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

excreted in relatively large amounts in rat bile. In the guinea pig the minimum molecular weight for significant biliary excretion appears to be about 400 ± 25 and in the rabbit it appears to be about 500 ± 50 .

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Some Aspects of the Metabolism of ^{14}C -Labelled ($\pm$)-2,3-Dehydroemetine in the Rat

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Female Sprague-Dawley rats of the same age and weight (200g) were operated upon under sodium pentothal anaesthesia for cannulation of the common bile duct close to the liver, to afford continuous collection of bile. After the operation the animals were placed in restraining cages with access to chow and tap water. They were injected intravenously with ^{14}C -labelled (at the 3'-position) racemic 2,3-dehydroemetine dihydrochloride in freshly prepared saline solution (0.5ml) at a dose of $4\mu\text{Ci}/\text{mg}$ per 200g rat, and samples of bile and urine were collected continuously over a period of 3 days. Faeces were also collected.

Under these conditions radioactivity was detected in the urine and in the bile, which accounted for 33% of the administered dose in 3 days. The rate of excretion of radioactivity in bile and urine diminished rapidly after the first 24h. The almost complete absence of radioactivity from the faeces suggested that 2,3-dehydroemetine was not excreted through the intestinal wall, as might have been expected from the high yields of radioactivity in the faeces of non-cannulated animals treated with this drug by Schwartz & Herrero (1965).

Ascending t.l.c. (Gelman Instrument Co. chromatography media ITLC type SA) with solvent A [chloroform-acetone-diethylamine (5:4:1, by vol.)] and solvent B [benzene-ethyl acetate-diethylamine

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(8:2:1, by vol.)] as described by Randerath (1966), followed by scintillation counting of excised areas of the chromatograms in Bray's (1960) solution, established that the radioactivity in bile in all samples other than those collected in the first hour was not equivalent to free 2,3-dehydroemetine (R_F values 0.88 and 0.63 respectively), but appeared to be some kind of highly polar metabolite (R_F 0).

Treatment of bile with bacterial β -glucuronidase at pH 5 under the conditions described by Robinson, Smith & Williams (1953) released an appreciable amount of radioactivity migrating with the original compound, indicating that an *N*-glucuronide of 2,3-dehydroemetine is formed and excreted via the bile. Two apparent metabolites of 2,3-dehydroemetine are also released, with R_F values 0.60 and 0.20 in solvent A, representing approx. 21 and 12% respectively of the biliary radioactivity. The chromatography of enzyme-treated bile revealed that some 50% of the total radioactivity did not migrate (R_F 0), which suggests that there may be other conjugates or very polar metabolites. To put this to the test, portions of bile were hydrolysed with arylsulphatase from limpets at pH 5, but no sulphate conjugation could be demonstrated in this way.

Urine contained appreciable amounts of free 2,3-dehydroemetine. Chromatography with solvent A led to the detection of radioactive peaks with R_F values 0.56, 0.30 and 0.10, and radioactivity at the origin. Treatment of urine with β -glucuronidase or with arylsulphatase did not change the pattern of detectable radioactive compounds, which indicates that glucuronide and sulphate conjugates of 2,3-dehydroemetine or its metabolites are not present in the urine.

The possibility that 2,3-dehydroemetine might be *O*-demethylated was examined by incubating liver microsomal preparations from control and stimulated sodium phenobarbital-pretreated (80 mg/kg body wt. daily for 3 days) rats *in vitro* with 2,3-dehydroemetine as substrate and the full supporting system described by Jondorf, Simon & Avnimelech (1966), and measuring formaldehyde formed as a product of metabolism. We found no evidence for oxidative demethylation under these circumstances. Further, by incubating each of the microsomal preparations with chromatographically purified labelled 2,3-dehydroemetine under the same scaled-down general conditions and chromatographically analysing the end products of the metabolic reaction, we found that unchanged 2,3-dehydroemetine could be recovered in 95-100% yield.

This appears to indicate that the polar metabolites detectable with our chromatographic procedures were not likely to be *O*-demethylation products.