The Role of Gut Bacteria in the Aromatization of Quinic Acid in Different Species

By R. H. ADAMSON,* J. W. BRIDGES,† M. E. EVANS and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Adamson, Bridges & Williams (1966) showed that (—)-quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid) given orally was excreted in the urine as hippuric acid in man and some Old-World monkeys but not in some New-World monkeys and lemurs. This work has now been extended to 22 species. Oral doses (0.3 g./kg.) of quinic acid were aromatized to the extent of 20–70% in man, rhesus monkey, baboon and green monkey, but only to 0–10% (usually near zero) in the other species including spider monkey, squirrel monkey, capuchin, bush-baby, slow loris, tree shrew, dog, cat, ferret, rabbit, rat, mouse, guinea pig, hamster, lemming, fruit bat, hedgehog and pigeon.

Further studies were made in the rhesus monkey and rat. No aromatization occurred in the monkey if the quinic acid was injected intraperitoneally. If the monkeys were pretreated for 4 days orally with neomycin (2 g./day) to suppress the gut flora, the excretion of hippuric acid after oral doses of quinic acid was decreased to almost zero, but the ability to aromatize slowly returned over 20–30 days. These results strongly suggest that gut bacteria were responsible for the aromatization of quinic acid. When three rhesus monkeys were given [^{14}C]quinic acid orally, 20–25% of the dose was excreted in the urine as labelled hippuric acid. In two of the monkeys there was no unchanged quinic acid in the urine and in the third about 5% was excreted unchanged. In six rats, 20–50% of the orally administered quinic acid was oxidized to CO_2 ; the hippuric acid output as determined by isotope dilution was 0, 0.05, 0.13, 0.6, 3.6 and 11.3% for the six animals, and the output of unchanged quinic acid was 0% in two rats and 5–33% in the other four.

The results obtained suggest that the species differences found in the aromatization of quinic acid are dependent in part on gut bacteria. However, quinic acid is aromatized aerobically *in vitro* by faecal homogenates from man and rat; this aspect is being investigated.

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* Present address: National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014, U.S.A.

† Present address: Department of Biochemistry, University of Surrey, Falcon Road, London, S.W. 11.

The Role of Gut Flora in the Enterohepatic Circulation of Stilboestrol in the Rat

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

In the rat, stilboestrol is eliminated mainly in the bile as a monoglucuronide and after enterohepatic circulation is excreted in the faeces (Hanahan, Daskalakis, Edwards & Dauben, 1953; Fischer, Millburn, Smith & Williams, 1966). The recirculation depends on hydrolysis in the intestine of the monoglucuronide to stilboestrol, which is reabsorbed (Fischer *et al.* 1966). This hydrolysis has now been found to be enzymic, since intraduodenal infusion of [^{14}C]stilboestrol monoglucuronide (10 mg./kg.) together with glucaro-1,4-lactone, a β -glucuronidase inhibitor (Levy, 1952), decreases the rate of absorption of the ^{14}C by 90% compared with that in the absence of the inhibitor. Glucaro-1,4-lactone did not affect the intestinal absorption of free [^{14}C]stilboestrol (2 $\mu\text{g.}/\text{kg.}$).

Rats were given 100 mg. of neomycin sulphate orally twice daily for 4 days to suppress gut bacteria. On the fifth day [^{14}C]stilboestrol monoglucuronide (10 mg./kg.) was injected intraduodenally. The rate of intestinal absorption of ^{14}C was 50 times smaller than that in rats not given neomycin. However, neomycin treatment did not affect the absorption of free [^{14}C]stilboestrol (2 $\mu\text{g.}/\text{kg.}$). Similar results were found in rats treated in an identical fashion with kanamycin. These experiments suggest that the gut micro-organisms are the source of β -glucuronidase for the intestinal hydrolysis of stilboestrol monoglucuronide.

Fischer *et al.* (1966) showed that, apart from the monoglucuronide, three other metabolites, amounting to 25% of the dose, appear in the bile of rats given stilboestrol intraperitoneally. ^{14}C -labelled samples of these metabolites have now been obtained from rat bile by paper chromatography. When these metabolites were given intraduodenally, their rate of intestinal absorption was less than one-tenth that of stilboestrol monoglucuronide. They are therefore poorly absorbed and pass out in the faeces.

Stilboestrol is thus excreted in the rat mainly in the bile as its monoglucuronide together with three minor metabolites. The glucuronide is apparently hydrolysed by gut bacteria to stilboestrol, which is reabsorbed, further metabolized and re-excreted in the bile. The other three metabolites are poorly reabsorbed and appear to be important in the final elimination of stilboestrol in the faeces.

* Present address: Department of Pharmacology, Oakdale Toxicology Center, University of Iowa, Oakdale, Iowa, U.S.A.