containing 1.25% of water. Sand (1 cm.) was placed on top of the column to prevent the gel floating during elution with solvent of specific gravity higher than that of the Sephadex. This also facilitated application of the lipid samples (dissolved in 2 ml. of solvent) without disturbing the column.

When chloroform only was used as eluent, tristearin, tributyrin, stearate, *n*-decanoate, *n*-butyrate and acetate were separated into well defined peaks [elution volumes (V_e) 65, 85, 225, 320, 450 and 575 ml. respectively]. Linolenate was not separated from stearate. The recovery of both fatty acids and triglycerides was approx. 85%. Two peaks, each containing less than 10% of the phospholipid applied, were eluted; one near the void volume of the column (V_e , 60 ml.) and the other (V_e , 200 ml.) before the stearate peak. After elution of the fatty acids by chloroform, a third peak containing 50% of the phospholipid applied was eluted when the column was washed with methanol. Thin-layer chromatography (Vaskovsky & Kostetsky, 1968) indicated marked differences in the major phospholipid components of each peak.

When these fractionations were carried out on a column equilibrated with 20% methanol in chloroform containing 1.25% of water, very poor resolution of the polar lipids was obtained. The phospholipids were eluted as a single peak (V_e , 55 ml.) near the void volume, closely followed by the fatty acids. Stearate, *n*-decanoate and *n*-butyrate were eluted as three ill-defined peaks (V_e , 70, 85 and 96 ml. respectively).

The separation of the individual fatty acids and phospholipids is apparently effected mainly by the adsorptive characteristics of Sephadex LH-20, whereas the triglycerides are fractionated by molecular sieving. This preliminary investigation indicates that the combined molecular-sieving and adsorptive properties of Sephadex LH-20 render it a versatile material for the chromatography of both polar and non-polar lipids.

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Amino Acids Involved in Chromophore-Protein Linkages in Phycoerythrins

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The phycoerythrins contain the covalently-bound bile pigment, phycoerythrobilin, as their chief

prosthetic group. Recent evidence indicates that this tetrapyrrolic chromophore is doubly linked to the apoprotein; one bond, probably ester-type (Ó Carra, Ó hEocha & Carroll, 1964), involves a propionic acid side-chain of the chromophore and the other involves the lactam grouping of its ring A (Rüdiger & Ó Carra, 1968).

To investigate the amino acids bound to the pigment, R-phycoerythrin was digested with proteolytic enzymes, the chromopeptide material was purified, and the attached amino acids were identified.

The best results were obtained by digestion of the reduced aminoethylated phycoerythrin (Raftery & Cole, 1963) first with pancreatin (Tong & Chaikoff, 1958) in the presence of mercaptoethanol (0.14 M), then with hog intestinal peptidases (Sigma Chemical Co.) and finally with carboxypeptidase A, leucine aminopeptidase and prolidase. The chromopeptide fraction was isolated and purified by chromatography on talc (Dus, Bartsch & Kamen, 1962) followed by gel filtration on Sephadex G-50 in phenol-acetic acid-water (1:1:1, by vol.) (Synge & Youngson, 1961). The chromophore was then removed by preliminary hydrolysis (11N-HCl for 6 hr. at 15°) followed by adsorption on talc, and the released peptide material was completely hydrolysed (6N-HCl for 20 hr. at 110°). The resulting amino acids, after dinitrophenylation, were identified by thin-layer chromatography (Brenner, Niederwieser & Pataki, 1961).

The following amino acids were found: glutamic acid, serine, aspartic acid, threonine, glycine and alanine. It seems unlikely that glycine or alanine could be involved in the linkage, but repeated efforts to eliminate them from the chromopeptide preparations by further proteolytic digestion failed. Glutamic acid and serine were always present in higher concentration, and in some experiments in much higher concentration, than aspartic acid and threonine. Preliminary results with B- and C-phycoerythrins indicate a similar pattern. It seems likely, therefore, that at least the majority of the phycoerythrobilin units are linked through glutamic acid and serine. This is consistent with the results of Rüdiger & Ó Carra (1968) mentioned above, since serine could form an ester bond with a propionic acid side-chain of phycoerythrobilin, and the γ -carboxyl group of glutamic acid could be bound to the lactam grouping.

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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 ¹⁴C-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