Intestinal Azo Reduction and Glucuronide Conjugation of Prontosil

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After oral administration to adult female Wistar albino rats, the lipid-soluble azo-dye Prontosil (2,4 - diamino - 4' - sulphonamidoazobenzene) was largely excreted in the urine as total sulphanilamide (free and *N*⁴-acetylated). Repeated treatment with antibiotics, which decreased both the numbers of caecal micro-organisms and the azo reductase activity of the whole caecal contents, markedly decreased the output of urinary sulphanilamide (Gingell, Bridges & Williams, 1971).

The effect of this antibiotic treatment on the absorption of [³⁵S]Prontosil by everted rat intestinal sections was investigated by the method of Wiseman (1956). After incubation of everted sacs for 1 h in oxygenated Krebs saline saturated with [³⁵S]-Prontosil, the sac contents were chromatographed and assayed (Gingell *et al.* 1971). The major metabolite present in duodenal sections was Prontosil *N*-glucuronide (59% of the total ³⁵S absorbed), together with unchanged dye (32%) and traces of sulphanilamide. This mucosal conjugation probably plays an important role in the absorption process and constitutes a detoxication mechanism for the dye. Prontosil *N*-glucuronide is also formed in the liver and excreted in the bile after intravenous administration of Prontosil to rats (Gingell *et al.* 1971).

The absorption of Prontosil (both unchanged and conjugated) from everted duodenal sacs obtained from the antibiotic-treated rats was significantly diminished (51–61% of control values), indicating that the effects of oral antibiotic treatment may not be confined to the intestinal flora. Antibiotic mixtures containing neomycin are known to interfere with the absorption of some compounds (Rothfeld & Osborne, 1963).

The intestinal site of bacterial and/or mucosal azo reduction, and the relative importance of mucosal and hepatic glucuronide conjugation of Prontosil, were determined by measuring the metabolites in bile and urine hourly for 6 h after intravenous, intraduodenal and intracaecal administration of 5 mg of [³⁵S]Prontosil to anaesthetized rats with bile-duct and bladder cannulae. Over the initial 3 h 2–3 times as much Prontosil *N*-glucuronide appeared in the bile after intraduodenal administration as that after intravenous administra-

tion, suggesting that at this dose intestinal mucosal conjugation is quantitatively more important than hepatic conjugation. Little dye appeared in the urine or bile after intracaecal administration of Prontosil, but total sulphanilamide appearing in the urine was much greater than after intraduodenal (45% of the value after intracaecal administration) or intravenous (10% of the value after intracaecal administration) administration. Since little sulphanilamide was excreted in the urine after intravenous administration of Prontosil to these bile-duct-cannulated rats, hepatic azo reduction appears to be quantitatively insignificant. These results imply that, although some azo reduction of Prontosil does occur in the duodenum, most of the sulphanilamide appearing in the urine after oral administration is produced in the large intestine, probably after enterohepatic circulation, by the action of the caecal microflora.

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The Biliary Excretion of Hydroxyethyl-rutinosides and other Flavonoids in the Rat

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Recent studies of flavonoid metabolism have centred on the role of the intestinal microflora and the elimination of phenolic metabolites in the urine (Nakagawa, Shetlar & Wendar, 1965; Das & Griffiths, 1969). However, it has now been observed that intraperitoneal administration to albino rats of 3',4',7-tri-*O*-(β -hydroxyethyl)rutoside and 3',4',5,7-tetra-*O*-(β -hydroxyethyl)rutoside, which are the major components of the vascular therapeutic agent Paroven, results in their aglycones being extensively voided in faeces. As this suggested a high rate of biliary excretion, studies have been carried out with bile-duct-cannulated rats on the routes of excretion of 3',4',7-tri-*O*-(β -hydroxyethyl)rutoside, 3',4',5,7-tetra-*O*-(β -hydroxyethyl)rutoside, phlorrhizin, naringin, rutin and (+)-catechin.

After oral or intravenous administration of 3',4',7-tri-*O*-(β -hydroxyethyl)rutoside at least four metabolites were detected in the bile collected over the first 24 h. The major metabolite was identified by chromatographic and spectral studies as the

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administered glycoside. The corresponding aglycone, arising from microfloral degradation of the glycoside, was voided in the faeces after oral administration. Neither the glycoside nor the aglycone was detected in the urine of rats receiving 3',4',7-tri-*O*-(β -hydroxyethyl)rutoside orally. When 3',4',5,7-tetra-*O*-(β -hydroxyethyl)rutoside was administered either orally or intravenously the glycoside was detected in both bile and urine for at least 30h. The corresponding aglycone was voided in the faeces after oral dosage.

A quantitative procedure, utilizing band I of the u.v. spectra of these glycosides (Courbat, Uhlmann & Guerne, 1966), was employed to determine the amount of glycoside plus related metabolites voided in the bile and urine. After intravenous administration it was found that over a 24h period 50–60% of the 3',4'-7-tri-*O*-(β -hydroxyethyl)rutoside was voided in the bile, with 6–30% in the urine; and that 12–30% and 24–54% of the 3',4',5,7-tetra-*O*-(β -hydroxyethyl)rutoside were voided in the bile and urine respectively. The amounts voided over 24h in the bile and urine after oral administration of either glycoside were less than 10% of the dose.

Administration of naringin, phlorrhizin and (+)-catechin orally and intraperitoneally resulted in the excretion of flavonoid conjugates in both bile and urine. Unchanged naringin and (+)-catechin were also detected in the bile after dosage by either route, but phlorrhizin was only detected in the bile after intraperitoneal administration. Unchanged rutin was not detected in the bile or urine after oral or intraperitoneal administration. This may be attributable to the low solubility of rutin, resulting in retention of the compound at the site of injection or within the alimentary tract (Clark & Mackay, 1950). The findings indicate that biliary excretion, with subsequent elimination in the faeces, may be of major importance in the excretion of certain flavonoids.

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The Biliary Excretion of Anions of Molecular Weight 300–800 in the Rat, Guinea Pig and Rabbit

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Abou-El-Makarem, Millburn, Smith & Williams (1967) showed that anions of molecular weight less than about 300 were poorly excreted in the bile of all species examined. With compounds of molecular weight 300–500 there was a species variation in the extent of biliary excretion, the rat, dog and hen being good biliary excretors but the rabbit, guinea pig and rhesus monkey being poor biliary excretors. A perusal of the literature suggested that there might not be a marked species variation in the biliary excretion of compounds of molecular weight greater than about 500 and that such compounds would be extensively excreted in the bile (see Millburn, 1970; Smith, 1970).

The biliary excretion of 11 anions with molecular weights between 354 and 752 was examined in the female rat, guinea pig and rabbit. The compounds were injected intravenously into bile-duct-cannulated animals, and the bile and urine were collected for 3h, analysed colorimetrically and chromatographed to check that the compounds were excreted unchanged. The compounds examined and the molecular weights of their anions were succinylsulphathiazole (354), phthalylsulphathiazole (402), hexahydrophthalylsulphathiazole (408), tartrazine (465), phenolphthalein disulphate (494), phenolphthalein glucuronide (511), tetrachlorophthalylsulphathiazole (540), Bromochlorophenol Blue (580), Bromophenol Blue (669), Bromocresol Green (697) and Indocyanine Green (752).

For the first six compounds a marked species difference was found in the extent of their biliary excretion, which was in the order rat > guinea pig > rabbit, e.g. hexahydrophthalylsulphathiazole (% of dose in bile), rat (80), guinea pig (44), rabbit (8). With tetrachlorophthalylsulphathiazole the rat and guinea pig excreted 50–60% and the rabbit 31% of the dose in the bile. The remaining four compounds, which have the highest molecular weights, were excreted in the bile to the extent of 70% or more of the dose by all three species.

These results support the above suggestion that compounds with molecular weights of about 500 or more are extensively excreted in the bile of most species. Further, there appears to be a minimum molecular weight, which varies with species, above which biliary excretion becomes significant (i.e. greater than 10% of the dose). For the rat this value is about 325 ± 50 (Millburn, Smith & Williams, 1967) and all the above compounds were