**Discussion**

Our findings suggest that the large cells of the nucleus pulposus (notochordal?) influence the rate at which newly synthesised proteoglycans are incorporated into the extracellular matrix of both the annulus fibrosus and the nucleus pulposus of the intervertebral disc. The data also suggest that the large (notochordal?) cells of the non-chondrodystrophic nucleus pulposus synthesise a considerable amount of large, sulphated proteoglycan and that it is this cell that produces the matrix that is responsible for the biomechanical properties of the tissue. This is contrary to what has been published about the biosynthetic capabilities of the notochordal cell and suggests that it does have a significant role in maintaining homeostasis in the normal intervertebral disc. This study is the first to show that the cells of the annulus fibrosus and nucleus pulposus behave very differently in healthy and diseased intervertebral discs. It may be that in the discs of species where notochordal cells do not normally survive after birth (human), they are still extremely important in defining the composition of the nucleus pulposus.

**References**


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**Introduction**

Back pain and herniation of the intervertebral disc, associated with neurological dysfunction, is a common clinical problem. It is known that the intervertebral disc is an avascular tissue populated by a low content of poorly characterised cells, distributed in an extensive extracellular matrix. The matrix of the central nucleus pulposus is rich in proteoglycans, whereas, that of the annulus fibrosus is predominantly collagenous. The intervertebral discs of the chondrodystrophoid breeds are reported to demonstrate a chondroid metamorphosis in the nucleus pulposus within two months of birth. This developmental process also occurs in non-chondrodystrophoid breeds, but it is a more gradual process requiring almost the full life span of the dog for it to be completed. Beagles and other chondrodystrophoid breeds are more disposed too early intervertebral disc prolapsed than non-chondrodystrophoid breeds such as the Greyhound and Labrador. We have tested the following hypothesis:

The rate of assembly of aggrecan in the extracellular matrix of the nucleus pulposus from the chondrodystrophoid dog (Beagle) is different to that of a non-chondrodystrophoid breed (Labrador).

**Methods**

The nucleus pulposus and annulus fibrosus were isolated from the intervertebral discs of a Beagle, a Labrador and a Labrador Crossbreed, all aged 12 months. The isolated cells were incorporated into alginate beads at 20,000 cell/bead and cultured for 3 days in Dulbecco’s Modified Eagles medium (DMEM) containing 10% FCS. The alginate beads were washed with Hams F-12 and pulsed with 100µCi/ml 35S-sulphate for 6 hours and the beads and culture medium were collected on the day of the pulse and after 5 days, 10 days and 15 days in chase culture (DMEM). The supernatant (intercellular compartment) and culture medium were collected on the day of the pulse and after 5 days, 10 days and 15 days in chase culture (DMEM).  The supernatant (intercellular matrix) and cell pellet with its associated pericellular matrix were separated, after disrupting the gel with citric acid. The distribution of proteoglycan between the two pools was determined by calculating the 35S-sulphate incorporated into glycosaminoglycan chains. Quantitative separation of 35S-sulphate labeled proteoglycan and unincorporated isotope was achieved using a Multiscreen Filtration system. Large (notochordal?) cells and small cells in each preparation were determined by counting them using a ruled graticule.

**Results**

The proteoglycans synthesised by the nucleus pulposus and the annulus fibrosus cells of the Beagle (fig 1) were distributed similarly between the pericellular (open symbol) and intercellular (solid symbol) compartments. Whereas, those synthesised by the cells of both the annulus fibrosus and those of the nucleus pulposus, from the Labrador (Fig 3) were incorporated into the intercellular compartment more quickly, but the rate of incorporation is faster in the nucleus pulposus than in the annulus fibrosus. The crossbreed Labrador (Fig 2) also incorporated newly synthesised proteoglycan into the extracellular matrix at a faster rate than the chondrodystrophoid dog (Beagle) and showed the same difference in rate of incorporation between the annulus fibrosus and nucleus pulposus that was observed for the Labrador. However, it should be noted that the rate of incorporation of proteoglycan by both tissues was faster than that of the Beagle, slower than that of the Labrador. When the ratio of large (notochordal?) and small cells was measured in the preparations of the nucleus pulposus from all breeds of dog, the Labrador crossbreed had a value that was greater(6.14) than that of the Beagle (0.087), but less than that of the Labrador (10.63).

**Discussion**

Our findings suggest that the large cells of the nucleus pulposus (notochordal?) influence the rate at which newly synthesised proteoglycans are incorporated into the extracellular matrix of both the annulus fibrosus and the nucleus pulposus of the intervertebral disc. The data also suggest that the large (notochordal?) cells of the non-chondrodystrophic nucleus pulposus synthesise a considerable amount of large, sulphated proteoglycan and that it is this cell that produces the matrix that is responsible for the biomechanical properties of the tissue. This is contrary to what has been published about the biosynthetic capabilities of the notochordal cell and suggests that it does have a significant role in maintaining homeostasis in the normal intervertebral disc. This study is the first to show that the cells of the annulus fibrosus and nucleus pulposus behave very differently in healthy and diseased intervertebral discs. It may be that in the discs of species where notochordal cells do not normally survive after birth (human), they are still extremely important in defining the composition of the nucleus pulposus.