THE USE OF SEM TO STUDY DRUG-GLYCOPROTEIN INTERACTIONS

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Scanning electron microscopy (SEM) studies on cervical mucus have shown that a honeycomb structure exists with interconnecting channels separated by thin, membranous walls (Chretien & David 1978) which represent glycoprotein bundles or sheets. The channels were considered to represent the actual pores existing in the native gel and changes in channel size were observed at different stages of the menstrual cycle(Chretien & David 1978). Forstner et al (1977) have shown that intestinal goblet cell mucin produces a similar picture and gross changes could be induced by the addition of calcium ions. In this work we evaluate the use of SEM to study drug mucus interactions.

50 µl aliquots of a 5% mucus gel (pH 7.5) purified from human sputum (Brown & Marriott 1979), with or without additives, were introduced into hollow rivets with an internal diameter of 2 mm. Samples were then either fixed by immersion in 1% glutaraldehyde or quench frozen in either boiling liquid nitrogen or melting dichlorodifluoromethane [Freon] to provide slow or fast rates of freezing respectively. Samples were freeze dried at a vacuum of 3×10^{-4} Pa at 193° K for 18 hours, sputter-coated with gold and examined in a Philips model 501 SEM. The drugs added were 0.5% oxytetracycline HCl (OT), 1.0% disodium tetraborate (DST), 1% DNA, 1% N-acetylcysteine (NAC) and 100 mM calcium ions.

By examination of a natural fissure adjacent to the sample surface a suitable reference point could be obtained. Fixing in glutaraldehyde did not affect the results and was omitted in subsequent experiments. The nature of the pores varied markedly according to the freezing process; the liquid nitrogen frozen samples showed irregularly shaped pores, 3-10 µm across, whereas the Freon frozen samples had more regularly shaped pores, 0.2 µm across. The rate of freezing in liquid nitrogen produces larger crystals (Gressel & Robards 1975) which results in larger pore sizes on the micrograph. The difference in pore sizes observed by other workers may well be due to the use of dissimilar freezing conditions and casts doubt on the actual existence of pores in the natural gel state since it appears that ice crystals push the glycoproteins into bundles or sheets as the ice crystals form. However, when the preparation was rigorously controlled, then highly reproducible results were obtained and consequently liquid nitrogen freezing was used to evaluate the effect of additives. Surprisingly, NAC produced no change in pore size and since it is known to break disulphide bridges then presumably the resultant glycoprotein subunits are still sufficiently large to permit ice crystal nucleation and growth. The thickening agents OT, DST and DNA also had little effect, although the former two did produce more elongated pores. However, with calcium, changes were produced both on the surface and in the internal structure of the gel; the septa between the pores increased to 1-2 µm in thickness. The thickening effect of calcium on tracheal mucus (Marriott et al 1979) was confirmed with the purified mucus by creep compliance testing where η_o increased progressively from 98 to 263 Nsm^{-2} on addition of calcium ion concentrations of up to 30 mM. It is thought that neutralization of the negatively charged polar head groups on the glycoprotein sugar side chains is responsible and that the electron micrographs may reflect the induced conformational change. However, it does appear that SEM can be used to demonstrate glycoprotein-drug interactions but only if properly controlled.

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