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*

By P. W. KENT, I. J. MOLINEUX, M. R. HAEGELE and F. K. STEVENSON. (*Department of Biochemistry, University of Oxford*)

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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