Countercurrent Studies of some Porphyrins of various Tissues

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Recent studies of the ether-soluble porphyrins in the bile of variegate-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 aetioporphyrin 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 aetioporphyrin spectra. All 'S411' porphyrins had rhodoporphyrin 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 $BaSO_4$ converted the rhodoporphyrin spectrum into an aetioporphyrin 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with mass-spectrophotometric and spectrophotometric findings (C. Rimington, D. Nicholson, J. M. French & A. H. Jackson, unpublished work).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.

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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, Harrap & 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 $NaBH_4$ 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

compound was generously supplied by Dr S. G. Waley).

Two characteristics of cataractous lenses favour mixed-disulphide formation. First, the protein thiol groups become more accessible and, secondly, there is relatively little glutathione present to reduce mixed disulphides. Huisman & Dozy (1962), working on haemoglobin, predicted that mixed-disulphide formation would occur when the concentration of glutathione was low.

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Diquat Cataract in the Rat

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Rats fed on a diet containing 0·05–0·075% of the herbicide diquat (1,1′-ethylene-2,2′-dipyridylium dibromide) slowly develop lens opacities while remaining healthy in other respects (Howe & Wright, 1965; A. Pirie & J. R. Rees, unpublished work). The lens opacity starts in the posterior cortex, spreads to the central core and finally becomes complete. Mitosis in the lens epithelium is initially depressed, but recovers and no cellular damage remains. Intraperitoneal injection of diquat labelled with ^{14}C in the ethylene bridge leads to appearance of radioactivity in the lens and other parts of the eye.

The herbicidal action of diquat is considered to be due to its reduction to a bright-green free radical in photosynthesizing cells (Homer, Mees & Tomlinson, 1960; Davenport, 1963). The reaction is reversed by O_2 with formation of H_2O_2. Gage (1968) has described several enzyme systems in liver that can form the free radical, and Orr (1966) found that ascorbic acid can reduce diquat to the free radical in air while being oxidized itself. Present experiments have shown that the ascorbic acid of the lens and of intraocular fluids was less in diquat-fed rats than in litter-mate controls. We confirmed the finding by D. G. Clark (personal communication) that the GSH concentration of the lens remained at the normal value during cataract development. This marks diquat cataract off from many other types in which the GSH concentration of the lens decreases.

We have investigated enzymic and non-enzymic reactions of diquat with constituents of lens, aqueous humour and vitreous humour. A freshly prepared lens extract will reduce diquat to the free radical anaerobically in the dark, probably largely through the action of glutathione reductase and NADPH. The free radical is also formed photochemically in sunlight and can be demonstrated anaerobically in the presence of electron donors such as EDTA or amino acids, or with aqueous humour. Sunlight will also catalyse aerobic oxidation of ascorbic acid by diquat in aqueous humour. H_2O_2 is formed. The maintenance of GSH in the cataractous lens may be connected with the ability of the diquat free radical to reduce GSSG in the presence of glutathione reductase and NADP.

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Spontaneous Reversal of Arsenate-Induced Swelling of Mitochondria

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Hunter & Ford (1955) and Packer (1961) have described mitochondrial swelling induced by arsenate and prevented by EDTA. Ter Welle & Slater (1967) found that arsenate produces a time-dependent appearance of oligomycin-insensitive respiration and that this also is prevented by EDTA.

Addition of arsenate (1·25mM) to rat liver mitochondria oxidizing β-hydroxybutyrate in an Mg^{2+}-free medium results in the following events: a lag phase in which the rate of oxygen uptake is approximately doubled and during which little or no swelling occurs; a phase of marked swelling and acceleration of oxygen uptake; a phase of temporary reversal of swelling (by about 20% of the total) during which the high rate of oxygen uptake is maintained; a phase of slow steady swelling during which the rate of oxygen uptake declines; a small amplitude burst of swelling when the medium goes anaerobic (or if antimycin A or an uncoupling agent