Acta orthop. scand. 48, 32–40, 1977

ULTRASTRUCTURE OF PROLAPSED DISC

Jesper Sylvest[1], Bent Hentzer & Takasi Kobayasi

University of Copenhagen Department of Dermatology (with Connective Tissue Research Laboratories), Rigshospitalet, Copenhagen, Denmark.

Prolapsed tissue and removed interspace contents were obtained during hemilaminectomy for disc prolapse. The ultrastructure of the tissue was studied. Division of the material into *annulus fibrosus* and *nucleus pulposus* proved to be inaccurate. Chondrocytes were always the predominant cell type and could be classified into normal, cloning and necrotized types. Matrix vesicles were found in relation to the haloes of chondrocytes and seemed to be products of disintegrated chondrocytes. Findings of crystals in the vesicles were rare. The intercellular substance showed degraded collagen fibrils, which could be seen in spindle shaped cells as well. A dense amorphous material interpersed with collagen fibrils seemed to be a glycoprotein. A few elastic fibres were found, but without any evidence of severe degeneration. These findings represent changes occurring in cells and in the intercellular substance.

Key words: intervertebral disc displacement; ultrastructure; chondrocytes; matrix vesicles; collagen; proteoglycan; glycoprotein: elastic fibres

Accepted 22.xi.76

Knowledge of the ultrastructure of undisplaced human discs is based on examinations of necropsies from normal spines (Sylvén et al. 1951, Watanabe 1960, Dahmen 1963, Gomibuchi 1964, Render 1971, Inoue 1973, Takeda 1975, Inoue & Takeda 1975) and biopsy material from scoliotic spines obtained during surgical operation for wedge resection (Cornah et al. 1970, Meachim & Cornah 1970, Meachim 1972).

Hence, the interpretation of the ultrastructural findings on a prolapsed disc is hampered by incomplete knowledge of the structure of the fresh normal disc, and, furthermore, by specific difficulties due to the mixed nature of prolapsed specimens (Brown 1971).

These may be the reasons why no comprehensive study on the ultrastructure of the displaced intervertebral disc has been carried out since 1963–64 when Dahmen, Happey et al. and Gomibuchi reported their findings. Because of the less-developed preparation technique they used, a more detailed ultrastructural picture of prolapsed discs is still unavailable.

MATERIAL AND METHODS

Specimens were obtained during hemilaminectomy for disc prolapse from six patients (three males and three females) ranging from 31 to 70 years of age. The patients had had significant clinical symptoms for 2 to 9 months. The level

[1] Supported by a fellowship from the Danish Rheumatism Association.

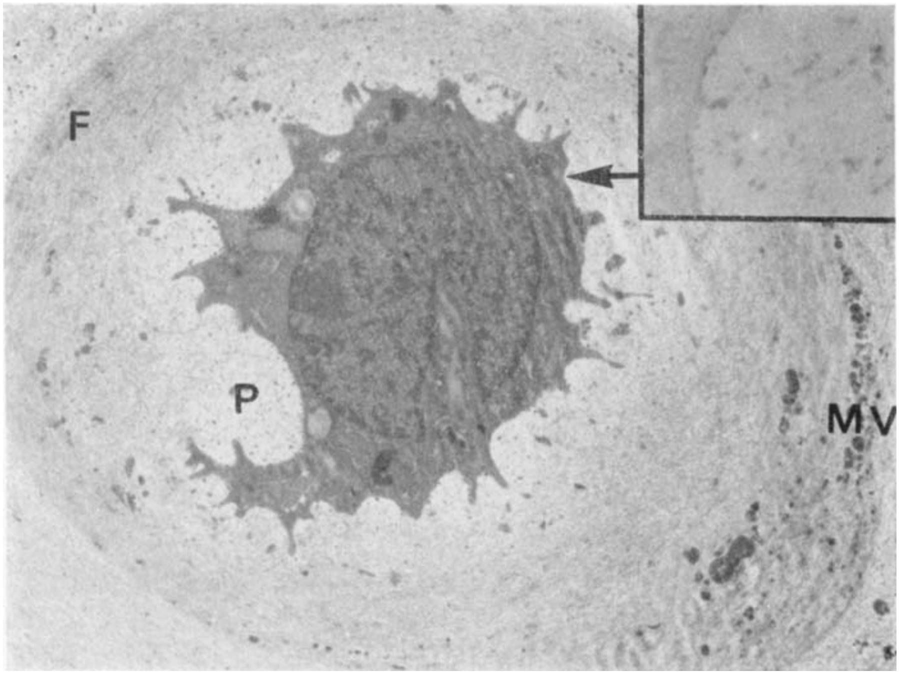

Figure 1. A chondrocyte surrounded by a halo consisting of a pericellular lacuna (P) and fine collagen fibrils (F). Outside the halo a collection of matrix vesicles (MV). × *6,000.*
Inset: Proteoglycan granules are seen in the pericellular lacuna. Ruthenium-red stain. × *60,000.*

of herniation was L_4 or L_5. According to surgical findings, five patients had a protruded disc. One disc was found to be ruptured.

According to their anatomical position, the tissue specimens were classified into a herniated part and an intervertebral part.

The tissue specimens were fixed in a 6 per cent glutaraldehyde solution in 0.1 M cacodylate buffer at pH 7.4 with 7.5 sucrose at 4° C, and osmicated with 1 per cent osmic acid. After dehydration of the specimens in graded alcohols, they were embedded in Epon 812 and ultrathin sections were stained with uranyl acetate and lead citrate. Some sections were also stained with ruthenium red (Kobayasi & Asboe-Hansen 1971) and periodic acid silver proteinate (Thiéry 1967).

A Siemens electron microscope was operated at 80 kV with double condensors.

OBSERVATIONS

The chondrocytes were surrounded by a halo consisting of a pericellular lacuna and an outer fibrillar ring. Three different types of arrangements could be identified. In one type, a single oval cell was found to be surrounded by a thick fibrillar ring made up of collagen fibrils with a diameter of 100–200 Å (Figure 1). Inside this, the pericellular lacuna contained numerous granules with delicate branches. The granules stained with ruthenium red, representing high contents of proteoglycans (Figure 1 inset). The chondrocyte showed a large nucleus with one nucleolus. The cytoplasm contained Golgi cisternae, distinct granular endoplasmic reticulum, a few mitochondria, lysosomes and fat droplets.

A second type of arrangement consisted of one or several cells encircled by one common halo (Figure 2). The halo was formed of an outer narrow fibrillar part and an inner broad proteoglycan part. The cells had short cytoplasmic processes which did not reach the fibrillar ring. The cells contained a well-developed granular endoplasmic re-

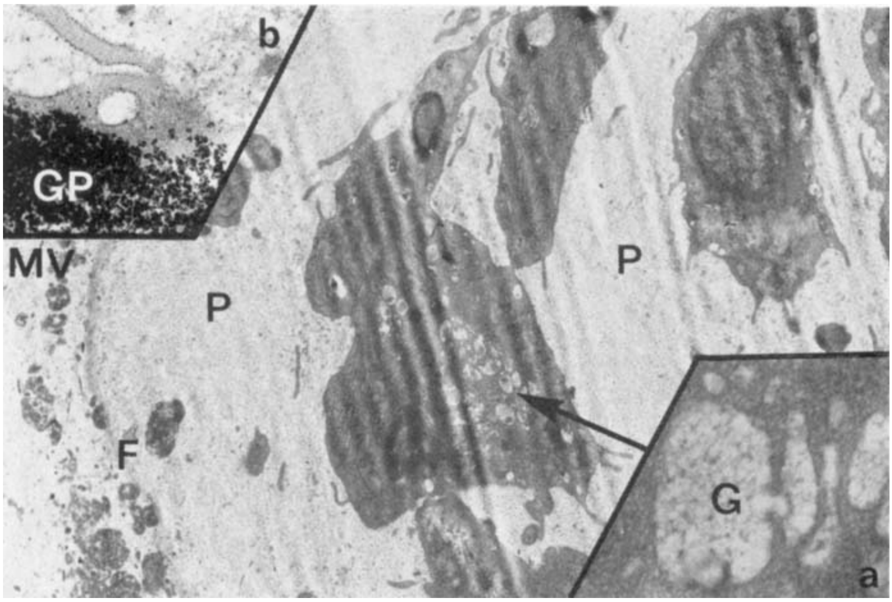

Figure 2. Chondrocytes surrounded by one common halo. Broad pericellular lacuna (P). Thin fibrillar ring (F). Matrix vesicles (MV). × *6,000.*

Inset a: Detail of dilated Golgi cisternae containing proteoglycan figures. × *60,000.*

Inset b: Glycogen particles (GP) in the cytoplasm of a chondrocyte. Periodic acid silver proteinate. × *30,000.*

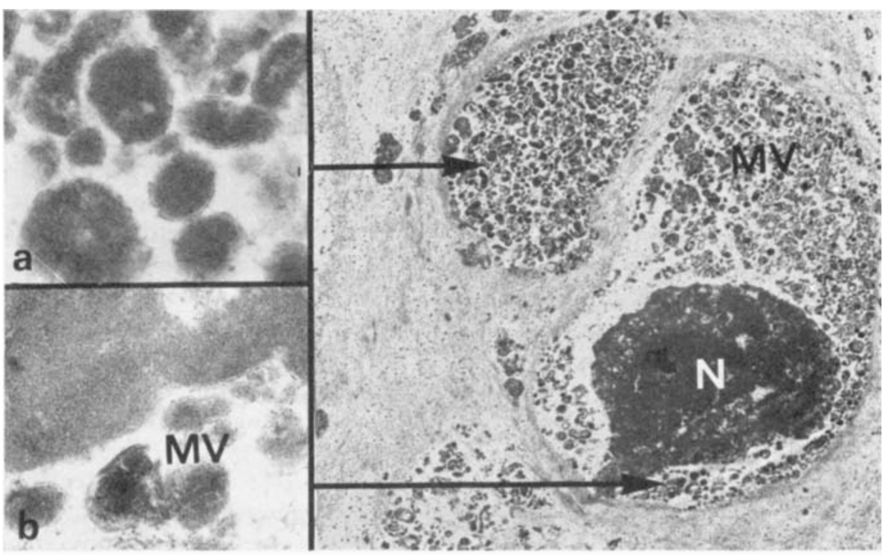

Figure 3. Necrotic chondrocyte (N). Lots of matrix vesicles (MV) are seen in the pericellular lacuna. × *6,000.*

Inset a: Pleomorphic matrix vesicles lying in cluster formation. × *60,000.*

Inset b: Matrix vesicles at the surface of necrotic chondrocyte. The plasma membrane is disrupted. × *60,000.*

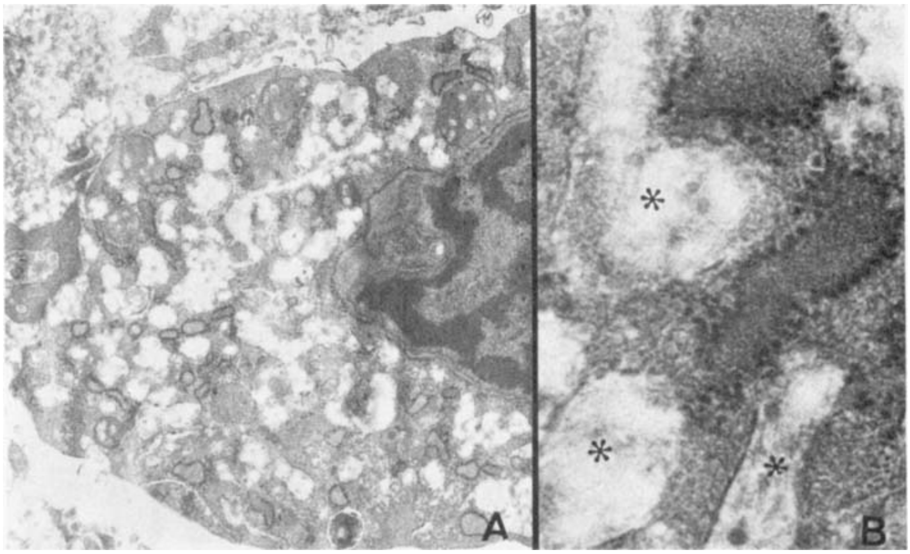

Figure 4 A. A spindle shaped cell containing collagen fibrils. × *15,000.*

Figure 4 B. Detail of engulfed collagen fibrils (asterisks). × *60,000.*

ticulum, mitochondria, lysosomes, and dilated vesicles. The Golgi apparatus was dilated with proteoglycan figures in the cisternae (Figure 2 inset a). Fat droplets and numerous PAS-positive glycogen particles were deposited in the cytoplasm of some of the cells (Figure 2 inset b).

The third type of arrangement consisted of one or occasionally two cells surrounded by an often poorly defined fibrillar ring. The cells were necrotic and had a dark cytoplasm with lucent spaces containing proteoglycan figures (Figure 3). The cytoplasmic details could not be identified except for the large fat droplets. In the immediate vicinity of the cell surface, large numbers of membrane-invested vesicles were found (Figure 3 inset b).

A common finding in all the observed haloes was the presence of membrane-bounded vesicles located immediately outside the fibrous ring. They often appeared at one side of the periphery, lending the group a semilunar shape in section. In the first two of the above-described chondrocyte arrangements, the vesicles were never found inside the halo. The individual vesicle was round, oval or kidney shaped (Figure 3 inset a). The size varied from 500 to 3,000 Å with a modal value of approximately 1,000 Å. It was bounded by a double membrane and contained a material of varying density. However, empty vesicles were also found. Sometimes the limiting membrane was disrupted, and extrusion of vesicle contents could be seen. Rarely, fine crystalline formations could be identified inside the vesicles, but only provided no counterstaining had been used.

A minority of cells had no chondrocytic appearance. These cells were spindle shaped without a halo and were always found in the dense fibrillar tissues (Figure 4). They held a distinct granular endoplasmic reticulum, lysosomes and often phagocytized collagen fibrils.

The intercellular substance consisted of collagen fibrils, proteoglycans, dense amorphous material, filament bundles with cross bands, and occasionally, elastic fibres.

In the prolapsed part of the discs, the

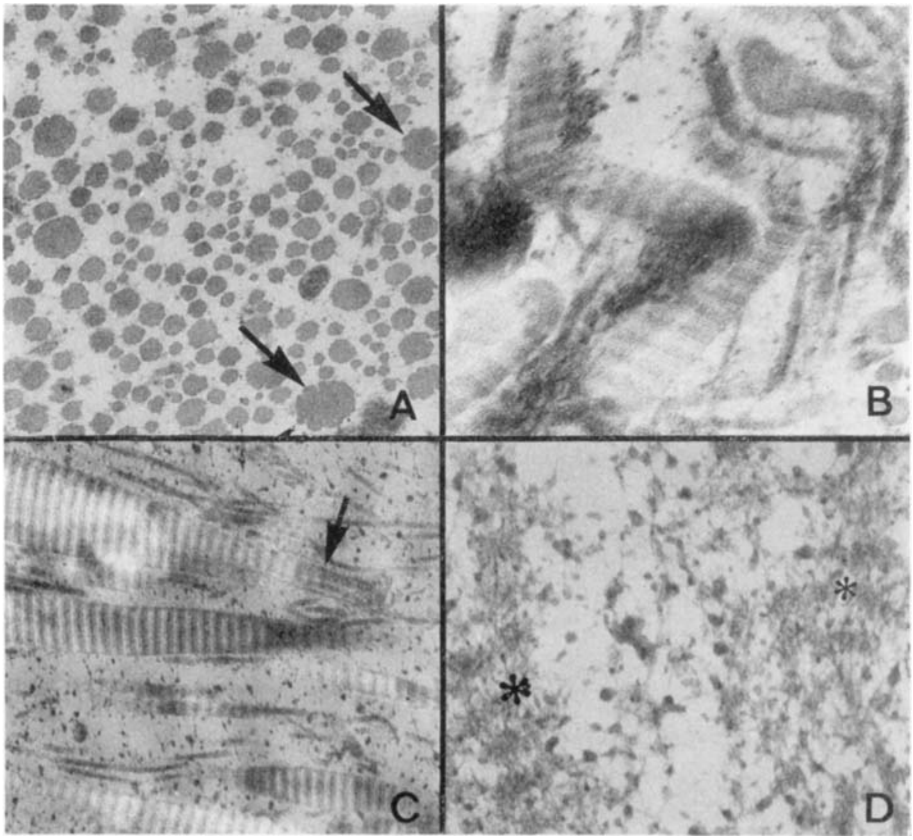

Figure 5 A. Abnormal collagen fibrils showing varying diameters and irregular margins (arrows). × 30,000.

Figure 5 B. Disarranged collagen fibrils, showing curled and bent shapes. × 60,000.

Figure 5 C. Thick collagen fibrils, showing tapered ends (arrow). × 30,000.

Figure 5 D. Cross-banded filamentous bundles (asterisks). × 60,000.

most densely packed collagen was noted, while in the intervertebral part, more proteoglycan figures and less collagen fibrils were present. An absolute correlation between fibrillarity and the presumed contribution of annulus fibrosus to the tissue, as estimated by the *in situ* location of the specimen was, however, not found.

The collagen fibrils when tightly packed were running parallel to each other in firm bundles. In the case of looser arrangement, no predominant direction of the fibrils was found. The axial periodicity was always normal. The range of the diameters was 100–4,000 Å. However, most fibrils had diameters within the range of 300-2,500 Å, but they varied considerably between the fibrils even within small areas (Figure 5 A). Pathological phenomena, such as irregular cut surfaces, curling, bending and tapered ends were common (Figure 5 A, B, C).

The dense amorphous material was seen in large compact masses or spread out, interspersed between the collagen fibrils or bundles (Figure 6 A). Numerous proteoglycan figures were seen in this material. The material was periodic acid silver proteinate positive (Figure

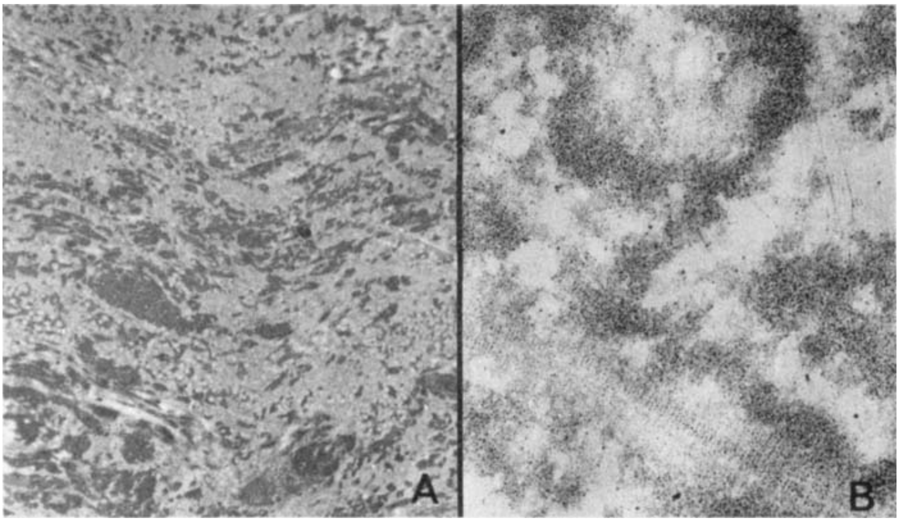

Figure 6 A. Dense amorphous material interspersed with collagen fibrils. × *6,000.*

Figure 6 B. The dense amorphous material surrounding collagen fibrils shows staining with periodic acid silver proteinate. × *60,000.*

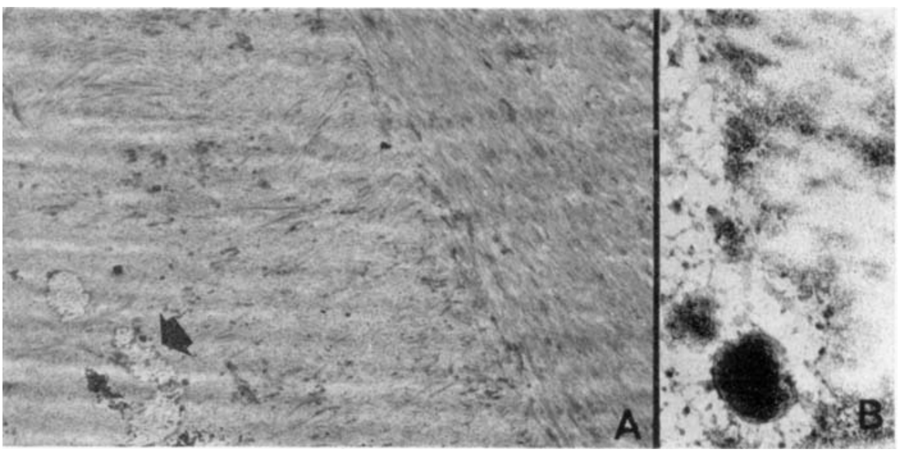

Figure 7 A. Tightly packed collagen fibrils on the right hand side of the micrograph, while, left, the micrograph shows a loose arrangement of the collagen fibrils with proteoglycan figures and elastic fibres (arrow). × *6,000.*

Figure 7 B. The arrow-pointed elastic fibre in Figure 7 A. A matrix vesicle is seen. × *60,000.*

6 B), while the ruthenium-red staining turned out to be more faint.

Filamentous bundles with cross bands with a periodicity of 800–900 Å (Figure 5 D) were usually located at the haloes of the chondrocytes. However, they were never found in the first described chondrocyte type.

Elastic fibres were identified especially at the borderline between the *annulus fibrosus* and the *nucleus pulposus* (Figure 7). They appeared without fibrils but were coated with a PAS-positive material.

DISCUSSION

In the present study, the prolapsed tissue and removed contents of the disc interspace have been described.

The first chondrocyte arrangement described represents the healthy cells found in discs of scoliotic spines (Meachim & Cornah 1970, Meachim 1972) and the chondrocytes in the middle layer of normal articular cartilage (Weiss 1973, Lane & Weiss 1975). The cells are believed to take part in the normal turnover of the matrix.

The second type of chondrocyte arrangement shows cloning cells and evidence of increased secretion. It is found in degenerating cartilage (Weiss 1973) and is interpreted as an attempt at repair, compensating for loss of intercellular substance.

The third chondrocyte type characterizes a stage of cell death. Necrotic chondrocytes are a frequent observation in discs of scoliotic spines and are found in osteoarthritic cartilage as well (Weiss 1973). Surrounding the necrotic cell remnant, we found numerous membrane-bounded bodies which seem to be identical to the matrix vesicles first described by Anderson (1967) and Bonucci (1967). They are believed to play a major role in calcification of the tissue in which they are found. Their origin is still a matter of debate (Rabinovitch & Anderson 1976).

As necrotic cells in our material were the only cells having matrix vesicles in their pericellular lacuna, it seems likely that they are produced only by these cells.

The rare ocurrence of crystals inside or in the vicinity of matrix vesicles disagrees with the finding that the major function of matrix vesicles should include calcification of the prolapsed disc. Whether the matrix vesicles in the prolapsed disc play a role in the degeneration of the matrix or merely reflect necrosis of chondrocytes seems unclear.

Our finding of varying diameters of collagen fibrils has formerly been described in prolapsed tissue, but in undisplaced necropsy tissue as well (Sylvén et al. 1951, Dahmen 1963, Render 1971) and, therefore, it can hardly be recognized as a pathological phenomenon. However, a greater range of diameters was found in our material. The cross sections of fibres most frequently showed irregular margins. This has been described in collagen after exposure to collagenases (Keech 1954, Kobayasi et al. 1977) and is supposed to represent an effect of a collagenolytic agent.

The tapered ends of fibrils are believed to represent the same phenomenon. This type of collagen change has been described in the prolapsed disc by Dahmen (1963).

The pathological curling of fibrils has also been observed in skin disease, such as necrobiosis lipoidica (Kobayasi et al. 1974) and shagreen patch (Kobayasi et al. 1973). No causative mechanism of the change is known.

Cross-banded filamentous bundles appear in the intervertebral disc of humans (Cornah et al. 1970) and in rabbits (Smith & Serafini-Fracassini 1968). They are described most frequently to be located in the pericellular matrix, and we believe they are a type of collagen. They have been found in other connective tissues as well, and in organ cultured skin, they have turned out to be degradative products of collagen due to collagenolytic activity (Hentzer & Kobayasi 1975). The same mechanism may be responsible for their formation in the disc.

The dense amorphous material is supposed to be a glycoprotein, as it stains heavily with periodic acid silver proteinate. Hence, the substance might be identical to a glycoprotein identified in increased concentrations in older discs (Pearson et al. 1972). In our observations, the affinity of the collagen for this material agrees with its preferred location between collagen fibrils.

Elastic fibrils in discs have been described recently by Buckwalter et al. (1976). In our specimens, elastic fibres were primarily located at what we believe to be the borderline between the annulus and the nucleus. A concentration at this location is reasonable and fits with the theory that a necessary condition for their formation is a tensile force combined with a cross force (Little 1973).

ACKNOWLEDGEMENT

We are indebted to the Department of Neurosurgery, Rigshospitalet, for providing the surgical material.

REFERENCES

Anderson, H. C. (1967) Electron microscopic studies of induced cartilage development and calcification. *J. Cell Biol.* **35**, 81–101.

Bonucci, E. (1967) Fine structure of early cartilage calcification. *J. ultrastruct. Res.* **20**, 33–50.

Brown, M. D. (1971) The pathophysiology of disc disease. *Orthop. Clin. N. Amer.* **2**, 359–370.

Buckwalter, J. A., Cooper, R. R. & Maynard, J. A. (1976) Elastic fibres in human intervertebral discs. *J. Bone Jt Surg.* **58**-A, 73–76.

Cornah, M. S., Meachim, G. & Parry, E. W. (1970) Banded structures in the matrix of human and rabbit nucleus pulposus. *J. Anat. (Lond.)* **107**, 351–362.

Dahmen, G. (1963) Submikroskopische Untersuchungen an Wirbelbandscheiben. *Z. Rheumaforsch.* **22**, 192–213.

Gomibuchi, R. (1964) Electron microscope studies on the fine structure of the intervertebral disc, with special reference to intervertebral disc herniation. *J. Jap. orthop. Ass.* **37**, 1027–1041.

Happey, F., Johnson, A. G., Naylor, A. & Turner, R. L. (1964) Preliminary observations concerning the fine structure of the intervertebral disc. *J. Bone Jt Surg.* **46**-B, 563–567.

Hentzer, B. & Kobayasi, T. (1975) Ultrastructural changes of human skin in organ culture. *IRCS Med. Sci.* **3**, 211.

Inoue, H. (1973) Three-dimensional observation of collagen framework of intervertebral discs in rats, dogs and humans. *Arch. histol. Jap.* **36**, 39–56.

Inoue, H. & Takeda, T. (1975) Three-dimensional observation of collagen framework of lumbar intervertebral discs. *Acta orthop. scand.* **46**, 949–956.

Keech, M. K. (1954) The effect of collagenase and trypsin on collagen. *Anat. Rec.* **119**, 139–154.

Kobayasi, T. & Asboe-Hansen, G. (1971) Ruthenium red staining of ultrathin sections of human mast-cell granules. *J. Microsc.* **93**, 55–60.

Kobayasi, T., Wolf-Jürgensen, P. & Danielsen, L. (1973) Ultrastructure of shagreen patch. *Acta dermatovener.* **53**, 275–278.

Kobayasi, T., Danielsen, L. & Asboe-Hansen, G. (1974) Ultrastructure of necrobiosis lipoidica diabeticorum. *Acta dermatovener.* **54**, 427–431.

Kobayasi, T., Hentzer, B. & Asboe-Hansen, G. (1977) Degradation of dermal fibrillar structures. Effects of collagenase, elastase, dithioerythritol and citrate. *Acta dermatovener.* (In press).

Lane, J. M. & Weiss, C. (1975) Review of articular cartilage collagen research. *Arthr. and Rheum.* **18**, 553–562.

Little, K. (1973) Observations on the nature and production of proteins in the intercellular matrices of connective tissues. *J. Path.* **110**, 1–12.

Meachim, G. (1972) Meshwork patterns in the ground substance of articular cartilage and nucleus pulposus. *J. Anat. (Lond.)* **111**, 219–227.

Meachim, G. & Cornah, M. S. (1970) Fine structure of juvenile human nucleus pulposus. *J. Anat. (Lond.)* **107**, 337–350.

Pearson, C. H., Happey, F., Naylor, A., Turner, R. L., Palframan, J. & Shentall, D. (1972) Collagens and associated glycoproteins in the human intervertebral disc. *Ann. rheum. Dis.* **31**, 45–53.

Rabinovitch, A. L. & Anderson, H. C. (1976) Biogenesis of matrix vesicles in cartilage growth plates. *Fed. Proc.* **35**, 112–116.

Render, R. M. (1971) An investigation using the electron microscope into the structure of the human intervertebral disc. M. Sc. Thesis, University of Bradford.

Smith, J. W. & Serafini-Fracassini, A. (1968) The distribution of the protein-polysaccharide complex in the nucleus pulposus matrix in young rabbits. *J. Cell Sci.* **3**, 33–40.

Sylvén, B., Paulson, S., Hirsch, C. & Snellman, O. (1951) The ultrastructure of bovine and human nuclei pulposi. *J. Bone Jt Surg.* **33**-A, 333–340.

Takeda, T. (1975) Three-dimensional observation of collagen framework of human lumbar discs. *J. Jap. orthop. Ass.* **49**, 45–57.
Thiéry, J.-P. (1967) Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microscopie* **6**, 987–1018.
Watanabe, S. (1960) Ageing of the human intervertebral disc from the aspect of histochemistry and submicroscopic structure. *Shinshu Med. J. (Japanese)* **9**, 16–32.
Weiss, C. (1973) Ultrastructural characteristics of osteoarthritis. *Fed. Proc.* **32**, 1459–1466.

Correspondence to: Jesper Sylvest, M.D., Department of Dermatology, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark.