stabilities of the polymeric collagen increased up to the age of 25–30 years. After treatment with cold alkali, however, the stability of the polymeric collagen appeared to increase up to the age of 60 years. The trends were similar whether values from normal individuals alone were taken or whether the whole group was considered, including patients with various connective-tissue disorders. There is thus evidence for two distinct biochemical processes leading to an increased stability of polymeric collagen; one of these may continue throughout life. The relation of the changes to the known inter- and intramolecular cross-links present in collagen fibres is being investigated.

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Fractionation of Water-Soluble Pig Gastric Mucus by Equilibrium Density-Gradient Centrifugation in Caesium Chloride

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The application of the technique of equilibrium density-gradient centrifugation in CsCl (Creeth & Denborough, 1970) to the separation of protein from mucoprotein blood-group substances has been followed to fractionate further the water-soluble gastric mucus preparation (Snary & Allen, 1971). This water-soluble mucus has previously been shown from gel filtration, ultracentrifugation and electrophoretic studies to consist of two apparently homogeneous, though polydisperse, mucoprotein components. Because these two mucoprotein components have very similar chemical, physical, biosynthetic and immunological properties, the high-molecular-weight component A is thought to be a polymer of the lower-molecular-weight component B/C.

Equilibrium density-gradient centrifugation in CsCl of the unfractionated water-soluble mucus produced a clear separation between a protein fraction at the top of the tube ($\rho = 1.38\,g\cdot ml^{-1}$) and the mucoprotein fraction, containing all the bound hexose and fucose, at the bottom of the tube ($\rho = 1.56\,g\cdot ml^{-1}$). Sedimentation analysis of this mucoprotein fraction showed two components of $s^0_{25,w}$ 33S and 5.7S that are shown to correspond to the high-molecular-weight and low-molecular-weight components A and B/C ($s^0_{25,w}$ 18.7S and 4.3S respectively) of the unfractionated water-soluble mucus. The two mucoprotein components from the equilibrium density-gradient centrifugation were separated in the excluded and included volumes respectively of a Sepharose 4B column. The carbohydrate analysis of the high-molecular-weight 33S component was hexose 26%, fucose 11.3%, glucosamine 19.5% and galactosamine 8.3%. Purified 18.7S mucoprotein A isolated by gel filtration of the water-soluble mucus, which contained 21.7% protein, was fractionated by equilibrium density-gradient centrifugation, producing a protein fraction and the 33S mucoprotein containing 16.5% protein. Amino acid analysis of this 33S mucoprotein showed an enriched serine, threonine and proline content and a lower aspartic acid and glutamic acid content compared with the parent 18.7S mucoprotein fraction A. The protein content, however, is still higher than blood-group substance isolated by proteolytic digestion of pig stomach (Pusztai & Morgan, 1963). These results show that equilibrium density-gradient fractionation in CsCl removes non-covalently bound protein from gastric mucoprotein that was judged to be homogeneous by other methods.

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The Effect of Caesium Chloride on the Tertiary Structure of the Water-Soluble Pig Gastric Mucus

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The two mucoproteins isolated from water-soluble pig gastric mucus by using equilibrium density-gradient centrifugation in CsCl (Starkey *et al.*, 1972) have $s^0_{25,w}$ values of 33S and 5.7S respectively in 0.2M-KCl buffer, pH 5.5. These values compare with 18.7S and 4.3S for the same mucoproteins (A and B/C) isolated by gel filtration (Snary & Allen, 1971). The viscosity of the water-soluble mucus decreased 63% between 1mM-CsCl and 0.2M-CsCl but then remained constant up to 3.5M-CsCl, the concentration

used for density-gradient fractionation. The 18.7S mucoprotein, which accounts for most of the viscosity of the water-soluble mucus (Snary *et al.*, 1970), had an intrinsic viscosity of 320 $ml \cdot g^{-1}$ in 0.2M-KCl buffer, pH5.5, compared with 160$ml \cdot g^{-1}$ for the same mucoprotein isolated by CsCl-density-gradient centrifugation. Further, the considerable shear-dependence of the viscosity of the 18.7S mucoprotein was completely eliminated after CsCl-density-gradient centrifugation. Since the molecular weights of the 18.7S and 33S mucoproteins were shown to be the same and the ratio of the areas of the two sedimentation peaks for the mucoproteins in the water-soluble mucus before and after CsCl centrifugation was constant, the changes in $s^0_{25,w}$ values and in viscosity imply changes in shape of the mucoproteins. The value for $K_s/[\eta]$ (Creeth & Knight, 1965) was 0.8 for the 18.7S and 33S mucoproteins and their respective frictional ratios, f/f_0, were 10.4 and 1.9. This decrease in f/f_0, together with the constancy of $K_s/[\eta]$, indicates a decrease in the expansion and hence the hydration of the mucoprotein, giving a relatively compact structure similar in shape to that of the highly hydrated shear-dependent 18.7S mucoprotein present in the water-soluble mucus. It is noteworthy that 0.2M-CsCl produces an even higher degree of contraction of the mucoprotein than that induced by 1.5M-KCl (Snary *et al.*, 1971). These results show that the use of CsCl in density-gradient centrifugation of mucoproteins can cause large and irreversible changes in their tertiary structure. In the case of gastric mucoproteins their native tertiary structure is necessary for their function *in vivo* (Snary *et al.*, 1972).

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Glycoproteins in the Cell Envelope of *Halobacterium halobium*

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Halobacterium halobium is a rod-shaped Gram-negative bacterium that grows optimally at above 4M-NaCl and lacks the peptidoglycan component found in the cell envelopes of most other bacteria. Cell envelopes prepared from this organism contain approx. 10% of their dry weight as hexose and 3–4% as hexosamine. A glycolipid has been isolated from this organism (Kates *et al.*, 1967), and the results now presented indicate that glycoproteins are also present.

When cell envelopes were treated with aq. 50% (w/v) phenol the carbohydrate-containing material was extracted into the aqueous phase, from which it could be precipitated by the addition of ethanol. The precipitate was washed with ethanol and then treated with nucleases before being exhaustively dialysed and freeze-dried. The yield was approx. 70mg/g of cells.

This fraction was resolved by electrophoresis in 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulphate into three bands that could be stained by both periodate–Schiff reagent and Coomassie Brilliant Blue. The positions of the bands indicate molecular weights of 41000, 56000 and 79000, on the basis of pure protein standards. The mobilities of the periodate–Schiff-positive materials in this system is at least partly due to covalently attached proteins, since treatment of the preparation with Pronase lowers the electrophoretic mobilities of these components. These results support the suggestion that at least a proportion of the periodate–Schiff-positive material is glycoprotein.

The carbohydrate moieties present in the cell envelopes were isolated after exhaustive Pronase treatment. Mannose was the only hexose present in appreciable quantities. Molecular weights of these carbohydrate moieties were determined by gel filtration on a 2:1 mixed bed of Sephadex G-100 and G-200. About 85% of the hexose content was eluted in a broad peak at a position corresponding to an average molecular weight of 15000. The remainder of the hexose appeared to be present in compounds of molecular weights approx. 30000 and 60000.

When concanavalin A was added to washed-cell suspensions of *H. halobium*, agglutination was observed but there was no loss of u.v.-absorbing material from the cells in excess of that lost by control cells. This suggests that the glycoproteins are probably surface components with the carbohydrate moiety accessible to the phytagglutinnin.

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