Countercurrent Studies of some Porphyrins of various Tissues

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Recent studies of the ether-soluble porphyrins in the bile of variegated porphyria-gene carriers (Smith, Belcher, Mahler & Yudkin, 1968, 1969) has demonstrated the absence of the 'S411' porphyrin, discovered in bile and meconium by French & Thonger (1966). In addition to protoporphyrin and coproporphyrin, porphyrins running on lutidine chromatograms in the positions corresponding to those of porphyrins with 2 or 3 carboxyl groups (designated below as 2COOH and 3COOH respectively) were detected.

Countercurrent and chromatographic studies showed the 'S411', 2COOH and 3COOH porphyrins to be present in bile from living patients, post-mortem bile, bovine sternal bone marrow and human meconium. Porcine liver extracts contained 2COOH and 3COOH porphyrins, but not 'S411' porphyrin. Porcine blood contained none of these porphyrins, but in one collection an unidentified 1COOH porphyrin with an aetioporphyria spectrum and a Soret band at 400nm. in 5% HCl was found.

All 2COOH and 3COOH porphyrins investigated except those from liver had Soret bands at 404.5 and 404nm. respectively in 5% HCl and aetioporphyria spectra. All 'S411' porphyrins had rhodoporphyria spectra, and chromatographed as a single band in the coproporphyrin III position, as noted by French & Thonger (1966). Catalytic hydrogenation of 'S411' porphyrin with 5% Pd on BaSO₄ converted the rhodoporphyria spectrum into an aetioporphyria spectrum and the Soret band from 411nm. to 402nm. in 5% HCl. The product also chromatographed as coproporphyrin III. These changes indicate a conversion of the unsaturated 4-carboxyl 'S411' porphyrin into the saturated more symmetrical 4-carboxyl coproporphyrin III. It was shown by chromatography that hydrolysis of the tetramethyl ester of 'S411' porphyrin with 25% HCl removed three of the four methyl ester groups. The remaining group could only be hydrolysed after catalytic hydrogenation. This evidence suggests that one of the acid groups of 'S411' porphyrin is not a propionic acid but is reduced to a propionic acid group by catalytic hydrogenation. This indicates that the 'S411' porphyrin is a monoacrylic acid derivative of coproporphyrin III, and this conclusion is in agreement with mass-spectrophotometric and spectrophotometric findings (C. Rimington, D.

Nicholson, J. M. French & A. H. Jackson, unpublished work).

The atypical 2COOH and 3COOH porphyrins in porcine liver are being further studied and characterized.

We thank the Abbotshill Trust for generous support.

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Glutathione-Protein Mixed Disulphides in Human Lens

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Protein-bound glutathione and cysteine have been identified by several authors (Mize, Thompson & Langdon, 1962; Huisman & Dozy, 1962; Révész & Modig, 1965; Huisman, Dozy, Horton & Nechtman, 1966; Modig, 1968; Jackson, Harrop & Smith, 1968). Glutathione-protein mixed disulphides were found in ox and rabbit lens (Herrmann & Moses, 1945) and their formation and function have often been discussed (van Heyningen, 1962; Kinoshita, 1964). In the present investigation such mixed disulphides have been identified in normal and cataractous human lenses.

Normal and cataractous human lenses were ground and carboxymethylated *in vacuo* in 7M-urea. Excess of reagent, low-molecular-weight disulphide and carboxymethylated thiols were dialysed away. Disulphides present in the protein solution or suspension were reduced by NaBH₄ in 7M-urea, pH 8.8. After deproteinization with trichloroacetic acid, total thiol was estimated by the method of Ellman (1959), and glutathione by the method of Ball (1966), in which cysteine is prevented from reacting with Ellman's reagent by prior reaction with glyoxylic acid. The results indicated that mixed disulphides of proteins with glutathione and other small molecules are present in normal human lens, that they increase in amount in the cataractous lens and that they increase with deepening nuclear colour of the cataractous lens. Classification by nuclear colour is according to the system of Pirie (1968).

The occurrence of glutathione as a low-molecular-weight partner was confirmed by paper electrophoresis of the same deproteinized supernatants. Further confirmation was provided by oxidation of a metaphosphoric acid precipitate of whole lens with performic acid, followed by deproteinization and identification of the released sulphonic acid of glutathione by paper electrophoresis (the reference