Vertebral End-Plate Changes With Aging of Human Vertebrae

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The present study describes the sequential age changes within the growth and articular layers of the cartilaginous end-plates of vertebrae from humans varying in age from birth to 73 years. There is a gradual reduction in the width of the growth cartilage up to 16–20 years of age. During adulthood and progressing into old age (60–73 years), the end-plates consist of only articular cartilage which undergoes calcification followed by resorption and replacement by bone. Age changes are observed in the arterioles, capillaries, and venules found in the nutrient canals or spaces of the bone adjacent to the cartilage or disc. The calcification of the articular cartilage and vascular changes seen in the older vertebrae would impede the passage of nutrients from the blood to the disc proper. Collagen fibers are observed arising from the older vertebral end-plates to course into the midregion of the disc. [Key words: age changes, end-plates, microvasculature, calcification]

PREVIOUS STUDIES confirming the avascularity of the intervertebral disc have indicated that the nutrition of the disc is mainly dependent upon the diffusion of nutrients and other substances through the central part of the cartilaginous end-plates.[1,4,5,9] It has also been shown that the permeability of the articular cartilage decreases with age.[9,11,12] In spite of these studies, there has been only speculation of the morphologic basis to explain the loss of diffusion through the end-plates. It has been suggested that there may also be an undefined reduction in blood flow to the bone, end-plates, and disc proper.

In a recent study by the authors,[3] sequential changes within the layers of the end-plate cartilage of vertebrae were described in marmosets from birth to old age, showing a gradual narrowing with alternate disappearance of the growth cartilage and simultaneous histochemical changes in the articular cartilage, leading to mineralization of this layer. It was assumed that the calcification of the articular cartilage would impede the diffusion of nutrients from the bone microvasculature through the calcified cartilage zone to reach the disc.

The present investigation was undertaken to demonstrate the age changes in human lumbar spine from birth to old age, with special emphasis on the end-plates and the microvascular components in the bone adjacent to the articular cartilage.

MATERIALS AND METHODS

Lumbar vertebrae from humans varying in age from birth to 73 years were studied. Specimens were obtained from both male and female individuals. Two adjacent lumbar vertebrae and the intervertebral disc were removed at the time of autopsy and fixed in an alcohol–formol acetic acid solution. Following decalcification in 10% nitric acid in 10% formalin, the specimens were embedded in nitrocellulose in the routine manner. The embedded blocks were sectioned 14 to 20 μm in thickness, and stained with hematoxylin and triosin, PAS-hematoxylin, alcian blue (pH 2.5) PAS, and silver nitrate impregnation.

RESULTS

In the following description of the findings, a positive PAS reaction (red in color) indicates the presence of neutral polysaccharides (proteoglycans) and siliac
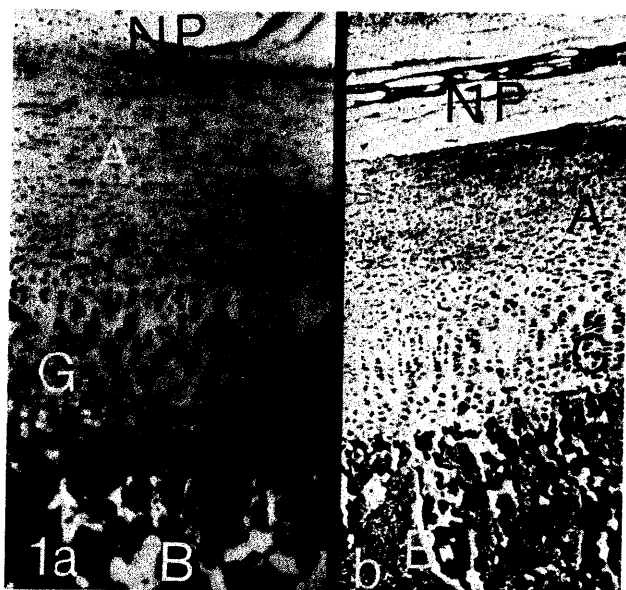
Fig 1. Lumbar vertebra from a newborn individual. The cartilaginous end-plate consists of an inner growth zone that stains pink in color following the PAS reaction (**a**) and negative after silver nitrate impregnation (**b**). The articular region appears slightly red with PAS and black with silver nitrate impregnation. A = articular cartilage; B = bone; G = growth cartilage; N.P. = nucleus pulposus (×50).

Fig 3. Lumbar vertebra from an 18-year-old male. The grow cartilage stains basophilic and is calcified. The articular cartilag exhibits areas of intense PAS-positive staining. A = articular car lage; B = bone; G = growth cartilage (PAS, hematoxylin ×50).

acid in the matrices of cartilage or bone. A pink-staining matrix with the PAS reaction confirmed by a positive alcian blue staining demonstrates the existence of glycosaminoglycans (chondroitin −4 (6)-sulfate). An argyrophilic fiber as visualized by silver nitrate impregnation is a collagen fiber.

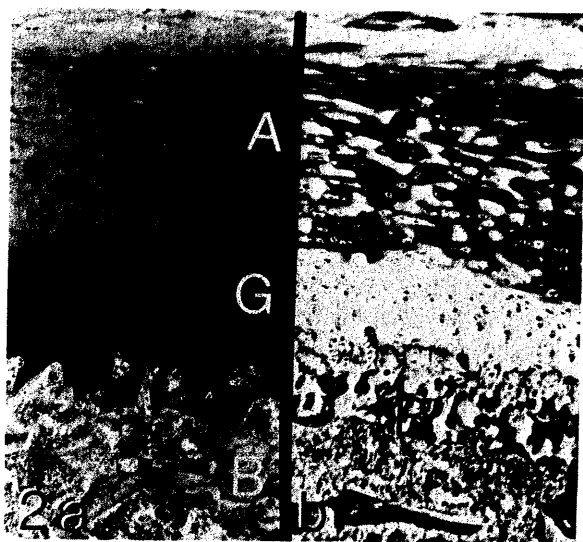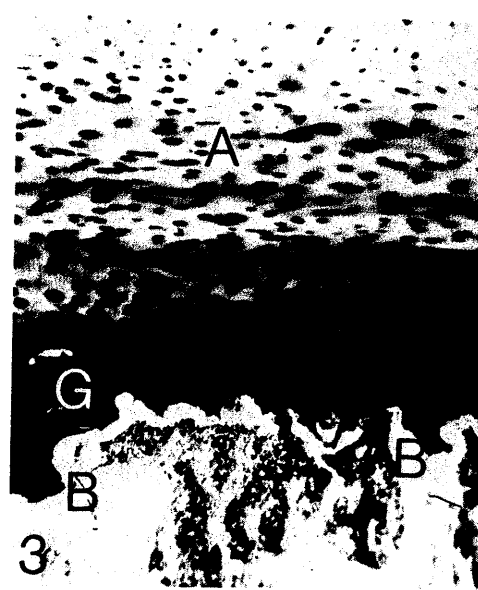The end-plates of the lumbar vertebrae from new-

borns consisted of an inner growth zone and a articular region bordering on the intervertebral dis(The growth cartilage was characterized by parall(columns of proliferating and hypertrophic cells. , thin, calcified cartilage zone was present adjacent t the diaphyseal trabeculae. The outer articular cartilag consisted of spindle-shaped chondrocytes scattere throughout the matrix. There was a difference in th staining response between the growth and articula cartilages following exposure to the PAS reaction. Th growth cartilage appeared pink, whereas the articula zone was red in color, representing the variations ii the type of mucopolysaccharide contents of the matri: in each zone (Figure 1a). This variance in stainin; response was also demonstrated with silver nitrat(impregnation, in which the growth cartilage exhibite(negative staining in contrast to the grayish-black ap pearance of the articular cartilage (Figure 1b).

In vertebrae from individuals 10 to 15 years of age there was a reduction in the width of the growth zon(with a decrease in the number of irregularly arrange(proliferating cells. The matrix still stained pink in colo following exposure to the PAS reaction. The articulat(cartilage was relatively thicker and stained intensel) PAS-positive (Figure 2a). The articular cartilage wa: argyrophilic in nature whereas the growth cartilag(still stained negative after silver nitrate impregnatior (Figure 2b).

Specimens obtained from individuals 17 to 20 year: of age exhibited a sealing-off of the cartilage by bone. The remnants of the growth cartilage stained basophil· ic with hematoxylin and appeared homogenous ir nature. Cracks were found within the calcified mass.

Fig 2. Lumbar vertebra from a ten-year-old male. The growth zone is reduced in width and irregular in outline. It stains basophilic in nature and negative in silver nitrate impregnation. The articular cartilage, in contrast, stains positive with PAS and black following silver impregnation (**b**). A = articular cartilage; B = bone; G = growth cartilage (×50).
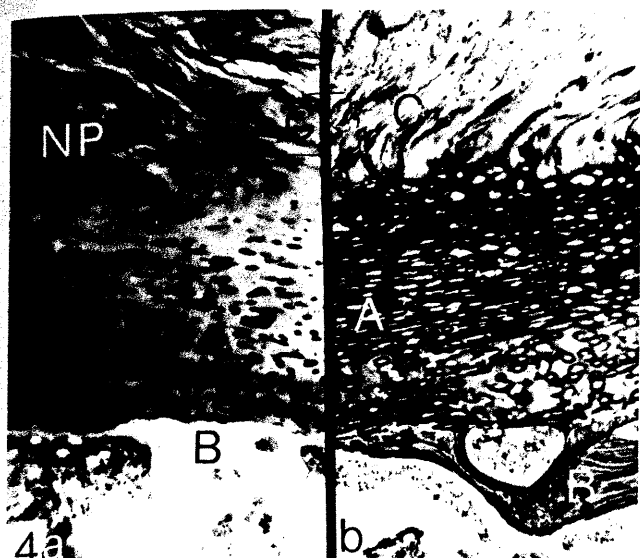
Fig 4. Lumbar vertebra from a female in her middle 30s. Notice that the bone is directly adjacent to the articular cartilage. The articular cartilage appears deep red after PAS exposure (**a**). At the cartilage disc border, a pinkish material containing chondrocyte-like cells is present in the nucleus pulposus. In addition, pink colored fibers are radiating into the nucleus. **b.** An alternate section exposed to silver nitrate impregnation reveals that the articular cartilage is strongly argyrophilic, and the fibers are collagenous in nature. A = articular cartilage; B = bone; C = collagen; N.P. = nucleus pulposus (photographically reproduced at 85% of original magnification ×50).

Fig 5. Higher magnification of the end-plate illustrated in Figure 4b. Notice the presence of (A) fine collagenous fibers, (B) silver granules that are associated with the collagenous fibers as well as being free in the matrix, and (C) the heavy concentration of granules and fibers in the capsular areas (silver nitrate impregnation) (photographically reproduced at 82% of original magnification ×250).

The articular cartilage, on the other hand, stained intensely with the PAS reaction (Figure 3).

The end-plates of vertebrae from adults 20 to 40 years of age consisted only of an articular cartilage layer that stained deep red after the PAS reaction. A pink matrix containing chondryocytes was observed in the region of the disc adjacent to the articular cartilage. Pinkish-staining fibers were arising from the articular cartilage and coursing through the pink matrix of the disc (Figure 4a). The matrix of the articular cartilage, following silver nitrate impregnation, showed fine silver granules deposited throughout intercellular regions. The pinkish-staining fibers seen in Figure 4a were found to be argyrophilic, indicating that they were collagenous in nature (Figure 4b). With higher magnification, fine collagenous fibers were demonstrable in the matrix; other areas showed the intimate relationship between the collagen fibers and silver granules; and in other regions only heavy deposits of silver granules were seen "masking" over the collagenous elements as well as the ground substance (Figure 5).

No staining differences were noted in the articular cartilage in vertebrae from individuals 45 to 73 years of age. In the 45 to 60 year age group, there was resorption of the calcified articular cartilage with replacement by bone (Figure 6). By the age of 65 years, only a narrow strip of articular cartilage separated the
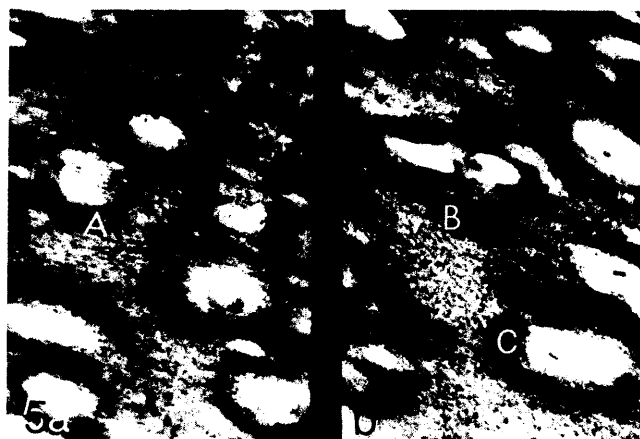
disc from the underlying bone (Figure 7a). With silver nitrate impregnation, collagenous fibers were seen arising from both the cartilage and bone passing into the disc in an interweaving pattern (Figure 7b).

Figure 8 shows an end-plate and underlying bone from a 73-year-old female. Only a very thin, incomplete bony plate was observed adjacent to the narrowed calcified articular cartilage. Severe osteoporosis of the body of the vertebra was apparent.

Vascular changes were seen in the bone adjacent to the articular cartilage and disc in individuals over 45 years of age. The nutrient canals and spaces in the bone adjacent to the cartilage were partially or completely occluded with an intensely PAS-positive material (Figure 9). The walls of the arterioles and venules within these spaces were homogenous in nature and deep red in color. In contrast, the canals and spaces in
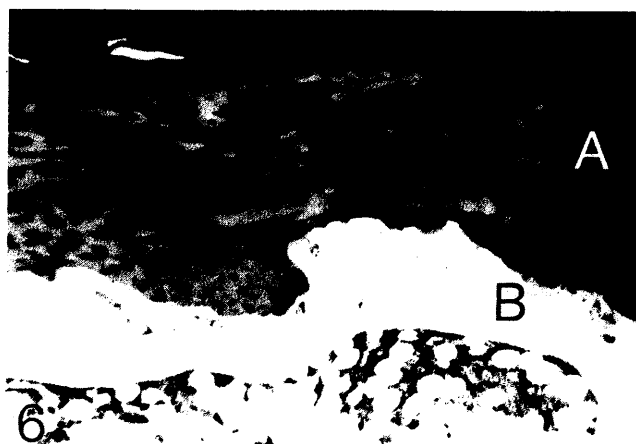

Fig 6. Lumbar vertebra from a 55-year-old male. Note the invasion of bone into the articular cartilage. A = articular cartilage; B = bone (PAS hematoxylin; photographically reproduced at 96.5% of original magnification ×50).
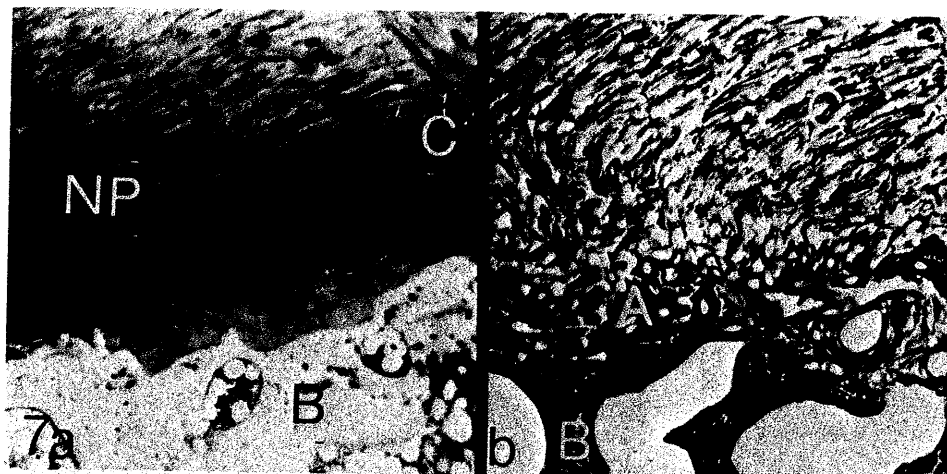
Fig 7. Lumbar vertebra from a 65-year-old individual. **a.** In one area, a thin sliver of cartilage separates the bone from the disc; in adjacent regions, bone abuts against the disc (PAS, hematoxylin). A silver-nitrate-impregnated alternate section illustrates collagenous fibers arising from bone to radiate into the nucleus pulposus. **b.** A = articular cartilage; B = bone; C = collagenous fibers; N.P. = nucleus pulposus (×50).

the vertebrae from young adults were still open and filled with either fat, loose connective tissue, or myeloid elements (Figure 10).

DISCUSSION

Recently, there has been a recognition of the importance of the role played by the vertebral end-plates in transporting nutrients to the avascular intervertebral discs. It is generally agreed that the discs in adults are nourished by diffusion of substances through the cartilaginous end-plates and that there is a decrease in permeability through the end-plates in vertebrae from aging animals and man.[4,9,11,12] Questions prevail about whether the loss of permeability is due to physical and/or chemical change in the articular cartilage or due to changes in the vascular supply of the underlying bone.


Fig 8. Section of lumbar vertebra from an 83-year-old female. Notice that only a very thin layer of bone is abutting against the narrowed intensely PAS-positive articular cartilage. Severe osteoporosis of the body of the vertebra is evident. A = articular cartilage; B = bone; D = disc (PAS, hematoxylin; photographically reproduced at 83% of original magnification ×50).

Bernick et al[3] have recently demonstrated progressive age changes in the marmoset vertebrae from birth to old age. In the old animal, as demonstrated by histochemical staining, there was a gradual decrease in the stainable sulfated glycoaminoglycan, chondroitin sulfate, and keratin sulfate in the articular cartilage. In adult and aged animals, the articular cartilage became intensely PAS-positive, indicating the presence of neutral mucoproteins and siliac acid. The cartilaginous matrix of the end-plate in growing animals contained fine fibrillar collagen elements (type 2) that were masked by the carbohydrate moiety so that the matrix appeared homogenous following silver nitrate impregnation. At the time of vertebral maturity, fine argyrophilic collagen fibers were demonstrable with silver nitrate impregnation. The presence of argyrophilic collagen fibers, neutral mucoproteins, and a depolymerization of GAG provided the environment for mineralization of the matrix. The mineralization of the articular cartilage not only makes it susceptible to osteoclastic activity but would restrict the diffusion of substances.

Similar changes were observed in the present study on human vertebrae. The sequence in the aging of the human lumbar spine from birth to old age is summarized in Figure 11. Beginning in adults (age 20–40 years), there was a gradual mineralization of the articular cartilage. As with the marmoset vertebrae, there was resorption of the calcified cartilage during middle and old age and a replacement with bone so that in the vertebrae from individuals over the age of 60 years only a thin layer of calcified cartilage or bone separated the disc from the body of the vertebrae. The presence of calcified cartilage would therefore interfere with movement of nutrients through the end-plates and substantiates the findings of Marasdas et al,[7] Marasuda et al,[8] and Brown[5] that there is a decrease in diffusion of substances in aging end-plates.
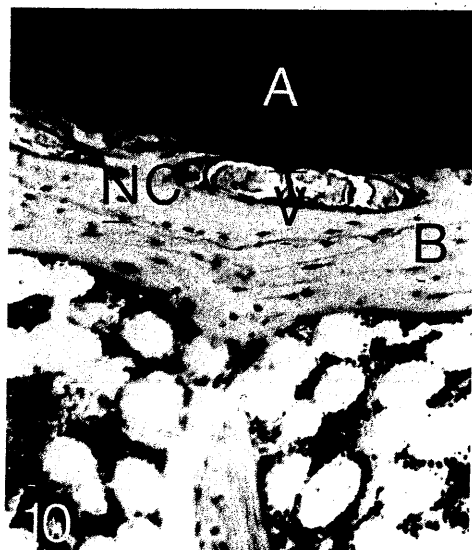
One can subscribe to the thesis that calcified articu-

9. Section of the bone–artic-cartilage interface of a lum-vertebra from a 50-year-old :. PAS-positive material fills nterstitial spaces of nutrient ils. Note the small nutrient il that is obliterated by PAS :rial (**a**). A high magnification ə nutrient canal (x) is seen in ite that the lumen of the canal obliterated by Schiff-positive rial. A = articular cartilage; B one; N.C. = nutrient canal , hematoxylin ×50).
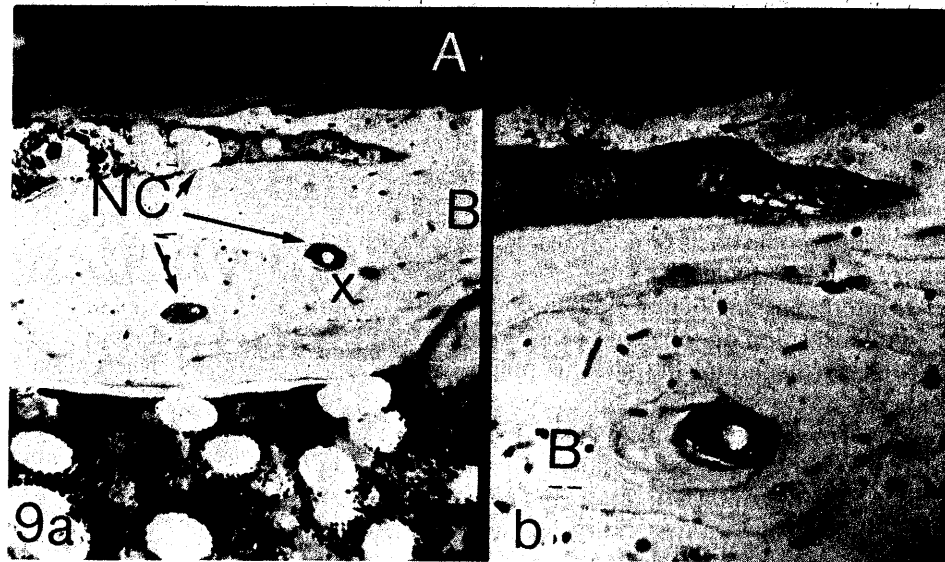
cartilage in aged vertebrae impedes diffusion of stances into the nucleus pulposus. Other factors t be considered. In animals and man, before etal maturity, blood vessels are in contact with ilage at the bone cartilage interface, permitting age of substances through the cartilage. These :ular buds allow nutrients to pass from the blood the cartilage and into the nucleus pulposus. In the ng'' vertebrae, the progressive resorption of the ified cartilage allows the invasion of bone to 'oach the border of the disc. The microvasculature d within the nutrient spaces or canals would le materials to pass eventually into the nucleus osus. Examination of the boy nutrient spaces or

canals adjacent to the disc in the aged vertebrae revealed that the walls of the arterioles, capillaries, and venules within these spaces were thickened and intensely PAS-positive. In addition, the spaces themselves contained varying degrees of PAS-positive material. Many were completely obliterated with the carbohydrate moiety or even calcified. The changes to the microvasculature as well as obliteration of the
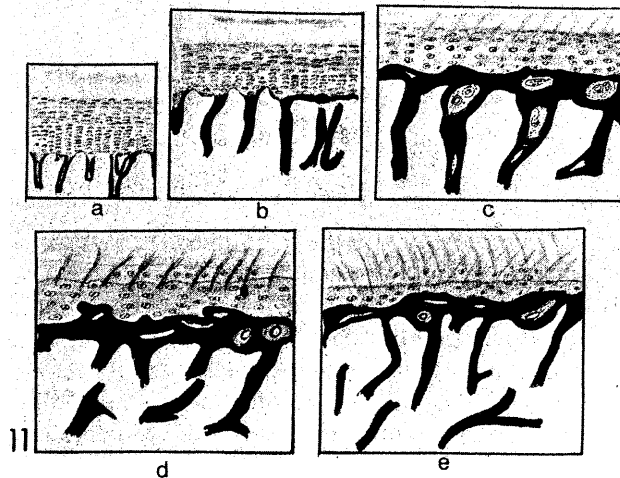

Fig 11. Schematic drawing summarizing age changes of the lumbar vertebral end-plates. **a.** The growth stage consisted of an active growth cartilage zone characterized by regular columns of proliferating and hypertrophic chondrocytes. **b.** The maturation stage. There is a decrease in the number of proliferating chondrocytes, leading to a narrower, irregularly arranged growth cartilage. Bone is sealing off the cartilage border. The growth cartilage has completely disappeared by stage **c**, and bone is directly apposed to the articular cartilage. The articular cartilage is undergoing calcification. Collagen fibers arising from the articular cartilage course into the nucleus polposus. In stages **d** and **d** there is a progressive resorption of the articular cartilage with replacement by bone so that during stage **e** only a very thin layer of cartilage separates the underlying bone from the disc.

0. Section of the bone–articular cartilage interface from a ir vertebra of a 38-year-old male. Note the absence of PAS ial in the nutrient canals. A = articular cartilage; B = bone; = nutrient canal; V = venules (PAS, hematoxylin ×150).

nutrient spaces or canals would retard passage of substances from the blood into the bone, cartilage, and disc. These age changes in the vasculature are not restricted to the vessels of bone but are found throughout the soft tissues, as shown by Ballard et al[2] and deMignard and Bernick.[6] The findings of the present study contradict the views of Brown et al[5] and Amato et al,[1] who, on the basis of their own studies, found no change in the vasculature.

An interesting incidental finding of this study was the demonstration of collagen fibers radiating from the articular cartilage into the nucleus pulposus. The appearance of these collagen fibers were first noted in the vertebrae having reached skeletal maturity. These collagen fibers were independent of the anchoring fibers of the annulus. The attachment of these fibers into the disc may give stability to the disc at times when the articular cartilage is calcified or replaced by bone.

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