THE USE OF SCANNING ELECTRON MICROSCOPY TO EVALUATE THE EFFECT OF MUCOLYTIC AGENTS ON MUCUS GEL STRUCTURE

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Scanning electron microscopy (SEM) of mucus from human conjuctiva (Greiner et al 1982) and both respiratory (Parish et al 1982) and cervical (Chretien et al 1973) epithelia has suggested a honeycomb structure with many interconnecting channels. Changes in the honeycomb network of cervical mucus through the menstrual cycle have been observed (Chretien & David 1978), but may be an artefact created by the freezing technique (Parish et al 1982). The pores and channels are due to formation of ice crystals as the gel freezes and their size depends on both the water content of the sample and the rate of freezing. However, cervical mucus from women taking oral contraceptives is reported to show a change in appearance of the artefact, which may parallel in vivo alteration of mucus structure. In order to define the gel changes and freezing artefacts a method has been developed using rigidly controlled preparation techniques to examine mucus structure and the effects of mucolytic agents.

The glycoprotein from crude porcine gastric mucus was isolated by gel filtration, dialysed and concentrated by ultrafiltration. Aliquots of the gel, mounted between two rivets, were frozen in slushed nitrogen, fractured by breaking the rivets apart and transferred immediately to the SEM stage ('Emscope' SP2000). The temperature of the stage was increased for a set time so that partial sublimation of the sample occurred. The surface was then sputter coated with gold and examined by SEM (Philips PSEM 501B).

Gels coated immediately after fracture, without sublimation, showed only a slightly 'scaley' surface, the water being present as ice forming a solid mass. Significantly there was no apparent difference between a broken down gel with low viscosity and a 'normal' viscous gel. Partial sublimation showed a porous channelled network with filaments of two different thicknesses: an inner small pore network with pore diameter of 0.2-0.8μ and an overlying structure of larger pores with filaments 0.1-0.5μ thick and pores 1-2μ wide. Other techniques have demonstrated pores of only one size. In this work the pore size was found to be reproducible and of uniform appearance over the entire fractured surface of the sample.

Porcine gastric mucus gels treated with acetyl-cysteine or mercaptoethanol which reduce disulphide links in the glycoprotein showed considerable thickening of the larger filaments up to 1μ and breakdown of the overlying structure in some areas. However, the small pores remained apparently unaltered. Mucus glycoprotein reduced with mercaptoethanol and reacetylated with iodoacetamide also showed the characteristic thickening of some filaments. However a gel treated with mercaptoethanol and then dialysed over 24 hours regained the structure of the original gel. This would support the hitherto theoretical proposal that reformation of disulphide linkages between glycoprotein molecules previously reduced by mercaptoethanol or acetyl-cysteine is possible.

The porous network seen by the SEM procedure is related to the location of water molecules in the gels and ice crystal formation in the freezing process. However, reproducibility of results with rigorously controlled freezing conditions allows changes in the mucus gels and associated water to be detected. The previously observed artefacts can therefore be controlled such that the SEM appearance could reflect in vivo changes in the mucus and may be valuable in determing the mode of action of putative mucolytic drugs.

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