Purification of Bile Salts on Columns of Sephadex LH-20

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Bile salts, whether alcohol monosulphate esters, taurine conjugates or glycine conjugates (all as anions), can easily be separated from impurities originally present in the bile or collected by elution from t.l.c. plates. Sephadex LH-20 (0.5 g; Pharmacia, Uppsala, Sweden) was swollen overnight in chloroform-methanol (1:1, v/v) and then packed by gravity into columns (length about 9.5 cm, diam. about 0.64 cm) in the same solvent mixture. Bile salts [0.1–1.0 mg, crude from bile or eluted with methanol (after location with iodine) from t.l.c. plates of silica gel] were dissolved in methanol (50 μ l), which was diluted with chloroform (50 μ l) immediately before transference of the mixture to the top of the column. Chloroform-methanol (1:1, v/v) (about 200 μ l in all) was used to complete the transfer, and as soon as the mixture had entered the column elution with the same solvent was begun. Eight fractions (1 ml each) were collected manually into tared tubes, evaporated and weighed. T.l.c. showed bile salts in fractions 4 and 5, with minor amounts in fractions 3 and 6. The products in fractions 4 and 5 were sufficiently pure for i.r. spectroscopy, but, except for some segregation of glycine conjugates in fraction 4, separation of individual bile salts did not occur.

The method is rapid, reproducible and gives a quick estimate of the bile salt content of very impure samples. Since the exclusion volume of these columns (determined with polyethylene glycol in methanol) is close to 1.2 ml, gel filtration and/or partition is probably taking place. Different and less satisfactory results with methanol alone suggested that the chloroform in the solvent mixture might have decreased the size of the bile salt micelles.

Chemical and Biological Aspects of Certain Bacterial Endotoxins

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Spectral shifts caused by reactions involving certain groupings occurring in some endotoxic materials (Janda, 1971, 1972) are interpreted for *Escherichia coli* A.T.C.C. 12408.

The shifts appear to detect active toxic groupings of endotoxins, since the treatments that are generally

used to eliminate toxic effects prevented their spectral responses (Janda, 1971), and the grouping content in solvolysed materials was related to mouse lethality (Janda, 1972).

I.r. spectra of lipopolysaccharide preparations permit tentative identification of some components as analogues of sugar sulphates and alkyl hydrogen sulphates, and the spectra of solvolysed materials were altered. The solvolysis provides 'selective means' for removing sulphate esters. This and other evidence (Janda, 1971) may suggest that endotoxins possess labile regions comprised of the groupings attached at their sulphur-containing sites to a polyhydroxy sugar derivatives.

The liberation of sulphur might result in the formation of deoxy sugars from these derivatives. Indeed, discrepancies are implied in reports (Osborn, 1963; Droge *et al.*, 1970) on the 3-deoxy-2-oxo-octonic acid content in various lipopolysaccharide intact cores and their hydrolysates. In my preparations deoxy sugars became detectable after alkali treatment (Janda & Work, 1970), with no observable precipitation but with concurrent decrease (by about two-thirds) in the groupings content. Some of the groupings became available for an ether extraction.

Thus the hypothetical regions might occur in the zone known as '3-deoxy-2-oxo-octonic acid trisaccharide' rather than in lipid A. However, whether or not the groupings are portions of lipopolysaccharide/glycolipid and whether they occur in some or all of their units is still open to question. The known lipopolysaccharide of *E. coli* is obscure and is not compatible with structures proposed for those of *Salmonella* sp. The preparations analysed were chemically heterogeneous (S/heptose ratio approx. 1:10), and procedures estimating the reaction groupings and the lipopolysaccharide revealed heterogeneity in purified preparations as well as in primary materials released by the cells (Janda, 1972). This indicates that not all lipopolysaccharides/glycolipid units in cell envelopes possess toxic properties and deserve the term endotoxins. The reactive groupings might participate in an interface phenomenon and in the spatial conformation of endotoxins.

Spectral variations (Janda, 1972) thus indicate that the endotoxins are populations of closely related molecules and that their heterogeneity is caused primarily by biochemical events occurring during normal cell syntheses. The endotoxin biosynthesis does not appear to be linked with protein synthesis, but endotoxins have an effect on the cell division (Janda, 1972).

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Spectral Evidence for the Presence of Cytochrome P-450 in Microsomal Fractions Obtained from some Higher Plants

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Cytochrome P-450 has been detected in mitochondrial and microsomal fractions of a variety of mammalian tissues (Boyd *et al.*, 1971; Ernster & Orrenius, 1965). Studies on bacteria indicate the involvement of cytochrome P-450 in camphor hydroxylation by *Pseudomonas putida* (Gunsalus, 1971). In fungal metabolism a role for cytochrome P-450 has been demonstrated in *Claviceps purpurea* and *Candida tropicalis*. In the former it is involved in alkaloid biosynthesis, which may be stimulated by pretreatment with phenobarbital (Ambike & Baxter, 1970; Orrenius, 1971). In the yeast there is involvement in ω -hydroxylation of fatty acids (Lebean, 1971).

Studies on spermatophyta are limited. Murphy & West (1969) showed that the conversion of kaurene into kaurenol required NADPH as well as O₂, and was inhibited by CO. Similar results were obtained for the cinnamate 4-hydroxylase system in *Pisum sativum* (pea) by Russell (1971). The importance of cytochrome P-450 in higher-plant metabolism is still a matter for conjecture, other modes of hydroxylation having been described, e.g. phenolase (Butt & Vaughan, 1970) and peroxidase (Rathmell & Bendall, 1972).

Moore (1967) obtained evidence for the characteristic cytochrome P-450-CO binding spectra in microsomal fractions from *Pisum sativum* cotyledons (Chance *et al.*, 1968; D. S. Bendall, personal communication). We have obtained spectral evidence for cytochrome P-450 in a variety of etiolated plant tissues. The selected region of the plant was homogenized in 0.1 M-phosphate buffer, pH 7.4, containing 2M-glycerol and 0.02M-2-mercaptoethanol. The 220000g-min supernatant was recentrifuged for 1 h at 105000g and the resulting pellet was resuspended in the homogenizing medium.

Investigations of CO-binding spectra indicated the presence of material giving a 450nm peak within *Phaseolus vulgaris* (bean) cotyledons, *Pisum sativum* seedlings and *Zea mais* (maize) cotyledons. The concentration in bean and pea tissue was about 0.02nmol/mg of microsomal protein, whereas that in monocotyledons was about 0.007nmol/mg (ϵ taken to be 91mm⁻¹.cm⁻¹). These values are far lower

than those encountered in rat liver (0.8–1.2nmol/mg of microsomal protein). We have not obtained spectral evidence for cytochrome P-450 in *Phaseolus aureus* (mung bean), citrus fruit, microsomal preparations or in any of the 220000g-min pellets. The 450nm peak was transient, especially in the absence of the thiol, and was always accompanied by a peak at 420nm.

Preliminary studies *in vivo* and *in vitro* have shown no biphenyl hydroxylase activity in a variety of plant tissues. However, a trace of *p*-nitroaniline *O*-demethylase activity has been found in both the cotyledon and seedling of *Pisum sativum* (less than 0.012 μ mol of *p*-nitrophenol/h per mg of microsomal protein). So far pretreatment with phenobarbital or 2,4-dichlorophenoxyacetate has failed to influence cytochrome P-450 concentrations in *Pisum sativum* or *Phaseolus vulgaris*. Binding studies have produced no distinct spectra with biphenyl or aniline, but metyrapone [2-methyl-1,2-di-(3-pyridyl)propan-1-one] gave evidence of a type II complex in preparations from *Phaseolus vulgaris*.

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Structure-Activity Relationships for the Induction and Inhibition of Hepatic Microsomal Enzymes in the Rat by Organohalogen Compounds

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A number of organochlorine pesticides, such as DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane], cause the induction of several hepatic