INTRODUCTION
The inflammatory response around herniated tissue in the epidural space is believed to play a major role in the spontaneous regression of herniated lumbar disc. Numerous macrophages invade the herniated tissue along with newly formed blood vessels which influence oxygen gradient. Inflammatory cytokines such as interleukin-1 are produced by macrophages. These chemical mediators could stimulate disc cells to produce proteases such as MMPs which degrade the intervertebral disc matrix and could hence influence regression of the herniation. Here we have examined the influence of IL-1β and oxygen tension on proteoglycan turnover using a three-dimensional disc-cell culture system.

METHODS.
Cells were isolated from the nucleus pulposus of 18-24 month bovine caudal discs by enzyme digestion. They were initially cultured for 14 days in alginate beads in DMEM containing 6% FBS (50 beads/50 ml) at 4.10^6 cells/ml under 21% oxygen to accumulate matrix. The medium was not changed. They were then cultured for 6 days (10 beads/2ml-DMEM containing 6% FBS) under 0% or 21% oxygen and with or without IL-1β (2.5 ìg/ml). The medium was changed every third day. The cell viability profile was determined by manual counting using trypan blue staining and by light microscope using immuno-histochemical techniques (ABC methods). Lactate production and glucose consumption were measured enzymatically as markers of energy metabolism. Glycosaminoglycan (GAG) accumulation (as a measure of proteoglycan) were measured using a DMB assay (Enobakhare et al, 1996). Rates of sulphated GAG synthesis were measured using a standard 35S-sulfate incorporation method (Maroudas, 1980). MMP activity (MMP-2/ MMP-7) was measured using coumarin fluorescent assay (Knight et al, 1992).

RESULTS
The viability profile was already established by 16-20 days of culture and remained constant since the results after 18 and 20 days were very similar. There was no difference in the cell viability with or without oxygen and IL-1β. In the immunohistochemical study, the NP cells inside the alginate beads were positive for IL-1β, MMP-2, 3, and 7 after culture for 6 days under 21% O₂ (Fig.1). Glucose consumption decreased in the presence of IL-1β. Glucose consumption was lowest if both IL-1β and oxygen were present. At cell densities found in vivo in the disc nucleus viz. 4.10^6 cells/ml and at 21% oxygen the concentration of GAG in the bead reached 0.91 ± 0.2 mg/ml in 14 days i.e. around 1% of the GAG concentration found in situ. Total GAG/million cells was 0.027 ± 0.009 mg after 14 days. The results showed that IL-1β had a significant effect on GAG accumulation and production and that its effect was dependent on oxygen tension. Total GAG/million cells rates decreased in the presence of IL-1β at high oxygen but low oxygen inhibited the effects of this cytokine. Sulphate incorporation rates decreased in the presence of IL-1β at high oxygen but low oxygen inhibited the effects of this cytokine. Treatment with IL-1β showed a significant increase in the rate of MMP activity with APMA (p-aminophenyl mercuric acetate). MMP activity increased with IL-1β under 21% oxygen, but not at low oxygen (Fig.2,3). IL-1β is effective in causing an inhibition in the rate of GAG production in alginate beads.

DISCUSSION: IL-1β is known to have catabolic effects on matrix turnover as it increases rates of MMP production and activity and reduces rates of matrix macromolecule production. Here we show that the influence of IL-1β depends on oxygen tension. Under 21% oxygen (air) and in the presence of IL-1β GAG synthesis and accumulation fall significantly and Total MMP production and activity increase significantly. Under 0% oxygen and in the presence of IL-1β, there is little effect on GAG synthesis and accumulation and MMP activity and production increases only slightly.

CONCLUSION: Exogenous IL-1β will activate MMP activity and will digest the extracellular matrix of intervertebral disc but only if oxygen is present. Vascularisation is necessary for IL-1β to exert its effects.

REFERENCES