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.

We thank Professor R. T. Williams for his interest in this work, and Roche Products Ltd. for a grant.

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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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