those obtained with succinylsulphathiazole (mol. wt. 355) and sulphadimethoxine N^1 -glucuronide (mol. wt. 487), both of which are excreted extensively in rat bile unchanged (Millburn, Smith & Williams, 1967b).

3-Aminophenyl sulphate (100mg./kg.), 4-aminophenyl glucuronide (100mg./kg.), succinylsulphathiazole (50mg./kg.) and sulphadimethoxine N^1 -glucuronide (15mg./kg.) were injected intraperitoneally into biliary-cannulated rats. The concentration of each compound in the plasma, liver and bile was determined at various times up to 3 hr.

The plasma concentration of 4-aminophenyl glucuronide and 3-aminophenyl sulphate was always three to ten times greater than that of the liver, which was about the same as that of the bile. This suggests that both compounds have difficulty in entering the liver and are not excreted into bile against a concentration gradient. The distribution of succinylsulphathiazole and sulphadimethoxine N^1 -glucuronide was the reverse of that found above; at all times their concentration was in the order bile > liver > plasma. Thus, succinylsulphathiazole and sulphadimethoxine N^1 -glucuronide appear to enter the liver readily and are excreted against a concentration gradient from plasma to bile.

A comparison of the pK_a values, plasma-protein binding and the surface tension of 0.01M solutions of the four compounds showed no significant difference. However, the distribution coefficients of succinylsulphathiazole and sulphadimethoxine N^1 -glucuronide between 0.1M-phosphate buffer, pH 7.4, and the following solvents: benzyl alcohol, ethyl acetate and butan-1-ol were 20–300 times greater than those found for 4-aminophenyl glucuronide and 3-aminophenyl sulphate. It is suggested therefore that the low biliary excretion of the latter two compounds may be partly due to their poor ability to penetrate the liver and this may be related to their low lipid solubility.

M.M.A.-E.-M. is grateful to the United Arab Republic Education Bureau for a scholarship.

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The Effect of Phenobarbitone on the Biliary Excretion of Foreign Compounds by the Rat

By W. G. LEVINE, P. MILLBURN, R. L. SMITH and R. T. WILLIAMS (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Molecular size, polarity and metabolism appear

to be important factors in the biliary excretion of foreign compounds (Abou-El-Makarem, Millburn, Smith & Williams, 1967; Millburn, Smith & Williams, 1967a,b). The possible role of the endoplasmic reticulum in this process has been investigated by studying the effect of phenobarbitone pretreatment on the biliary excretion of succinylsulphathiazole, stilboestrol glucuronide and phenolphthalein glucuronide, which are known to be excreted unchanged in bile (Millburn *et al.* 1967a,b). Phenobarbitone induces a marked proliferation of the endoplasmic reticulum (Remmer & Merker, 1965).

Female albino Wistar rats (200 ± 20 g.) were injected intraperitoneally with phenobarbitone (70mg./kg.) once daily for 4 days. The bile ducts were cannulated 24 hr. after the last injection. Succinylsulphathiazole (20 or 200mg./kg.), stilboestrol glucuronide (18mg./kg.) or phenolphthalein glucuronide (81.5mg./kg.) was injected intraperitoneally, and then bile samples were collected at 15 or 20 min. intervals for 2–3 hr. The rates of biliary excretion of these compounds were unchanged after phenobarbitone pretreatment. Phenobarbitone pretreatment increases the weight and protein content of the liver (Conney, Davison, Gastel & Burns, 1960), and therefore, if the biliary excretion of the above compounds is calculated per g. of liver, the rates are actually less after pretreatment.

Phenobarbitone pretreatment, however, was found to increase the rate of biliary excretion of free stilboestrol and phenolphthalein, which are metabolized to their glucuronides before appearing in bile. This accelerated excretion is most marked immediately after the administration of the drugs. Thus in pretreated rats the biliary excretion of stilboestrol (10mg./kg. intraperitoneally) was 264% of that of the controls in 20 min., 171% in 60 min., 138% in 120 min., and 120% in 180 min. That of phenolphthalein (50mg./kg. intraperitoneally) was 142% of that of the controls in 15 min., 137% in 30 min., 120% in 60 min. and 110% in 120 min. Phenobarbitone pretreatment enhances the activity of glucuronyl transferase (Zeidenberg, Orrenius & Ernster, 1967), and therefore the enhanced rate of biliary excretion of stilboestrol and phenolphthalein appears to be related to an increased rate of conjugation with glucuronic acid.

The biliary excretion of stilboestrol and phenolphthalein can be considered to occur in at least two steps: (a) metabolism to their glucuronides, and (b) the transfer of these glucuronides from liver to bile. Our results show that phenobarbitone pretreatment stimulates (a) but not (b). Thus, although phenobarbitone readily induces the formation of microsomal membranes and their associated drug-metabolizing enzymes, it appears that the newly

formed membranes do not participate in the transfer of compounds from liver to bile.

W.G.L. is a Research Career Development Awardee of the United States Public Health Service, K3-GM-8102.

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The Effect of a Structural Modification on the Metabolism and Mode of Excretion of Naphthyl 2[³⁵S]-sulphate

By D. J. HEARSE, GILLIAN M. POWELL and A. H. OLAVESSEN (*Department of Biochemistry, University College, Cardiff*)

Previous investigations (Hearse, Powell, Olavesen & Dodgson, 1967, 1968) showed that after the administration of the potassium salts of biphenyl 4[³⁵S]-sulphate or cyclohexylphenyl 4[³⁵S]-sulphate to the rat the injected radioactivity is rapidly recovered in the urine. Hawkins & Young (1954) obtained similar results after the administration of naphthyl 2[³⁵S]-sulphate. Further investigations with biphenyl 4[³⁵S]-sulphate and cyclohexylphenyl 4[³⁵S]-sulphate showed that, in both cases, the ultimate urinary elimination is preceded by significant biliary circulation of radioactivity. However, the latter compound undergoes a much greater biliary circulation than the former. This difference in the metabolic behaviour of these two structurally very similar compounds is explicable only on the basis of the absence of aromatic character in the ring remote from the sulphate moiety of cyclohexylphenyl 4[³⁵S]-sulphate. It was decided to determine whether any biliary circulation of radioactivity could be demonstrated after the administration of naphthyl 2[³⁵S]-sulphate and, further, to investigate how the biliary excretion was effected by saturation of the ring remote from the sulphate moiety. Consequently, the metabolic fate and mode of excretion of the potassium salts of naphthyl 2[³⁵S]-sulphate and 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate were compared.

In preliminary experiments, free-ranging rats (male and female) received intraperitoneal injections (1mg./100g. body wt.) of either naphthyl 2[³⁵S]-sulphate or 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate, and urine and faeces were collected over 48hr. In all experiments up to 100% of the

injected radioactivity appeared in the urine and small amounts (5-13%) of the radioactivity appeared as inorganic [³⁵S]sulphate.

In subsequent experiments, the ³⁵S-labelled esters were administered (1mg./100g. body wt.) to male and female rats with bile-duct and ureter cannulae, and bile and urine were collected over 6hr. Over the experimental period up to 1.5% of the injected radioactivity appeared in the bile of rats receiving naphthyl 2[³⁵S]-sulphate and up to 4.7% in that of rats receiving 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate; up to 87% of the dose was recovered in the urine in both cases.

Chromatographic analysis of urine samples obtained from animals receiving naphthyl 2[³⁵S]-sulphate revealed the presence of only one major radioactive component, which was identified by isotope-dilution analysis (Dodgson, Powell, Rose & Tudball, 1961) as unchanged parent ester. Similar chromatographic analysis of urine obtained from rats receiving 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate revealed the presence of four major radioactive components, one of which has been identified as unchanged parent ester.

It seemed possible that rapid clearance of the injected radioactivity by the kidneys might account for the low biliary excretion observed with both esters. This possibility was explored by measuring the biliary radioactivity in rats in which renal function had been eliminated by ligation of the renal pedicles. Under these conditions, although the extent of biliary excretion was increased, it still represented less than 14% of the administered dose.

In experiments with biphenyl 4[³⁵S]-sulphate and cyclohexylphenyl 4[³⁵S]-sulphate, the presence of a saturated ring in the latter compound resulted in increased biliary elimination. In contrast, the present findings indicate that in the analogous situation with naphthyl 2[³⁵S]-sulphate and 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate, the presence of a saturated ring does not facilitate biliary excretion. It does, however, appear to render the molecule far more susceptible to metabolic degradation than was recorded with naphthyl 2[³⁵S]-sulphate. The results further indicate that the absence of any significant biliary elimination of the esters cannot be explained by their rapid renal clearance but may reflect the influence of certain physicochemical characteristics of the molecules on their ability to penetrate liver membranes or utilize hepatic excretory mechanisms.

D. J. H. is grateful to the Medical Research Council for a Research Studentship. The work was supported by U.S. Public Health Service Research Grant No. AMO1982 from the Arthritis and Metabolic Diseases Division.

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