Effect of phenobarbitone on the biliary reabsorption of [^{35}S]sulphobromophthalein sodium from the rat biliary tree

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Enhanced plasma disappearance and biliary excretion of organic acids, bases and neutral compounds including some that are conjugated before excretion and some that are excreted in an unaltered form have been observed after phenobarbitone treatment (Klaassen, 1970). Phenobarbitone treatment increases the rate of biliary flow in the rats (Berthelot, Erlinger & others, 1970; Klaassen, 1970; Paumgartner, Horak & others, 1971). The enhanced bile flow was related to a bile salt—independent fraction of bile which appears of hepatocytic origin (Berthelot & others, 1970). This increase in biliary flow is not a common effect produced by all microsomal drug-metabolizing enzyme inducers, phenobarbitone (Klaassen, 1969) and polychlorinated biphenyls (Grote, Schmoldt & Dammann, 1975) being the only effective agents of those tested. In this study the effect of phenobarbitone on the biliary reabsorption of [^{35}S]sulphobromophthalein sodium from the rat biliary tree was tested by the retrograde intrabiliary injection technique.

Male Wistar rats (285–295 g) were used (Zentralinstitut für Versuchstiere, Hannover, Germany). Phenobarbitone sodium (75 mg kg^{-1}, i.p.) was injected once daily for six days. Control rats received identical volumes of saline (1·0 ml kg^{-1}). Pair feeding was performed throughout. Rats were studied within 24 h after final injection. After laparatomy the common bile duct was cannulated with polyethylene 10 tubing. The volume of the bile duct cannula was 10 μl. Following surgery [^{35}S]sulphobromophthalein sodium (^{35}S-BSP) was administered by retrograde intrabiliary injection (RII) using a micrometer syringe joined to the bile duct cannula. This technique allowed accurate administration of μl quantities and free bile flow could be reinitiated 3–5 s after RII ^{35}S-BSP (1·2 μmol, in 20 μl aqueous solution) was washed in with 30 μl NaCl solution (0·9% w/v) giving a total retrograde volume of 40 μl. Bile flow was started immediately after the retrograde administration of the dye. Bile samples were collected at 0–2·5 min, 2·5–5 min and 10–15 min after the injection. An aliquot (20 μl) of each bile sample was investigated by thin-layer chromatography on silica gel (Dammann & v. Essen, 1976). Additionally 2·5 min, 5 min, 10 min and 15 min after RII ^{35}S-BSP blood samples were collected. The radioactivity in each bile and blood sample was counted in a liquid scintillation spectrometer. Each value represents the mean with s.d. of ten animals. The data obtained were analysed using a group comparison Students *t*-test, $P < 0.05$ was considered to be significant.

^{35}S-BSP and phenobarbitone sodium were purchased from Amersham Buchler, Braunschweig and E. Merck, Darmstadt, Germany respectively.

Table 1 summarizes the results obtained. Several features merit comment: (1) percent recoveries found in the bile during the first time interval (0–2·5 min) were significantly higher in the phenobarbitone-treated group. Since bile flow in the first 2·5 min after RII surpasses biliary tree volume (Dammann, 1977) it is suggested that percent recoveries in the first 2·5 min after RII of 1·2 μmol ^{35}S-BSP also reflect the amount of reabsorbed bile salts i.e. the difference between administered amount of the dye and percent recoveries in the first 2·5 min after retrograde administration indicates the extent of reabsorption. (2) The detection of BSP-conjugates at all times gives clear evidence that biliary reabsorption has really occurred after RII of the dye. In addition ^{35}S-radioactivity was found in the bloodstream in significant concentrations 2·5, 5, 10, and 15 min after RII. ^{35}S-BSP blood concentrations were two- to threefold lower in the phenobarbitone-treated group compared with controls.

In accordance with investigations of Klaassen (1969), Berthelot & others (1970) and Paumgartner & others (1971) phenobarbitone treatment induced a significant increase in bile flow per animal (20·5 ± 4·7 *vs* 11·6 ± 2·9 μl min^{-1}, $P < 0.01$). However with the experimental conditions of the present study (pair feeding) and in contrast to Paumgartner & others (1971), no increase of the bile flow g^{-1} liver wet weight could be demonstrated (1·79 ± 0·36 *vs* 1·71 ± 0·37 μl min^{-1}, not significant). Liver wet weights differed grossly (11·4 ± 0·9 *vs* 6·8 ± 1·3 g, $P < 0.01$). Also body weights of the rats

Table 1. *Group A (phenobarbitone—treated rats, 75 mg kg^{-1} body weight i.p. once daily for six days) and group B (controls) show percent recoveries of total [^{35}S]sulphobromophthalein sodium (BSP), BSP-conjugates (BSP-C) and unconjugated BSP (BSP-U) in bile fractions at 0–2·5 min, 2·5–5 min, 5–10 min and 10–15 min when free bile flow was restarted after retrograde intrabiliary injection of 1.2 μmol [^{35}S]sulphobromophthalein sodium.*

	0–2·5	2·5–5	5–10	10–15
Group A				
BSP	70·0 ± 8·7*	3·6 ± 1·0	7·6 ± 3·6	4·5 ± 2·0†
BSP-U	63·2 ± 7·0*	2·0 ± 0·8	2·7 ± 1·4	1·1 ± 0·5*
BSP-C	1·2 ± 0·2	1·6 ± 0·6	4·3 ± 3·0	3·3 ± 2·0
Group B				
BSP	55·3 ± 6·6	3·6 ± 1·8	9·7 ± 4·9	7·7 ± 2·7
BSP-U	52·3 ± 7·4	2·2 ± 1·4	4·0 ± 1·7	2·8 ± 1·1
BSP-C	1·0 ± 0·3	1·2 ± 0·7	4·3 ± 2·2	3·5 ± 1·9

*$P < 0.05$, † $P < 0.01$.

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

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