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Sheathing of collagen fibrils in human intervertebral discs

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INTRODUCTION

During a study of human intervertebral disc ultrastructure, we observed electron-dense cylinders sheathing collagen-like fibrils in the annulus fibrosus and nucleus pulposus. Previous studies of human, cow, cat and rabbit intervertebral discs do not mention such sheaths (Sylven, Paulson, Hirsch & Snellman, 1951; Dahmen, 1963; Happey, Johnson, Naylor & Turner, 1964; Smith & Serafini-Fracassini, 1968; Vasilev & Ruseva, 1969*a*, *b*; Cornah, Meachim & Parry, 1970; Meachim & Cornah, 1970; Butler & Fujioka, 1972*a*, *b*; Meachim, 1972).

MATERIALS AND METHODS

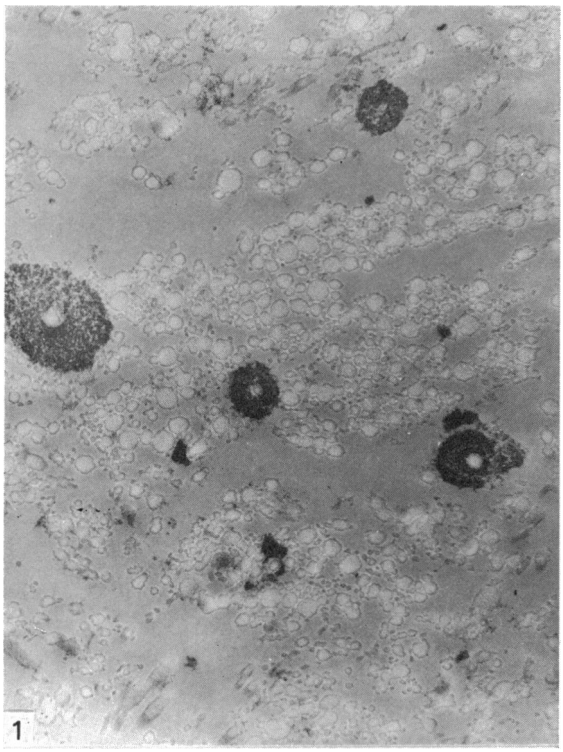
We examined intervertebral discs from 25 patients, 12 hours to 71 years old. There were discs from ten patients with scoliosis, and from seven with intervertebral disc herniation, while eight discs were removed at autopsies performed within 4 hours of death on patients without a history of disc disease. Annulus fibrosus and nucleus pulposus were fixed in cacodylate-buffered (pH 7·4) 3 % glutaraldehyde at 0 °C for 2 hours, post-fixed in buffered (pH 7·4) osmium tetraoxide, dehydrated in increasing concentrations of ethanol in water, and embedded in Epon 812. Thin sections were mounted on copper grids and stained by one of three methods: uranyl acetate alone; uranyl acetate and lead citrate; and ruthenium red.

Specimens treated with ruthenium red were processed as outlined above except that they were fixed in two parts buffered glutaraldehyde and one part ruthenium red stain (6 mg ruthenium red per ml distilled water) for two hours at 0 °C and post-fixed in one part buffered osmium tetraoxide and one part ruthenium red stain. The sections were examined with an RCA EMU 3H electron microscope.

OBSERVATIONS

We found electron-dense sheathing of some collagen-like fibrils in both annulus fibrosus (Figs. 1, 2) and nucleus pulposus (Fig. 3) in all 25 intervertebral discs. Although electron microscopy does not allow accurate estimation of the quantity of the sheaths, we identified them in every specimen. We found them most frequently in specimens from patients more than 25 years old, and in herniated intervertebral discs.

Commonly the electron-dense material surrounded a single collagen-like fibril in a field of hundreds. Occasionally sheaths enveloped two or three collagen-like fibrils (Fig. 2). Sheaths appeared circular in transverse section, and varied from 3 to 12



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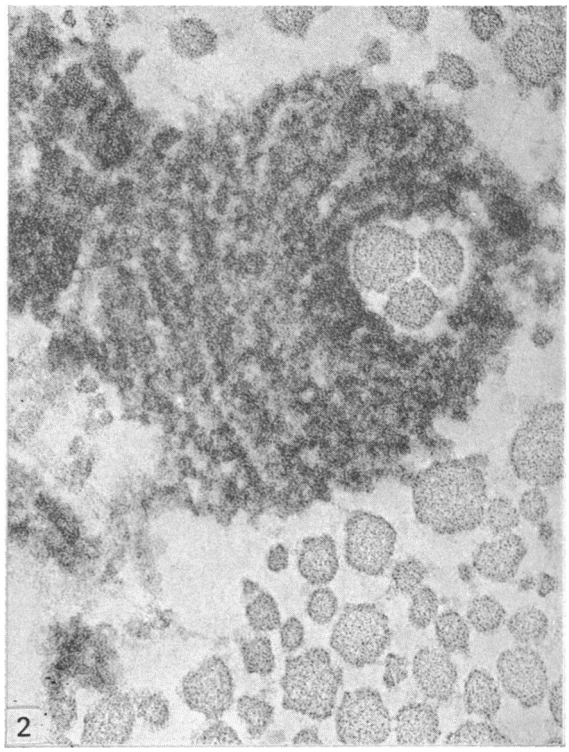

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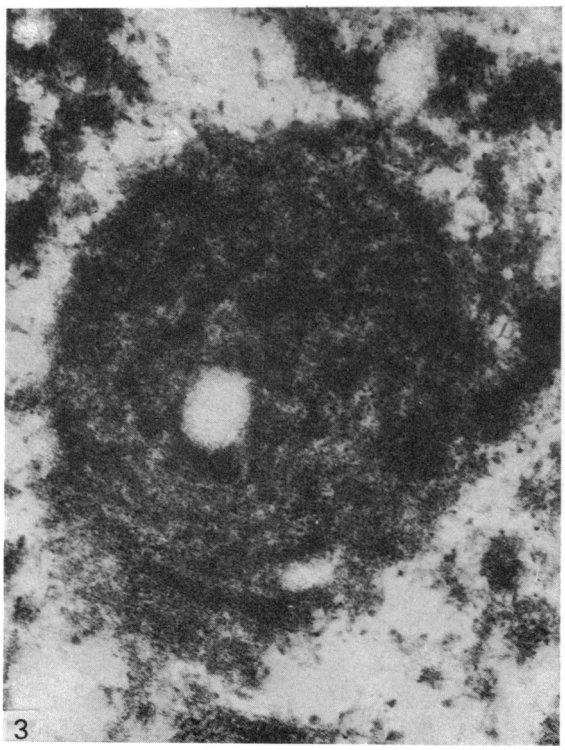

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times the diameter of the encased collagen-like fibril. Longitudinal sections of the sheaths demonstrated a central cross banded fibril (Fig. 4). The central sheathed fibril did not differ in size, pattern of cross banding or staining characteristics from the surrounding native collagen fibrils.

The internal structure of the sheaths appeared similar on transverse and longitudinal sections (Figs. 3, 4). They consisted of electron-dense granules or particles and occasional filaments. The dense particles resembled those seen in the extracellular matrix (Fig. 3). In many transverse sections the particles formed concentric layers around the central fibril.

Sheaths treated with ruthenium red (Fig. 4) resembled those stained with uranyl acetate or uranyl acetate and lead citrate. Sections stained only with uranyl acetate showed less contrast between the sheaths and the surrounding matrix than those treated with lead citrate, but were identical otherwise.

DISCUSSION

The precise appearance of the sheaths depended on which techniques of tissue fixation, embedding, and staining were employed. However, the consistency of their morphological features in all the specimens studied suggests that they are normal elements of intervertebral disc structure. The apparent increased frequency of the sheaths in sections from older discs and herniated discs may either reflect a change in the staining properties of the material or indicate an actual increase in the number of sheathed fibrils.

Electron-dense granular material surrounding cross banded fibres has been identified *within* myofibroblasts, and its presence has been attributed to some abnormality in the secretion, polymerization, or phagocytosis of collagen (Ryan *et al.* 1973). However, the sheaths of the intervertebral disc described here are extracellular, and large relative to the enclosed fibril.

Since collagen fibres and proteoglycans form the major macromolecular components of most connective tissues, it seems reasonable to consider that the sheaths may be a form of proteoglycan; but previous studies of proteoglycans in synovium, cartilage and nucleus pulposus have not demonstrated such sheaths. Meyers, Highton & Rayns (1969) studied acid mucopolysaccharides associated with synovial collagen fibrils, using ruthenium red staining. They identified approximately 20 nm thick, irregular, amorphous, distinctly granular coats covering collagen fibres. Collagen sheaths of the intervertebral discs are considerably thicker, and usually surround only one fibril among many. To demonstrate extracellular proteoglycans, Thyberg, Lohmander & Friberg (1973) stained guinea-pig cartilage with alcian blue and ruthenium red. Although they found proteoglycans closely related to collagen fibrils, they did not describe structures resembling the collagen sheaths. From

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Fig. 1. Sheaths surrounding simple random collagen fibrils in the annulus fibrosus from a 54 years old woman. Uranyl acetate and lead citrate. × 19964.

Fig. 2. A sheath enveloping three collagen fibrils in the annulus fibrosus from a 40 years old man. Uranyl acetate and lead citrate. × 103025.

Fig. 3. A sheath in the nucleus pulposus from a 71 years old man. Uranyl acetate and lead citrate. × 70374.

Fig. 4. Longitudinal section of a sheath from the annulus fibrosus of a 54 years old man demonstrating typical cross banded collagen fibril. Ruthenium red staining. × 54841.

selective enzymatic digestion studies they concluded that ruthenium red stains proteoglycans, but may not demonstrate all of the extracellular proteoglycans. Smith & Serafini-Fracassini (1968) studied the distribution and relationships of the collagen protein–polysaccharide complex in the extracellular matrix of rabbit nucleus pulposus, but did not identify structures similar to the sheaths. The sheaths may consist of protein or proteoglycan forming a previously unrecognized relationship with collagen. Possibly there is an increasing tendency for extracellular protein or proteoglycan to become attached to collagen fibrils as the intervertebral disc ages, or when collagen is subjected to mechanical stress or changes in its chemical or osmotic environment.

SUMMARY

In 25 human intervertebral discs studied by electron microscopy, sheathing of collagen-like fibrils by electron-dense cylinders was observed. The sheaths consisted of layers of dense granules 3 to 12 times the diameter of the enclosed collagen-like fibrils, and they appeared to be more frequent in older discs.

REFERENCES

BUTLER, W. F. & FUJIOKA, T. (1972*a*). Fine structure of the annulus fibrosus of the intervertebral disc of the cat. *Anatomischer Anzeiger* **132**, 454–464.

BUTLER, W. F. & FUJIOKA, T. (1972*b*). Fine structure of the nucleus pulposus of the intervertebral disc of the cat. *Anatomischer Anzeiger* **132**, 465–475.

CORNAH, M. S., MEACHIM, G. & PARRY, E. W. (1970). Banded structures in the matrix of human and rabbit nucleus pulposus. *Journal of Anatomy* **107**, 351–362.

DAHMEN, V. G. (1963). Submikroskopische Untersuchungen an Wirbelbandscheiben. *Zeitschrift für Rheumaforschung* **22**, 192–213.

HAPPEY, F., JOHNSON, A. G., NAYLOR, A. & TURNER, R. L. (1964). Preliminary observations concerning the fine structure of the intervertebral disc. *Journal of Bone and Joint Surgery* **46B**, 563–567.

MEACHIM, G. (1972). Meshwork patterns in the ground substance of articular cartilage and nucleus pulposus. *Journal of Anatomy* **111**, 219–227.

MEACHIM, G. & CORNAH, M. S. (1970). Fine structure of juvenile human nucleus pulposus. *Journal of Anatomy* **107**, 337–350.

MEYERS, D. B., HIGHTON, T. C. & RAYNS, D. G. (1969). Acid mucopolysaccharides closely associated with collagen fibrils in normal human synovium. *Journal of Ultrastructure Research* **28**, 203–213.

RYAN, G. B., CLIFF, W. J., GABBIANI, G., IRLE, C., STATKOV, P. R. & MAJNO, G. (1973). Myofibroblasts in an avascular fibrous tissue. *Laboratory Investigation* **29**, 197–206.

SMITH, J. W. & SERAFINI-FRACASSINI, A. (1968). The distribution of the protein–polysaccharide complex in the nucleus pulposus matrix in young rabbits. *Journal of Cell Science* **3**, 33–40.

SYLVEN, B., PAULSON, S., HIRSCH, C. & SNELLMAN, O. (1951). Biophysical and physiological investigations on cartilage and other mesenchymal tissues. II. The ultrastructure of bovine and human nuclei pulposi. *Journal of Bone and Joint Surgery* **33A**, 333–340.

THYBERG, J., LOHMANDER, S. & FRIBERG, U. (1973). Electron microscopic demonstration of proteoglycans in guinea-pig epiphyseal cartilage. *Journal of Ultrastructure Research* **45**, 407–427.

VASILEV, V. & RUSEVA, M. (1969*a*). Electron microscopic investigations of the annulus fibrosus in mammals. *Acta medica Instituti superiorus medici Sofia* **48**, 69–80.

VASILEV, V. & RUSEVA, M. (1969*b*). Electron microscopic investigations of nucleus pulposus in cat. *Acta medica Instituti superiorus medici Sofia* **48**, 81–89.