The Distribution of Acidic Mucopolysaccharides in Corneal Stroma

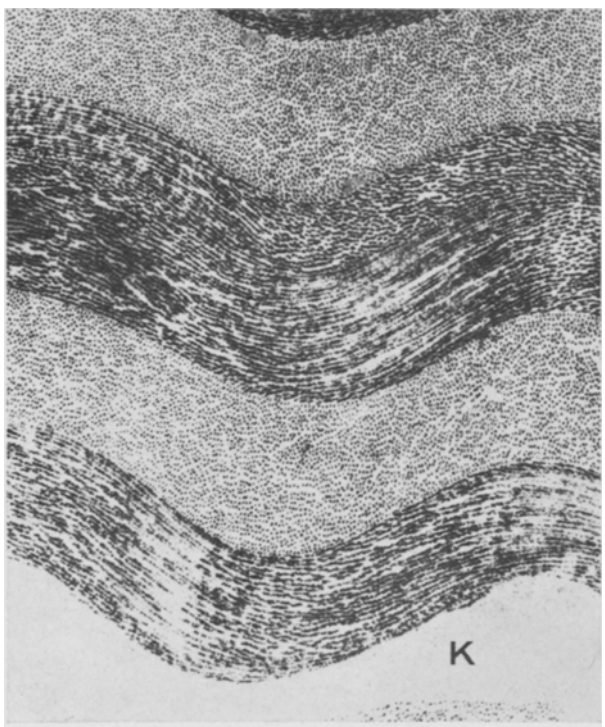
Corneal stroma, like most connective tissues, derives its mechanical properties from the collagen fibres which constitute most of its dry weight[1]. Corneal collagen fibres exhibit a special geometry[2]. In each lamella, or bundle of fibres, all the fibres are parallel and equidistant. Nearest neighbour separation is about 500 Å. This orientation has been shown by MAURICE[3] to be essential for corneal transparency. The other main macromolecular constituents of corneal stroma are the acidic mucopolysaccharides, chondroitin sulphate and keratan sulphate[4]. The position of the collagen fibres may be seen 'directly' in the electron microscope but the distribution of the acidic mucopolysaccharides (AMPS) is less certain and has been the subject of speculation. It has been claimed that the AMPS is a ground matrix in which the collagen fibres are embedded[3]. This seems to imply that the distribution of the AMPS is uniform throughout the stroma. SMITH and FRAME[5] have detected threads of material between neighbouring collagen fibres which they suggest is AMPS. Chondroitin sulphate is covalently bound to the collagen fibre[6] and its reported molecular weight[7,8] allows for about 100 repeating disaccharide units each measuring about 6 Å. It seems possible therefore that the AMPS may link neighbouring fibres.

Recently a new electron histochemical technique has been developed which forms a reaction product specifically over sites occupied by acidic groups of low pK[9]. It may therefore be used to localize the acidic groups of both chondroitin and keratin sulphates. Adult Dutch rabbits were killed by an i.v. injection of nembutal and the corneas processed for electron microscopy as before[10]. Fixation and processing extracts none of the AMPS from corneal stroma[11]. Thin sections were stained for low pK acidic groups by the periodic acid-silver methenamine technique and were examined in the electron microscope without further staining. The results are shown in the Figure. The collagen fibres are not visible after the staining technique. In some lamellae, the rows of crystals can be seen to follow the route of individual collagen fibres. In neighbouring lamellae, sectioned through the collagen fibres, the crystals are also clearly localized over the collagen fibres. (It may be noted that in these preparations the collagen fibres are not equidistant as required by MAURICE's lattice theory of transparency[3]. This is because chemical fixation of corneal stroma always causes swelling of the stroma and deformation of the fibre lattice. A regular fibre lattice is seen only after freezing and freeze substitution techniques[12].) Crystals are never found in the spaces between the collagen fibres. The absence of reaction product over the keratocyte cells indicates the specificity of the reaction.

The results show that AMPS is found only in close proximity to the collagen fibres. They do not support the concept of a ground matrix of AMPS nor that the AMPS bridge the gaps between neighbouring fibres.

AMPS is known to generate the swelling pressure of the cornea[13]. There are two ways in which it might do this. The first is by the Donnan potential associated with a non-diffusible (fixed) negative charge. The second is by electrostatic repulsion between the acidic group of the AMPS. Now that the distribution of AMPS is known it might be possible, with further data, to calculate the zeta potential around each fibre and, in principle, what amount the electrostatic repulsion contributes to the swelling pressure.

Résumé. La distribution des mucopolysaccharides acides dans le stroma cornéen est examinée. Tous les mucopolysaccharides se trouvent sur les fibrilles de collagene, tandis qu'il n'y en a pas entre les fibrilles.



Area of the corneal stroma. K, Keratocyte. × 7000.

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