Studies have been mainly concerned with the undegraded water-soluble mucoprotein fraction, which accounts for 22% of the non-diffusible radioactivity. The water-soluble fraction, on gel filtration, gives three radioactive and u.v.-absorbing peaks that have similar specific radioactivities (1050 d.p.m./mg.) and chemical analyses: protein, 20%; hexose, 30%; hexosamine, 32%; fucose, 9%; sulphate, phosphate and uronic acid all less than 0.3%. Except for the higher protein content, this analysis is similar to that of the blood-group substances isolated from peptic digests of porcine gastric mucosa (Kabat, 1956). Further, the water-soluble fraction possesses both A and H blood-group activity. Gel filtration, together with the above analysis, suggests that the faster-moving peak is a polymeric form of the two slower-moving peaks. The radioactivity in the water-soluble fraction is thus in a non-sulphated neutral mucoprotein that shows blood-group activity and comprises 83% by weight of the total water-soluble material.

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Properties of an α -D-Galactosyltransferase in Human Tissues obtained from Blood-Group B Donors

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A particle-bound α -D-galactosyltransferase that transfers D-[14 C]galactose from UDP-D-[14 C]galactose to low-molecular-weight acceptors containing β -linked D-galactose residues substituted at C-2 with L-fucose was demonstrated in a human submaxillary gland from a blood-group B donor and was not found in glands from donors of group A or O (Race, Zideman & Watkins, 1968). The presence of this enzyme has now been confirmed in eight group B and two group AB submaxillary-gland specimens and in the mucosal linings from three group B stomachs. The transferase was found in the tissues of both secretor and non-secretor persons belonging to group B, whereas tissues of group A and O controls consistently failed to yield evidence of the enzyme.

The acceptor specificity of the transferase was more clearly defined by examination of a range of low-molecular-weight fucose-containing oligosac-

charides isolated from human milk (cf. Kuhn, 1957; Montreuil, 1960). In agreement with the earlier findings, those containing an α -L-fucosyl residue (1 $\rightarrow$ 2)-linked to the terminal non-reducing galactose unit, namely 2'-fucosylgalactose, 2'-fucosyl-lactose and lacto-N-fucopentaose I, were good acceptors of galactose in α -linkage. Lacto-N-fucopentaose II, lacto-N-difucohexaose II and 3'-fucosyl-lactose, which have an L-fucose substituent on the subterminal sugar, and lacto-N-difucohexaose I and lactodifucotetraose, which are substituted with two L-fucose residues on adjacent sugars, were poor acceptors, or failed to accept, in the same system. The specificity requirements of the α -galactosyltransferase are therefore consistent with those postulated for the product of the blood-group B gene (cf. Race *et al.* 1968).

Attempts to solubilize the particle-bound enzyme by ultrasonic treatment or by extraction with 1% sodium deoxycholate resulted in loss of α -galactosyltransferase activity. Treatment with 1% digitonin or Triton X-100 increased the transferase activity of the particles obtained from either submaxillary glands or stomach mucosal linings and released soluble enzyme from the stomach preparations. The most effective method so far investigated for solubilization is extraction with digitonin of an acetone-dried powder prepared from the particles from stomach mucosal lining. The transferase activity of the particles is retained by the insoluble acetone-dried powder and treatment of this preparation with digitonin resulted in the release of about half of the activity in a soluble form.

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Properties of α -Galactosidase I from *Vicia faba*

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Dormant *Vicia faba* seeds contain two molecular forms of α -galactosidase (α -D-galactoside galactohydrolase; EC 3.2.1.22). Form I has a molecular weight of 209 000 and appears to be a glycoprotein whereas form II is substantially free of carbohydrate and has a molecular weight of 38 000 (Dey & Pridham, 1968, 1969a,b).

The specificity and mode of action of enzyme I

has now been examined. Changes in the galactosyl moiety of substrates result in a decreased hydrolysis rate and a decrease in the affinity of enzyme for substrate. With *p*-nitrophenyl derivatives, for example, $V_{\max}$ decreases and K_m increases in the following order: α -D-galactopyranoside, α -D-fucopyranoside, β -L-arabinopyranoside. The enzyme is strongly inhibited by D-galactose and some α -D-galactosides and to a smaller extent by L-arabinose and D-fucose.

The effect of pH on $V_{\max}$ and K_m values suggests that carboxyl and histidine imidazole groups are essential for hydrolytic activity. The presence of the latter group at the active site is substantiated by photo-oxidation studies. There is little evidence to support the involvement of thiol groups in the enzyme catalysis.

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Protein-Polysaccharide Complexes Secreted by the Calf Tracheal Epithelium *in vitro*

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Investigations of human foetal trachea (Lamb & Reid, 1965, 1968; Reid, 1967), respiratory excretions (Harvez, Roussel, Degard & Biserte, 1965, 1967; Harvez *et al.* 1967) and rat tracheal mucosa (Dalhamm, 1956; Formijne, Van der Schoot & Nie, 1964) by radiographic, histological and analytical techniques indicate that glycoproteins are formed by the epithelial tissue. The present work is concerned with the fractionation and characterization of such macromolecules from a defined region of the tract, namely calf trachea.

Organ culture of calf tracheal explants (in Eagle medium at 33° in the presence of streptomycin, penicillin and tetracyclin) (Hoorn, 1966) gives continued secretion of epithelial macromolecular products as long as ciliary motion can be observed or up to 20 days, initially at a rate of approx. 60–100 μ g./cm.² day. Fractionation of the spent culture fluids on Sephadex G-25 followed by Sephadex G-150 furnished three principal components (A, B and C). Complex A, found to be free from serum components by immunochemical techniques, had $\bar{S}$ 6.6–7.6s, average mol.wt. 2.5×10^5 (in 0.1M-NaCl, 2–7 mg./ml.). The complex gave evidence of homogeneity by a number of

techniques. It contained 18.5% of carbohydrate, which included galactose (46 residues/ 2.5×10^5 mol.wt. units), mannose (15), fucose (5–12), xylose (9–13), glucuronic acid (48), *N*-acetylglucosamine (23), *N*-acetylgalactosamine (41) and sialic acids (5.3). Ester sulphate was present and the peptide moiety was comprised of 17 amino acids, principally glutamic acid, aspartic acid and glycine. In collaboration with Dr D. A. J. Tyrrell, it was found the complex A possessed marked activity as an inhibitor of haemaggregation by influenza B virus and it was highly active in inhibiting haemaggregation of trypsin-treated erythrocytes (Tyrrell, Head & Dimic, 1967). Neither of these effects was shown to any appreciable extent by complex B or complex C.

Complex B had average mol.wt. 1.6×10^5 and complex C ($\bar{S}$ 2.8–3.4s, average mol.wt. 1.03×10^5 under the same conditions as used for complex A) contained smaller proportions of carbohydrate (9.2 and 6.2% respectively) composed of the same monosaccharides as complex A. Immunochemical examination of complexes B and C showed that some serum components were present, e.g. immunoglobulins and albumin. In no fraction could RNA or DNA be detected.

All three complexes are considered to be of epithelial origin, and no evidence of their presence was found in culture fluids from explants where the epithelium had been removed.

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