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Reabsorption from the Biliary System as a Factor Influencing the Biliary Excretion of Organic Anions

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Although the mechanism of biliary excretion is obscure (Sperber, 1959; Schanker, 1968; Wheeler, 1968), much is known about the properties of the compounds that are eliminated in the bile. Molecular size, polarity and structural features are important factors in determining the extent of biliary excretion of organic molecules (Abou-El-Makarem, Millburn, Smith & Williams, 1967; Millburn, Smith & Williams, 1967a,b; Hirom, Millburn, Smith & Williams, 1969). Thus large organic anions (mol.wt. > 300) are usually extensively excreted in the bile of the rat whereas small ones (mol.wt. < 300) are not. It would seem reasonable to suppose that this difference may be related in some way to the mechanism of bile formation. It has been suggested that bile, during its passage down the biliary tract, may be modified in composition possibly by secretion and reabsorption of water and solutes (Andrews, 1955; Brauer, 1959; Sperber, 1959; Goldfarb, Singer & Popper, 1963; Wheeler, 1968). However, evidence for a reabsorption process in bile formation is scanty (Leong, Holloway & Brauer, 1955; Barber-Riley, 1963; Mallet-Guy, Michoulier & Baev, 1965). If small organic anions are reabsorbed from the primary bile this might explain their low biliary excretion. Therefore we have investigated the reabsorption from the biliary tree of small anions (mol.wts. 179–285), which are poorly eliminated in bile, and larger anions (mol.wts. 445–752), which are extensively excreted in the bile of the rat.

Aqueous solutions of the compounds (20 μ moles/kg.) were administered by retrograde injection during 1–2 min. into a short cannula inserted in the common bile duct of anaesthetized rats. After the infusion, the bile was collected for 30 min. and analysed. With the small anions (hippuric acid, 3-aminophenyl sulphate, succinylsulphanilamide and 2-aminophenyl glucuronide) only 7–15% of

the infused compounds was recovered in the bile whereas 50–60% was found in the urine collected from the bladder. However, with the larger anions (stilboestrol glucuronide, phenolphthalein glucuronide, phenolphthalein disulphate and Indocyanine Green) 50–80% was recovered in the bile and less than 2% in the urine. Thus small but not large organic anions appear to be readily absorbed from the biliary tree after their retrograde injection.

These observations suggest that the low biliary excretion of small organic anions may be due to their reabsorption from the primary bile.

A. G. C. is grateful to the New Zealand University Grants Committee for financial support and to the Wellcome Foundation for a Travel Grant.

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Bile Secretion in Relation to Decreases in Liver Adenosine Triphosphate Concentration in the Rat

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The relationship between the concentration of ATP in liver and bile secretion has been studied. In this paper the effects on bile flow of two agents that decrease the concentration of ATP in rat liver *in vivo* will be considered.

2,4-Dinitrophenol was studied since it is an active uncoupling agent *in vitro* and was expected to decrease liver ATP concentration after parenteral administration. Intraperitoneal doses up to 70 mg./kg. body wt., however, had little effect either on

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bile flow or on liver ATP concentration. Liver and blood analyses indicated that 2,4-dinitrophenol was not entering the liver in significant amounts, probably as a result of a tight binding to plasma albumin (Weinbach, Garbus & Claggett, 1966; Schimassek, 1968). Displacement from the albumin site was attempted by using anionic competitors such as ethyl α -(4-chlorophenoxy)- α -methylpropionate and sodium salicylate. The subsequent effects on bile flow with ethyl α -(4-chlorophenoxy)- α -methylpropionate as the displacing agent were inconsistent and were accompanied by a high mortality rate. Displacement of 2,4-dinitrophenol (20mg./kg. body wt.) by sodium salicylate (60mg./kg. body wt.) gave a decreased bile flow of 27% in the first hour and 12% in the second hour compared with the salicylate controls. At the end of the second hour the liver ATP concentration had decreased to 58% of the control value. As the maximal depression of bile flow occurred 30–45 min. after the injection of 2,4-dinitrophenol plus sodium salicylate, changes in total homogenate and supernatant ATP concentration were investigated at 30 min. after dosing, by using the micro filtration procedure of Delaney & Slater (1967). The results showed that the percentage of total homogenate ATP in the cell-sap fraction was not affected by treatment with 2,4-dinitrophenol plus salicylate, although total homogenate ATP and the total ATP content in the cell-sap fraction of the whole liver were decreased.

The intraperitoneal injection of 40m-moles of fructose/kg. body wt. after 24hr. starvation has been reported to produce a rapid depression of liver ATP concentration (Burch, Max, Chyn & Lowry, 1969). By using this dose it was found that a drop in bile flow of 46% occurred in the first hour and 37% in the second hour after injection; there was a decrease of 40% in total liver ATP by the end of the second hour. These results will be discussed in terms of the role of liver ATP in bile-flow mechanisms.

M.N.E. is grateful to the Science Research Council for a Research Studentship.

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Multiple Electrophoresis Peaks of Rat Liver Decarboxylases for 3,4-Dihydroxyphenylalanine and 5-Hydroxytryptophan

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Evidence is accumulating that catecholamines and indolylalkylamines are involved in the development of certain abnormal mental states. For example, Bunney *et al.* (1969) suggest that there are two types of 'depression', biochemically characterized by deficiency of either catecholamines or indolylalkylamines and possibly corresponding to the clinical states of retarded (inhibited) and agitated (psychotic) depression respectively.

The enzymic activities leading to the formation of these amines, 3,4-dihydroxy-L-phenylalanine (DOPA) decarboxylase and 5-hydroxy-L-tryptophan decarboxylase have been given different EC numbers (4.1.1.26 and 4.1.1.28 respectively), but have been reported as associated with a single protein (e.g. from rabbit kidney cortex; Rosengren, 1960). On the other hand, Marmaras, Sekeris & Karlson (1966) have shown simultaneous induction of 3,4-dihydroxyphenylalanine decarboxylase and repression of 5-hydroxytryptophan decarboxylase activities by ecdysone in insect larvae.

Before the significance of catecholamine and indolylalkylamine concentrations in human depression can be evaluated, the nature of the decarboxylase systems that produce them in various mammalian tissues must be clarified.

A sensitive radioisotopic assay for the decarboxylases has been developed in this laboratory, based on the procedure of Karlson & Sekeris (1962), with 3,4-dihydroxyphenyl[2- ^{14} C]alanine and 5-hydroxy-[methylene- ^{14} C]tryptophan. The products are separated from the substrates by one-dimensional paper chromatography, and the radioactivity on the paper is measured by liquid-scintillation counting, the paper being placed directly into toluene scintillator.

Male rat liver homogenate was centrifuged at 10⁵g for 60 min. In this supernatant there was a marked pH-dependence of enzymic activity, with 3,4-dihydroxyphenylalanine decarboxylase activity maximal at pH 6.8 and 5-hydroxytryptophan decarboxylase activity maximal at pH 7.8, in agreement with other workers (Reid, Riley & Shepherd, 1963).

The supernatant was subjected to electrophoresis by the preparative technique of Brownstone (1969), with a 5% total monomer polyacrylamide gel at pH 8.7 in glycine-tris buffer, and with a potential difference of 170 v across a 45 mm.-thick gel. Four peaks of decarboxylase activity were recovered.