RELATIONSHIPS BETWEEN CONNECTIVE TISSUE PROTEOGLYCANS AND FIBROUS PROTEINS, AS SHOWN BY ELECTRON MICROSCOPY

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Our study on proteoglycans of the extracellular matrix was conducted directly on tissues, utilizing techniques of histochemical electron microscopy. The submicroscopic pattern of proteoglycans and their interactions with other fibrous components were the objects of our research.

We examined cartilage from different origins (nasal, articular, tracheal, and costal), the aortic wall, the derma, the cornea and fibroblasts and condrocytes taken from an 18-day-old chick embryo and cultured in vitro under different experimental conditions.

The histochemical technique was based on the use of alcian blue dissolved in critical electrolyte concentrations of $MgCl_2$ and used between treatment with glutaraldehyde and osmium respectively. Similar treatment, preceded by incubation with testicular hyaluronidase and condroitinase ABC, was performed, as was normal fixation with glutaraldehyde and osmium. Preparations for the freeze-etching techniques were carried out together with the histochemical procedures.

Proteoglycans of various connective tissues are difficult to visualize at the electron microscope after usual glutaraldehyde and osmium fixation. However, in cartilage, where a large amount of proteoglycans are present, they appear as electron dense granules. These granules, can be more easily demonstrated by the use of cationic stains such as alcian blue, ruthenium red, iron, bismuth nitrate, etc.

The treatment with alcian blue dissolved in critical electrolyte concentrations of $MgCl_2$ (Scott and Dorling: Histochemie 5, 221, 1965) permits the visualization, both in cartilage and connective tissue, of long linear electron dense structures uniformly distributed in the intercellular and pericellular areas. These particles have been identified enzymatically as proteoglycan monomers and/or aggregates.

The differences in the molecular composition of the proteoglycans, found in various tissues, do not have a direct correlation with the electron dense particles. Nevertheless, from our observations at least two different types of particles can be distinguished. One is filament-like with a uniform diameter of about 80 Å. The other is leaf-like in appearance and has a diameter that varies from 100 to 250 Å. Both particles are resolvable into two thin subunits. In proximity to the collagen fibrils, the subunits may diverge from each other and assume a singular relation with the fibrils. An attempt to identify such proteoglycan particles in replicas of freeze-etched connective and cartilage tissues was made.

As stated above, in tissues treated with alcian blue, the cartilage proteoglycans are kept as distended particles between the other fibrous components and are not coiled in granules. In respect to the granules, the particles are more uniformly electron dense and occupy wider areas in the electron microscopic pictures. These characteristics suggest the possibility of a morphometric evaluation of proteoglycans and, therefore, of a volumetric determination, both absolute and relative to the collagen fibrils.