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

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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 (0·3g./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 (2g./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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The Role of Gut Flora in the Enterohepatic Circulation of Stilboestrol in the Rat

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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 (10mg./kg.) together with glucaro-1,4-lactone, a β-glucuronidase inhibitor (Levvy, 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 (2μg./kg.).

Rats were given 100mg. of neomycin sulphate orally twice daily for 4 days to suppress gut bacteria. On the fifth day [^{14}C]stilboestrol monoglucuronide (10mg./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 (2μg./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 metabolities, 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.

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Fractionation of Nucleic Acids by the use of Poly-L-lysine–Kieselguhr Columns

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Nucleic acids have been separated and fractionated by a wide variety of methods, the most versatile of which is column chromatography.

Benzolated DEAE-cellulose (Sedat, Kelly & Sinsheimer, 1967) I.R.C.-50a (a polycarboxylic resin, in the Mg^{2+} form), (Frankel & Crompton, 1962), hydroxyapatite (Bernardi, 1965) and methylated albumin-coated kieselguhr (Mandell & Hershey, 1960) are among the methods that have been used to try to obtain some degree of resolution between DNA and RNA and to differentiate between DNA molecules differing in some physical or chemical property.

Ayad & Blamire (1968) showed that elution of the DNA of *Bacillus subtilis* strain Marburg from poly-L-lysine kieselguhr (PLK) columns with a linear gradient of increasing salt concentration gave an elution profile that, when continuously monitored at 257mμ, contained three distinct peaks.

In the present study PLK fractionation, which appears to depend mainly on the base composition of the nucleic acids and to a lesser extent on the secondary structure, was used to separate mixtures of RNA and DNA prepared by the method of Marmur (1961) from *B. subtilis* strain Marburg. The RNA peak appeared as a separate component, at 1·0–1·1M-NaCl, well separated from the main DNA peak, at 2·0M-NaCl, and was identified by assaying the fractions obtained from a standard PLK elution by using the orcinol assay for RNA (Albaum & Umbreit, 1948) and the diphenylamine assay for DNA (Burton, 1956). Treatment of the mixture, or fractions, with ribonuclease removes this peak, leaving only the standard DNA elution profile.

The region between the RNA and DNA peaks is being investigated and appears from the initial results to contain 'protected' DNA. The form this protection takes is not certain, but RNA and protein are both present and the amount of each can be decreased by extensive deproteinization with agents such as chloroform–3-methylbutan-1-ol, followed by further ribonuclease treatment.

The main DNA peak, whose elution commences at 2·0M-NaCl, was assayed for transforming activity by using *B. subtilis* strain 31, which is auxotrophic for histidine and tryptophan. It was found that when individual fractions across this peak, which are successively higher in guanine–cytosine content, were assayed for both markers the histidine gene was found to be eluted earlier than the tryptophan gene. Thus the results suggest that at least a partial separation has been achieved of DNA corresponding to the genes associated with the synthesis of histidine and tryptophan.

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Quenching of Fluorescence of Deoxyribonucleic Acid-Bound Hydrocarbons

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When a polycyclic hydrocarbon such as benzo[*a*]pyrene is solubilized in aqueous DNA solution, the fluorescence maxima are shifted to longer wavelengths compared with those in free solution and the overall intensity is considerably decreased (Boyland & Green, 1962, 1964; Liquori, De Lerma, Ascoli, Botré & Trasciatti, 1962).

Under conditions favouring hydrocarbon solubilization (i.e. with DNA dissolved in glass-distilled water) the intensity of benzo[*a*]pyrene or perylene fluorescence in such solutions consistently decreases as the pH is lowered from 7·5 to 5, though the extent of the quenching varies (generally three- to seven-fold). The ultraviolet-absorption spectrum of the DNA indicates that cytosine residues are starting to be titrated over this pH range under these conditions of low ionic strength.

Addition of Ag^+ ions to such DNA–benzo[*a*]pyrene solutions (Green, 1967) at a silver nitrate/DNA phosphate ratio $< 0{\cdot}15$ causes a similar quenching of hydrocarbon fluorescence. The