# In vitro mucopolysaccharide metabolism of epithelial tissue cells

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# IN VITRO MUCOPOLYSACCHARIDE METABOLISM OF EPITHELIAL CELLS

SUMMARY

Intercellular protein/polysaccharide matrices have been implicated in the regulation of diffusion and transport mechanisms in biorheology, biocements and in the maintenance of tissue integrity.

Biochemical identification of macromolecular polysaccharide corresponding to proteoglycans (mucopolysaccharides) in an amnion cell line, and evidence of de novo synthesis have been achieved. Primary cell cultures of disaggregated human gingival epithelium and short term gingival slice incubations revealed that these cells were also capable of synthesising intercellular proteoglycan.

Techniques to localize such materials both within intercellular sites and as retained intracellular components have been developed. They include modifications of conventional histochemistry and autoradiographic methods. The specificity of the incorporation of radioactive precursors during 'pulse' incubation has been improved by utilisation of controlled precipitation with detergents such as cetylpyridinium chloride, together with sequential elution with increasing salt solution.

Some preliminary resolution of the biosynthetic events which are involved in elaborating the sulphated polysaccharides in both amnion and gingival epithelial cells also relied on detergent precipitation techniques, followed by cellulose acetate electrophoresis, paper chromatography and high voltage electrophoresis. Amnion cells grown to different degrees of monolayer development have been assayed for uronic

acid and sulphate as well as for protein associated with cetylpyridinium chloride precipitable material.

Cells of fragmented human gingivae were fractionated according to the size of the cell clumps that passed sequentially through nylon filters of decreasing gauge. This procedure provided cells ranked with respect to their intercellular surface contact. Uronic acid, sulphate and associated protein was assayed on representative culture of these categories. Following one interpretation of the data presented in this thesis, an hypothesis has been advanced that the degree of sulphation of intercellular matrix macromolecules which contain chondroitin sulphate reflects the degree of the cell-cell interface contact.

The addition of hyaluronate to gingival-epithelial cell cultures inhibited both proteoglycan synthesis and secretion similarly to that previously described by the author for chondrocytes. The implication that the initial mode of action of hyaluronate on gingival epithelial cells was due to passive binding at the cell surface was tested with fluorescently labelled hyaluronate. This material would bind to epithelial cell surfaces and was shown to be indistinguishable from unlabelled hyaluronate with respect to biological activities (namely ~~proteoglycan~~ inhibition of biosynthesis of proteoglycans by chondrocytes).

Associative and dissociative extractions of proteoglycans from separated gingival epithelium and connective tissue provided material which was fractionated on Sepharose 2B-CL gel chromatography. Reassociation of a connective tissue proteoglycan fraction in the absence

of extraneous hyaluronate resulted in the identification of a species of macromolecule, distinct from a fraction extracted from the epithelium. This latter fraction had the capacity to interact with hyaluronate.

The results emphasise the importance of the intimate extracellular matrix in the regulation of the maintenance of tissue integrity. Not only does the amount (total uronic acid) appear to be important, the composition (sulphate/protein levels) and conformation (molecular size and extractability) of the intact proteoglycan are arguably fundamental in determining whether tissue is susceptable to degradative disease processes. Macromolecular interactions within extracellular matrices are thus regarded as arbiters of structural as well as functional tissue integrity.