Age-related changes in the beagle spine

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Age-related changes were evaluated in the spines of beagle dogs by radiology, histology, and mechanical testing. Thirty healthy beagle dogs were divided into five groups having mean ages of 2–14 years. Radiographs were taken of intact spines at the time of death and of defleshed spines at necropsy. Cervical, thoracic, and lumbar segments were tested in compression to calculate peak stress, peak strain, and elastic modulus. Adjacent spine segments were graded grossly for osteophyte development, and sections of the intervertebral discs were evaluated histologically. Histologic evidence of disc degeneration and changes in the mechanical properties of the intervertebral disc joint preceded radiographic changes. Changes in the mechanical properties of the disc space were probably a result of the disc degeneration rather than the spondylitic lesions.

Intervertebral disc disease is a common lesion in the dog, with an increased incidence reported in chondrodystrophic breeds, such as the dachshund, pekinese, and beagle (Goggin 1970, Gage 1975, Walker 1981). Chondrodystrophic breeds are characterized by a disturbance of epiphyseal chondroblastic growth referred to as achondroplasia (Braund 1975). The achondroplasia is believed to be responsible for premature disc degeneration, as reflected by chondroid transformation of the nucleus pulposus prior to 1 year after birth (Ghosh 1976a).

The alterations present in the discs of chondrodystrophic breeds show many similarities to the aging pattern reported for the human disc. The nucleus pulposus in man changes from a watery gel in youth to a fibrous solid in old age, characterized by a decrease in total mucopolysaccharide, water content, and light-fraction protein polysaccharide (Gower 1969). In both species, these alterations in the intervertebral disc are thought to result in changes in mechanical properties that then render the intervertebral disc susceptible to herniation or prolapse (Gower 1969, Ghosh 1977).

The term *spondylosis* is often used nonspecifically to describe any lesion of the spine of a degenerative nature. In this manuscript, spondylosis refers specifically to the syndrome "spondylosis deformans" as defined by Morgan (1967b) and Forestier and Lagier (1971). Spondylosis in both dog and man has been associated with disc degeneration (Morgan 1967b, Schneck 1985). The pathogenesis of spondylosis is not known, but it is thought to be related to degenerative changes within the intervertebral disc and resultant failing of the mechanical properties.

This investigation was designed to study the effects of age on the compressive mechanical properties of the beagle intervertebral disc and to correlate these changes with pathologic and radiographic examination of the spine.

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Methods

Thirty, healthy, age-matched beagle dogs from the ITRI colony were divided into five groups having mean ages of 2, 5, 8, 11, and 14 years. An equal number of male and female dogs in each age group were included in the study. The dogs were anesthetized with halothane, and a complete series of spinal radiographs was taken. Dogs were then killed by an intravenous injection of pentobarbital, and a complete necropsy was performed. Defleshed intact spinal columns were again radiographed. The spinal columns were then sectioned transversely with a band saw into segments composed of three adjacent intervertebral disc spaces. Spinal segments C4–7, T9–12, and L2–5 were placed in 10 percent buffered formalin for histopathologic studies.

Spinal segments C1–4, T6–9, and T12–L2 were shipped to investigations at Case Western Reserve University (refrigerated and stored at 4 °C) The specimens were coded so that all the measurements were performed blindly. The specimens were cut transversely through the midsection of the vertebral bodies. Pedicles and articulating facets were removed. The test specimen consisted of an isolated intervertebral disc with one half the vertebral body above and below. The external disc dimensions were measured and approximate cross-sectional areas calculated assuming an elliptical shape. Intervertebral discs C3–4, T6–7, T8–9, T12–L1, and L1–2 were then used for mechanical testing in compression. The prepared specimens were placed between parallel compression plates contained within a temperature and humidity controlled environmental chamber. Test specimens were equilibrated for 30 min in the test chamber at 37 °C and 100 percent humidity. The mecanical tests were conducted on a universal testing machine (Instron model 1122) with a cross head speed of 5 mm/min.

The five spinal segments C3–4, T6–7, T8–9, T12–13, and L1–2 were tested in compression to determine peak stress, elastic modulus, and strain at peak stress, and the results analyzed in relation to age. In addition, results were analyzed when intervertebral discs were grouped based on cervical (C3–4), thoracic (T6–7 and T8–9), and thoracolumbar (T12–13 and L1–2) locations. For statistical analysis, mutiple comparisons were done by analysis of variance and Newman-Kuels multiple range test if the distributions were normal and Kruska-Qallis multiple comparison test if they were not. The criterion for significance was $P < 0.05$ for all the comparisons. Linear regression analyses were performed to test correlations between age and the mechanical variables..

Radiographs of the spines were evaluated in a blind fashion for osteophyte formation, and each intervertebral space was graded into one of five categories according to Morgan (1967b). Spinal segments retained for histology were examined grossly and also scored blindly for osteophyte formation by this method.

For histopathologic studies, a transverse section of the intervertebral disc of the proximal disc space of each spinal segment was prepared according to Hansen (1952). Midsagittal sections of the second cranial disc space of each segment were decalcified. Both tissue sections were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Results

Examination of spinal radiographs revealed marked variation in the incidence and severity of spondylosis in individual dogs (Figure 1). Calcified disc material and narrowed disc spaces were rarely observed. If a narrowed disc space was present, it was invariably associated with a grade 4 or 5 spondylosis. The highest frequency of spondylosis occurred in the lower thoracic and upper lumbar spinal segments and at C6–7 (Figure 2). A clear age-related progression was found in the incidence and severity of spondylosis (Table 1). Five of the six dogs in both the 11- and 14-year-old age groups exhibited radiographic signs of spondylosis. Sex-dependent differences were not apparent. Examination of radiographs of defleshed spines resulted in increased detection of milder spondylitic lesions as compared with radiographs taken antemortem.

Gross observation of osteophyte formation paralleled the radiographic findings (Table 1). The number of intervertebral disc spaces involved and the severity of the lesion increased with advancing age. Cervical and lumbar segments were more severely affected than the thoracic segment. Palpable projections from the

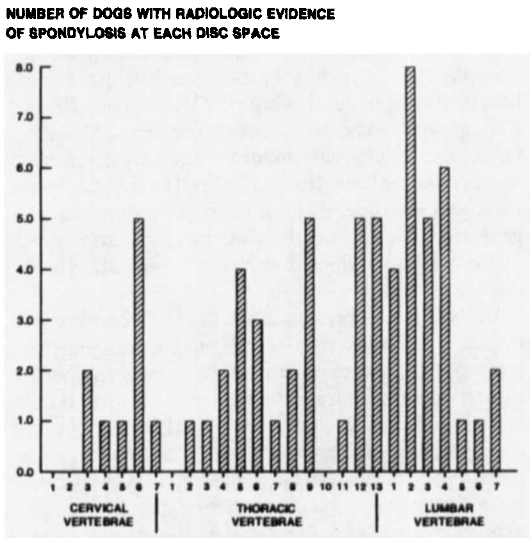

Figure 2. Frequency of antemortem radiographic evidence of vertebral spondylosis according to intervertebral disc space in 30 beagle dogs. All age groups combined.

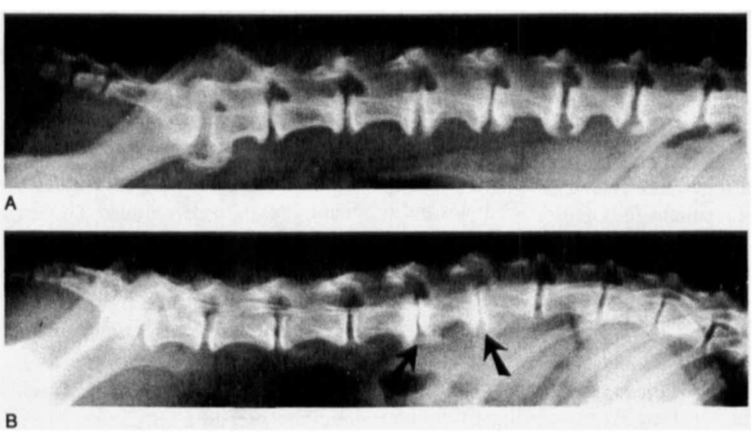

Figure 1. The lumbar vertebral column of two 14-year-old, beagle dogs.
A. End-plate sclerosis and ventral osteophyte lipping at multiple intervertebral discs.
B. In contrast, the primary lesion is collapse of the disc spaces at L2–3 and L3–4 (arrows) while adjacent spaces show no visible lesions.

Table 1. Incidence and severity of spondylosis as graded radiographically (R) and by gross observation (G) in Beagles of different ages

	Age in years									
	2		5		8		11		14	
	R	G	R	G	R	G	R	G	R	G
Number of dogs	6	6	6	6	6	6	6	6	6	6
Number affected	0	6	0	6	2	6	5	6	5	6
Number of intervertebral discs affected (sum of all dogs)	0	26	0	42	4	34	19	44	44	44
Spondylosis stage										
1	0	9	0	16	0	11	0	10	0	6
2	0	8	0	9	3	2	6	3	12	8
3	0	6	0	6	1	9	3	12	20	9
4	0	2	0	9	0	4	7	6	6	9
5	0	1	0	2	0	8	3	13	6	12

Only spinal segments C4–7, T9–12 and L2–5, for a total of 9 intervertebral discs, were evaluated by gross observation per dog.

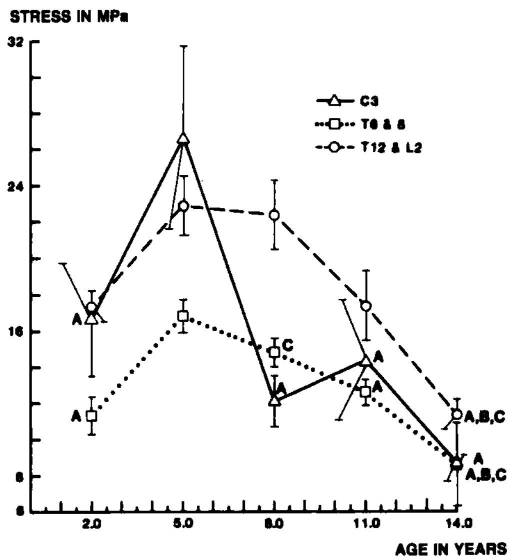

Figure 3. Peak stress (mean and standard error) among age groups with spinal segments grouped according to cervical (C3), thoracic (T6 and T8), or thoracolumbar (T12 and L2) location.
A indicates difference from the 5-year-old age group.
B indicates difference from the preceding age group.
C indicates difference from two preceding age intervals ($P < 0.05$).

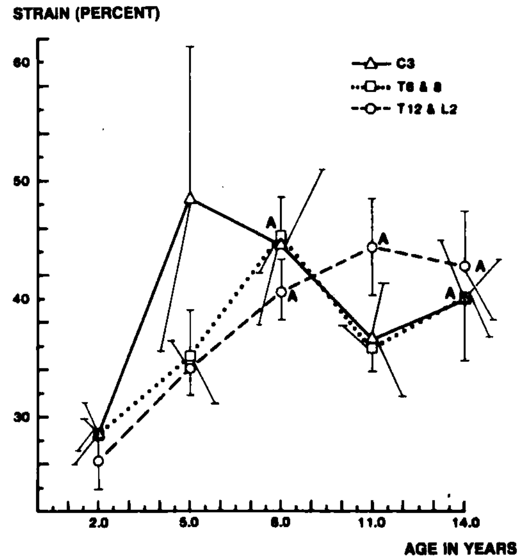

Figure 4. Peak strain (mean and standard error) among age groups with spinal segments grouped according to cervical (C3), thoracic (T6 and T8), or thoracolumbar (T12 and L2) location. A indicates difference from the preceding age group ($P < 0.05$).

vertebral body were present prior to the radiographic detection of osteophytes.

Analysis of mechanical data showed similar age-related effects regardless of how the spinal segments were grouped based on anatomic location. However, mechanical changes in the cervical regions were more variable than in the thoracic and lumbar vertebrae. Peak stress to failure increased from 2 to 5 years of age and then declined with increasing age (Figure 3). A similar trend occurred at all the spinal levels. Although not significant, a general trend toward increasing peak strain with age was observed (Figure 4). The most distinct age-related effects were reflected in the elastic modulus (Figure 5); for all the spinal levels, elastic modulus decreased with increasing age ($r = -0.7$). The mechanical changes indicated that the intervertebral disc space became less strong and less stiff with increasing age.

Comparison of mechanical data with antemortem radiographic findings showed no clear trends.

Early degenerative changes of the intervertebral disc were detectable histologically in the 2-year-old age

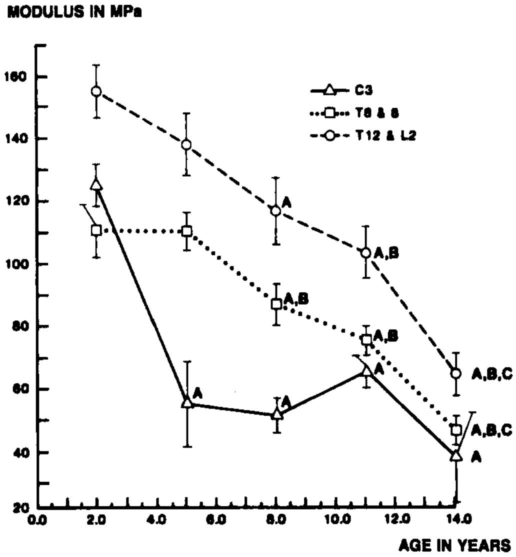

Figure 5. Elastic modulus (mean and standard error) among age groups with spinal segments grouped according to cervical (C3), thoracic (T6 and T8), or lumbar (T12 and L2) location.
A indicates difference from 2-year-old group.
B indicates difference from 5-year-old age group.
C indicates difference from preceding age group ($P < 0.05$).

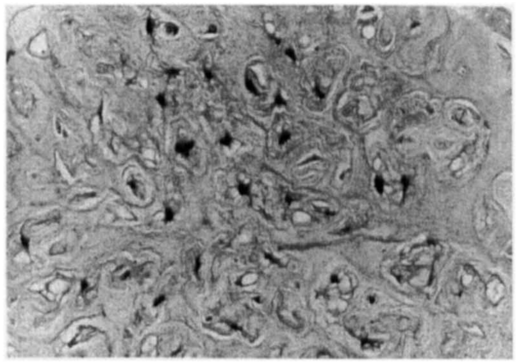

Figure 6. Photomicrograph of the nucleus pulposus of an intervertebral disc from a 2-year-old beagle dog. Note the extensive vacuolar degeneration of the fibrocartilage present even at this young age. x480.

Figure 7A

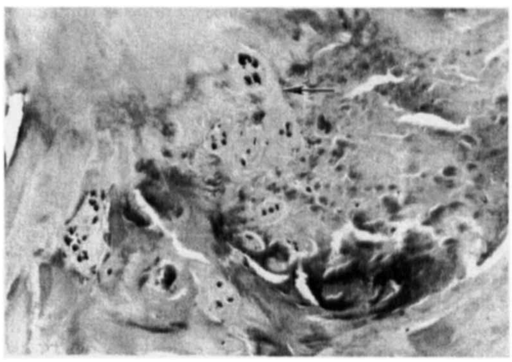

Figure 7B

Figure 7. Photomicrograph of a sagittal section of an intervertebral disc space from a 14-year-old beagle dog (7A; x32). The ventral borders of the two adjacent vertebral bodies are indicated (thick arrows). Extensive fibrocartilaginous tissue proliferation is present between the outer lamellae of the disc and the longitudinal ligament. New bone formation is also present (thin arrow). Higher magnification of the area indicated by "B" shows degeneration of the fibrocartilage with cloning of chondrocytes. (7B; x270).

group. The earliest and most frequent change noted was the presence of randomly distributed basophilic foci within and between the lamellae of the annulus fibrosis. Separation of lamellae and proliferation of cartilage within and between lamellae were rare at 2 and 5 years of age, but increasingly prominent with advancing age. Early changes within the nucleus pulposus included vacuolar degeneration of the fibrocartilaginous tissue and cloning of cartilage, which were present even at 2 years of age (Figure 6). Necrosis and calcification of the chondroid tissue occurred in more severely involved discs in the older age groups. Little recognizable disc tissue was present in the majority of the transverse disc sections examined by 14 years of age. The tissue was primarily composed of fibrocartilage. Early lesions identified on sagittal sections of the intervertebral disc space consisted of proliferation of fibrocartilage from the ventral tip of the vertebral body.

with areas of cartilage and bone formation, and notable neovascularization (Figure 7). Bridging of the disc space by fibrous tissue or, more rarely, by cartilage was common by 11 years of age; complete bony bridging of the intervertebral disc space was rare, and was present in only two of the 90 intervertebral disc spaces examined.

Discussion

The spinal distribution of osteophytes in our study, as measured radiographically, is similar to that found in man, the highest incidence occurring in the lower thoracic and lumbar regions (Morgan 1967b, Schneck 1985). Thus, the distribution of osteophytes is similar in these two species despite the marked difference in posture and mode of ambulation.

higher incidence and in younger dogs than radiographic observation because the earliest periosteal proliferation is composed of radiolucent fibrocartilage. Degenerative changes in the disc as determined through histologic investigation agree with the descriptions reported by Morgan (1967b). Although the histologic changes and mechanical changes followed similar trends throughout all the spinal levels, the incidence of osteophytes was distinctly different throughout the spinal column. These findings suggest that the degree of mobility in different segments of the spinal column may be of importance for the eventual formation of osteophytes. Previous investigators have speculated that mechanical factors are responsible for the degeneration of the discs (Morgan 1967b). However, our study suggests that degenerative changes predate and probably produce the changes in mechanical properties seen with age.

Mechanical testing indicated that the intervertebral disc space became less strong and less stiff with increasing age. The disc was attached to the adjacent vertebral bodies during mechanical testing. However, because the strength and modulus (stiffness) of the vertebral bone are substantially greater than that of the disc material, the changes in mechanical properties observed experimentally were interpreted as being the result of changes in the disc. In addition, no evidence of trabecular bone damage was observed following testing. Attempts to correlate mechanical parameters with radiographic changes at specific intervertebral disc spaces were unsuccessful unless a bony fusion of the joint was present. As has been previously reported, bony ankylosis from spondylosis is rare; large osteophyte projections frequently interdigitate, but rarely unite (Morgan 1967b). Despite the radiographic evidence of large osteophyte projections that might be anticipated to contribute to the mechanical strength, these appear to be relatively uninvolved in the mechanical properties of the disc space. Previous investigators have suggested that the osteophyte projections that characterize spondylosis are a result of changes in the biomechanics of the spine (Morgan 1967b). Although this study does not definitively prove this hypothesis, the time course of the appearance of mechanical alterations and subsequent appearance of spondylosis does give supporting evidence.

Because water and proteoglycans provide the resilience of the disc, a decrease in these components would be anticipated to impair resilience and impose additional stresses on the annulus fibrosis (Cole 1986). The mechanical tests in this study measured average properties of the disc space and did not specifically measure the true material property of the disc material alone. Therefore, these changes reflect a sum of the degenerative processes occurring in the intervertebral space, not just in the nucleus pulposus. This study does, however, support the hypothesis that the degenerative changes in the disc alter the mechanical properties of that tissue.

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