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Fibrous Structure in the Intervertebral Disk: Correlation of MR Appearance with Anatomic Sections

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To correlate the MR appearance of the disk with its fibrous structure, we studied the lumbar intervertebral disks in 10 cadavers with MR, CT, cryomicrotome anatomic sections, and, in selected disks, with histologic and dried sections. In MR images the predominantly fibrous tissues such as Sharpey's fibers had a low signal intensity while the fibrocartilagenous tissues with a mucoid matrix in the intervertebral disk had a high signal intensity. In the equator of the adult disk was a well-defined fibrous plate that contained collagenous, elastic, and reticular fibers with little ground substance. This plate appeared to develop progressively from the periphery of the nucleus toward the center, starting in the second decade of life. The fibrous plate was also distinguished as a lower signal intensity in the MR images.

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In previous anatomic/radiologic correlative studies MR has demonstrated an internal structure of the intervertebral disk [1–3]. Sharpey's fibers, which are predominantly collagen [4–7], have a low signal intensity, as do fibrous tissues elsewhere in the body. MR distinguishes Sharpey's fibers from the remainder of the anulus and nucleus, which contain less collagen and more ground substance [8, 9]. Degeneration of the disk, in which the ground substance in the nucleus pulposus is progressively replaced by collagen, is also reflected in MR images as a change in signal intensity [2].

The progressive development of the nucleus pulposus has been characterized in previous studies [1, 2]. In the newborn the fibrocartilage of the nucleus pulposus contains little collagen. Fibrous layers develop first in the anulus fibrosus and then in the peripheral nucleus pulposus. Development of fibers, which progresses toward the center of the nucleus, appears to be related to the curvature of the spine, since in the kyphotic regions of the spine the fiber content appears to progress more rapidly on the dorsal aspect of the nucleus and in the lordotic region of the spine on the ventral aspect. Fiber content in the nucleus pulposus, especially near the equator, increases with age. Fiber content in the fibrocartilage of the nucleus pulposus near the endplates remains sparse throughout life. The most common anatomic techniques do not effectively demonstrate the fibers within fibrocartilage. Except where collagenous fibers are plentiful, they are not observed on gross inspection or in many histologic stains, unless polarized light is used. The purpose of this study was to evaluate the fibrous structure of the intervertebral disk by means of specialized histologic and anatomic techniques.

Materials and Methods

Ten cadavers (two newborns and eight others ages 2, 10, 19, 44, 50, 54, 65, and 66 years old) were selected for this study. Of the 10 cadavers, three were embalmed and seven were fresh (within 48 hr of death). The cadavers were processed as follows:

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MR Imaging

In the case of the fixed cadavers, the spine was removed from the body by dissection prior to imaging. The fresh cadavers and fixed specimens were imaged with a 1.5-T GE imager. A 4-in. butterfly or 3.5-in. sphenoid surface coil* placed posterior to the spine or cadaver was used as a radio frequency receiver. Spin-echo images were obtained with long TR (2500 with TEs of 20, 40, 60, 80; or 4000 with TEs of 20 and 70), short TR (600 or 800 with TEs of 20 or 40), 256 ×256 matrix, two excitations, and 3-mm slice thickness in the sagittal plane. Supplementary gradient-echo and axial spin-echo images were obtained in some cases.

Freezing and Harvesting

After MR imaging, the cadaver or specimen was frozen at −70°C for 3 days. A block of tissue including the entire lumbar spine was removed and embedded in carboxymethylcellulose solution in a Styrofoam box.

CT Scanning

The frozen block was imaged in a GE 9800 CT scanner. Direct sagittal images were obtained with 1.5-mm slice thickness, 3–8 sec scan time, 200 mA, 120 kV, and 512 × 512 matrix. The locations of the first and last scans were marked on the surfaces of the Styrofoam box with a laser localizer light.

Cryosectioning and Photography

A block of tissue determined by lines marked on the box was removed. The block was then sectioned on the stage of a heavy-duty cryomicrotome† in the sagittal plane, as described previously [3]. Photographs of the surfaces of the specimens were taken every 1 mm as they were sectioned. Slices of tissue 6- and 750-μm thick were obtained from the midline of the specimen for histologic and dried sections, respectively, by means of the cryomicrotome or a diamond bandsaw.

Histologic and Dried Sections

The 6-μm histologic sections were stained with H and E (for fibers in the disk), Van Gieson's and trichrome technique (for collagenous fibers), Verhoeff's stains (for elastic fibers), and Wilder's stain (for reticular fibers). The 750-μm sections, removed from the frozen specimen with a diamond bandsaw, were pressed between two pieces of glass and dried at 37°C for 24–48 hr.

The MR images were correlated with their corresponding CT, cryomicrotomic, histologic, and dried sections. The disks were classified as immature, transitional, adult, early degenerated, or severely degenerated on the basis of their cryomicrotome appearance.

Results

Fibrous structure was characterized in 50 disks. Immature, transitional, and normal adult lumbar disks had different fibrous patterns. The degenerated adult disks are not described in this report.

In the newborn disk (Fig. 1), the only fibrous tissue evident with any of the techniques was in the laminated fibers in the periphery of the disk (i.e., Sharpey's fibers). The remainder of the disk contained predominantly ground substance. Microscopically, little fiber was found in the ground substance. On MR images (2500/60), the low signal intensity of Sharpey's fibers contrasted with the remainder of the anulus and the nucleus, which had higher signal except for a thin plate of weak signal related to a plate of notochord remnant lying midway between the endplates.

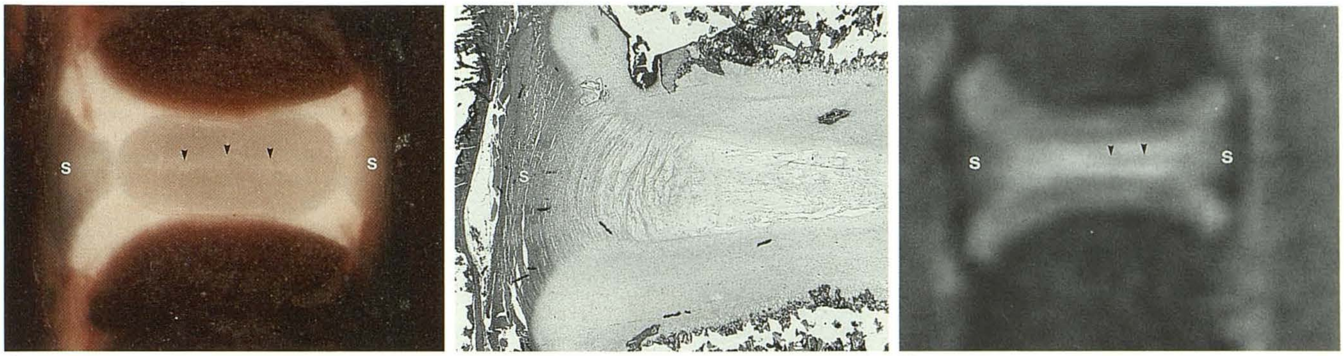
In the immature disk, at 2 years of age (Fig. 2), an increase in fibrous tissue in the midportion of the nucleus was demonstrated in the anatomic and histologic sections. The Van Gieson's, trichrome, Verhoeff's, and Wilder's stains showed that it was composed mainly of collagen. In the 2-year-old's disks, MR demonstrated Sharpey's fibers, as in the neonate.

By 10 years of age (Fig. 3), fibers in the lumbar nucleus pulposus were evident. Well-organized fibers appeared on the ventral or dorsal aspect of the nucleus near the anulus and extended toward the center. The location of these fibrous structures seemed to be related to the curvature of the spine. They were anteriorly located in the upper part of the spine and posteriorly located in the lower part. Clumped fibers were visible in the ground substance of the nucleus pulposus on gross inspection. On histologic sections, the fibers in the ventral or dorsal aspect of the nucleus were predominantly vertically oriented. On MR images (2500/60), these laminated fibers like Sharpey's fibers had a low signal intensity. Except for darker spots, which represented the scattered clumps of fibers in the ground substance, the nucleus and anulus had a relatively high signal intensity in CT images. The fibrous portions of the anulus fibrosus and nucleus pulposus appeared as high-density regions; the mucoid region as lower density.

By 19 years of age (Fig. 4), the disks had a solid, opaque, and fibrous-appearing nucleus pulposus and lacked a sharp demarcation between nucleus and anulus. The disks had a relatively uniform yellowish color except in the periphery where Sharpey's fibers were present and in the nucleus where pigmented regions were located. Multiple herniations of the disk through the endplates into the vertebral bodies (Schmorl nodes) were present in this case. Histologic sections revealed a plate of collagenous fibers in the disks. It stained dark red in Van Gieson's and blue-green in trichrome stains. Verhoeff's and Wilder's stains showed a small amount of elastic and reticular fibers present with the collagenous fibers. Fibers were both vertically and horizontally oriented. Sharpey's fibers in the 19-year-old were thicker than in the newborn or child. Sagittal MR images (spin-echo 2500/20 or 2500/80; gradient-echo 200/30) demonstrated a band of low signal intensity that correlated with the plate of fibrous tissue in the equator of the disk.

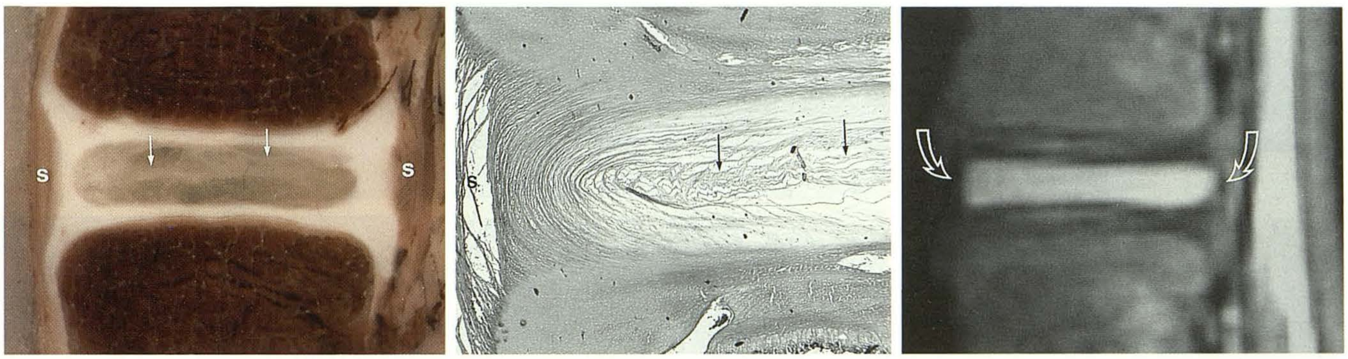
Adult disks (Fig. 5) had an opaque and fibrous nucleus. The pigmented regions in the nucleus were conspicuous. Concentric and/or transverse tears were frequent in the anulus fibrosus and Sharpey's fibers. A plate of tissue in the equator of the disk, slightly darker yellow than the rest of the disk, was evident in some cases. Histologic sections showed that

* Medical Advances, Inc., Milwaukee, WI.
† LKB 2250, LKB Gaithersburg, MD.



A B C

Fig. 1.—Fibrous structure in L1-L2 intervertebral disk of a newborn.
A and *B*, Sagittal cryomicrotome (*A*) and histologic (*B*) sections, stained with H and E, show Sharpey's fibers (S) and notochord remnants (*arrowheads* in *A*).
C, Corresponding MR image (2500/60) shows Sharpey's fibers (S) and notochord remnants (*arrowheads*) as regions of low signal intensity. Cartilage of unossified portion of vertebral body has an intermediate signal partially obscured by a dark line at the junction. Ventral side of spinal column in each figure is to the reader's left.



A B C

Fig. 2.—L3-L4 intervertebral disk of a 2-year-old.
A and *B*, Sagittal cryomicrotome (*A*) and histologic (*B*) sections, stained with H and E, illustrate Sharpey's fibers (S) and fibers developing in nucleus pulposus (*arrowheads*).
C, Corresponding MR image (4000/70) illustrates the low signal intensity of Sharpey's fibers (*curved arrows*). Remainder of anulus and nucleus have high signal.

the plate in the equator was composed of collagen with some elastic and reticular fibers. The dried sections of all normal adult disks revealed compact fibrous tissue in the region of Sharpey's fibers and the plate in the equator of the nucleus and the anulus where the darker yellow tissue was noted in cryotome section. The spaces in the dried sections corresponded to the mucoid, nonfibrous regions of the disk (Fig. 5C). On MR images (2500/80), the regions of compact fibrous tissue had lower signal intensity than the mucoid regions in the remainder of the disk. In some cases, very bright signal regions were noted where fissures in the nucleus were present near the center of the disk. Direct sagittal CT images showed the fibrous structure in the disk as high-density zones; the mucoid regions as lower-density zones.

Discussion

This study clarifies the sources of high and low signal intensities from within the disk. The lower signal intensity regions in T2-weighted MR images of the disk correlated with fibrous structures; the higher signal intensity with the mucoid regions or ground substance. Collagen fibers within fibrocartilage are not well demonstrated in the cryomicrotome sections or gross anatomic sections used in this and in previous correlative studies of disks. Even in histologic preparations, the sparse collagen fibers in a fibrocartilagenous ground substance may be difficult to distinguish without polarized light. Dried sections effectively show the fibrous structure of the disk because the dehydration eliminates the gelatinous and mucoid portions of the disk. The fibrous tissue was characterized as predominantly collagenous (positive staining with Von Grieson's and trichrome), with some elastic (positive staining with Verhoeff's), and reticular fibers (positive staining with Wilder's).

The band of fibrous tissue in the equator of the disk has not been emphasized previously. It has been referred to inexactly as the "intranuclear cleft" [10]. The band is characterized by compact collagenous, reticular, and elastic fibers

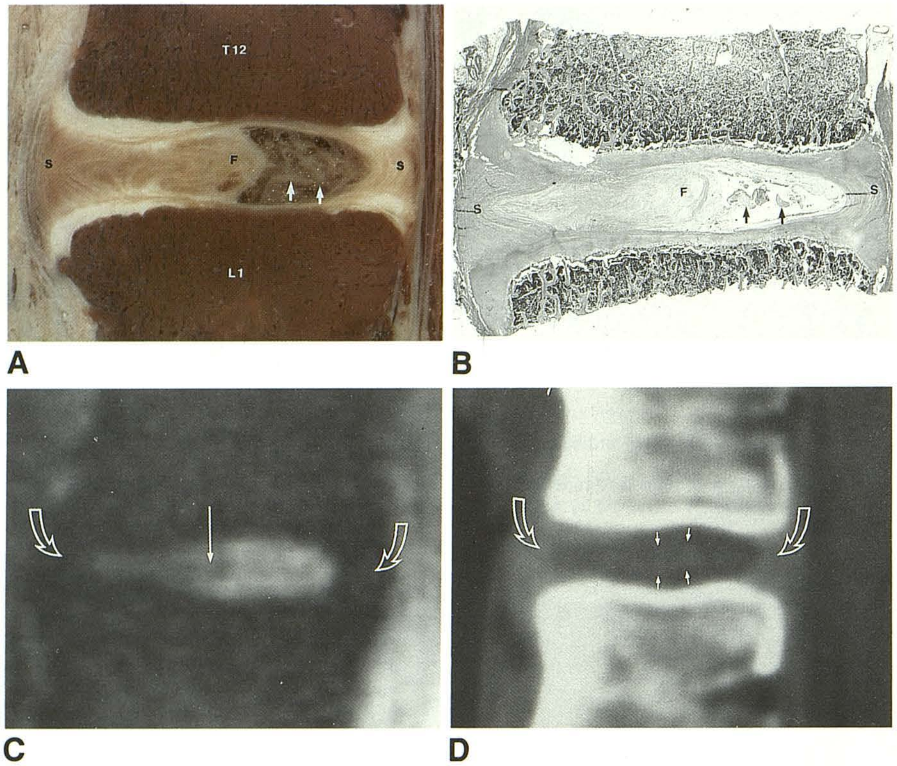


Fig. 3.—L1–L2 intervertebral disk of a 10-year-old.

A and *B*, Cryomicrotome (*A*) and histologic (*B*) sections demonstrate a transitional intervertebral disk. Well-organized fibers (F) appear on ventral aspect of nucleus near anulus and extend toward center. Sharpey's fibers (S) and clumps of fibers in the nucleus pulposus (*arrows*) are clearly seen.

C, Sagittal MR image (2500/60) shows low signal intensity of Sharpey's fibers (*curved arrows*) and fibers in nucleus (*straight arrow*).

D, CT image shows Sharpey's fibers (*curved arrows*) and fibers in nucleus (*small arrows*) as higher density.

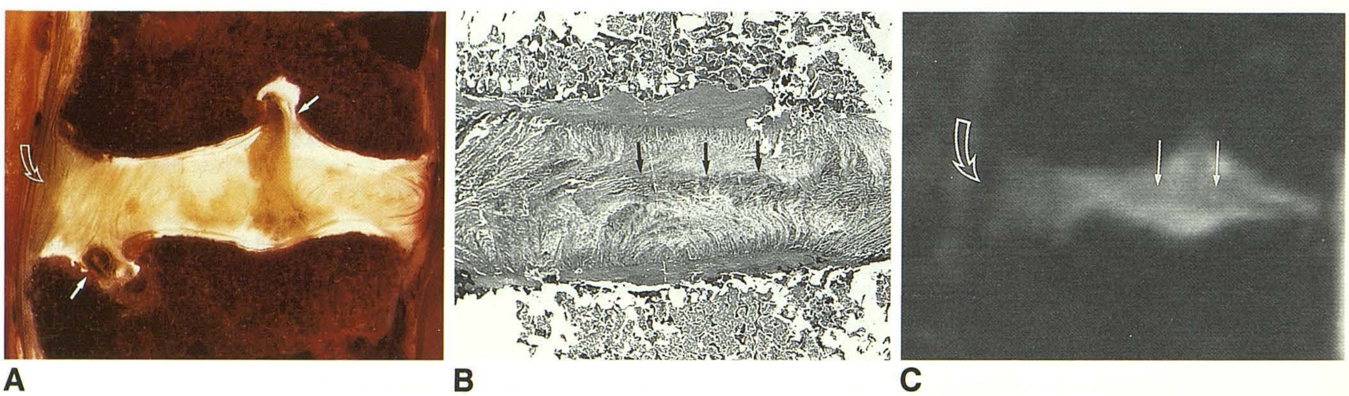


Fig. 4.—L4–L5 intervertebral disk of 19-year-old man.

A, Sagittal cryomicrotome section demonstrates a mature adult disk. Disk is opaque and yellow with more pigmented regions in nucleus. Sharpey's fibers (*curved arrow*) are darker. Multiple herniations of disk through endplates into vertebral bodies (*straight arrows*) are present.

B, Corresponding histologic section (Van Gieson's stain) reveals a dark band (*arrows*) in midportion of nucleus pulposus and anulus that represents collagenous fibers.

C, Gradient echo MR image (200/30) shows collagenous band (*straight arrows*) and Sharpey's fibers (*curved arrow*) as regions of low signal intensity.

oriented predominantly transversely and less frequently vertically. The band is not present at birth and appears to develop in adolescence. It may develop as a result of stresses on the disk, as fiber develops in other cartilagenous tissues in response to stress [11]. It has not been emphasized in anatomic texts, probably because it is not conspicuous unless appropriate stains are used. This compact fibrous tissue corresponds with the dark band seen on T2-weighted MR images. The development of fibrous tissue in lumbar disks is presented schematically in Figure 6.

Other causes for a dark band through the intervertebral disk have been described [12, 13]. In the immature nucleus pulposus of the newborn, the dark band corresponds to a platelike region in the disk that contains degenerating notochordal cells and ground substance [1, 2]. Notochord tissue can be seen in children's disks but not in adults'. In some disks, when the intervertebral disk height is equivalent to 4 pixels with the imaging parameters chosen, a thin, regular dark line may appear transversely, midway between the endplates, as a result of the truncation artifact [12, 13]. The artifact is characteristically found in thoracic disks, which have a relatively narrower disk space. In contrast to the artifact,

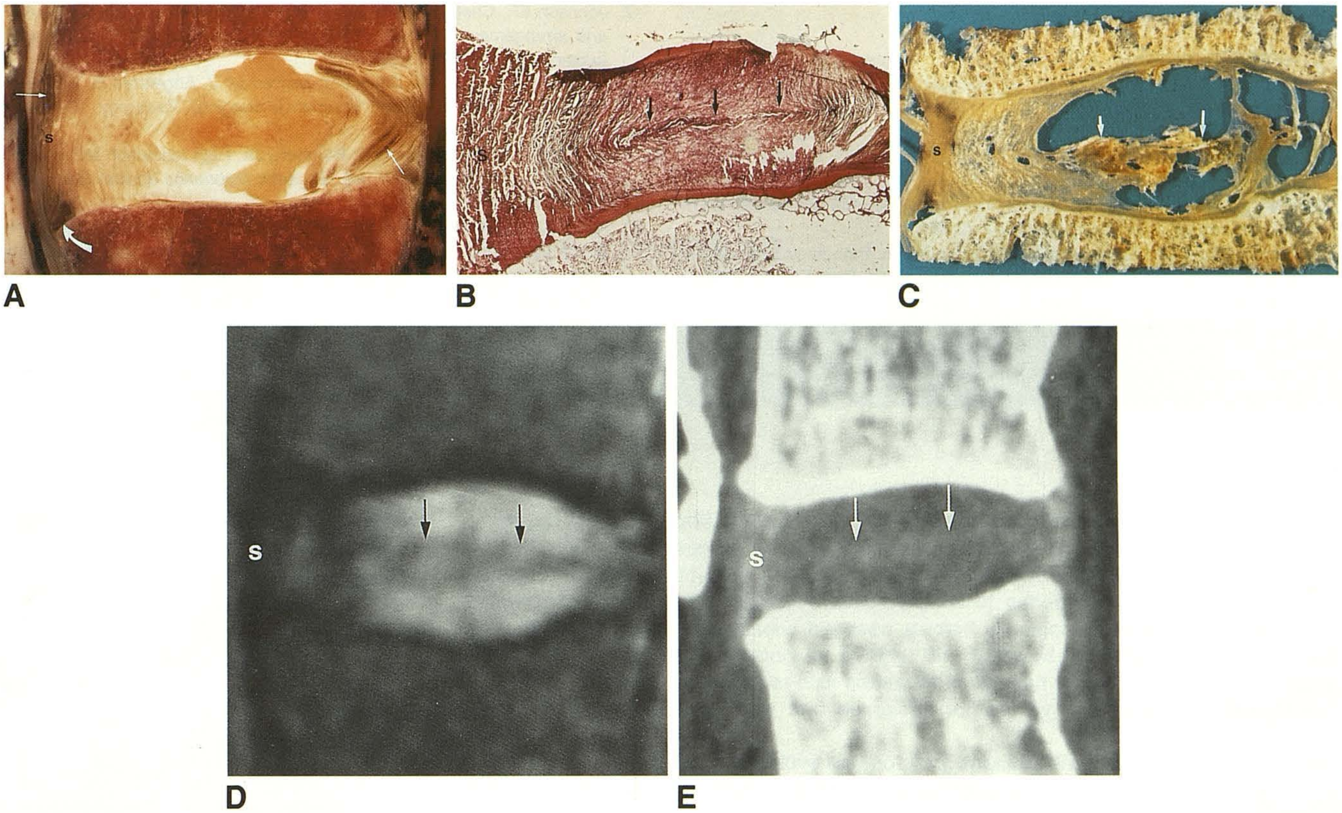


Fig. 5.—L4-L5 intervertebral disk of 54-year-old.

A, Sagittal cryomicrotome section shows a mature adult disk. Concentric tears (*straight arrows*) and transverse tear (*curved arrow*) in Sharpey's fibers (S) and anulus are shown.

B, Histologic section with Van Gieson's stain reveals a dark red band representing collagenous fibers (*arrows*) in middle of disk.

C, Dried thick section reveals a compact fibrous tissue in midportion of disk (*arrows*) and in Sharpey's fibers (S).

D, MR image (2500/80) demonstrates fibrous band (*arrows*) and Sharpey's fibers (S) as regions of low signal intensity.

E, Corresponding CT image shows the band (*arrows*) and Sharpey's (S) as regions of higher density.

the dark band representing fibrous tissue is thicker, slightly indistinct, often irregular, and not always precisely halfway between the two endplates.

The customary designations for disk tissues may be confusing. The nucleus has been referred to as a remnant of primitive notochord, as mucoid material, or as fibrocartilage. In the newborn, the nucleus and anulus both contain fibrocartilage, which, except for Sharpey's fibers, has very sparse fibers. The paucity of fibers gives the disk a mucoid appearance. During maturation and especially in adolescence, the collagenous fiber content in the nucleus pulposus and anulus fibrosus increases, giving it a more opaque, less mucoid appearance. During this process, a transverse fibrous band appears in the disk. The fibers develop from tropocollagen secreted by cells into the matrix or ground substance. During growth and development the fibrocartilage of the disk becomes progressively more fibrous. Ground substance of fibrocartilage correlates with a relatively high signal intensity in T2-weighted MR images. Compact collagen produces a low intensity signal. Contrast in MR images of the disk is produced by the proportion of ground substance and collagen in the fibrocartilage.

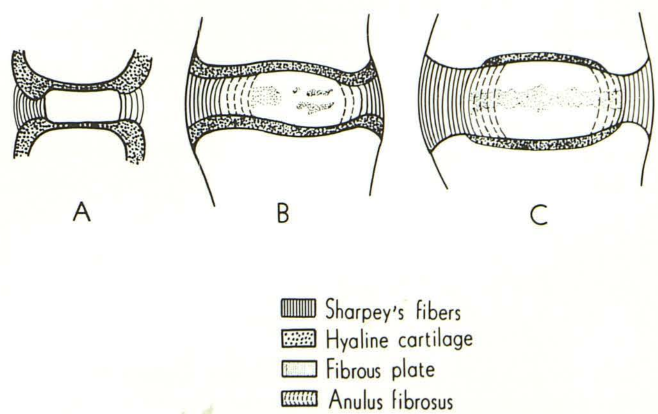


Fig. 6.—Schematic shows development of fibrous tissue in lumbar disks. In newborn (*A*), the fibrous tissue is evident only in Sharpey's fibers. In 10-year-old (*B*), vertically oriented fibers appear on ventral or dorsal aspect of the nucleus. Clumped fibers are visible in the ground substance of nucleus pulposus. In adult (*C*), there is a fibrous plate in equator of disk.

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