four. It is thus clear that in these animals, uptake of both compounds from the abdominal cavity is a rate limiting process.

A further observation is that after injection of phenolphthalein intravenously, the rate of excretion is significantly higher than after injection of its glucuronide by the same route. This suggests that uptake of the glucuronide from the blood imposes a rate limitation which outweighs the fact that no conjugation is required before excretion.

Table 1. *Excretion of phenolphthalein in the bile of rats after injection of phenolphthalein or its glucuronide via either the intraperitoneal or intravenous routes.* Experimental methods were as described in the text. Results are expressed as cumulative percentages of the administered dose. The results were analysed statistically by the Student's *t*-test as follows: data in PP(i.p.) column were tested against those in the PP(i.v.) column as control; data in the PPG(i.v.) column were tested against those in the PP(i.v.) column, and data in the PPG(i.p.) column were tested against those in the PPG(i.v.) column.

Time	PP i.v. (8)	PP i.p. (6)	PPG i.v. (10)	PPG i.p. (6)
5	18·3 ±5·1	0·74 ±0·54**	10·2 ±2·0*	1·9 ±2·7**
10	38 ±7·4	5·4 ±2·2**	22·8 ±3·6**	5·9 ±4·8**
15	50 ±8·1	12·4 ±3·6**	33·6 ±4·0**	11·4 ±5·0**
30	70 ±8·8	31·2 ±7·3**	53·4 ±7·0**	30·2 ±6·1**
45	79·8 ±9·4	42·5 ±10·5**	64·4 ±8·1*	45·5 ±7·4**
60	86·8 ±9·8	48·5 ±14**	71·2 ±9·4*	58·5 ±6·6*
Mean bile volume (ml)	1·040 ±0·12	1·06 ±0·14	1·02 ±0·20	1·09 ±0·14

Significantly different from control at * $P < 0.01$, ** $P < 0.0025$.

The above trends were observed if the results were analysed in groups of the same sex.

From these experiments, it becomes clear that it is essential, by means of preliminary experiments, to establish the likely rate determining step in the processes leading up to excretion. If this is not done, the mechanistic interpretation of experimental results will be insecurely based.

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