- Raftery, M. A. & Cole, R. D. (1963). *Biochem. biophys. Res. Commun.* **10**, 467.
- Rüdiger, W. & Ó Carra, P. (1968). *Europ. J. Biochem.* (In the Press).
- Synge, R. L. M. & Youngson, M. A. (1961). *Biochem. J.* **98**, 31p.
- Tong, W. & Chaikoff, I. L. (1958). *J. biol. Chem.* **232**, 939.

Bile and Faecal Porphyrins in the Quiescent Phase of Variegate Porphyria

By S. G. SMITH, R. V. BELCHER, R. MAHLER and J. YUDKIN. (*Tenovus Institute for Cancer Research, and Department of Metabolic Medicine, Welsh National School of Medicine, Cardiff*)

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By using lutidine chromatography on paper (Eriksen, 1958) and on silica gel (S. G. Smith & R. V. Belcher, unpublished work), and also by measuring the Soret maxima in 5% HCl and noting the tube position in the counter-current apparatus, it was possible to identify the first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 ¹⁴C-labelled fluorodinitrobenzene method of Sanger (1945).

- Eriksen, L. (1958). *Scand. J. clin. Lab. Invest.* **10**, 319.
- French, J. M. & Thonger, E. (1966). *Clin. Sci.* **31**, 337.
- French, J. M., England, M. T., Lines, J. & Thonger, E. (1964). *Arch. Biochem. Biophys.* **107**, 404.
- Rimington, C. & Belcher, R. V. (1967). *J. Chromat.* **28**, 112.
- Rimington, C., Lockwood, W. & Belcher, R. V. (1968). *Clin. Sci.* (In the Press).
- Sanger, F. (1945). *Biochem. J.* **39**, 507.
- Smith, S. G., Belcher, R. V., Mahler, R. & Yudkin, J. (1968). *Biochem. J.* (In Preparation).

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

By T. F. SLATER, B. C. SAWYER, V. B. DELANEY and G. BULLOCK. (*Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1, and Research Division, CIBA Laboratories Ltd., Horsham, Sussex*)

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