Our study establishes a rabbit model of disc degeneration which requires neither a chemical nor physical injury to the disc. Disc degeneration similar to that seen in man was created at levels proximal (L4-L5) and caudal (L7-S1) to a simulated lumbar fusion and was studied for up to nine months after arthrodesis.

Loss of the normal parallel arrangement of collagen bundles within the annular lamellae was observed in intervertebral discs adjacent to the fusion at three months. By six months there was further disorganisation as well as loss of distinction between the lamellae themselves. By nine months the structure of the disc had been replaced by disorganised fibrous tissue, and annular tears were seen. There was an initial cellular proliferative response followed by loss of chondrocytes and notochordal cells in the nucleus pulposus. Degeneration was accompanied by a decrease in the monomer size of proteoglycans. Narrowing of the disc space, endplate sclerosis and the formation of osteophytes at adjacent disc spaces were observed radiologically.

Materials and Methods

We used 28 skeletally mature New Zealand white rabbits weighing 2 to 3 kg. All handling, housing and feeding were approved by the University Animal Care Committee. Fifteen rabbits had a simulated posterolateral arthrodesis as described below, six had sham surgery and six unoperated rabbits were used as a control group. One operated rabbit died on the night of surgery and was not included. All the rabbits were older than 12 months at operation and between 18 and 24 months at the time of death.

Operative technique. Under general anaesthesia a dorsal midline skin incision was made followed by two para-median fascial incisions. The transverse processes of the fifth, sixth and seventh lumbar vertebrae were exposed and cleared of soft tissue. We performed a bilateral posterolateral intertransverse arthrodesis using methylmethacrylate and wire. Stainless-steel 20-gauge wire was used to interconnect the fifth, sixth and seventh transverse processes in a figure-of-eight fashion. Methylmethacrylate was then applied to encircle the transverse process taking care to prevent the cement from contacting the adjacent, non-arthrodesed, facet joints. The cement was cooled by saline irrigation while curing. The wound was closed in layers. In the sham rabbits, the same surgical exposure of the transverse processes was carried out, but no arthrodesis was performed. After operation all the animals were allowed unlimited activity.

The rabbits were killed at three (n = 5), six (n = 5) and nine months (n = 5) after arthrodesis. Standard anteroposterior and lateral lumbar radiographs were obtained from all the rabbits immediately after surgery and after they had been killed. The radiographs were digitised and the...
height of the disc at the level of the mid-vertebral body was measured on both lateral films.

After death, the lumbar spines were harvested en bloc from T12 to the sacrum. They were inspected visually for evidence of cracks in the methylmethacrylate or breakage of the wire. The spines were manipulated manually to determine if there was any gross movement across the simulated fusion. The methylmethacrylate and wires were then removed. The intervertebral discs were harvested by dividing the vertebral endplates immediately adjacent to the disc in order to preserve the architecture of the disc.

The intact discs were fixed in formaldehyde solution, decalcified, sectioned in either the transverse or longitudinal axis and stained with haematoxylin and eosin. They were assessed by light microscopy.

Discs from nine-month (n = 2) and age-matched control (n = 2) specimens were analysed for proteoglycan. The dry weight of the disc tissue was obtained. Tissue slices were diced, dissociatively extracted and separated into A1 and A1D1 fractions as described previously. The content of uronic acid was measured in the extract and in the papain-digested residual disc tissue by an automated procedure of the modified carbazole reaction.

The A1 and A1D1 fractions were applied to a Sepharose CL-2B column and eluted in 0.05 mol/l of sodium acetate at a flow rate of 4 ml/hr. The fractions were collected and analysed for uronic acid.

The findings were compared with those in the sham-operated and the control groups.

Results

Figure 1 shows lumbar radiographs obtained immediately after operation and after death. At three months slight narrowing of the disc space was observed at the L4-L5 and L7-S1 adjacent levels (Table I). By nine months, further narrowing of approximately 50%, endplate sclerosis, and the formation of osteophytes were noted at these levels in all rabbits. The radiographs of the age-matched sham-operated group at three, six and nine months showed no loss of disc height or evidence of disc degeneration.

The lumbar arthrodeses were assessed at the time of sacrifice. In all 15 rabbits the cement mass remained intact and connected to the transverse processes without any full-thickness cracks. All of the wires were intact. There was no detectable movement at the levels of fusion on manual manipulation.

In a normal annulus the collagen bundles within the lamellae are parallel and at right angles to the bundles of the adjacent lamellae (Fig. 2a). In the three-month specimens the most striking finding was loss of this organised parallel arrangement of the annular fibres in the discs at adjacent levels (Fig. 2b). Distinct annular lamellae were preserved. By six months there was further disorganisation within the lamellae, as well as loss of distinction between

<table>
<thead>
<tr>
<th>Specimen</th>
<th>L3-L4</th>
<th>L4-L5*</th>
<th>L7-S1*</th>
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<tr>
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<td></td>
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</tr>
<tr>
<td>Rabbit 1</td>
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<td>0.88</td>
<td></td>
</tr>
<tr>
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<td>0.89</td>
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<tr>
<td>Rabbit 4</td>
<td>0.91</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Rabbit 5</td>
<td>0.90</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>6-month</td>
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<td></td>
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</tr>
<tr>
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<td>0.75 (S)</td>
</tr>
<tr>
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<td>0.82 (S)</td>
<td>0.75 (S)</td>
</tr>
<tr>
<td>Rabbit 3</td>
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<td>0.83</td>
<td>0.75 (S)</td>
</tr>
<tr>
<td>Rabbit 4</td>
<td>1.00</td>
<td>0.82 (S)</td>
<td>0.75 (S)</td>
</tr>
<tr>
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<td>0.74 (S)</td>
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<tr>
<td>9-month</td>
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<tr>
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<td>0.50 (S,O)</td>
<td>0.47 (S,O)</td>
</tr>
<tr>
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<td>0.48 (S,O)</td>
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<td>0.52 (S)</td>
<td>0.50 (S,O)</td>
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<tr>
<td>Rabbit 5</td>
<td>0.93</td>
<td>0.52 (S)</td>
<td>0.51 (S,O)</td>
</tr>
</tbody>
</table>

* S, endplate sclerosis; O, osteophytes
Figure 2a – Cross-sectional photomicrograph showing annular fibres within lamellae parallel to one another and at right angles to the fibres of the adjacent lamellae (haematoxylin and eosin ×20). Figure 2b – Photomicrograph showing loss of the organised arrangement of the lamellar fibres in a three-month specimen (haematoxylin and eosin ×20). Figure 2c – Photomicrograph showing disorganisation within the annular lamellae with loss of the distinct lamellar borders. A radial cleft extending outwards towards the periphery of the disc is seen (clear arrow). The solid arrow points to the centre of the nucleus pulposus (haematoxylin and eosin ×15).

Figure 3a – Photomicrograph of the normal nucleus pulposus with chondrocytes embedded in an amorphous extracellular matrix, with central clumped notochordal cells (arrow) (haematoxylin and eosin ×20). Figure 3b – Photomicrograph of a six-month specimen showing proliferation of chondrocytes with loss of notochordal cells (haematoxylin and eosin ×20).
the lamellae themselves. These changes were more prominent in the middle and inner annular zones. Cellular proliferation was also present in the annulus. By nine months, clefts and tears had developed and extended towards the periphery of the disc. The normal annular architecture was extensively disrupted and was replaced by disorganised fibrous tissue (Fig. 2c). At nine months marginal osteophytes and formation of new bone were seen extending from the vertebral endplate into the disc matrix. At no point was there evidence of nuclear herniation through the annulus. The annular architecture of the sham group was normal.

The normal rabbit nucleus pulposus is composed of sparse chondrocytes embedded in an amorphous extracellular matrix with central clumped notochordal cells (Fig. 3a). The nucleus in the three-month specimens appeared to be similar to that of the control group with respect to overall architecture, cellularity and cell morphology. In the six-month specimens proliferation of chondrocytes in the nucleus was seen, with an associated loss of notochordal cells (Fig. 3b). By nine months the nuclear-annular distinction blurred and the entire disc was composed of a cellular fibrous matrix (Fig. 4). Few chondrocytes were present. The nucleus of the sham group at three, six and nine months was normal. The nuclear and annular histological changes at the L7-S1 disc were more advanced than those seen at the L4-L5 disc at each period studied.

Because of the loss of nuclear-annular distinction with degeneration entire discs were used for the analysis of proteoglycans. The total concentration of uronic acid was similar in the control and adjacent intervertebral discs nine months after arthrodesis. The proteoglycans (A1D1 fractions) from the latter were smaller, eluting later than those from the A1D1 fractions of control discs (Fig. 5). The elution profiles for the A1 fractions of control and degenerated discs were similar.

Discussion

The study of the pathogenesis of intervertebral disc degeneration has been limited by the difficulty of establishing a clinically relevant animal model. Such models of human disease should reproduce the pathological and biomechanical features of human disc degeneration without requiring an artificial injury to initiate these events. This has not previously been accomplished. The best characterised animal models of disc degeneration involve the creation of a direct physical (annulotomy) or chemical (chymopapain) injury to the disc to initiate degeneration.

Previous studies of degeneration adjacent to lumbar fusion have shed little light on the degenerative process. In an achondroplastic dog model, Taylor et al reported that...
although posterior spinal arthrodesis using autograft induced alterations in adjacent disc biochemistry at six months after fusion, no change in the architecture of the disc was seen. This may be attributable to the fact that fusion by autograft in dogs heals inconsistently, and may take months to consolidate, so that morphological changes in adjacent segments may not have occurred by six months. Olewski et al\(^7\) performed incision of the disc at levels adjacent to instrumented lumbar fusions in non-chondrodystrophic dogs. They reported significant degenerative changes in the injured discs by six months after fusion and incision of the disc. The clinical relevance of this study, however, is limited since the model involved an annulotomy and thus created a non-physiological model of degeneration of an adjacent segment.

Our study supports the concept that loss of the normal annular architecture, in response to abnormal mechanical stresses, may be an early event in the degenerative process (Fig. 6). In the normal rabbit annulus the collagen fibres are orientated parallel to those within the same lamella and at right angles to those of adjacent lamellae. In our study early loss of the organised arrangement of collagen fibres within lamellae was followed by loss of the discrete lamellar arrangement. These changes may result in impaired load transmission of the annular forces. The importance of the annular architecture in maintaining normal loading characteristics is supported by previous studies which have shown that the properties of the disc of maximum elongation to failure, residual deformation, and energy dissipation are dependent on the orientation of the fibres and the number of annular lamellae.\(^{15,16}\) In a recent study in which mouse tails were loaded in vivo with an external compression device similar changes to those seen in our study were observed.\(^{17}\)

Proliferation of chondrocytes, which probably represents an attempted reparative process, was observed, but with advancing degeneration loss of both chondrocytes and notochordal cells was seen. Lotz et al\(^{17}\) recently reported a correlation between artificial mechanical loading and apoptosis of chondrocytes in rat intervertebral discs. Altered loading of the disc has been suggested to be the mechanism of regression of postnatal notochordal cells in the human nucleus pulposus.\(^{17,18}\)

The initiating events in the degenerative process remain controversial. Hirsch and Schajowicz\(^{19}\) found that no ruptures occurred in the annulus fibrosus in the absence of advanced structural changes. By contrast, Vernon-Roberts and Pirie\(^{20}\) observed early peripheral annular tears and concluded that these were likely to be the result of primary trauma to the disc. In our study disruption of the annular organisation preceded the formation of tears and clefts, suggesting that tears and rupture occur in the structurally altered annulus rather than representing an inciting injury in the process of disc degeneration.

In our study nuclear herniations were not seen. This appears to be clinically relevant since disc degeneration without nuclear herniation is often observed in human MRI studies. These observations suggest either that annular
tears, which may allow for herniation of nuclear material, occur later in the degenerative cascade or that such herniation may require an additional inciting event. The response of the disc to the altered mechanical environment imposed by this model may also be different from that leading to nuclear herniation.

Proteoglycans are thought to play an important role in maintaining normal disc mechanics. With advancing human disc degeneration, the proteoglycan content and aggregability with hyaluronic acid decrease.\(^{21-23}\) In a rabbit model of disc injury, Lipson and Muir\(^{8}\) noted a trend of loss of proteoglycans with significant decrease in their aggregation beginning six weeks after disc incision. In an experimental model involving disc incision adjacent to a posterior lumbar fusion, Olewski et al\(^7\) reported no change in the concentration of proteoglycans at six months after injury to the disc. Melrose et al\(^24\) showed that loss of proteoglycans occurred eight months after experimental injury to the disc. In our study the total proteoglycan content at adjacent levels was unaltered at nine months after arthrodesis, but there was a decrease in monomer size.

The model described in our study is a reproducible technique for creating intervertebral disc degeneration at mobile segments adjacent to a lumbar fusion. Previous investigators have attempted this using techniques of autograft fusion.\(^{14}\) This is, however, limited by the unreliability of healing of the fusion and its variable stiffness. By using cement-wire two-level stabilisation a solid ‘fusion’ is created immediately. A two-level arthrodesis was chosen in the hope that this would lead to more rapid disc degeneration of adjacent segments than may be anticipated after a single-level arthrodesis. In this rabbit model, disc degeneration occurred more rapidly than may be expected in man.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References