S20.4
The Architecture of Respiratory Mucin Macromolecules

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Mucus glycoproteins, as found in mucus, are assembled from subunits via the agency of disulphide bonds. Our goal is to understand this assembly in terms of the organisation and interaction of the distinct glycosylated and naked domains within the subunit. We have studied the fragmentation of whole mucins and subunits by a variety of protein core cleaving reagents. The distribution of fragment size and molecular weight after degradation has been assayed with light scattering, rate zonal-centrifugation, electron microscopy and agarose gel electrophoresis. A novel feature of our data is evidence for a protease sensitive domain, present in macromolecules of $M_r > 10 \times 10^6$ which is involved in a supramolecular assembly resulting in molecules of M_r $20-30 \times 10^6$. Antibodies raised to the intact mucin are primarily targeted at this site which is also susceptible to reduction of disulphide bonds, though it is not disrupted by chaotropic agents such as 6 M guanidinium chloride or detergents such as 0.1 M SDS. The presence of two other distinct proteinase susceptible domains can also be detected in trypsin digestion experiments. One domain is cleaved over a period of 10 h leaving a fragment M_r $2.5-5 \times 10^6$ whereas further extremely slow degradation observed over 30–40 h yields a disperse group of fragments of average M_r 1.3×10^6. A coherent model for the architecture of these mucins, based on these data, will be presented.

S20.5
Changes in the Biosynthesis of Mucin Oligosaccharides in Human Cancer Cells

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The biosynthetic mechanisms leading to structural and antigenic changes of mucins in colon cancer are poorly understood. We are interested in determining the glycosylation and sulfation abnormalities of mucin oligosaccharide chains that specify cancer cells. As a model we chose mucin-secreting human colonic polyposis cells at three stages during their development to cancer. We assayed Gal-, GlcNAc-, sialyl-, fucosyl- and sulfotransferases that have previously been found by us to be present in normal colonic tissue and are predicted to be involved in assembling *O*-glycans of human colonic mucins. Assays were carried out by HPLC and other methods using specific glycosyltransferase substrates. Several glycosyltransferases were found to be abnormal in all three cell lines compared to normal human colonic tissue indicating that mucin biosynthesis in polyposis cells differs from normal cells. The normal colonic enzyme core 3 β3-GlcNAc-transferase is detectable in polyposis cells but appears to be turned off in cancer cells. Several glycosyltransferase and sulfotransferase activities show significant changes in activities and specificities that accompany carcinogenesis. We are hoping to elucidate the cancer-specific control of these activities. (This work was supported by grants from the CCFF and MRC of Canada, and the CRC and MRC, UK)

S20.6
Human Middle Ear Mucins

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Otitis media with effusion is the commonest cause of deafness in children and is characterised by the accumulation of a mucin rich fluid in the middle ear cleft. Middle ear effusions from children undergoing myringotomy were classified into three groups; (1) thick (mucoid) and (2) thin (serous), from anatomically normal children and (3) those from cleft palate patients. Mucin was purified from each of the three groups using CsCl equilibrium density gradient centrifugation. The purified mucins were all excluded from Sepharose CL-2B and therefore of large molecular weight. Intrinsic viscosity measurements demonstrated that the intact mucins could be ranked in order of molecular space occupancy; cleft palate ($0.33\ mlmg^{-1}$) > thick ($0.21\ mlmg^{-1}$) > thin ($0.17\ mlmg^{-1}$). The total thiol contents of the mucin pools were 61, 27 and 14 $nmolmg^{-1}$ for cleft palate, thick and thin respectively demonstrating a possible difference in polymerization which may explain the size differences. Amino acid analysis exhibited differences in the protein cores of the three pools of mucin in particular the content of serine, threonine and proline (33%, 24% and 41% for pools 1, 2 and 3 respectively). Several genes for mucins have now been described (1) and these results suggest that at least 2 genes are expressed in the middle ear and that these 3 effusion pools could result from differential expression of these genes.

(1) Kim, Y. S., Gum, J. R., Byrd, J. C. and Toribara, N. W. *Am. Rev. Respir. Dis.* (1991), **244**: S10–S14.

S20.7
Characterisation of Colonic Mucin

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The structure of pig and human colonic mucin was investigated by physical and chemical methods. Mucin was isolated from the adherent mucus gel in the presence of proteolytic inhibitors to prevent any *in vitro* proteolysis. Polymeric mucin was purified by CsCl gradient centrifugation followed by gel filtration to ensure the removal of any contaminating protein or degraded material. Polymeric mucin was fragmented into digested and reduced subunits. The molecular weight of the mucin was measured using sedimentation equilibrium and light scattering. The distributions of polymeric and reduced mucin were polydisperse, their mean Mw were 5.7×10^6 and 3.3×10^6 respectively. The proteolytically digested mucin was a single size species of Mw 5×10^5. This size distribution was confirmed by intrinsic viscosity studies. Polymeric mucin contained 84 disulphide bridges and 42 free thiol groups, reduced mucin 9 disulphide