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ORIGINAL ARTICLE

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Immunolocalization of major interstitial collagen types in human lumbar intervertebral discs of various ages

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Abstract We used complete transverse sections through 65 samples of human lumbar intervertebral discs for immunolocalization of the major interstitial collagen types I, II, III, V, VI and IX. The samples were selected from 47 patients ranging in age from 0 (fetuses) to 86 years. The results were compared with the histological findings in disc tissue degeneration and/or reparative alterations as indicated by tear and cleft formation, chondrocyte proliferation, mucous degeneration, granular matrix changes and fibrocartilage fibrillation. We observed a typical pattern for each antibody and each anatomical structure, with, however, remarkable inter- and intraindividual variability, which could be monitored only by use of the complete transverse sections. Accordingly, collagen I was seen in the normal annulus fibrosus and in the degeneratively altered nucleus pulposus, but not within the end-plate, regardless of degenerative changes. Collagens II and IX were found in the normal nucleus pulposus, the inner annulus fibrosus and the end-plate. The collagen II (and IX) staining seemed to be enhanced in areas of minor degenerative lesions, but reduced in advanced lesions and in the degenerated end-plate. Collagens III and VI were significantly increased in areas of minor to advanced degeneration in all anatomical settings, while collagen V showed only minor changes in its staining pattern. In general, histological signs of tissue degeneration coincided with significant quantitative, but also with certain qualitative, changes in the composition of the collagenous disc matrix. These observations indicate the association of degenerative and/or reparative alterations of the intervertebral disc and changes in the collagenous matrix, but document the variability in the extent of the abnormalities observed.

Key words Intervertebral disc · Interstitial collagens · Cartilage · Nucleus pulposus · Annulus fibrosus

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Introduction

The intervertebral disc can be separated macroscopically into three different components: a centrally located gelatinous mass (the nucleus pulposus) is enclosed in concentrically organized layers of collagen fibrils (the inner and outer annulus fibrosus), which are framed by hyaline-like cartilaginous end-plates forming a transition zone to the adjacent vertebral bodies. These structures form a unit in which all elements have to remain intact to provide normal function. Any disturbance of structural integrity leads to functional impairment and may cause tissue destruction [10, 17]. The pathoanatomical and biochemical alterations of the intervertebral discs with ageing [4] and/or degeneration are assumed to be the predominant cause of low back pain.

Within the disc structure, collagen plays a pivotal role. So far, seven different types of collagens have been identified in disc material [8, 9, 23]. Normal intervertebral discs contain collagen types I, II, III, V, VI, IX and XI. The proportions of the different collagen types vary between the different tissue structures. Previous biochemical studies showed that there is an inverse "gradient" of collagen types I and II from the outer annulus fibrosus via the inner annulus to the nucleus pulposus [11]. Accordingly, the annulus fibrosus contains more collagen type I than type II, while the nucleus pulposus is mainly composed of collagen type II.

Much less is known about other collagen types, and even less about their exact localization in normal and abnormal disc tissues. Except for a recent study on

small samples of different regions of discs [23] and experimental studies in animal models [13, 14] no comprehensive studies are available on the immunolocalization of various collagen types in completely analysed intervertebral discs. This may be due to difficulties in handling larger samples of the heterogenous intervertebral disc tissue.

It was the aim of the present investigation to analyse the distribution of the major interstitial collagen types in whole cross sections of intervertebral discs obtained from individuals of various ages and with varying extents of morphological signs of disc degeneration. This study is the first to unravel the zonal heterogeneity of collagen type distribution within one given intervertebral disc and to correlate these findings with the morphological signs of disc degeneration.

Materials and methods

Whole lumbar spines were obtained from 3 fetuses (27–35 weeks of gestation), 15 children (1 day to 13 years of age) and 16 young adults (16 to 25 years of age) at autopsy and served as a pool of "normal" intervertebral discs. In 13 further individuals (31–86 years of age) similar specimens were obtained to find degenerated intervertebral discs. All lumbar spines were removed within 24 h of death during routine post-mortem examinations. All individuals had died suddenly and none was known to have had any spinal disorder.

The lumbar spines were removed by an anterior approach. Osteotomy was performed at each pedicle level (L1 to S1) and at the vertebral levels of L1 and S1. Thus, only the anterior column with the anterior and posterior longitudinal ligament remaining intact was obtained, without mutilation of the cadaver. The specimens were then cut into motion segments, and a midsagittal slice of each segment was obtained containing parts of the adjacent vertebral body.

All slices were fixed in buffered 4% formaldehyde, pH 7.4, for 2× 24 h and subsequently decalcified in 0.1 M EDTA (pH 7.2). The decalcified complete sagittal disc slices were then embedded in paraffin as performed routinely. From the resulting blocks, paraffin sections (2–4 μm thick) were cut and placed on silanized glass slides for routine staining (HE, Masson-Goldner, Alcian blue–PAS) and subsequent immunohistochemistry.

The appropriate tissue sections were deparaffinized for immunohistochemistry and subsequently enzymatically pretreated to enhance immunoreactivity (0.2% trypsin/0.1% hyaluronidase or 0.4% pepsin as pretested [18–20] (Sigma, Deisenhofen, Germany). Following washing steps, the type specific antibodies were applied as previously tested [18, 19]. In this study, we used polyclonal rabbit antibodies against collagen type I (kindly provided by Dr. P.K. Müller, Lübeck), type II (Dr. P.K. Müller, Lübeck), type III (kindly supplied by Dr. E. Schleicher, Munich), type V (kindly provided by Dr. R. Brenner, Bonn), collagen type VI (donated by Dr. R. Timpl, Martinsried) and collagen type IX (Dr. P.K. Müller, Lübeck). The specificity of each antibody had previously been tested by ELISA, showing exclusive type specificity [25], and by immunohistochemical analysis [18–20]. The tissue sections were finally treated with a coupled secondary antibody, and the resulting reaction product was visualized by chromogene substances. Thus, we applied the avidin–biotin system [12] (Vector, Burlingame, Calif.) and the APAAP system [7] (Dako, Hamburg, Germany). The chromogenes were DAB in the case of the ABC method and fast red for the APAAP reaction (Sigma, Deisenhofen, Germany).

For negative controls, we used parallel sections that had been treated with normal rabbit serum instead of the specific antibodies. In addition, prior control experiments had been carried out using pre-application of the respective purified antigen [18–20]. All control experiments yielded negative results.

All intervertebral discs had been evaluated macroscopically and the degree of disc degeneration was evaluated from grade I (normal, juvenile discs) to grade V (severely degenerated discs) according to the grading scheme by Thompson et al. [24]. After tissue processing, each intervertebral disc was examined for histological features of disc degeneration. These comprised chondrocyte proliferation, granular matrix changes, mucous degeneration, tear and cleft formation and fibrocartilage fibrillation.

Results

A total of 229 transversely dissected lumbar intervertebral discs was available for histological analysis. For the present immunohistochemical study we particularly used the paraffin-embedded whole tissue blocs from the motion segment L3/L4 of each individual. In addition, we analysed a variable number of additional disc specimens, particularly from older individuals or degeneratively transformed intervertebral discs to obtain further information on degenerated intervertebral discs. Therefore, a total of 65 samples was immunohistochemically analysed.

Macroscopical signs of disc degeneration were present in 36 of all motion segments analysed. No macroscopic evidence for disc degeneration was seen in the fetal and juvenile specimens, so that a percentage of 90% of the adolescent/adult/senile specimens showed disc degeneration. According to the macroscopic grading, 4 discs were classified as normal (grade I according to Thompson et al. [24]), while 15 were ranked as grade II, 15 as grade III, 4 as grade IV and 2 as grade V discs (Fig. 1).

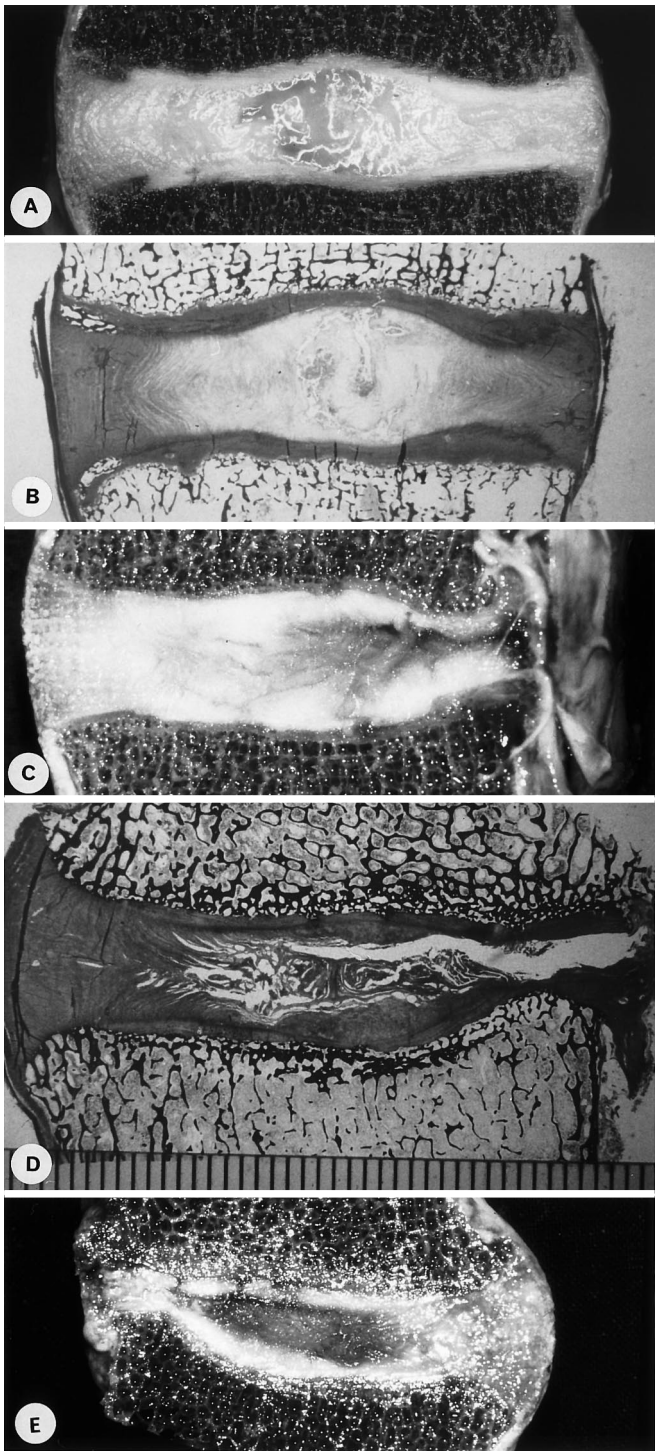
Histologically, tissue alterations consistent with degenerative and reparative lesions were seen in the intervertebral discs from all adolescent, adult and senile individuals. As expected, fetal and infantile discs were free of significant tissue alterations (Fig. 2A). There were significant changes in the tissue pattern both intra- and interindividually which could be recorded only by analysis of the whole disc sections.

Minor lesions were seen in the discs from two children (10 and 13 years old), all adolescents (16–18 years) and some of the younger adults (20–25 years). These comprised the formation of clefts and tears in the nucleus pulposus, sometimes extending into the inner annulus fibrosus. Those tears and clefts were often filled with large amounts of amorphous granular material (granular changes). Chondrocyte cloning as a sign of cell proliferation was focally seen in the specimens from somewhat older individuals (20–25 years), mostly in conjunction with tears and clefts. Focally, minor mucoid stroma degeneration and fibrocartilage fibrillation were present.

Advanced signs of degenerative and reparative alterations were seen in older adults and in the specimens from the senile individuals analysed. Here, huge clones of large chondrocytes were present (Fig. 2B), mostly associated with major tissue defects caused by large tears and clefts. These were seen in both the nuclear and the annu-

lar region, and they were also associated with extensive granular changes (Fig. 2C), extensive mucoid degeneration (Fig. 2D) and fibrillation of the fibrocartilage.

The end-stage of disc degeneration was seen in some of the senile specimens with macroscopic grade V lesions. These presented with huge tissue defects, scar-like tissue transformation and extensive vascular ingrowth, so that the intervertebral disc structure was completely destroyed (“burnt-out appearance”/scar tissue).



The immunolocalization of the various collagen types revealed a typical pattern for each antibody and each anatomical structure, with, however, remarkable inter- and intraindividual variability associated with the features of degenerative and reparative disc alterations. Accordingly, the extent of staining abnormalities was evident only in the whole tissue sections. The major findings are summarized in Table 1.

In general, the adjacent bone tissue consisted of collagen I (with collagens III, V and VI at the endostium and collagen V and VI around the osteocytes), while the longitudinal ligaments were composed of a mixture of collagens I, III, V and VI. The composition of the nucleus pulposus, the annulus fibrosus and the cartilaginous end-plate, however, differed considerably with age and/or signs of degeneration.

In fetal and infantile discs, collagen I was seen in the outer annulus fibrosus (Fig. 3A), the anterior/posterior ligaments and in the subchondral bone. Only traces were found in the inner annulus fibrosus, but no collagen I was observed in the nucleus pulposus (Fig. 3B). The outer annular staining intensity was significantly less than that in the bone. Collagen II was regularly seen in the cartilaginous end-plate and in the nucleus pulposus (Fig. 3C) and (mainly inner) annulus fibrosus, although with minor staining intensity. There was no collagen II staining in the outer annulus fibrosus (Fig. 3D). Collagen III was found with slight staining intensity in the annulus fibrosus and collagen V was present both in the nucleus pulposus and the annulus fibrosus. Collagen VI staining was present both in the nucleus pulposus (Fig. 3E), the annulus fibrosus (Fig. 3F) and the cartilaginous end-plate, in the latter region and in the nucleus pulposus mostly associated with the pericellular matrix of the chondrocytes, while in the annulus fibrosus it was found associated with the interstitial matrix. Collagen IX was found in association with the collagen II distribution. Accordingly, this collagen type covered the nucleus pulposus and the end-plate matrix, but with pericellularly enhanced staining.

Immunostaining for the various collagen types in discs from adolescents and young adults revealed a pattern comparable to that described for the infantile discs in those areas without any histological evidence for disc

Fig. 1A–E Macromorphologic aspects of intervertebral disc specimens from subjects of various ages. **A** A cross section through the intervertebral disc of an adolescent (17 years) shows a typical gelatinous nucleus pulposus centrally, framed by the annulus fibrosus. **B** The corresponding histological slide reveals well-arranged annulus fibrosus fibres, but centrally sited irregular nuclear clefts are signs of minor alterations. **C** This section through a disc from an adult (66 years) reveals a loss in regular structure, dislocation of the nucleus pulposus and extensive tear formation of the posterior annulus. **D** The corresponding histological section shows extensive cleft and tear formation, nuclear protrusion and loss of tissue arrangement. Note also slight bony sclerosis adjacent to both end-plates. **E** The macroscopical aspect of the disc (L5/S1) of a senile individual (85 years), with severe loss of disc height and deformation of the disc structure. Note also the browing of the nuclear connective tissue matrix

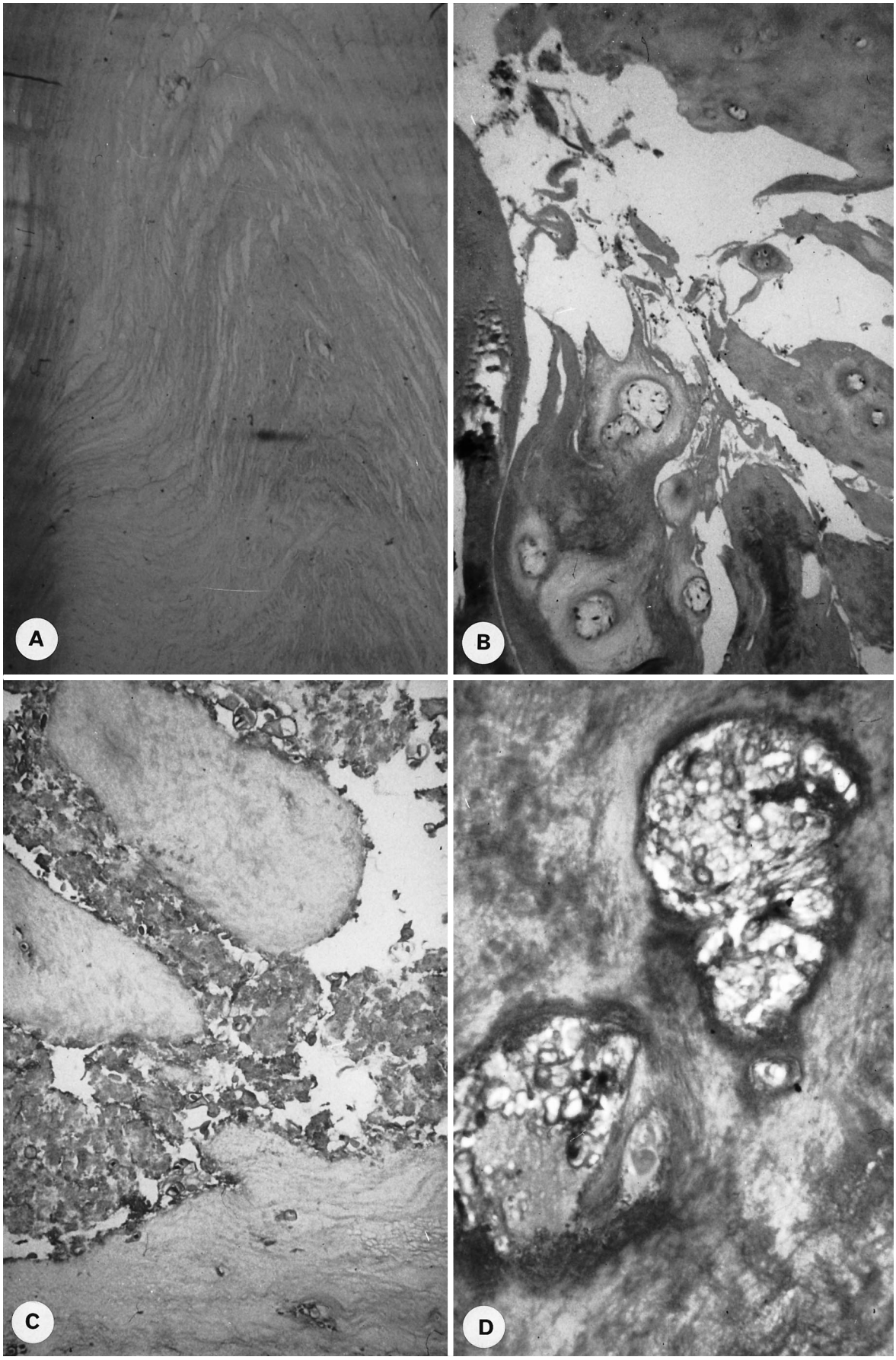

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Table 1 Immunohistochemical distribution of major collagen types in normal and diseased intervertebral human discs (*Coll* collagen, *NP* nucleus pulposus, *AF* annulus fibrosus, *EP* end-plate, – absent, (+) weakly positive, + positive, ++ strongly positive)

Discs from	Ages	Degeneration	Coll I	Coll II	Coll III	Coll V	Coll VI	Coll IX
Fetuses	27–35 weeks	–						
NP			–	+	–	+	+	+
AF			+	+	+	+	+	+
EP			–	+	(+)	–	+	+
Infants/juveniles	1 day to 13 years	–						
NP			–	+	(+)	+	+	+
AF			+	+	+	+	+	+
EP			–	+	(+)	+	+	+
Adolescents/young adults	16–25 years	(+)/+						
NP			–	++	++	+	++	++
AF			+	+	+/++	+	++	+
EP			–	+	+	+	+	++
Adults	31–58 years	(+)/++						
NP			(+)/+	(+)/+	++	++	+/++	(+)
AF			++	(+)/+	+/++	+	++	(+)
EP			–	(+)	+	+	+/++	(+)
Old people	66-86 years	+/++						
NP			(+)/+	–/(+)	+	++	+	–/(+)
AF			+	–/(+)	(+)	+	+	–/(+)
EP			–	–/(+)	+	+	+	–/(+)

degeneration/regeneration (Fig. 3G). In regions with minor signs of disc alteration, however, the collagen type pattern differed. In areas with minor degenerative alteration, we observed enhanced staining for collagen II (Fig. 3H) – mostly accentuated in the pericellular matrix – associated with significantly increased staining for collagen III and VI. Collagens V and IX did not reveal major alterations either in their normal staining pattern or in their intensity. The collagen I staining was restricted to the outer and inner annulus fibrosus, with some banding pattern according to the orientation of the annular fibers. The end-plate showed pericellular expression of significant amounts of collagen III (Fig. 3I) in addition to collagens VI and IX. The end-plate contained mainly collagens II and IX, which, however, seemed to be reduced in areas of degenerative alteration (Fig. 4A) compared with normal tissue.

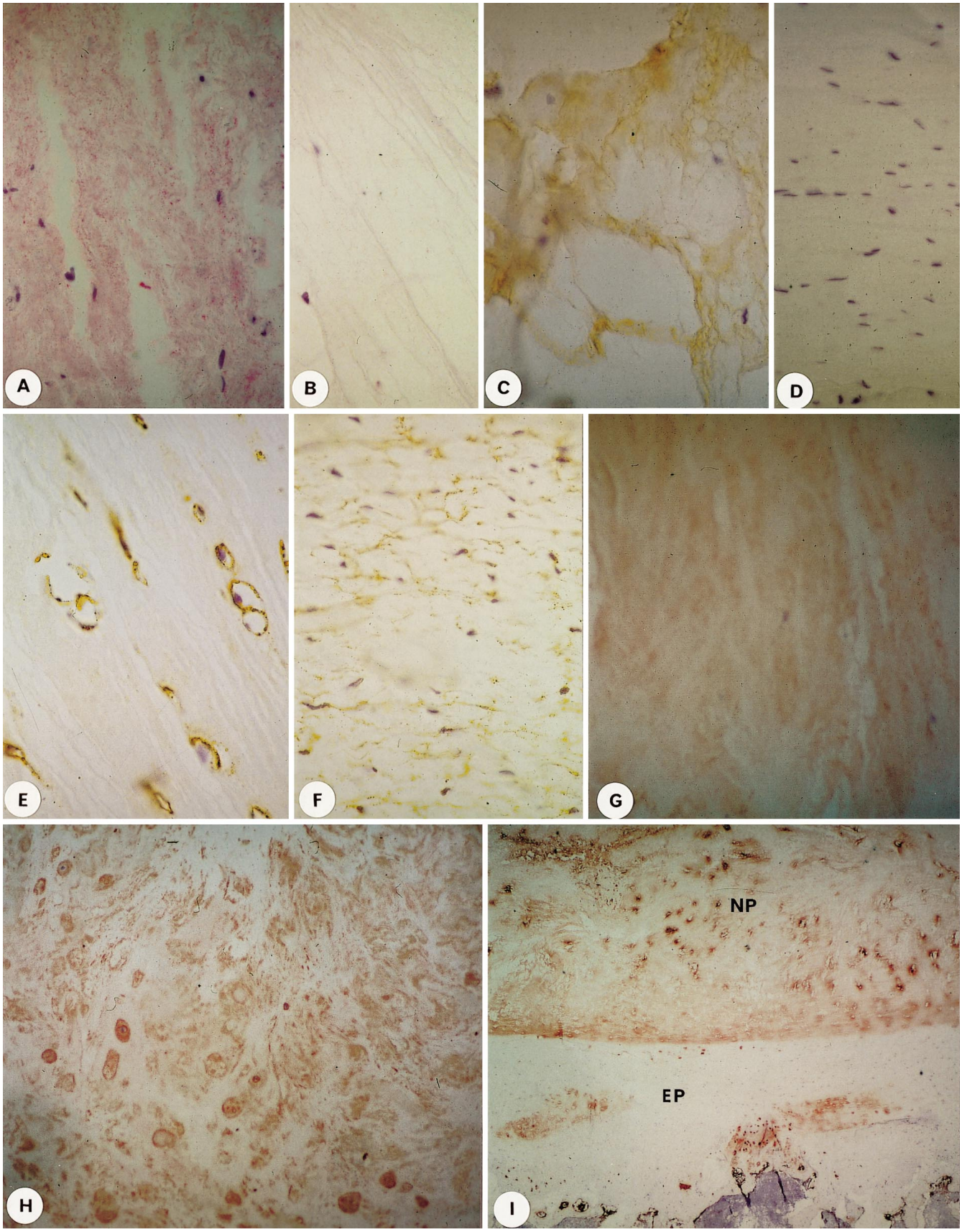
In discs from adult and senile individuals, we observed the most extensive abnormalities of the collagen-type pattern compared with discs from younger controls. Again, the severity of the alterations was highest in areas with significant tissue degeneration/regeneration. Accordingly, we found expression of small amounts of immunochemically detectable collagen I in the nuclei pulposi of individuals with extensive cleft formation (Fig. 4B). Enhanced staining for collagen I was also seen in some inner annular areas (Fig. 4C), even without cleft or tear formation. These discs apparently contained reduced amounts of collagen II (Fig. 4D) and IX in the nucleus pulposus. The staining for collagen III (Fig. 4E) and VI (Fig. 4F) seemed to be increased in areas of major disorganization, while collagen V remained relatively unaltered. In the nucleus pulposus with severe lesions, however, the staining for collagens III and VI was reduced. In end-plates with major degenerative lesions, a reduced residual and often only focally detectable staining for collagen II was seen, along with an enhanced staining for collagen III and VI. Additionally, collagen V occurred within this tissue. However, no collagen I staining was seen even in the severely altered end-plate.

The specimens with complete destruction of the tissue architecture revealed only small residual areas of retained and often only minimal positive staining for collagens II (Fig. 4H) and IX, mostly in the interterritorial matrix of retained chondrocytes, while the major part of this tissue and its matrix were positively labelled for collagen I (Fig. 4G), III, V and VI. The mostly sclerotic subchondral bone showed typical collagen I staining (Fig. 4G).

◀ **Fig. 2A–D** Histological aspects of normal and altered disc structures. **A** The annulus fibrosus of juvenile and adolescent individuals shows a regular orientation of the annular collagen fibres without disruption of the architecture. Alcian blue-PAS, ×100. **B** Signs of disc degeneration are represented by extensive cleft formation and associated reactive chondrocyte proliferation focally forming huge chondrocyte clones. HE, ×250. **C** In some of the clefts, an amorphous and strongly eosinophilic "granular" matrix is seen indicating matrix degradation. HE, ×250. **D** Foci of mucous degeneration are seen within nuclear and annular areas. Alcian blue-PAS, ×400

Discussion

The intervertebral disc is a complex tissue structure which is composed of a variety of different connective tissue types: fibrocartilage in the nucleus pulposus and inner annulus fibrosus, hyaline cartilage at the end plate, fibrous tissue in the outer annulus fibrosus, and finally the adjacent bone of the vertebral bodies. This complexi-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ty implicates that any change in the composition or structure of one of these tissues may ultimately lead to a general tissue disarrangement and thus to a loss of the proper function of the disc. Since the collagenous framework is essential for the biomechanical stability and the overall property, the correct structure and composition of the collagen is a prerequisite for the maintenance of the biomechanical function.

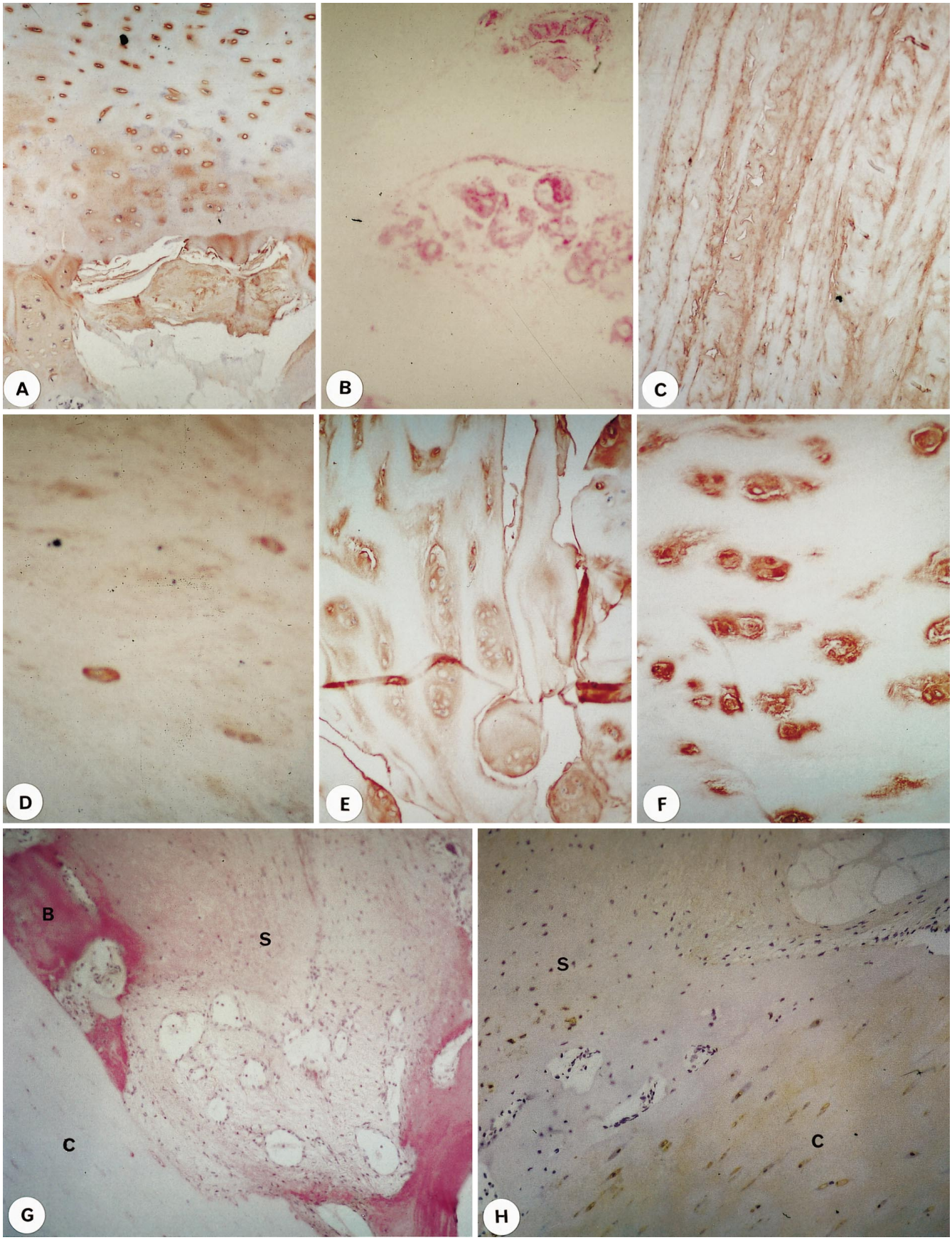
With respect to clinical symptoms, it is widely accepted that disc herniation or internal disc disruption is a major cause of low back pain. Recent investigations, however, have clearly provided evidence that there is a high rate of asymptomatic disc herniation [5], ranging between about 20% and 30% of an unselected population. By the use of magnetic resonance imaging it has furthermore been shown that both symptomatic and asymptomatic disc herniation is accompanied by structural abnormalities of the intervertebral discs [5, 6]. At the moment, it is therefore impossible to define a clinically symptomatic from an asymptomatic condition on the basis of clinical or morphological criteria. Accordingly, no clear-cut distinction can be drawn between truly "pathologic" and "non-pathologic", merely age-related alterations of the discs. With these restrictions in mind, we performed our study on a series of intervertebral discs from individuals without known spinal disorder to evaluate the spectrum of morphological disc alterations with increasing age and to correlate our routine morphological observations with the composition of the collagenous matrix.

Few data are available on the biochemical composition and the structure of the various intervertebral disc tissues [8, 9, 16, 27] under normal and abnormal conditions. The paucity of these data is mainly caused by significant difficulties in tissue preparation and collagen extraction, with yields leading to limited and incomplete data. We therefore chose an immunohistochemical approach to analyse the distribution of various collagen isotypes in the intervertebral disc tissues to overcome the difficulties of collagen extraction and to correlate morphological indicators of tissue degeneration and/or regeneration with a qualitative analysis of major collagen types.

Several previous studies have investigated the localization of distinct collagen types in normal and diseased disc tissue [2, 3, 14, 21–23, 26]. These studies were all done on small samples using mainly frozen section and immunofluorescence techniques. Accordingly, the representative nature of those samples is uncertain. In addition, it is well known that the use of the immunofluorescence technique often involves major difficulties in attributing a positive signal to a distinct morphological subset. In our study, we therefore used the avidin–biotin complex technique and the APAAP technique on formalin-fixed, decalcified and paraffin-embedded whole transverse sections through a series of lumbar discs, in order to obtain not only insight into the heterogeneity of the intervertebral tissue composition at various ages, but also to correlate histological signs of degeneration and regeneration with the pattern of collagen types. This procedure is time-consuming during the tissue preparation; however, it provides information that is potentially superior to that obtained hitherto. Our analysis clearly shows that current immunohistochemical techniques offer reproducible and reliable data on the distribution of various proteins, as we have previously shown in other tissues, including joint cartilage [19, 20]. One critical point may be antigen masking of the collagen isotypes despite pretreatment of the sections. Since, however, we have found a very similar staining pattern to that shown in previous studies and our own extensive pretesting did not provide evidence for significant masking or demasking by different pretreatments, we assume that our repeatedly performed observations reflect the real changes in the disc tissues.

As previously reported, we observed a distinct staining pattern for each collagen type analysed in the intervertebral discs of our series. Thus, collagen I is absent from the juvenile/adolescent nucleus pulposus and only faintly seen in the annulus fibrosus, mainly the outer annulus fibrosus [2, 23]. The fact that the annular collagen I staining is rather weak has also been reported by others [2, 3, 23], although biochemically collagen I represents the major bulk of annular collagen [11]. The reason remains unclear, but it may well be that the closely parallel orientation of the annular collagen I fibres leads to some antigen "masking". Collagen II is the main collagen component of the nucleus pulposus, but it is also seen in the (inner) annulus fibrosus and a major constituent of the end-plate. The collagen types III and VI – although forming different fibre networks – show some codistribution with a typical pericellular arrangement, which is not only been seen in the nucleus pulposus and the annulus, but also the end-plate [21, 22]. To the best of our knowledge there are no reports on the immunolocalization of collagen V in the human intervertebral disc. This collagen type was found in the normal annulus fibrosus and scarcely in the nucleus pulposus, but not at all in the end-plate. Accordingly, there is some overlap in its tissue distribution with the localization of collagen I and II, which is at variance with the known codistributional pattern of collagen types I and V [15]. Finally, in the normal discs we found the minor cartilage collagen type IX not only in the end-plate, but also in the nucleus, mainly in asso-

◀ **Fig. 3A–I** Immunohistochemical findings of the collagen type distribution in intervertebral disc tissues with normal appearance or minor degenerative alterations. **A** In the juvenile annulus fibrosus collagen I is slightly stained in the parallel oriented fibre bundles, but not in the nucleus pulposus (**B**). ×400. **C** Collagen II is seen in the nucleus pulposus, but not in the annulus fibrosus (**D**). ×400. **E** Collagen VI is observed pericellularly in the nucleus pulposus and both pericellularly and interstitially in the annulus fibrosus (**F**). ×400. **G** The normal adult nucleus pulposus shows a significant positive staining for collagen II. ×400. **H** In the nucleus pulposus with some signs of disc degeneration enhanced staining for collagen II can be seen, mostly in the pericellular, territorial matrix, which may indicate enhanced de novo synthesis of collagen II. ×250. **I** The end-plate (*EP*) with minor degenerative alterations shows a focal expression for collagen III, which is also seen enhanced in the nuclear region (*NP*)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ciation with collagen type II, but with a typically pericellularly enhanced staining. In contrast, Roberts et al. [23] did not find collagen IX in human disc tissue but did detect it in bovine and rat disc tissue. It remains unclear whether their antibody reacted sufficiently with the human antigen or whether there are certain splicing variants for collagen IX that may have escaped their analysis.

In our series from fetal to senile disc tissue, we did not find significant changes in the collagen type distribution between morphologically unaltered discs from subjects of various ages. The collagen type pattern was comparable between fetal, infantile and juvenile discs and similar to that seen in unaltered adult discs.

In contrast, we observed significant changes in the collagenous matrix in the discs from younger and older adults and in senile samples, which have not previously been described. The extent of the (semi-)quantitatively estimated collagen type alterations correlated with the degree of tissue disarrangement. Accordingly, the discs with the severest lesions showed the most advanced collagen alterations. In particular, the formation of tissue clefts was often associated with an abnormal collagen staining pattern, such as the occurrence of collagen I in the nucleus pulposus in cases with extensive cleft formation, cell proliferation and matrix fibrillation. Our observations thus support the concept that intervertebral disc degeneration is associated with significant changes in the collagenous matrix. These changes are mainly quantitative rather than qualitative in nature, and they show considerable intra- and interindividual variability. In general, it seems that disc degeneration is associated, at least initially, with enhanced staining for the "regular" collagen types of the corresponding anatomical structure. We found enhanced staining for collagen types II, III, V and VI in the nucleus pulposus with degenerative alterations, and for collagen I in degenerated annulus fibrosus tissue. This is agreement with the observations made on osteoarthritic joint cartilage in early stages [1]. The consequences of this increase in collagen matrix seem to be significant, although the cause remains unclear. It may result in a change of the biomechanical tissue properties and facilitate tissue disruption. It is assumed that adjacent cells are destroyed either by mechanical force or secondarily by the blocking of a nutritional pathway. We speculate that this increase in collagen staining may either be due to a loss of proteoglycans, with a relative increase in collagen, or result from a response to abnormal biomechanical stress, or both. We cannot excluded the possibility that enhanced staining may be the consequence of better access of the antibodies to the antigens by unmasking of antigenic structures. In the final stage of "burnt-out" discs the collagenous matrix shows a scar-like pattern, as expected on the grounds of the histological features.

Besides these quantitative changes in collagen type staining which may be significantly influenced by the accessability of the antibodies to the antigens, we observed qualitative changes, which reflect tissue disarrangement during disc degeneration and the associated reparative processes. It is of interest that collagen I occurred in the degenerated nucleus pulposus indicating severe tissue disarrangement, as seen in experimental scarring of the disc [14]. Similarly, the loss of collagen II in the degenerated end-plate indicates major disarrangement. It seems fair to assume that this leads to altered biomechanical and biophysical properties of the end-plate, and this may be one cause of abnormal nutritional fluid transport into the otherwise unvascularized disc.

In general, our findings are the first on the collagen distribution in whole sections of intervertebral discs. This approach seems necessary to evaluate the ample heterogeneity in abnormalities of the disc tissue structure, which can be correlated with certain distinct alterations in the collagen type distribution. This observation explains how discrepant findings can easily occur when the analysis is based on only small tissue samples. These findings underline the pathobiological significance of collagen type analysis. It remains to be proven, however, whether these observations are of any value for the evaluation of degenerative lesions in biopsy specimens.

We conclude that the matrix changes observed are the consequence of alterations of cellular function, leading to the matrix and tissue disarrangement observed. Detailed analysis of chondrocyte function and phenotype in the various disc tissues and conditions is projected.

◀ **Fig. 4A–H** Immunohistochemical findings in intervertebral discs with advanced signs of disc degeneration. **A** In the end plate with minor lesions, the staining for collagen IX is focally reduced. ×250. **B** In more severely affected discs in the nucleus pulposus collagen I is deposited around chondrocytes. ×400. **C** The annulus fibrosus of degenerated discs shows an increased staining pattern for collagen I, even without local cleft and tear formation. ×250. **D** In areas of advanced disc degeneration, the nuclear staining for collagen II is significantly reduced. ×400. **E** Significantly disturbed nuclear areas show a strongly positive reaction for collagen III, and enhanced pericellular staining for collagen VI (**F**). ×250. **G** Severely altered disc specimens with burnt-out appearance show collagen I staining in the bony exostoses (*B*) and slightly in the scar-like tissue formation (*S*), but not in the residual cartilage (*C*), while collagen II (*H*) can be found only faintly in the cartilage (*C*) and minimally in scar-like areas (*S*). ×140

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