diethylstilboestrol *in vivo* 24 hr. before testosterone injection does not appear to affect the overall pattern of metabolism.

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Changes in Glutathione Reductase and Glutathione Peroxidase Activities in Rat Liver Related to Age and Sex

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Enzyme activity of many enzymes has been studied during the development of the rat (Herrmann & Tootle, 1964; Dawkins, 1966; Taylor, Bailey & Bartley, 1967; Vernon & Walker, 1968a). Differences in activities of some enzymes due to sex are also known (Glock & McLean, 1953; Taylor *et al.* 1967; Roux & Tordet-Caridroit, 1967; Vernon & Walker, 1968b), although glutathione reductase and glutathione peroxidase have not been studied. Glutathione reductase (Rall & Lehninger, 1952) is an important enzyme for the maintenance of glutathione in the reduced form and possibly for controlling the redox state of NADP⁺/NADPH in tissues through glutathione peroxidase (Mills, 1960). Glutathione oxidation (Pinto, 1961) depends on xanthine oxidase activity (Jocelyn, 1964; Christophersen, 1966) or other enzymes associated with the formation of hydrogen peroxide or organic peroxides (Christophersen, 1966) that are possible substrates for glutathione peroxidase (O'Brien & Little, 1968).

This communication reports changes in glutathione reductase and glutathione peroxidase activities in rat liver from foetal to adult life and during the oestrous cycle, and also differences of enzyme activities between pregnant and non-pregnant females.

The average glutathione reductase activities in male and female rat livers were 10.4 ± 0.8 and 9.0 ± 1.3 units respectively. Glutathione peroxidase activities were 48 ± 5 units (males) and 81 ± 15 units (females). The larger scatter in females is due to the oestrous cycle. Glutathione reductase activity in dioestrus was 10.3 ± 1.1 units and in oestrus 8.1 ± 0.7 units. Glutathione peroxidase activity in dioestrus was 64 ± 7 units and in oestrus 96 ± 7 units. Intermediate values for both enzymes activities were found during pro-oestrus and metoestrus.

Pregnant rats had about the same activities as in oestrus.

Glutathione reductase activity in the foetus increased till birth (7 units). After birth the activities showed two maxima at about 2 (11 units) and 32 days old (13 units), and one minimum at about 16 days (5.8 units). Glutathione peroxidase activity in the foetus increased till birth (18 units) and continued to increase till maturity when there was a sharp increase to adult values in the female. Differences due to sex appeared after 32 days.

These enzyme changes are associated with changes of diet and hormones during development.

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Lipid Solubility and the Low Biliary Excretion of 3-Aminophenyl Sulphate and 4-Aminophenyl Glucuronide by the Rat

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Previous studies on the biliary excretion of foreign compounds indicate the importance in this process of several factors, namely molecular size, polarity and metabolism (Abou-El-Makarem, Millburn, Smith & Williams, 1967; Millburn, Smith & Williams, 1967a). For extensive biliary excretion in the rat a minimum molecular weight (about 325 ± 50) and a polar ionizable group appear to be necessary.

We decided to try to find out why low-molecular-weight benzene derivatives are excreted poorly by rats in bile. We therefore studied the biliary excretion and the distribution between plasma, liver and bile in biliary-cannulated rats of two such compounds, namely 3-aminophenyl sulphate (mol.wt. 189) and 4-aminophenyl glucuronide (mol.wt. 285). The findings were compared with

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.

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

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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