Reagents for the electron microscopical demonstration of connective tissue proteoglycans

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Our aim is to demonstrate tissue proteoglycan structure in 3 dimensions, as it relates to the other molecules and cells in the tissue, by electron microscopy. Since proteoglycans are of a similar electron density to that of their in vivo environment, electron 'stains' must be applied (in practice to the polyanion) to enhance contrast. There is a complete dearth of relevant specific chemical reactions, and ionic interactions currently offer best prospects. Staining with electron dense cationic dyes nevertheless cannot display the complete 3 dimensional structure, as present in vivo, since staining is a precipitation reaction (Scott, 1973) which necessarily involves a considerable collapse of the polyanion domain (Scott, 1974), and probably translocation of the polymer in the process. Moreover, conventional processing through non-solvents into plastic media for electron microscopy inevitably results in a deflation of the polyanion domain. However, the staining molecule helps determine the morphology of the polyanion (Scott, 1974, 1976), in that small-sized precipitants (e.g. La^{+++}) cause the greatest degree of collapse and greatest contrast, whereas larger molecules (Alcian Blue) produce more diffuse, less contrasted complexes. Intermediate sized molecules (e.g. cinchomeronic phthalocyanin (Scott, 1976) characteristically give electron dense complexes with recognisable features of the native proteoglycan. Used according to critical electrolyte concentration principles (Scott, 1973), this reagent is able to pick out proteoglycans in connective tissues. Contrast intensification using sodium tungstate, (Scott & Jones unpublished, see poster) permits the clear localisation of proteoglycans in e.g. rat tail tendons in relationship to collagen fibres. Some form of fixation is essential. It appears that there is a regular relationship, in which the proteoglycan is present like an external 'fishnet' around the collagen fibrils.

Scott, J.E. (1972) in "Current Developments in Rheumatology" p.10-29 Ed. H.Boström, B.Tillander, Stockholm.

Scott, J.E. (1973) Trans.Biochem.Soc. 1, 787-806.

Scott, J.E. (1974) in "Normal and Osteoarthritic Articular Cartilage" p. 19-33 Ed. S.Y.Ali, M.W.Elwes & D.H.Leaback, published by Institute of Orthopaedics, London.

Scott, J.E. (1976) Ann.Méd.Reims, 13, 2-3 63-67.