slowly, but anaerobically both D(-)- and L(+)-lactate were rapidly oxidized to acetate by the shikimate $\rightarrow$ dihydroshikimate reduction. The reaction proceeded stoichiometrically as follows:

A flavin compound may be involved since addition of riboflavin, FMN or FAD accelerated the reaction. Also, addition of oxalate, an inhibitor of NAD-independent lactate dehydrogenases (Snoswell, 1963), initially strongly inhibited oxidation of L-lactate by the shikimate reductase system. It was not found possible to bring about the reverse process, oxidation of dihydroshikimate to shikimate aerobically, even in the presence of phenazine methosulphate.

The suggestion that quinate $\rightleftharpoons$ shikimate $\rightleftharpoons$ dihydroshikimate might act similarly to malate $\rightleftharpoons$ fumarate $\rightleftharpoons$ succinate as an oxidation-reduction system in plant tissues was made earlier (Hulme, 1951).

Homofermentative *lactobacilli* showed a more complex pattern of quinate and shikimate metabolism. Adaptive enzyme systems resulted in the formation of an acidic product (I) and of catechol. The acid (I) was obtained with m.p. 150° and $[\alpha]_D^{18.5} -18^\circ$ (c 1.0 in water). It was identified by mass and n.m.r. spectra, together with acetyl lactone formation, as all-*cis*-3,4-dihydroxycyclohexane-1-carboxylic acid. Dihydroshikimate also was reduced to acid (I), but no catechol was formed. Both D- and L-lactate were oxidized anaerobically by adapted cells with concomitant reduction of quinate, shikimate or dihydroshikimate to acid (I).

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Metabolites of Sulphadimethoxypyrimidine in the Urine of Various Species

By S. R. WALKER* and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London W.2*)

2,4-Dimethoxy-6-sulphanilamidopyrimidine (sulphadimethoxine) is excreted by man and other primates mainly as an N^1 -glucuronide (Adamson, Bridges & Williams, 1966) in which glucuronic acid is attached to the sulphonamide nitrogen (N^1)

* Present address: Department of Clinical Pharmacology, Institute of Diseases of the Chest, Brompton, London S.W.3.

(Bridges, Kibby & Williams, 1965). This glucuronide is formed in only minor amounts in several lower mammals and not at all in rabbits (Bridges, Kibby, Walker & Williams, 1968). Further, if the positions of the methoxy groups in the pyrimidine ring of the drug are altered or if one is removed from the 2- or 4-position, the ability to form an N^1 -glucuronide *in vivo* in man or the rhesus monkey is considerably diminished or abolished (Bridges, Kibby, Walker & Williams, 1969). If the 2- and 4-methoxyl groups are replaced by methyl groups N^1 -glucuronide formation is abolished, but if the 2-methoxyl group only is replaced by a methyl group, as in 4-methoxy-2-methyl-6-sulphanilamidopyrimidine (sulphamethomidine), the N^1 -glucuronide formation still occurs in man and the monkey (Bridges, Walker & Williams, 1969). The formation of N^1 -glucuronides in man and monkey seems to be associated with the presence of certain groups (e.g. methoxyl) in the two positions *meta* to the attachment of the sulphanilamido group to the pyrimidine ring. This arrangement is found in the drug sulphadimethoxypyrimidine or 4,6-dimethoxy-2-sulphanilamidopyrimidine.

This drug was administered orally to monkey, rabbit, rat (each 100 mg./kg.) and man (30 mg./kg.). In the rabbit 66% of the dose was excreted in the urine in 24 hr., but no N^1 -glucuronide was excreted. The main metabolites were the unchanged drug and its N^4 -acetyl derivative in roughly equal amounts (25–30%). In two rhesus monkeys 50 and 70% of the dose was excreted in 24 hr. and the main metabolites now were the N^4 -acetyl derivative and the N^1 -glucuronide in roughly equal amounts (20–25% of the dose). In man and the rat the drug was very slowly excreted, man excreting 10% and the rat 6% of the dose in 24 hr. In man the main metabolite was the N^1 -glucuronide, which amounted to 2.9% of the dose in 24 hr., whereas in the rat more than half the 24 hr. excretion was unchanged drug, the N^1 -glucuronide amounting to 0.4% of the dose.

Thus the N^1 -glucuronide is a major metabolite of sulphadimethoxypyrimidine in the urine of the rhesus monkey, but this metabolite is not formed at all in the rabbit.

A comparison of the physical properties of the three drugs forming N^1 -glucuronides in primates, i.e. 2,4-dimethoxy-6-sulphanilamidopyrimidine, 4-methoxy-2-methyl-6-sulphanilamidopyrimidine and 4,6-dimethoxy-2-sulphanilamidopyrimidine, provided no information on the structural specificity of this conjugation.

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

By A. G. CLARK,* P. C. HIROM, P. MILLBURN, R. L. SMITH and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London W.2*)

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.

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

By M. N. EAKINS, T. F. SLATER and V. B. DELANEY. (*Department of Chemical Pathology, University College Hospital Medical School, London W.C.1*)

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

* Present address: Department of Biochemistry, Institute of Psychiatry, Maudsley Hospital, Denmark Hill, London S.E.5.