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Bile and Faecal Porphyrins in the Quiescent Phase of Variegate Porphyria

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Recent studies of bile in the quiescent phase of variegate porphyria (Smith, Belcher, Mahler & Yudkin, 1968) by the counter-current technique of French & Thonger (1966) separated the ether-soluble fraction of bile into four porphyrin peaks.

By using lutidine chromatography on paper (Eriksen, 1958) and on silica gel (S. G. Smith & R. V. Belcher, unpublished work), and also by measuring the Soret maxima in 5% HCl and noting the tube position in the counter-current apparatus, it was possible to identify the first peak as coproporphyrin III and the third peak as protoporphyrin.

The second peak was resolved into two porphyrins, a dicarboxyl porphyrin with a Soret maximum at 404.5 m μ , and a tricarboxyl porphyrin with a maximum at 404 m μ . The fourth peak consisted of protoporphyrin and also a monocarboxyl porphyrin with a maximum at 423 m μ , which was therefore identified as a chlorophyll derivative.

Further work on the residue after ether extraction by Sephadex G-25 fractionation (Rimington & Belcher, 1967) of the hydrophilic 'X' porphyrins (Rimington, Lockwood & Belcher, 1968) separated out four fractions with Soret maxima at 407, 406, 405 and 403 m μ in probable order of molecular weight. A further insoluble fraction showed characteristic pink fluorescence and was also assumed to be a high molecular weight 'X' porphyrin.

Counter-current studies of a 24 hr. specimen of faeces from the period immediately before the bile collection was next done by the technique of French, England, Lines & Thonger (1964). The ether-soluble faecal porphyrins also separated into four peaks. Coproporphyrin III and protoporphyrin were again found, this time as the first and fourth peaks respectively. The second peak consisted of a dicarboxyl porphyrin with a Soret maximum at 403 m μ . The third peak contained two porphyrins, one being readily identified as protoporphyrin, the other being a tricarboxyl porphyrin with a Soret maximum at 406.5 m μ .

Preliminary work on the residue after ether extraction indicated fractions similar to those found in the bile and is so far in agreement with the findings of Rimington, Lockwood & Belcher (1968).

All the porphyrins described, including the chlorophyll derivative, gave aetioporphyria spectra in the visible range. None of the ether-soluble porphyrins from bile or faeces were shown to be protein-bound by the ^{14}C -labelled fluorodinitrobenzene method of Sanger (1945).

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The Effects of Sporidesmin and Icterogenin on Bile Flow in the Isolated Perfused Rat Liver

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Administration of the fungal toxin sporidesmin to sheep (Mortimer & Taylor, 1962), rats (Slater & Griffiths, 1963) and other species is known to cause severe disturbances in the secretion of bile. Bile flow virtually stops 24 hr. after the administration of 6 mg. of sporidesmin/kg. body wt. to rats, but returns to the normal value 3 days after dosing. The plant triterpene acid icterogenin likewise produces a severe inhibition of bile flow both in rabbits (Heikel, Knight, Rimington, Ritchie & Williams, 1960) and in rats (T. F. Slater & B. E. Cooper, unpublished work). Sporidesmin and icterogenin are only slightly soluble in biologically inert solvents, and investigation of their mechanisms of action on biliary flow are hampered by slow and incomplete absorption from the gut and peritoneal cavity (or both). This absorption factor has been overcome by the use of the isolated perfused rat liver preparation.

The techniques used for the operation and perfusion of rat liver were similar to those described by Brauer, Pesotti & Pizzolato (1951). Rats used were albino females, body wt. 250 g.; they were anaesthetized with Nembutal. Bile was collected by a nylon cannula (external diameter 0.62 mm.) in preweighed tubes cooled in ice. The perfusing medium was Krebs-Ringer bicarbonate buffer

containing 5 g. of bovine plasma albumin/100 ml., 11 mM-glucose, amino acids as listed by Scholz & Bücher (1965), 1.5 mM-sodium lactate and 25 ml. of fresh heparinized whole rat blood/100 ml. Bile was analysed for bilirubin, β -glucuronidase and acid phosphatase by methods described by Slater & Griffiths (1965); total bile acids were measured by using 3α -hydroxy steroid dehydrogenase. Representative samples of the median lobe were fixed in 4% glutaraldehyde-0.1M-cacodylate buffer, pH 7.2, post-fixed in 1% osmic acid in Millonig's buffer, and examined in an AEI EM6B electron microscope.

The introduction of approx. 10μ moles of either sporidesmin or icterogenin into the perfusion medium (120 ml.) resulted in a rapid decrease in bile flow, which had virtually stopped within 30–45 min. of dosing. Electron-microscopic examination of sporidesmin-treated livers showed swelling of the microvilli in the biliary canaliculi and alterations in the endoplasmic reticulum. These results, together with changes found in bile acid excretion and in the acid hydrolase content of bile, will be discussed.

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Isoenzymes of β -Hydroxybutyrate Dehydrogenase in *Rhizobium* spp.

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Poly- β -hydroxybutyrate is one of the principal storage products in several bacterial species, including *Rhizobium* spp. (see Dawes & Ribbons, 1964). D(-)- β -Hydroxybutyrate, a key intermediate in the conversion of poly- β -hydroxybutyrate into cell material, is converted into acetoacetate by β -hydroxybutyrate dehydrogenase (Gavard, Combre & Tuffet, 1960; Shuster & Doudoroff, 1962).

In the present investigation, isoenzymic forms of β -hydroxybutyrate dehydrogenase were detected in several *Rhizobium* spp. The isoenzymic patterns of β -hydroxybutyrate dehydrogenase from fourteen strains of *Rhizobium*, representing six species, were compared. All the *Rhizobium* strains studied nodulated their respective host legumes. Some of the legume-*Rhizobium* combinations, however, were capable of fixing nitrogen whereas others were not.

Starch-gel (Smithies, 1955, 1959) and disc electrophoresis (Davis, 1964) were used to compare β -hydroxybutyrate dehydrogenase isoenzymes from different *Rhizobium* strains. Also, the rate of reaction of β -hydroxybutyrate dehydrogenase, from different rhizobial strains, with various coenzyme analogues (Kaplan, Ciotti, Hamolsky & Bieber, 1960) was compared with the rate of reaction of the enzyme with NAD. The following analogues of NAD were used: acetylpyridine-NAD, pyridine aldehyde-NAD, deamino-NAD, acetylpyridine deamino-NAD.

On both starch and polyacrylamide gels, β -hydroxybutyrate dehydrogenase from several rhizobial strains was separated into two or more isoenzymic forms. One strain of *R. leguminosarum*, for instance, had five isoenzymes when examined by starch-gel and disc electrophoresis and this pattern was reproduced on six different occasions. It was possible therefore to identify individual strains by their isoenzyme patterns. Likewise, the rate of reaction of β -hydroxybutyrate dehydrogenase with NAD, expressed as a ratio of the rate of reaction with one of the aforementioned coenzyme analogues, was characteristic of several strains. For example, the ratio of the reaction rates of β -hydroxybutyrate dehydrogenase with NAD and acetylpyridine deamino-NAD was twice as high for one strain of *R. leguminosarum* as that for another strain of the same species.

Zymograms of β -hydroxybutyrate dehydrogenase were also prepared from the bacteroid fraction of soya-bean root nodules. Zymograms obtained from the bacteroid fraction were very similar to zymograms prepared from the strain of *R. japonicum* used to inoculate the soya beans.

These investigations show that β -hydroxybutyrate dehydrogenase isoenzymes may have important applications in differentiating between *Rhizobium* strains and in studying the metabolism of *Rhizobium* bacteroids in legume root nodules.

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