# ANNULAR TEARS AND INTERVERTEBRAL DISC DEGENERATION

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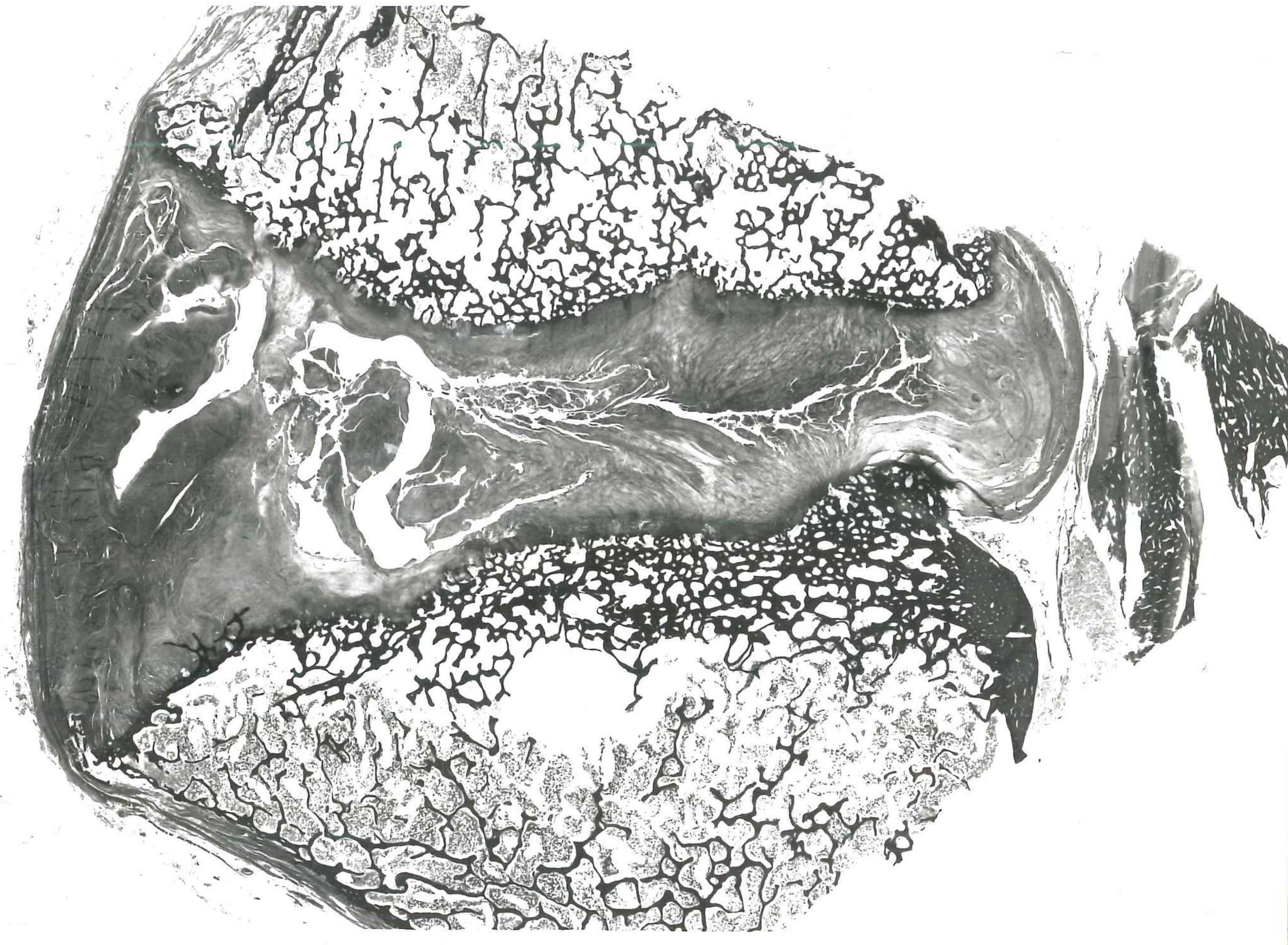
Figure 0 Low power micrograph of human lumbosacral disc. Note the marked clefting of the anterior annulus fibrosus.



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## SIGNED STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and that to the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due references made in the text of the thesis. The author consents to the thesis being made available for photocopying and loan if applicable, if accepted for the award of the degree of Doctor of Philosophy.

## AWARDS

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## ABSTRACT

In parallel with the dehydration and fraying of the nucleus pulposus the degenerating human intervertebral disc is characterised by the formation of tears within the annulus fibrosus.

Despite the likely relevance of annular tears in the pathogenesis of low back pain, their relationship with the biochemical degradation of the intervertebral disc remains uncertain. Furthermore, their association with mechanical stress is still controversial.

Pathological observations in young human lumbar discs have highlighted the possible role of discrete peripheral lesions of the annulus in the early phases of disc degeneration. The incidence of the various types of annular defects in the active adult population remains, at present, unknown.

The aims of the present study were to:

1. Analyse the characteristics and relative incidence of annular defects in the human lumbar spine;
2. Investigate their role in the pathogenesis of intervertebral disc degeneration.

1. A total of 260 human lumbar discs were examined. Serial macroscopic and microscopic observations were made and annular tears classified according to their morphology. The incidence of annular lesions was analysed in relation to age, level, area of the disc involved and association with degeneration of the nucleus pulposus.

The role of MRI and discography in imaging annular defects was analysed in a prospective study of 33 patients with low back pain. The results indicated that pain provocation at discography was strictly related to tears extending into the peripheal annulus fibrosus. Whilst all symptomatic discs had contrast pattern at discography consistent with marked degeneration, 12 had normal signal intensity at MRI. Of the 60 discs with normal signal intensity at MRI 18 had markedly abnormal discograms. None of the abnormal discs at MRI had normal discograms.

2. An animal model using the sheep was developed to investigate the role of peripheral tears of the annulus in the pathogenesis of disc degeneration. Using a retroperitoneal approach to expose the lumbar spine a cut of known depth was made in the left antero-lateral annulus fibrosus of randomly selected lumbar discs. This cut was parallel and adjacent to one end-plate and closely reproduced the rim lesion described by Schmorl and Vernon-Roberts. In 25 sheep the cut had a depth of 5mm and in the remaining 10 sheep of 2.5mm. These represented respectively two thirds and one third of the width of the anterior annulus, leaving the inner annulus and the nucleus pulposus intact.

Plain radiographs and discograms were performed at the time of operation. The sheep were sacrificed according to a randomised schedule at an interval from operation varying from one month to two years. Following sacrifice a series of experiments were carried out comprising MRI, discography, conventional and quantitive histology and biochemical analysis.

The results of the radiological investigations suggested that following acute annular failure, MRI would not show any pathological changes in the signal obtained by T2 weighted images from the nucleus pulposus for a period of up to 12 months.

Decreased signal intensity due to secondary dehydration of the nucleus pulposus was seen only in the later phases of degeneration and was paralleled by the biochemical data on moisture content and proteoglycan levels.

The results of the morphological analysis suggested that despite great care being taken to ensure that the inner annulus was not involved in the initial lesion, progressive failure of the inner annulus took place between four and twelve months after operation. Whilst microscopy showed healing of the outer one third of the annular defect no evidence of repair was seen of the inner two thirds of the original cut. Of the biochemical parameters examined, disc proteoglycans, proteoglycan aggregation and matrix protein levels were significantly affected in the operated discs. The biochemical findings were consistent with progressive disc degeneration.

These results indicate that discrete tears of the outer annulus may have a role in the formation of concentric clefts and in accelerating the development of radiating clefts in the annulus fibrosus and degeneration in the nucleus pulposus. Localised peripheral tears of the annulus fibrosus may be relevant in the pathogenesis of the early phases of degeneration of the intervertebral disc and in the production of discogenic pain.

# 2. INTRODUCTION

## 2.1 AIMS OF THE STUDY

This study was designed to test the hypothesis that discrete peripheral tears of the annulus lead to secondary degenerative changes in other components of the intervertebral disc and therefore are relevant to the sequence of events which lead to disc degeneration. In order to test this hypothesis 260 human lumbar intervertebral discs were examined and an animal model using the sheep was developed.

## 2.2 DEGENERATION OF THE INTERVERTEBRAL DISC - DEFINITION

In the 1976 edition of the Concise Oxford Dictionary the verb to degenerate is defined as to "have lost the qualities that are desirable or proper", to have "fallen from former excellence".

If the reader agrees on this rather broad definition then he or she would agree that as such intervertebral disc degeneration would appear to be a certainty in the human race. It would be more difficult to agree, however, on its possible causes.

A major obstacle in attempting to define the meaning of disc degeneration may be represented by its differentiation with ageing.

If these terms were to be considered synonymous, time would represent the single most important factor in the development of changes of the intervertebral discs.

I would like to use the word degeneration in relation to the intervertebral disc, however, with a more restricted meaning. This term as it was suggested by Friberg and Hirsch[66,67], could be used to imply premature ageing of the disc characterised by substantial disorganisation of the annular structure. This may not be seen in aged and otherwise normal discs.

If this definition is followed, degeneration would become a less universal phenomenon and may occur as early as during the second decade of life and be significantly associated with back pain.

The work that I present here is based on the hypothesis that discrete tears of the outer annulus fibrosus which may develop independently and occur following mechanical stress could influence the structural changes of the other components of the intervertebral disc associated with degeneration.

This hypothesis may be relevant for the identification of possible pain mechanisms from the intervertebral disc.

## 2.3 CHANGES IN THE DEGENERATING DISC AND THE FORMATION OF ANNULUS TEARS

Historically, if the intervertebral disc is considered in its separate components the annulus fibrosus seems to have been neglected in favour of the nucleus pulposus by many investigators engaged in the study of the pathology of the disc.

A possible explanation may be that the macroscopic changes which occur with time in the nucleus pulposus are easier to identify and appreciate.

The dessication and fraying of the nucleus pulposus or in biochemical terms, its dehydration and relative loss of proteoglycans have been considered by most authors as preceding structural changes in other parts of the intervertebral disc.

An example of the historically accepted pathological sequence undergone by the intervertebral disc can be found in the classic contributions on disc prolapse.

This entity first described by Luschka in 1858 and subsequently popularised by Mixter and Barr in 1934[137] implied a substantial failure in the lamellar arrangement of the annulus fibrosus. The disorganization and tearing of the annulus however, rather than being considered a primary feature related to possible mechanical damage were explained by Yunghanns[171] amongst others on the basis of outward extension of clefts from the degenerating nucleus pulposus and the inner annulus.

Schmorl[171] classified the typical pathological features of disc degeneration into two separate categories, spondylosis deformans and intervertebral chondrosis.

In spondylosis deformans it is the primary discrete failure of the outer annulus fibrosus which leads to formation of osteophytes. These, according to Schmorl, are an expression of endochondral ossification at the site of the detachment of the long perispinal ligaments from the vertebral body. The lifting of the long ligaments was considered a consequence of hypermobility induced by the peripheral annulus tears in association with nuclear displacement towards the site of the lesion (fig. 1).

This process required a hydrated nucleus pulposus and was therefore classified as a separate entity from intervertebral chondrosis which implies primary fraying of the nucleus pulposus with end plate sclerosis and subsequent narrowing of the intervertebral discs.

The concept of central degeneration of the intervertebral disc was advocated again by Hirsch and Schaiowitz in 1953[92].

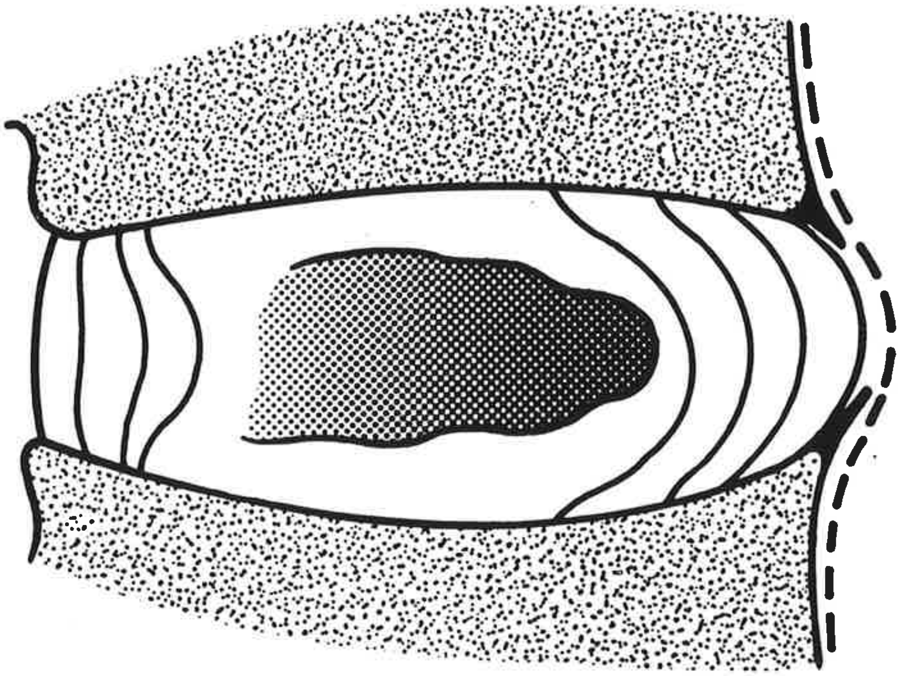
These authors stated that tears of the annulus would always follow primary degradation of the nucleus pulposus and proposed as a "new pathological basis for low back pain" the "appearance of a highly vascular granulation tissue within the intervertebral disc". Such tissue because of its location was considered an expression of attempted healing of the outer portion of what the authors referred to as "annular ruptures".

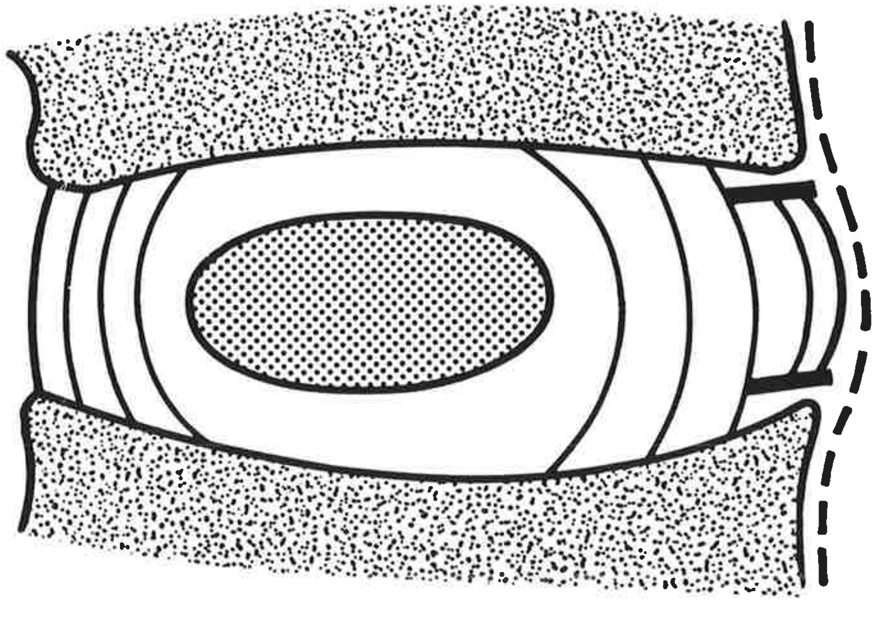
igure 1A

Schematic representation of osteophyte formation according to Schmorl and Junghanns. The primary lesion in spondylosis deformans is represented by discrete failure of the annular attachment into the vertebral bony rim.

'igure 1B

Peripheral annular failure leads to progressive nuclear displacement, lifting of the long perispinal ligament from the vertebral body and secondary endochondral ossification (osteophyte formation). (Modified from: Schmorl and Junghanns: The human spine in health and disease. Grune and Stratton New York 1971)





The concept of "radiating annular rupture" implies conventionally a progressive and outward extension of an inner annular lesion often as illustrated by Schmorl and Yunghanns[171] associated with clefts of the nucleus pulposus.

A hypothesis put forward to explain the primary central degradation of the intervertebral disc is based on diffusion studies[194]. Due to its avascular nature and nutritional dependance on diffusion from the outer annulus and the end plates, areas of disc tissue exist especially in the inner and middle annulus which are exposed to possible nutrient deficiencies. Recent studies have indicated that in the adult the mid annulus would contain the most active cell population for proteoglycan synthesis[15]. The nutritional risk of the central zone of the disc would be enhanced by decreased permeability of the end plates. Structural changes of the end plates which may affect their permeability are commonly seen at autopsy and have been explained by finite model analysis on the basis of compressional overload[176].

Whilst most authors agree on the central origin of radiating annular clefts in close association to the fraying of the nucleus pulposus, recent pathological observations[152] have confirmed the observation by Schmorl of the presence of discrete peripheral tears in young and otherwise normal discs. These tears were seen more frequently in the region of insertion of the outer annular lamellae into the vertebral body rim. Vernon-Roberts and Pirie[199] and Hilton and Ball[90,91] suggested that because of their microscopic characteristics these were likely to be the result of direct trauma to the disc rather than being an expression of degeneration. The fact that lumbar rim lesions were equally common above or below the age of twenty years suggested that they would preceed the secondary degenerative changes seen in the inner annulus and the nucleus pulposus.

The characteristics of the pathological changes seen in the annulus fibrosus of human lumbar intervertebral discs are discussed in more detail in chapter 3.

## 2.3 ANNULUS RUPTURES: POSSIBLE CAUSES

The understanding of the likely causes of failure of the annulus fibrosus is of great relevance for the analysis of the successive steps of disc degeneration.

If the close association between discrete peripheral annular tears and osteophytes proposed by Schmorl is accepted, repetitive trauma to the intervertebral disc would represent the single most important factor in the aetiopathogenesis of outer annular defects as indicated by osteophyte formation.

Despite this being still a very controversial issue a series of radiological studies[18,97,118,170] have indicated that spondylotic changes, that is to say osteophyte formation and disc narrowing, occur at a significantly younger age amongst heavy laborers.

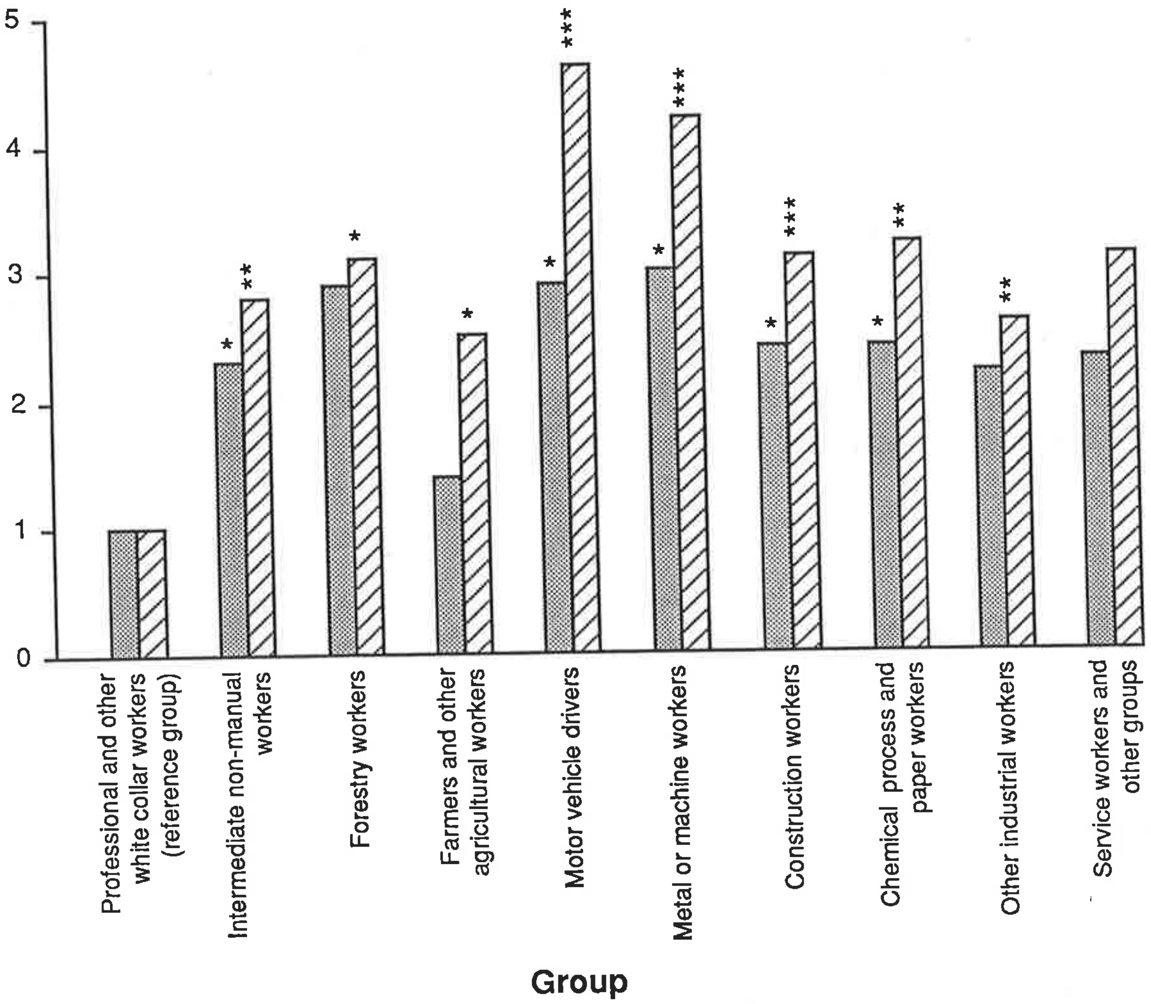
In a comparative radiological review of 216 concrete reinforcement workers and 201 house painters[165] a statistically significant difference was found in the presence of degenerative changes of the lumbar spine in relation to heavy physical work. Disc space narrowing, in fact, occurred at about 10 years and spondylophytes at about 5 years younger age in the concrete reinforcement workers group. In a recent epidemiological investigation[83] a strong correlation was found between "lumbar intervertebral disc disease" (herniated disc or typical sciatica) and occupational status with motor vehicle drivers and heavy laborers at highest risk (four times that of professional and other white collar workers) (fig 2).

An investigation of 142 young top Swedish atheletes[181] concluded that radiological abnormalities consistent with degenerative disc disease could be found in almost 50 percent of subjects. There was a significant correlation between intervertebral disc narrowing, change of configuration of the vertebral bodies and back pain.

Figure 2

Statistical correlation between "lumbar intervertebral disc disease" and occupation. [Modified from: Heliovaara, M: Epidemiology of sciatica and herniated lumbar intervertebral disc. Publications of The Social Insurance Institution Helsinki 1988]

**Relative risk of herniated lumbar intervertabral disc and that of herniated disc or sciatica in men by occupation**




Difference from the reference group: *$p<0.05$; **$p<0.01$; ***$p<0.0001$.

▩ Herniated lumbar invertebral disc
▨ Herniated disc

After examination of over 300 lumbar spines at autopsy Vernon-Roberts and Pirie[199] suggested that because of the occasional vascular ingrowth around the margins of the cleft, radiating lesions into the annulus fibrosus could be due to a tearing process rather than tissue breakdown from simple degeneration.

In 1984 Hilton and Ball[90] reported on the frequency, distribution and histological characteristics of vertebral rim lesions from 117 post-mortem spines. Most rim lesions showed evidence of attempted repair with fibrovascular and fibro-cartilaginous tissue in the region of the rim. Based on the histological appearance the authors concluded that a traumatic aetiology was likely and that the lesions could be relevant in the context of low back pain.

While repetitive trauma may explain the collagen disorganisation and tearing at the periphery of the intervertebral disc, the formation of radiating tears from the inner annulus and extending towards the periphery, as illustrated by Schmorl and Junghanns[171] is thought to be closely associated with the fraying of the nucleus pulposus. Friberg and Hirsch[67] in their study on the morphological characteristics of disc degeneration, suggested that annular ruptures started in the inner annulus and were directed either sagittally or laterally outwards toward the periphery. Galante[69] suggested that the formation of radiating tears in the annulus resulted from a combination of internal disc pressure and early failure of the inner annular fibres due to degenerative changes.

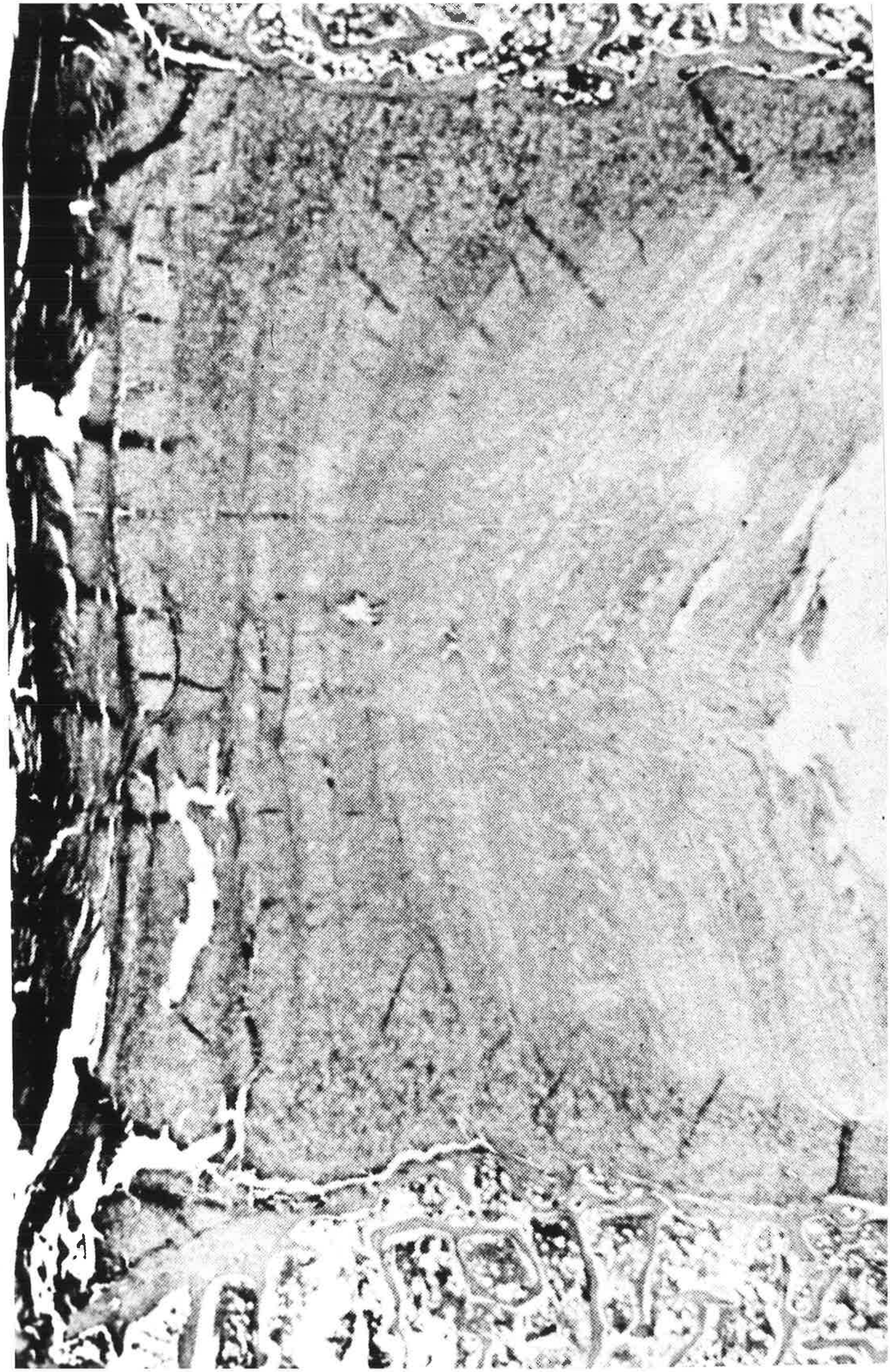
Most clinicians that recognize annular tears as an entity refer to Farfan's work[38,59,60,61,62,63,64] in which torsional stress was linked with the production of discrete tears of the peripheral annulus fibrosus (fig 3).

Based on a series of experiments in which 90 intervertebral joints were subjected to torsional loading, Farfan observed that degenerated discs had reduced resistence to torsion than normal discs.

Whilst experimental torsional failure did not produce any substantial damage to the vertebral bodies, annular defects induced experimentally by torsion produced changes similar to those seen in disc degeneration. The morphological features following torsional stress of the disc according to Farfan were the observation of extensive tearing in the peripheral layers of the annulus. In all specimens these lesions were limited to the outer lamellae with the inner annulus remaining intact. Farfan speculated that with successive injuries, deeper and deeper annular layers became compromised and eventually a communication between the nucleus and the outer annulus would form as seen in disc degeneration by radial tears.

Figure 3

Human lumbar intervertebral disc subjected to torsional loading. Clefts are seen parallel and adjacent to both end plates. (From : Farfan, H: Mechanical disorders of the low back. Lea and Febiger, 1973; reproduced with permission of the authors)



Because of the limited rotational motion possible in physiological circumstances in the lumbar spine the relevance of torsion in the pathogenesis of disc lesion was questioned by Adams and Hutton[2,4]. In a series of experiments using cadaveric lumbar intervertebral joints it was found that torsion was resisted primarily by the apophyseal joint in compression. Because of the protection offered by the facet, the intervertebral disc, according to Adams and Hutton, was subjected to relatively small stress in the physiological range of torsion. It was concluded that because greater angles of rotation were required to damage the intervertebral disc, torsion seemed unimportant in the aetiology of disc degeneration and prolapse. The same authors in a different series of experiments[3] were able to induce posterior disc prolapse by applying compressional load on the intervertebral disc using flexion only.

Their experiments confirmed the observation by Nachemson[143] of markedly increased tensile strain on the annulus fibrosus of discs tilted more than $5^o$ and subjected to vertical load, due to the significant increase in internal disc pressure.

## 2.5 ANNULUS FAILURE AND IMAGING

Following the introduction of discography by Lindblom (1948) Cloward in 1952[33] drew attention to its diagnostic value in identifying "ruptured lumbar intervertebral discs". He pointed out that discography would demonstrate intervertebral discs with substantial structural damage as outlined by the contrast medium. These pathological changes due to lack of posterior displacement into the spinal canal would not be detected by myelography.

Crock in 1970[44] introduced the term "internal disc disruption" to identify a clinical syndrome with prominent disabling back pain. The term implied marked disorganization of the intervertebral disc as seen at discography and which, according to the author, usually followed severe trauma inflicted on the disc, for example by sudden unprotected weight lifting or following high speed accidents. Discography in these cases may demonstrate radial tears in the substance of the annulus fibrosus short of complete extension to the outer layers. The macroscopic changes seen in the "disc tissue" according to Crock were indistinguishable from disc tissue in the early phases of degeneration.

One of the limitations that might exist in correlating the appearance of the intervertebral disc at discography with its possible pathological changes is that, if a discrete tear of the peripheral annulus were present especially anteriorly or anterolaterally, it would not necessarily communicate with the inner annulus and the nucleus pulposus and therefore would not be shown by the contrast medium (fig. 4 and 5). Furthermore, discography would not provide information on the reactive changes occuring in the outer lamellae which might play, as suggested by Hirsch and Schajowicz[92], a significant role in the production of symptoms from the intervertebral disc proper.

Whilst theoretically MRI could supply information on pathological tissue changes within the intervertebral disc, at this stage the presence of discrete tears of the annulus not associated with dehydration of the nucleus pulposus would go undetected in vivo because of the very poor signal obtained from the annulus fibrosus. In a recent report by Yu et al[206], however, tears of the annulus fibrosus were identified in a comparative study in cadavers using MRI and cryomicrotome sections of the specimens. Circumferential tears on $T_2$ weighted images appeared as fluid filled spaces giving rise to bright signal which replaced the normal low intensity signal of the annulus fibrosus. The authors reported on the visualisation of radiating ruptures and of rim lesions with MRI although they stated that these were less effectively seen at MRI as compared to the anatomic sections.

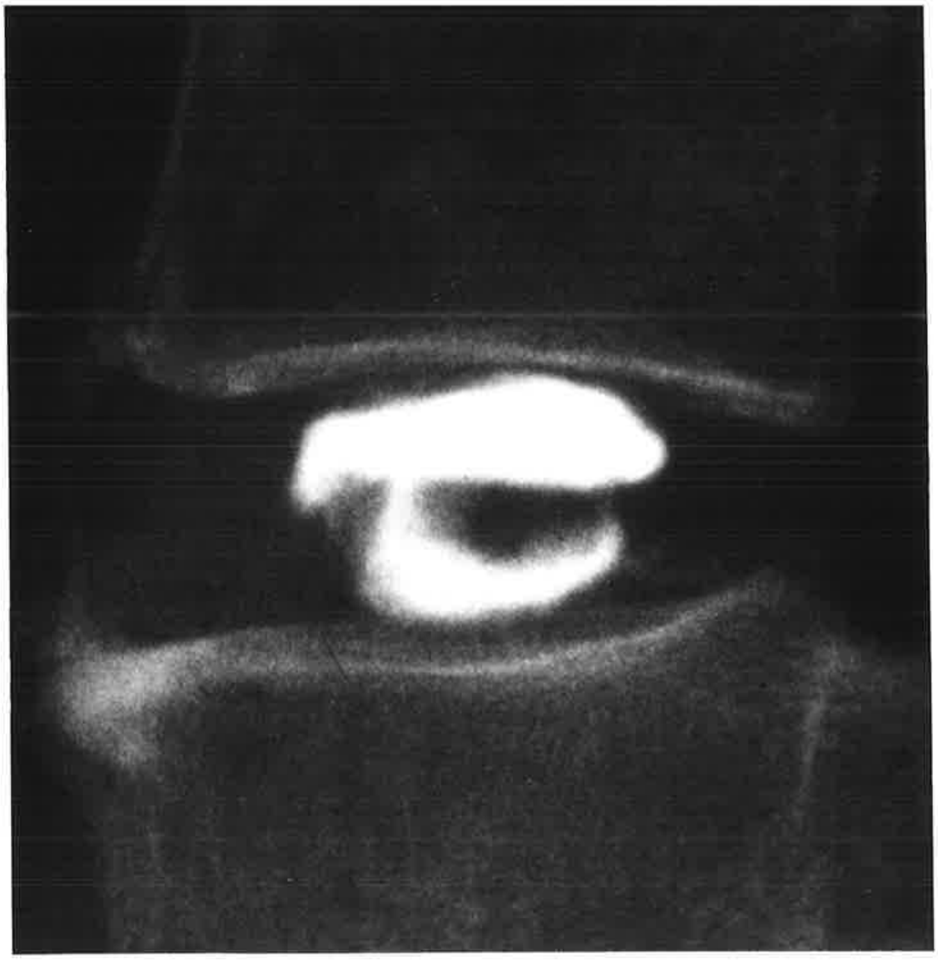
The relationship between annular rupture and investigative techniques such as discography and MRI is analysed in chapter 4.

igure 4

Example of normal discogram in the presence of marked osteophyte formation (from the anterior aspect of the vertebral body) and end plate sclerosis. This appearance is consistent with the presence of discrete failure of the peripheral annulus.

igure 5

Discogram demonstrating extravasation of contrast towards the anterior longitudinal ligament in association with osteophyte formation. Contrast is seen in the basivertebral venous plexus.



## 2.6 DEGENERATION OF THE DISC AND PAIN

The relationship between pathological changes of the intervertebral disc as seen in cadaver material, animal experiments or surgical specimens and back pain remains highly controversial.

Kirkaldy-Willis (1983)[110] tried to correlate degenerative changes of the intervertebral joint complex with the clinical presentation of patients suffering from back complaints. He proposed to divide the process of degeneration into three phases: dysfunction, instability and stabilisation. In phase one repetitive injury would result in interruption of normal function with the early changes in the disc represented by circumferential and radial annulus tears. In phase two abnormal increased movements would be noticed. This phase would be characterised by laxity of the annulus fibrosus. The progression of degenerative changes would result in the unstable segment regaining its stability (phase three) through tissue fibrosis and osteophyte formation.

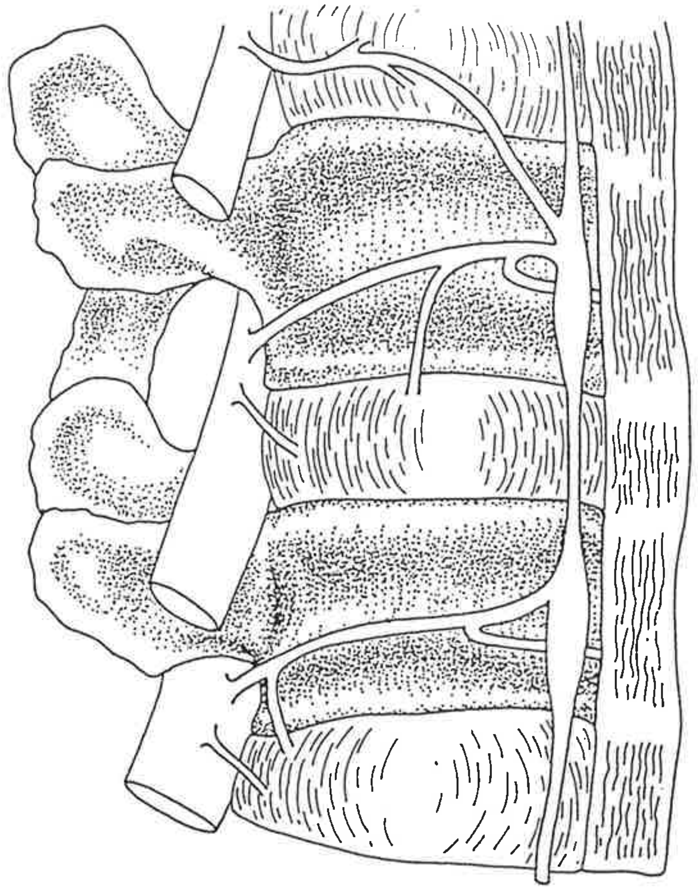


Figure 6

Drawing demonstrating the innervation of the intervertebral disc in the human lumbar spine. The sympathetic trunk is seen with branches to the intervertebral disc from the grey rami. Innervation to the disc is seen from the ventral ramus and to the anterior longitudinal ligament from the grey ramus communicans.

One of the reasons for the controversy which surrounds the concept of discogenic pain is the paucity of anatomical studies which have demonstrated innervation of the intervertebral discs. Whilst most authors agree on the existence of a well developed nerve supply along the longitudinal ligaments anteriorly and posteriorly, few studies to date have shown nerve endings within the intervertebral discs and these have always been confined to the very outer part of the annulus fibrosus. Advocators of the concept of pain arising from the intervertebral disc proper have used the well documented phenomenon of pain provocation by discography as one of the main supporting factors. Bogduk et al[19,20], based on original anatomical studies in human lumbar discs confirmed the observation made by Stillwell[180] in monkeys, of innervation of the posterior annulus by the sinu-vertebral nerves. In addition, a nerve supply by the recurrent branches of the grey rami comunicantes was established for the anterior longitudinal ligament (fig. 6). Yoshisawa et al[205], based on the histological analysis of human discs removed at operation described rich nerve supply within the outer half of the annulus fibrosus. A variety of nerve endings were described including free terminals with club like or bulbous expansion or less commonly terminals with glomerular type formations occasionally demarcated by a capsule like condensation of adjacent tissues.

Although little is known of the established nerve supply to the outer annulus fibrosus it seems reasonable to postulate that lesions of the peripheral annulus may lead to production of pain. This would be most likely to occur in the presence of high levels of intradiscal pressure as tensile strain on the peripheral annulus would be greater. In addition, outer annular damage would induce repair mechanisms with secondary granulation tissue formation and possible ingrowth of nerve fibres.

# 3. THE PATHOLOGY OF ANNULUS TEARS

## 3.1 SUMMARY

An attempt is made in this chapter to describe and classify the morphology of human annulus tears and their characteristics. This is based on the analysis of the relevant literature and on the personal review of 260 human lumbar discs collected at The London Hospital by Vernon-Roberts and in Adelaide during the last three years.

## 3.2 HISTORY

The first detailed description of the morphology of the "internal de-arrangement of the intervertebral disc structure" is from Schmorl and Yunghanns[171]. Closely related to the dessication of the nucleus pulposus characteristic of intervertebral chondrosis, these authors described the appearance of clefts extending from the nucleus pulposus into the annular layers with a transverse or oblique course. These clefts were considered to be associated with the development of disc prolapse. The formation of clefts within the annulus fibrosus could not only be due to radiation from the nucleus pulposus but according to Schmorl and Yunghanns could occur independently with clefts arranged mostly in a concentric fashion, both in the anterior and posterior regions.

Other discrete lesions of the annulus fibrosus were described in relation to osteophyte formation.

These lesions consisted of a separation of the annulus fibrosus from the vertebral body rim along a plane parallel and adjacent to the endplate and seen specifically at the very periphery of the disc. It is not clear, however, based on Schmorl and Yunghanns work to understand the relationship existing between rim lesions and the other features of annular damage.

In 1945 Coventry, Ghormley and Kernohan[39,40,41] described as a typical feature of ageing of the annulus fibrosus during the third decade the presence of concentric fissuring of the annular lamellae which would precede the more substantial changes seen in the nucleus pulposus. Friberg and Hirsch(67) in 1950 drew attention to the link between annular ruptures and disc degeneration proper as a separate entity from the normal physiological ageing of the intervertebral discs. Annular ruptures, according to them, were more commonly seen in the posterior annulus in the lower two lumbar discs whilst probably more frequently seen anteriorly and laterally in the upper lumbar segments. These ruptures radiated from the centre in the lower discs posteriorly and postero-laterally and were surrounded by smaller annular ruptures.

Three years later Hirsch and Schajowicz[92] described discrete changes of the annulus fibrosus which were seen as early as during the second decade of life. These changes were classified as mucous degeneration consisting of concentric cracks of varying size, more frequently seen in the posterior annulus and apparently not communicating with the nucleus pulposus. The authors therefore, differentiated these lesions from the radial ruptures which, as stated by Friberg and Hirsch, were described as extending in an outward direction from the nucleus more commonly in the posterior and postero-lateral annulus of the two lower lumbar discs. Hirsch and Schajowicz considered radiating ruptures as being a consequence of mechanical pressure. They also stated that if such ruptures extended to the outer annulus fibrosus a "vascular-connective tissue" was seen at histology in and around the radial rupture.

Morgan and King[141] in 1957 drew attention to the association of annulus tears, radiographic instability and low back pain. They observed the presence of "incomplete radial posterior tears" in the lower lumbar segments and of "anterior concentric fissures or slits" in the upper lumbar spine which they considered as the likely cause of lumbar instability.

Twenty years later Vernon-Roberts and Pirie[199] reviewed the results of over 300 lumbar spine autopsy observations. They supported the observation of Schmorl and Yunghanns where radiating lesions into the annulus fibrosus were considered as an extension of clefts forming originally in the nucleus pulposus. Because of the occasional vascular ingrowth around the margins of the clefts they suggested that these could extend into the annulus by a tearing process rather than tissue breakdown due to simple degeneration. Vernon-Roberts and Pirie confirmed the presence of circumferential clefts within layers of annular tissue and of tears directed at right angle to the direction of the collagen bundles seen near the attachments of the annulus to the vertebral body (rim lesions). The presence of cystic degeneration within the lamellae of the annulus fibrosus was described by Vernon-Roberts[198] as a separate entity amongst the microscopic features observed.

Hilton and Ball[90] in 1984 reported on the frequency, distribution and histological characteristics of vertebral rim lesion from 117 post mortem spines. Histological observations however were obtained systematically only from central sagittal slabs of the D11-12 and L4-5 intervertebral discs. Rim lesions were more frequently seen anteriorly and could be associated with major tears of the annulus fibrosus. Most rim lesions showed evidence of attempted repair with fibro-vascular and fibro-cartilaginous tissue in the region of the rim. The authors concluded that based on the histological appearance a traumatic aetiology was likely and that the lesion could be relevant in the context of low back pain.

Kirkaldy-Willis[110] postulated that radial tears may result from the coalescence of small circumferential tears which could occur initially in the outer annulus fibrosis. His hypothesis linked repetitive trauma and degeneration in the sequence of events which lead to annular damage.

## 3.3 MORPHOLOGICAL CLASSIFICATIONS OF ANNULUS TEARS

The classification which follows is based on my observations of 125 human adult lumbar discs from the material accumulated by Vernon-Roberts at the Bone and Joint Research Unit of the London Hospital and of 135 lumbar discs from 27 spines removed at autopsy in Adelaide between 1987 and 1989.

All subjects of the Adelaide series were aged between 17 and 50 years (average age: 31.5).

Annulus defects were classified into three types:

1) peripheral (rim lesions)

2) circumferential (concentric)

3) radial

Figure 7A

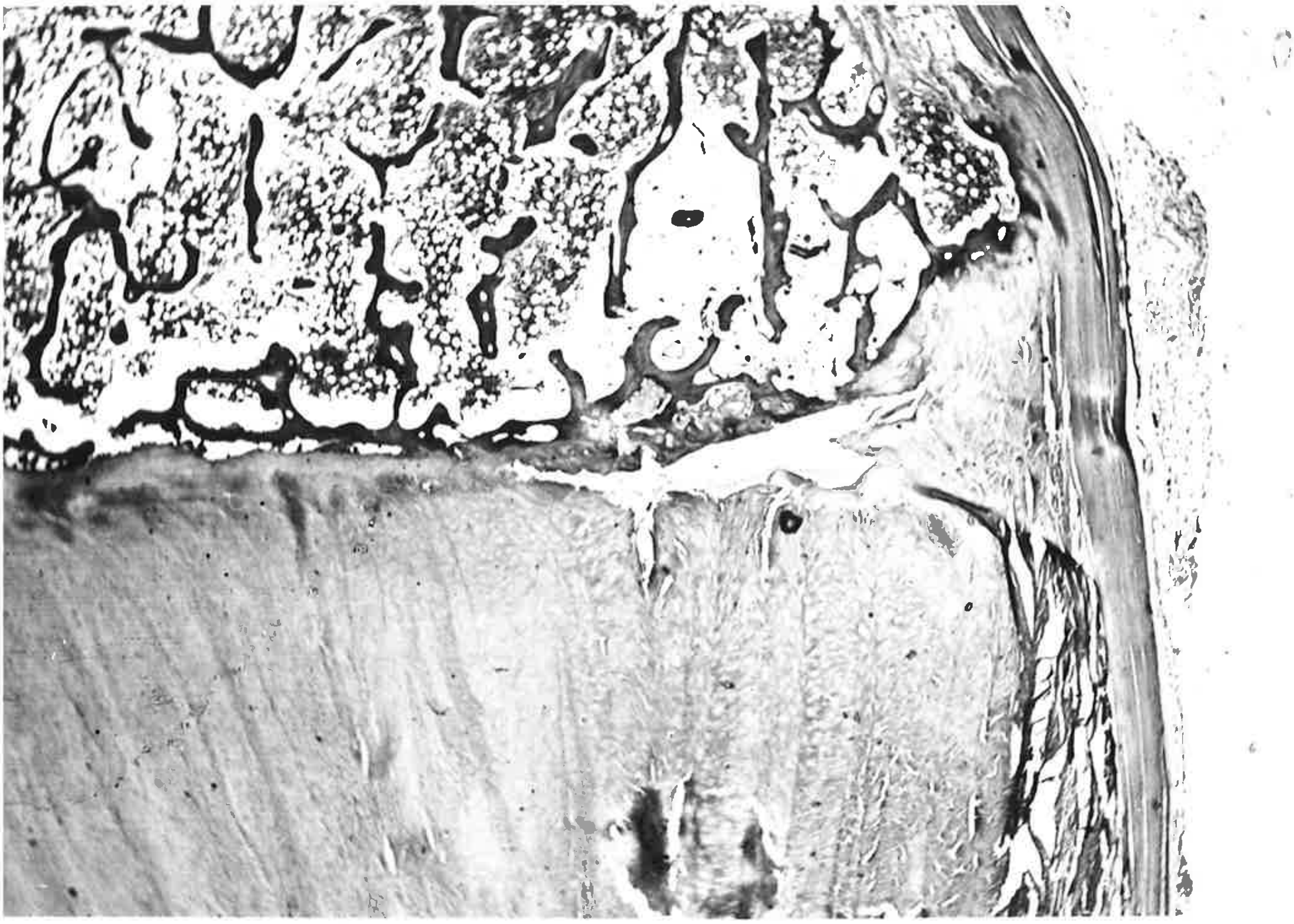
(Top) L2-3 intervertebral disc of a 39 year old woman. A rim lesion is visible adjacent to the upper anterior vertebral body rim of the lower vertebra. Initial osteophyte formation is seen adjacent to the rim lesion. The bone marrow is replaced by granulation tissue.

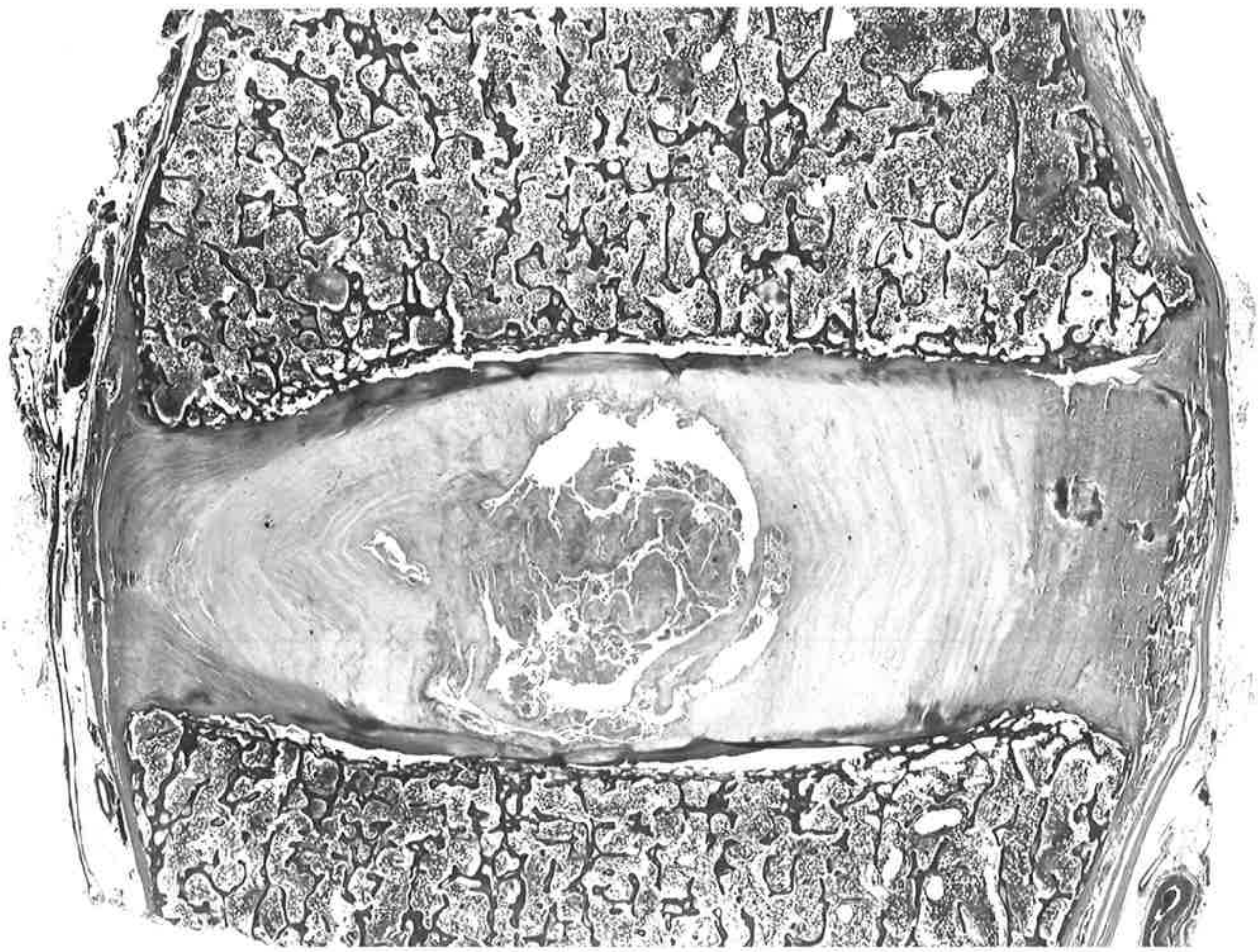
Figure 7B

(Bottom) High power micrograph of the rim lesion seen in figure 7A.

### 3.3.1 Rim Lesions

These are defined as discrete tears of the outer layers of the annulus fibrosus, parallel and adjacent to one or both endplates (figs. 7 and 8). Although significantly more frequent in the anterior annulus, they can seldom be seen posteriorly as well. Rim lesions appear to be frequently accompanied by ingrowth of vascular granulation tissue which may extend well into the mid layer of the annulus. Rim lesions generally give origin to circumferential tears which are seen as separation of the individual lamellae of the annulus. The adjacent bony rim may show replacement of the marrow by granulation tissue. Sclerosis may be observed of the subjacent bony trabeculae. Osteophytes are generally seen in association with rim lesions (fig 9). In discs with advanced degenerative changes radiating ruptures may be seen running into the rim lesions (fig. 10). As postulated by Vernon-Roberts[198] and Hilton et al[90,91], the morphology of these tears suggests a traumatic origin.



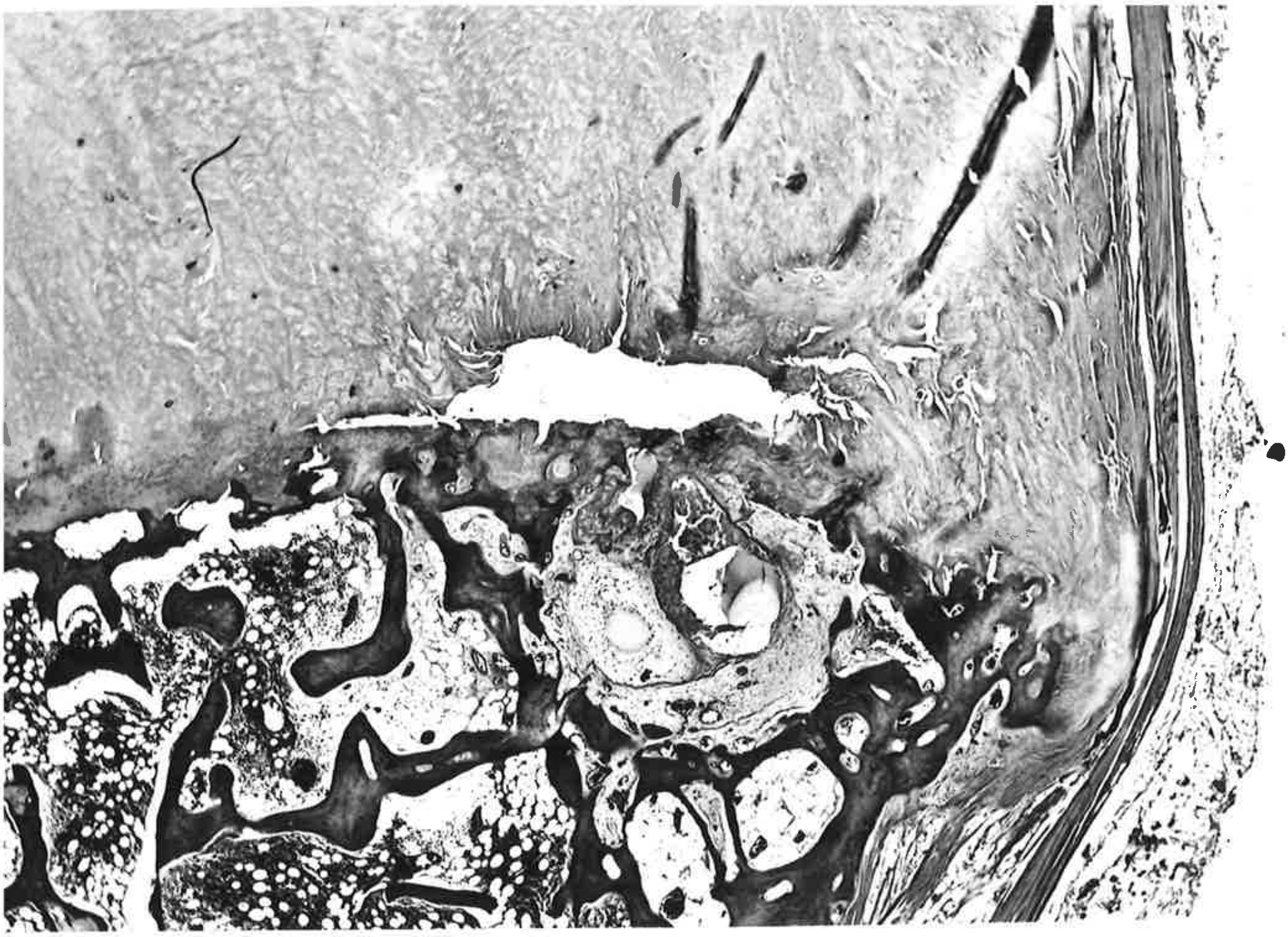


'igure 8A

(Top) Lower power micrograph of L2-3 intervertebral disc of a man aged 36. Rim lesions are seen in both the upper and lower end plates.

'igure 8B

(Bottom) Replacement of bone marrow with granulation tissue adjacent to the annular lesion. Initial osteophyte formation is seen at the infero-anterior corner of the vertebral body, just proximal to the limit of the bony rim.

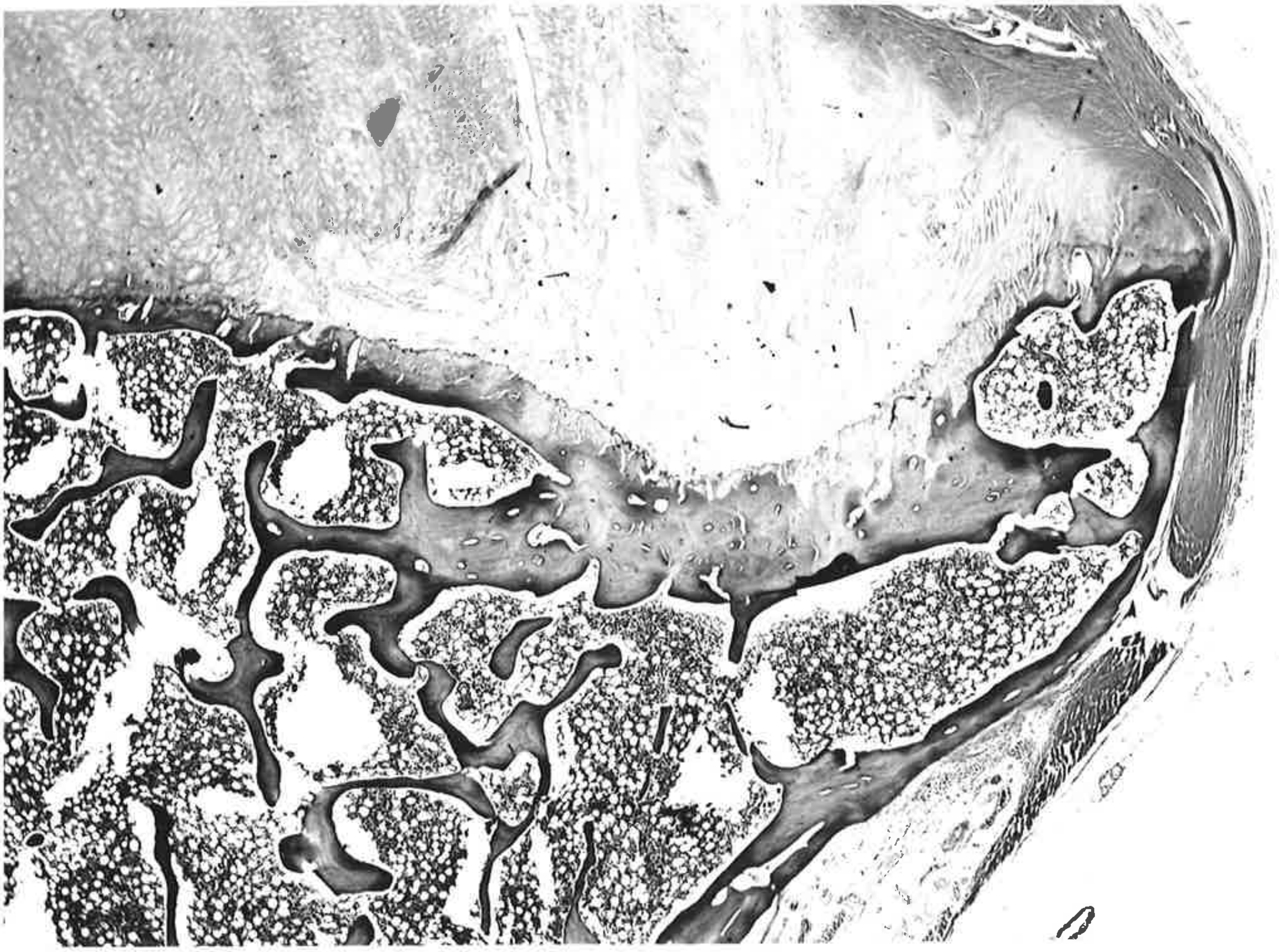


igure 9A

(Top) Low power micrograph of L4-5 intervertebral disc in a man aged 46. Advanced osteophyte formation of the lower end plate of the upper vertebral body in relation to disorganisation of the bony rim. This appearance is consistent with an old rim lesion.

Figure 9B

(Bottom) High power micrograph of osteophytes seen in figure 9A.



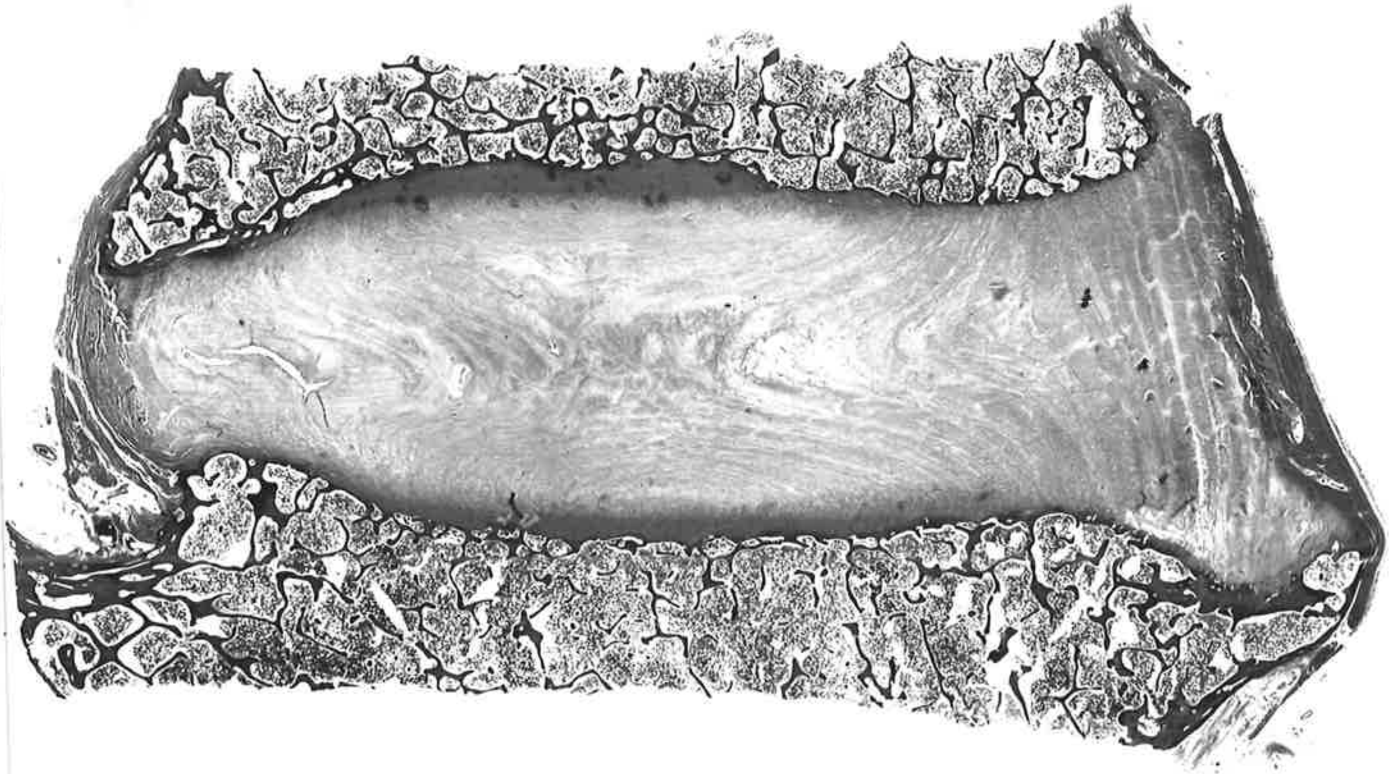
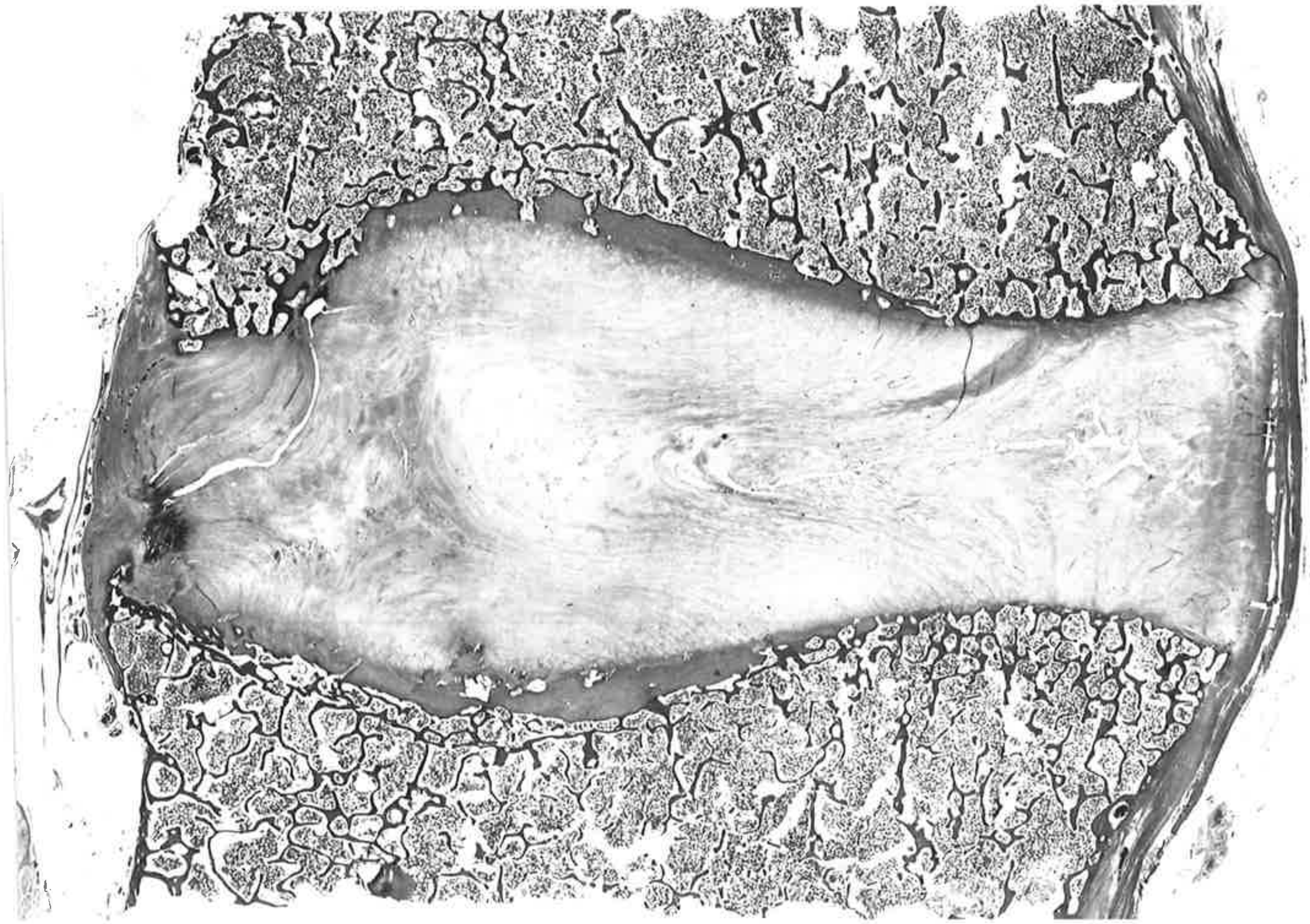


Figure 10A

[Top] Low power micrograph of L4-5 intervertebral disc in a woman aged 50. An oblique cleft is seen in the posterior annulus fibrosus extending from the upper end plate of the lower vertebra to the mid substance of the peripheral layers of the posterior annulus. The outer portion of the cleft is associated with marked vascularisation of the annulus.

Figure 10B

[Bottom] High power micrograph of the lesion seen in figure 10A. Note the similarities with the highly vascular granulation tissue seen in the sheep model (page 74).



### 3.3.2 Circumferential (Concentric) Tears

Circumferential cracks are frequently seen in the annulus, both anteriorly and posteriorly. Their frequency seems to be higher in the posterior annulus at the lower two levels (L4-5 and L5-S1) and anteriorly in the upper three lumbar segments. These tears, especially when in the outer layers, may be associated with vascular ingrowth which in some cases leads to cyst formation (figs. 11 and 12). It is of interest to note that cystic degeneration was seen in our animal model following discrete peripheral tears of the anterior annulus (Page 75). Circumferential tears, as already mentioned, are commonly seen in association with a rim lesion.

Figure 11

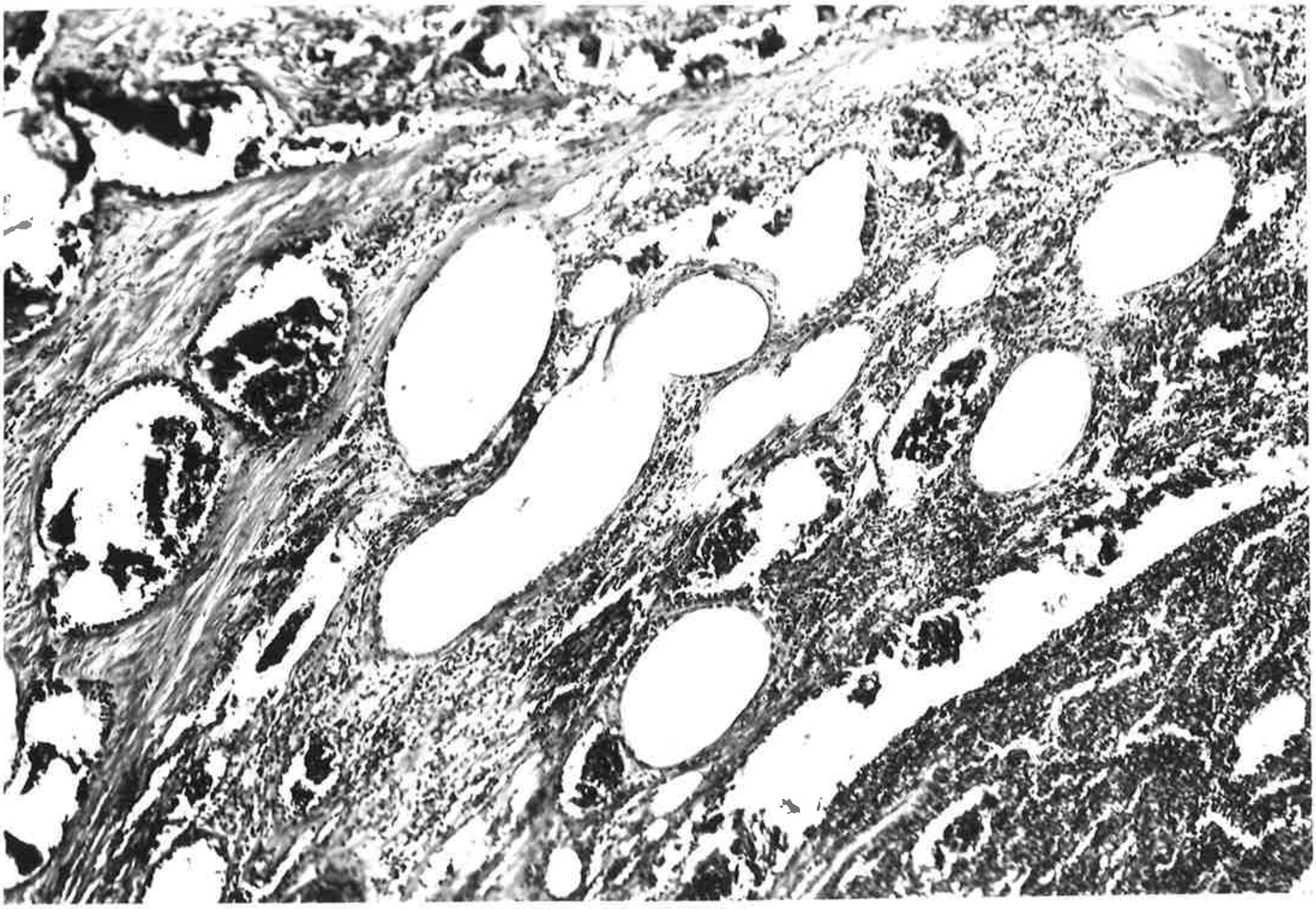
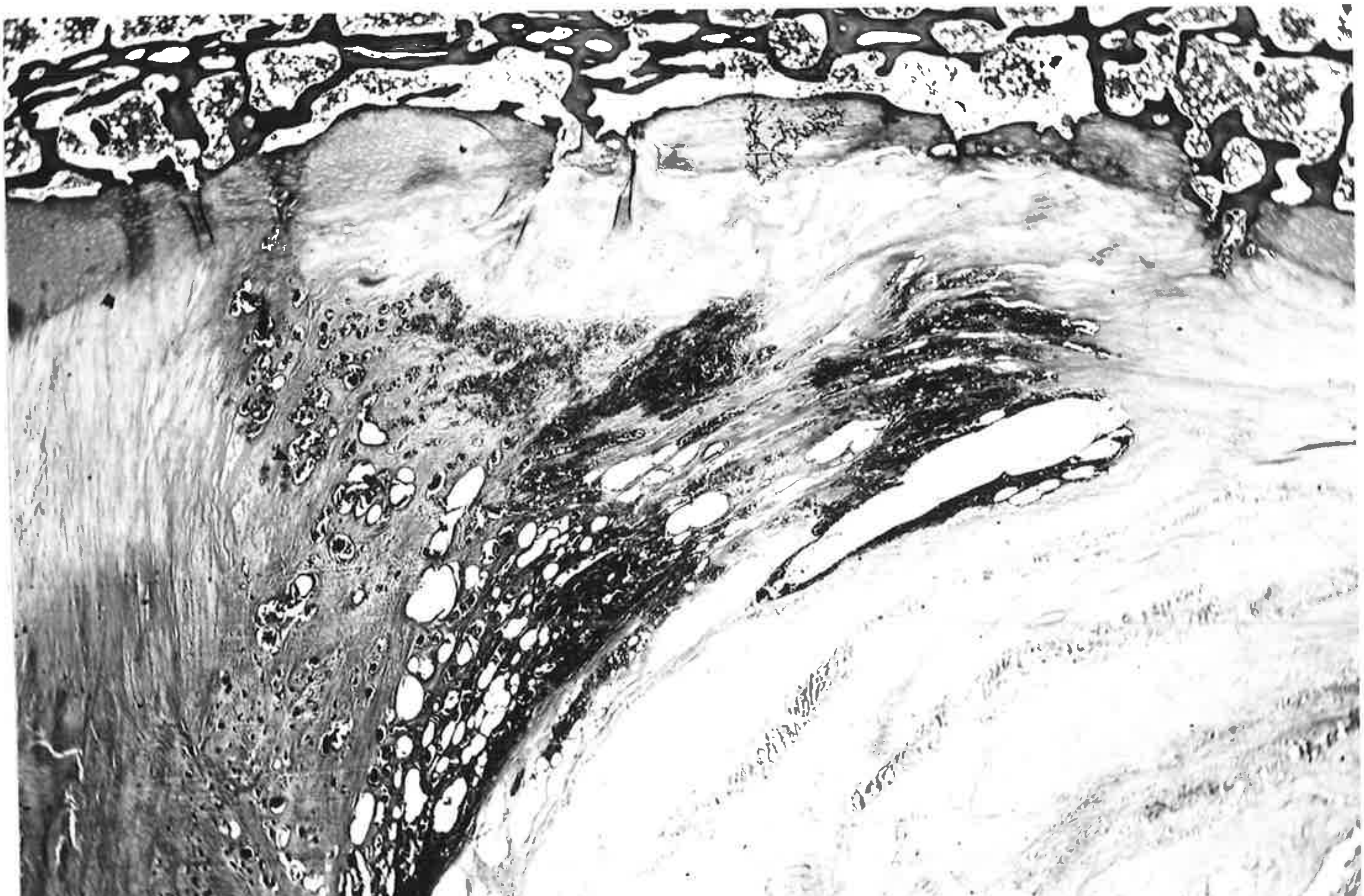
Low power micrograph of L3-4 intervertebral disc of a man aged 60. An area of cystic degeneration associated with highly vascular granulation tissue is seen separating the central portion of the disc from the mid layers of the posterior annulus fibrosus.

igure 12A

(Top) High power micrograph of the same lesion seen in figure 11.

Figure 12B

(Bottom) Higher magnification of the lesion seen in figure 11. The characteristic cystic formation of highly vascular granulation tissue seen within annular tissue is well documented in this case.

### 3.3.3 Radial Tears (Radiating Ruptures)

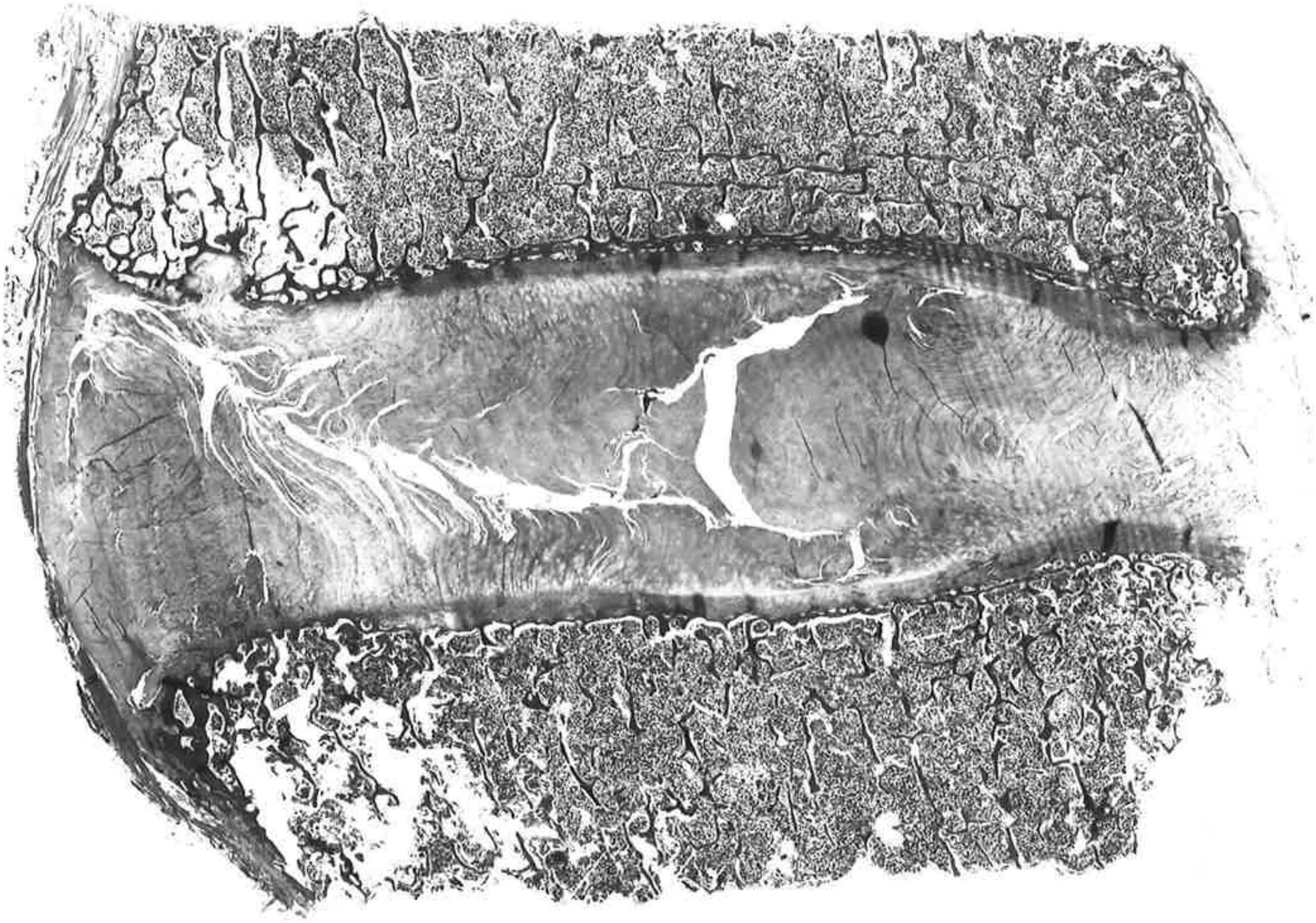
Radial tears as indicated by Friberg and Hirsch are a typical expression of advanced degeneration. They appear as clefts extending from the nucleus pulposus to the outer lamellae of the annulus on a plane parallel or oblique to the endplates (figs 13, 14, 15 and 16). In discs where clear demarcation still exists between annulus and nucleus radiating ruptures are associated with nuclear displacement. This is more commonly seen posteriorly in the lower two segments but is not infrequently seen in the anterior annulus especially in the upper lumbar segments. The displacement of the nucleus pulposus is accompanied by outward orientation of the margins of the annulus lamellae where they are separated by the cleft. In addition, folding of the contralateral inner annulus lamellae is seen towards the site of displacement. This is a constant feature and is seen well in our animal model. Whilst radiating ruptures, especially posteriorly, may be seen running in the mid substance of the annulus, they are more frequently seen running into rim lesions. As described by Hirsch and Schajowicz the outer portion of the tear is generally vascularised and granulation tissue at various stages of maturity is seen in relation to the peripheral part of the lesion. Although the presence of vascularised granulation tissue indicates an attempt at healing, this is, if present, generally confined to the very peripheral annulus layers only. This is in agreement with the results obtained from the sheep model.

Figure 13A

(Top) Low power micrograph of L4-5 intervertebral disc in a man aged 50. A radiating cleft extending from the central zone of the intervertebral disc to the outer layers of the anterior annulus fibrosus is seen. The radiating tear is associated with multiple concentric clefts and extends into a rim lesion adjacent to the bony rim of the upper vertebra. There is marrow replacement with granulation tissue in relation to the rim lesion.

Figure 13B

(Bottom) Same disc seen in figure 13A (more central slab). The characteristic infolding of the inner layers of the posterior annulus is seen in association with displacement of tissue from the central zone of the disc towards the anterior annulus. Note the similarities with the sheep model (page 80).

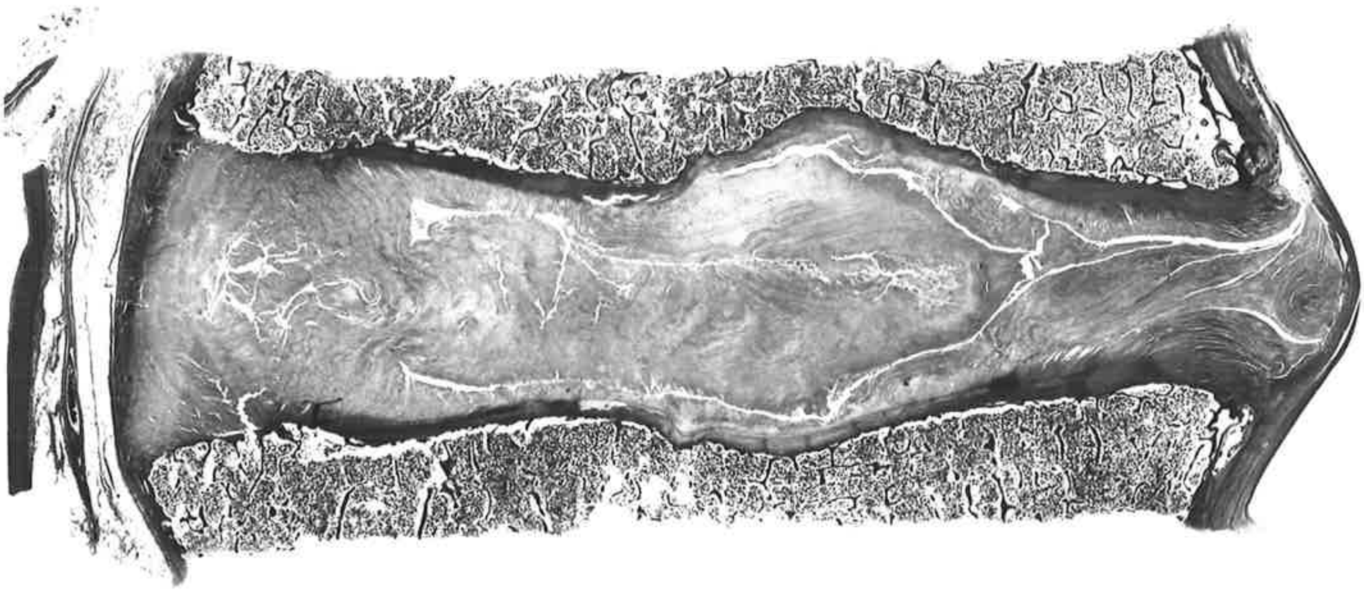


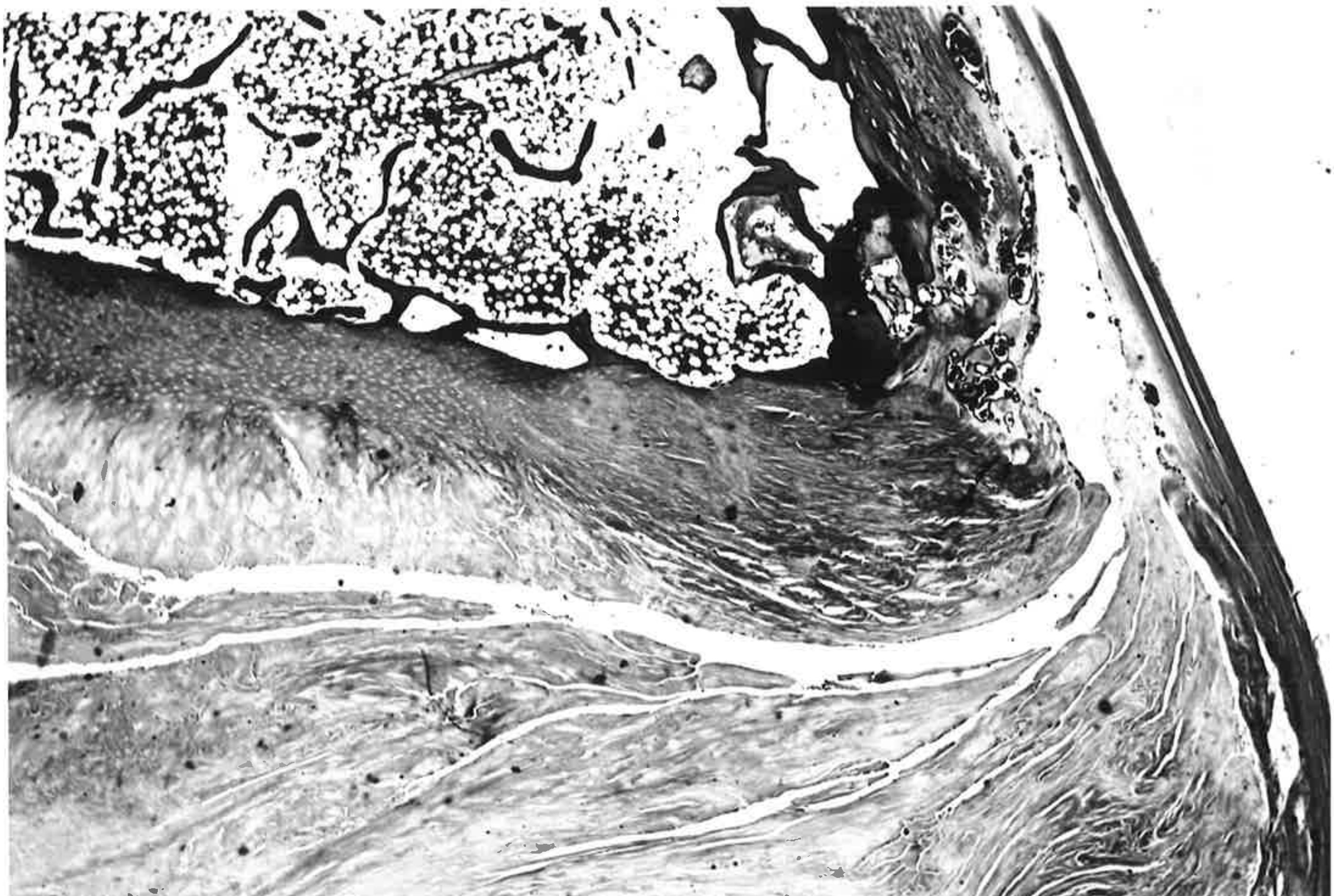
gure 14A

(Top) Low power micrograph of L5-S1 intervertebral disc of a woman aged 66. Multiple clefts are seen extending from the central zone of the disc towards the periphery of the posterior annulus. There is displacement of disc tissue posteriorly which is associated with infolding of the inner layers of the anterior annulus. A cleft is seen parallel to the end plate of the lower vertebra extending to the outer annulus fibrosus underneath the posterior longitudinal ligament.

igure 14B

(Bottom) High power magnification of cleft seen in figure 14A.



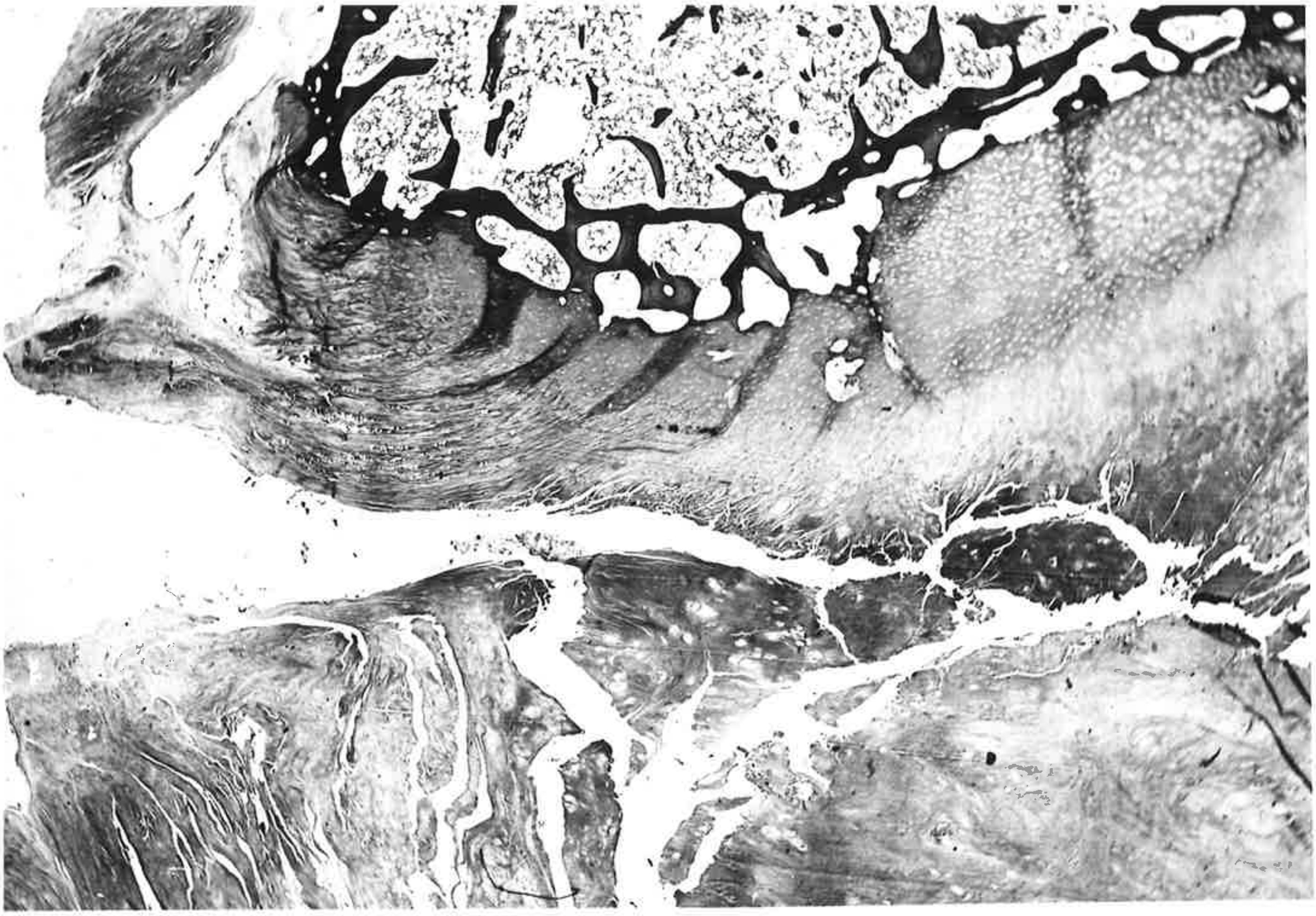


igure 15A

[Top] Low power micrograph of L3-4 intervertebral disc in a woman aged 56. Multiple clefts are seen in the central zone of the disc with a radiating tear extending to the periphery of the posterior annulus fibrosus. There is marked displacement of disc tissue towards the site of the tear with characteristic infolding of the inner layers of the anterior annulus towards the direction of the displacement. A relative retrolysthesis of the upper vertebra is seen.

Figure 15B

[Bottom] High power micrograph of the radiating cleft seen in figure 15A. The outward orientation of the margin of the annular lamellae is seen in relation to the cleft.



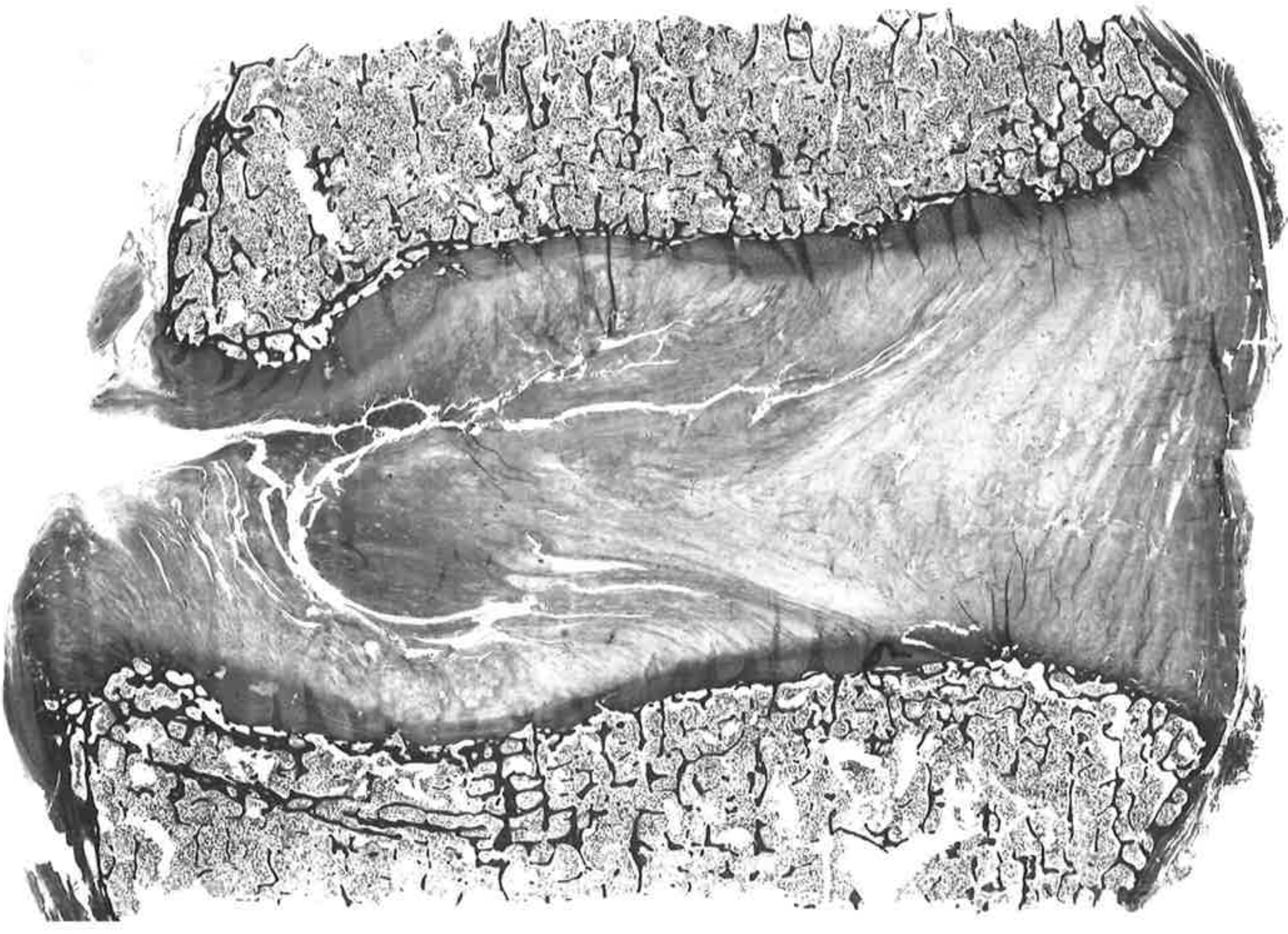


Figure 16A

[Top] Low power micrograph of L4-5 intervertebral disc in a man aged 44. Multiple clefts are seen in the central zone of the disc with radiating tears extending towards the periphery of the posterior annulus fibrosus. The typical finding of infolding of the inner annulus layers of the anterior annulus towards the site of disc displacement is again seen.

Figure 16B

[Bottom] High power micrograph of the radial tears seen in figure 16A.

## 3.4 THE INCIDENCE OF ANNULUS LESION IN THE HUMAN LUMBAR SPINE. THE RESULTS OF THE ADELAIDE STUDY

### 3.4.1 Material and Methods

135 lumbar discs from 27 spines removed at autopsy in Adelaide between 1987 and 1989 were studied. All subjects were aged between 17 and 50 years (average age 31.5). The relevant history of each subject was recorded and specifically if any skeletal or metabolic disorders were present at the time of death, and if any history of low back pain was known. In addition, the principal autopsy findings and the cause of death were included in the file. The fresh excised lumbar spines were radiographed and then fixed in formal saline. Following fixation individual joint complexes comprising the intervertebral disc, the adjacent end-plates and the posterior arch with the intact apophyseal joints were separated and decalcified. Decalcification was checked by daily radiographs. At completion of decalcification the intervertebral disc and the adjacent end-plates were cut into six parasagittal slabs of uniform thickness. Each slab was observed with the aid of a dissecting microscope, photographed and the height of the invertebral disc seen in each individual slab measured. Each slab was then embedded in wax and processed for histology.

Morphological Classification of Nucleus Pulposus Degeneration:

In addition to the observation of annular defects the morphological appearance of the nucleus pulposus was classified according to a three grade system. This was based on the macroscopic appearance of the nucleus as seen in central sagittal slabs (fig.17).

Figure 17 Morphological classification of the appearance of nucleus pulposus.

A (top) grade I Normal appearance. Clear demarcation is seen between the nucleus pulposus and the annulus fibrosus. No obvious clefting is observed.

B (centre) grade II Mild to moderate degeneration (see text).

C (bottom) grade III Marked degeneration (see text).

Grade I — Normal: Clear demarcation exists between the nucleus pulposus and the concentric lamellar structure of the annulus fibrosus. The cut surface of the nucleus appears to bulge with a gel-watery consistency. and a milky or cream like colour. No clefts are seen extending from the nucleus into the annular substance.

Grade II — Mild to moderate degeneration: The distinction between nucleus and inner annulus is less defined. The cut surface of the nucleus appears flatter and of more solid consistency. Its colour is slightly darker and initial clefting may be seen extending towards the outer areas of the disc.

Grade III — Marked degeneration: No distinction is seen between the nucleus and annulus fibrosus. The cut surface appears flattened and of frayable consistency. Yellow-brown pigmentation is commonly seen and extensive clefting is observed extending to the outer annulus fibrosus.

### 3.4.2 Results

These are summarised in table I, II and III.

Peripheral tears were more frequently observed anteriorly with the exception of the L5-S1 level where, out of nine rim lesions, four were seen anteriorly and five posteriorly.

Distribution of circumferential tears was similar between anterior and posterior annulus for the four upper levels. A different trend for the L5-S1 disc was observed where 18 circumferential tears were seen in the posterior annulus against seven anteriorly.

Radiating tears were almost exclusively seen in the posterior annulus and in almost half of the L5-S1 discs.

Eight L1-L2 discs had macroscopic evidence of nucleus degeneration against 18 L5-S1 discs.

A comparison was attempted between the 18 spines under the age of 35 and the 9 spines over the age of 35 (table II). Whilst no significant difference was seen in the incidence of peripheral and circumferential tears between the two groups, the incidence of radiating ruptures was markedly increased in the older age group. This correlated with the presence of nuclear degeneration.

Table I (see text)

Table II (see text)

Table III (see text)

## EPIDEMIOLOGY OF ANNULUS TEARS
(27 HUMAN LUMBAR SPINES AGE : 31.5 (Mean) (R:17-50)

| | | ANTERIOR ANNULUS | | | POSTERIOR ANNULUS | | | NUCLEUS DEGENERATION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DISC LEVEL | NO. | RIM LESIONS | CIRC. TEARS | RAD. TEARS | RIM LESIONS | CIRC. TEARS | RAD. TEARS | 1 | 2 | 3 |
| L 1-2 : | 27 | 7 | 11 | 1 | - | 10 | 6 | 19 | 7 | 1 |
| L 2-3 : | 27 | 6 | 12 | - | 3 | 13 | 3 | 17 | 9 | 1 |
| L 3-4 : | 27 | 9 | 9 | - | 2 | 12 | 5 | 19 | 7 | 1 |
| L 4-5 : | 27 | 12 | 12 | - | 13 | 13 | 6 | 15 | 11 | 1 |
| L 5-S1 : | 27 | 4 | 7 | 2 | 5 | 18 | 13 | 9 | 13 | 5 |
| TOTAL : | 135 | 38 | 51 | 4 | 13 | 66 | 33 | 79 | 47 | 9 |

## EPIDEMIOLOGY OF ANNULUS TEARS
(27 HUMAN LUMBAR SPINES AGE : 31.5 (Mean) (R:17-50)

### COMPARISON BETWEEN 90 DISCS OF SUBJECTS UNDER 35 YEARS OF AGE AND 45 DISCS OF SUBJECTS OVER 35

| | | ANTERIOR ANNULUS | | | POSTERIOR ANNULUS | | | NUCLEUS DEGENERATION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DISC LEVEL | NO. | RIM LESIONS | CIRC. TEARS | RAD. TEARS | RIM LESIONS | CIRC. TEARS | RAD. TEARS | 1 | 2 | 3 |
| L 1-2 : | 18 (9) | 4 (3) | 6 (5) | 1 (-) | - | 6 (4) | 2 (4) | 14 (5) | 4 (3) | - (1) |
| L 2-3 : | 18 (9) | 2 (4) | 6 (6) | - (-) | 1 (2) | 6 (7) | - (3) | 14 (3 | 4 (5) | - (1) |
| L 3-4 : | 18 (9) | 2 (7) | 3 (6) | - (1) | 1 (1) | 5 (7) | 1 (4) | 16 (3) | 2 (5) | - (1) |
| L 4-5 : | 18 (9) | 5 (7) | 6 (6) | - (-) | 1 (2) | 8 (5) | 3 (3) | 11 (4) | 7 (4) | - (1) |
| L 5-S1 : | 18 (9) | 2 (2) | 3 (4) | 1 (1) | 3 (2) | 10 (8) | 7 (6) | 8 (1) | 8 (5) | 2 (3) |
| TOTAL : | 90 (45) | 24(27) | 24 (27) | 2 (2) | 6 (7) | 35 (31) | 13 (20) | 63 (16) | 25 (22) | 2 (7) |

AGE <35 (AGE >35)

## EPIDEMIOLOGY OF ANNULUS TEARS
(27 HUMAN LUMBAR SPINES AGE : 31.5 (Mean) (R:17-50)

### DISCS WITH NO EVIDENCE OF ANNULAR TEARS

| | UNDER 35 (90 DISCS) | OVER 35 (45 DISCS) | TOTAL (135) |
|---|---|---|---|
| L 1-2 | 9 | 3 | 12 |
| L 2-3 | 11 | 3 | 14 |
| L 3-4 | 11 | 2 | 13 |
| L 4-5 | 6 | 2 | 8 |
| L 5-S1 | 8 | 2 | 10 |
| TOTAL | 45 | 12 | 57 |

Seven of the 45 discs from subjects over the age of 35 had marked nuclear degeneration, against two of the 90 discs from subjects under 35. Approximately half of the discs from the 18 spines under 35 years of age had no evidence of annular defects. 33 of the 45 discs from the group over 35, however, had marked annular pathology (table III).

### 3.4.3 Discussion

In their comprehensive description of intervertebral disc pathology Schmorl and Yunghanns[171] related the appearance of clefts extending from the nucleus pulposus into the annular layers to the dessication and fraying of the central part of the disc characteristic of intervertebral chondrosis. They added that the formation of clefts within the annulus fibrosus could not only be due to radiation from the nucleus pulposus but occur independently with defects arranged mostly in a concentric fashion and both in the anterior and posterior regions.

Other discrete lesions of the annulus fibrosus were described in relation to osteophyte formation. These lesions consisted of separation of the annulus fibrosus from the vertebral body rim along a plane parallel and adjacent to the end plates seen specifically at the very periphery of the disc.

Coventry, Ghormley, and Kernohan[39,40,41] in 1945 stated that during the third decade of life a typical feature of ageing of the annulus fibrosus would be the appearance of concentric fissuring of the annulus lamellae. This phenomenon would preceed the more substantial changes seen later in the nucleus pulposus.

Morgan and King[141] in 1957 drew attention to the association of annulus tears, radiographic instability and low back pain. They observed the presence of "incomplete radial posterior tears" in the lower lumbar segments and of "anterior concentric fissures or slits" in the upper lumbar spine which they considered as the likely cause of lumbar instability.

20 years later Vernon-Roberts and Pirie[199] reviewed the results of over 300 lumbar spine autopsy observations. They shared the observation made earlier by Schmorl and Junghanns of radiating annular tears being an extension of clefts originating from the nucleus pulposus. In addition, they confirmed the presence of circumferential tears within layers of annular fissure and of rim lesions seen near the attachment of the annulus to the peripheal vertebral body.

Hilton and Ball[90] in 1984 reported on the frequency distribution and histological characteristics of vertebral rim lesions of 117 post-mortem spines. Histological observations however, were obtained systematically only from central sagittal slabs of the D11-12 and L4-5 intervertebral discs.

Rim lesions were more frequently seen anteriorly and could be associated with major tears of the annulus fibrosus. Most rim lesions showed evidence of attempted repair in the fibro-vascular and fibro-cartilaginous tissue in the region of the rim. The authors concluded that based on the histological appearance a traumatic aetiology was likely and that these lesions could be relevant in the context of low back pain.

The results of our study suggest that radiating annular defects are closely associated with degeneration of the nucleus pulposus and more frequently seen in the lower lumbar spine and in the posterior annulus.

Discrete tears of the outer annulus fibrosus however, can occur in otherwise normal intervertebral discs and may preceed degenerative changes in other parts of the intervertebral joint complex.

Moreover, based on their histological characteristics it is likely that rim lesions are due to trauma to disc tissue rather than being the result of its biochemical degradation.

Friberg and Hirsch[67] suggested that annular ruptures would start in the inner annulus and direct either sagittally or laterally outwards towards the periphery. The concept of outward radiating ruptures was explained by Galante[69] as a combination of internal disc pressure and early failure of the inner annular fibres due to degenerative changes. This concept was challenged by Kirkaldy-Willis[110] who suggested that annular lesions would be first seen in the outer lamellae and would subsequently coalesce to extend inwards towards the nucleus pulposus.

Our observations on young and otherwise healthy lumbar intervertebral discs suggest that tears of the peripheral annulus fibrosus may develop independently and should be considered a separate entity from radiating clefts that form from the degenerating inner zone of the intervertebral disc.

The formation of discrete peripheral tears in relatively young discs may be interpreted as a result of mechanical strain on the annular lamellae. This would be in keeping with the results of Nachemson's experiment[142,143], where, in the presence of high values of intradiscal pressure, tensile strain would be highest in the peripheral annulus.

The relevance of peripheral annular lesions in the development of low back pain remains uncertain.

The commonly seen phenomenon in our series of vascular ingrowth and granulation tissue formation in association with discrete defects of the outer annulus would suggest that these tears may play a role in the production of back pain.

It is of interest to note in this context that pain provocation at discography is commonly associated with the presence of tears extending to the outer annulus fibrosus.

The relationship between annular tears, discography and pain provocation will be analysed in the following chapter.

# 4. INVESTIGATIVE TECHNIQUES

## 4.1 SUMMARY

In this chapter an attempt is made to correlate the morphological changes of the annulus fibrosus described in the previous pages with the results of investigative techniques such as MRI and discography.

These procedures are compared in relation to the staging of disc degeneration with special reference to annular ruptures.

Following the review of the literature the results of a comparative study between MRI and discography in 33 patients investigated by MRI and discography for low back pain are presented.

## 4.2 HISTORY

### 4.2.1 Discography

The use of discography for the diagnosis of disc prolapse was first reported by Lindblom in 1948. Numerous publications have since followed-[1,25,33,37,44,52,53,128,143,144,150,154,162,169,200].

Erlacher[52] in 1952 proposed to classify the pattern obtained by injecting radiographic contrast medium into the nucleus pulposus into five different forms. In form one and two the contrast appeared to be contained within the nucleus pulposus and according to Erlacher herniation or protrusion were unlikely. These forms were classified as normal. In form three, one or more radiating fissures were shown by the contrast but the nucleus was still clearly identifiable and "protrusion, herniation or pre-prolapse could frequently be diagnosed". In form four the appearance of multiple radiating tears in the presence of a small central shadow was referred to as a transitory form of degeneration in which "prolapse was likely to occur". In form five the contrast spread through the disc by multiple branches with no central shadow. This was considered by Erlacher as representing typical degeneration in association with narrowing of the disc. Herniation according to the author would have no longer been expected.

Cloward[22] in 1952 commented on the diagnostic value of discograms in "ruptured lumbar intervertebral discs". He felt that discography would highlight tearing and disorganization of the fibres of the annulus fibrosus which could occur in the absence of neurological signs of deficit. Cloward drew attention to the fact that in such patients myelography would often fail to disclose any lesions encroaching on the spinal canal and that annulus ruptures could present without herniation of disc material.

In 1965 Nachemson[143] observed the relationship between intradiscal pressure as measured by the injection of fluid into the nucleus pulposus and morphological patterns of discograms. He concluded that significant differences could be found between discs classified normal at discography and those labelled moderately or severely degenerated. In the normal discs in fact the vertical load on the annulus was found to be only 50% of the applied load per unit of area on the disc. In the moderately degenerated discs however, parallel to a decrease of intradiscal pressure the vertical load on the annulus would become the same as that applied on the disc per unit of area. In other words, moderate degeneration was associated with an increase by about 100% of the vertical load on the annulus fibrosus and a 50% decrease of tensile stress when compared to normal discs.

Crock[44] in 1970 drew attention to the phenomenon of typical pain reproduction by discography in patients with disabling back pain. The radiographic contrast pattern would show radiating ruptures of the annulus. He postulated, based on extensive studies of end plate vascularisation, that auto immune mechanisms could play a relevant role in the development of the patient's symptoms.

Brodsky and Binder[25] in 1979 proposed, as a way of explainining the phenomenon of pain production following intradiscal injection, the transmission of pressure through "a torn or weakened annulus" producing pain by stretching the annulus fiber.

Park et al[154] in the same year reported on 14 patients between 16 and 40 years of age with discographic evidence of posterior annular fissures extending backwards from an otherwise normally outlined nucleus. Eleven of them underwent surgery because of disabling pain, and at operation no evidence of disc prolapse was found but "a large rent was demonstrated penetrating through all layers of the annulus fibrosus". The authors postulated that radiating fissures of the annulus fibrosus could represent early evidence of disc injury. Two patients in the series failed to show pain reproduction by discography. In both cases the pattern of contrast demonstrated complete radial rupture of the annulus with rapid extravasation of contrast into the epidural space. The authors explained this on the basis that pain provocation at discography would be the result of "transference of increased nucleus tension to the outer annulus" which would not occur if a complete radial rupture was present.

In 1986 Adams, Dolan and Hutton[1] tried to correlate the pattern obtained by the contrast at discography with various stages of degeneration as seen in cadaver material. As for Erlacher's classification they identifed five different types with type one and two (cotton ball and lobular) considered as representing normal discs. In type three (irregular) distinct signs of degeneration were seen with clefts and small fissures extending into the inner annulus. In type four (fissured) the contrast would highlight the presence of radiating clefts extending to the outer edge of the annulus but with no leakage of contrast material from the disc into the epidural space. The authors stated that this pattern would be commonly found in patients with back pain and would be most often associated with symptomatic pain reproduced by the injection. Type five (ruptured) would show contrast material extending to the outer edge of the annulus and escaping from the disc space. This pattern would be found in all stages of degeneration and would be associated with a complete radial fissure, according to the authors, usually in the posterior annulus. Adams et al concluded that the discogram types one to four represented successive sequential stages of disc degeneration.

### 4.2.2 MRI

In the last few years Magnetic Resonance Imaging of the spine has been used as an alternative method of obtaining information on the state of the intervertebral disc. Modic and Weinstein[138] in 1984 reported on the results of their preliminary work suggesting that the spin echo technique with variation in the echo time and recovery (repetition) was very useful for the evaluation of the spine. They described three basic variations: the first, an abbreviated spin echo technique with an echo time (TE) of 30 milliseconds and recovery time (TR) of 0.5 seconds ($T_1$ weighted images) which would provide good anatomic delineation and contrast resolution between soft tissue structures. This sequence would, however, result in a homogeneous appearance of the normal intervertebral disc with no distinction between the annulus and the nucleus pulposus. The second spin echo technique involved a more prolonged TE (60 or 120 milliseconds) and TR (1 second). With this sequence (Proton density images) discrimination between nucleus pulposus and annulus fibrosus would be possible because of the higher signal intensity of the nucleus. With degeneration, parallel to the decrease in water content of the nucleus pulposus a decrease in intensity of the signal from the nucleus would be noticed with a more isodense image of the disc. The authors stated that normal aging of the disc would appear to result in similar changes.

A recent in vitro study[203] correlated the signal obtained by MRI with $T_2$ weighted images and proteoglycan content of the intervertebral disc. This study suggested that a strong relation was found between signal intensity and water content of the tissue samples analysed. Although a positive correlation was found between the water and proteoglycan content in the tissue samples, signal at MRI was not found to be directly correlated with proteoglycan content. The authors concluded that the method described in conjunction with MRI could be valuable in quantifying loss of water content from the intervertebral disc which may be associated with degenerative changes and low back pain.

The third sequence in Modic and Weinstein's study involved a further lengthening of the TR and TE (TE 120 milliseconds, TR 3 seconds,) which would result in an enhanced signal from the CSF (cerebro-spinal fluid) relative to the extradural structures ($T_2$ weighted images). The images obtained with this sequence according to the authors were comparable with a myelogram.

In 1986 Gibson et al[73] reported on a comparative study of 50 discs subjected to MRI and discography. They concluded that MRI seemed to be more accurate than discography in the diagnosis of disc degeneration. Their conclusion was based on the fact that four of the discs which were reported normal at discography showed some decrease in the signal at MRI from the nucleus pulposus.

In one disc, however, the needle at discography had been placed incorrectly in the annulus and in the other three which had been reported as normal, lateral fissuring as highlighted by the contrast material was present. The reason for the discrepancy between discography and MRI was attributed retrospectively to an error of interpretation of the discogram.

In 1987 Schneiderman et al[173] reported on the correlation between Magnetic Resonance Imaging findings and discography in 101 discs from 36 patients. They concluded that in 100 of the 101 levels MRI was accurate in predicting whether the disc morphology would be normal or abnormal at discography but stated that based on signal intensity alone the exact pattern of disc morphology seen at discography could not be determined by MRI The authors added that in patients with early acute symptoms due to acute herniation or tears in the annulus fibrosus, MRI may not show changes in signal intensity.

Zucherman et al[207] in 1988 reported on a series of 18 patients in which MRI did not accurately reflect internal disc morphology as outlined by discography. This series consisted of seven men and eleven women with a mean age of 33 years and a range between 24 to 52 years. 16 of the 18 patients had had no prior surgery and two had had previous surgery at different levels. The interval between MRI and discography was between one day to ten months with a mean value of 2.5 months. In all cases despite MRI reported as being normal, discography showed tears extending to the outer annulus fibrosus with or without extravasation of contrast into the epidural space. The authors stated that discography may allow detection of significant pathology not suggested by MRI scanning. They added that in patients with normal MRI and continuing symptoms consideration should be given for further investigation by discography. They concluded stating that discography was at present the most sensitive indicator of internal disc architecture.

Yu et al[206] in 1988 reported the results of a correlative study between MRI and macroscopic appearance of lumbar discs from twenty cadavers of various ages. They differentiated tears of the annulus into three categories: concentric tears, radial tears and transverse tears in the periphery of the annulus. They concluded that MRI provided an accurate means for investigating tears of the annulus, with the tear appearing on $T_2$ weighted images as an area of bright signal replacing the normal low intensity signal of the annulus.

## 4.3 ANNULAR TEARS AND DISC DEGENERATION, CORRELATION BETWEEN MRI AND DISCOGRAPHY. A PROSPECTIVE STUDY

The following study was carried out to analyse in a prospective fashion the correlation between MRI appearance and patterns of disc degeneration at discography in a consecutive series of patients with low back pain.

### 4.3.1 Material and Methods

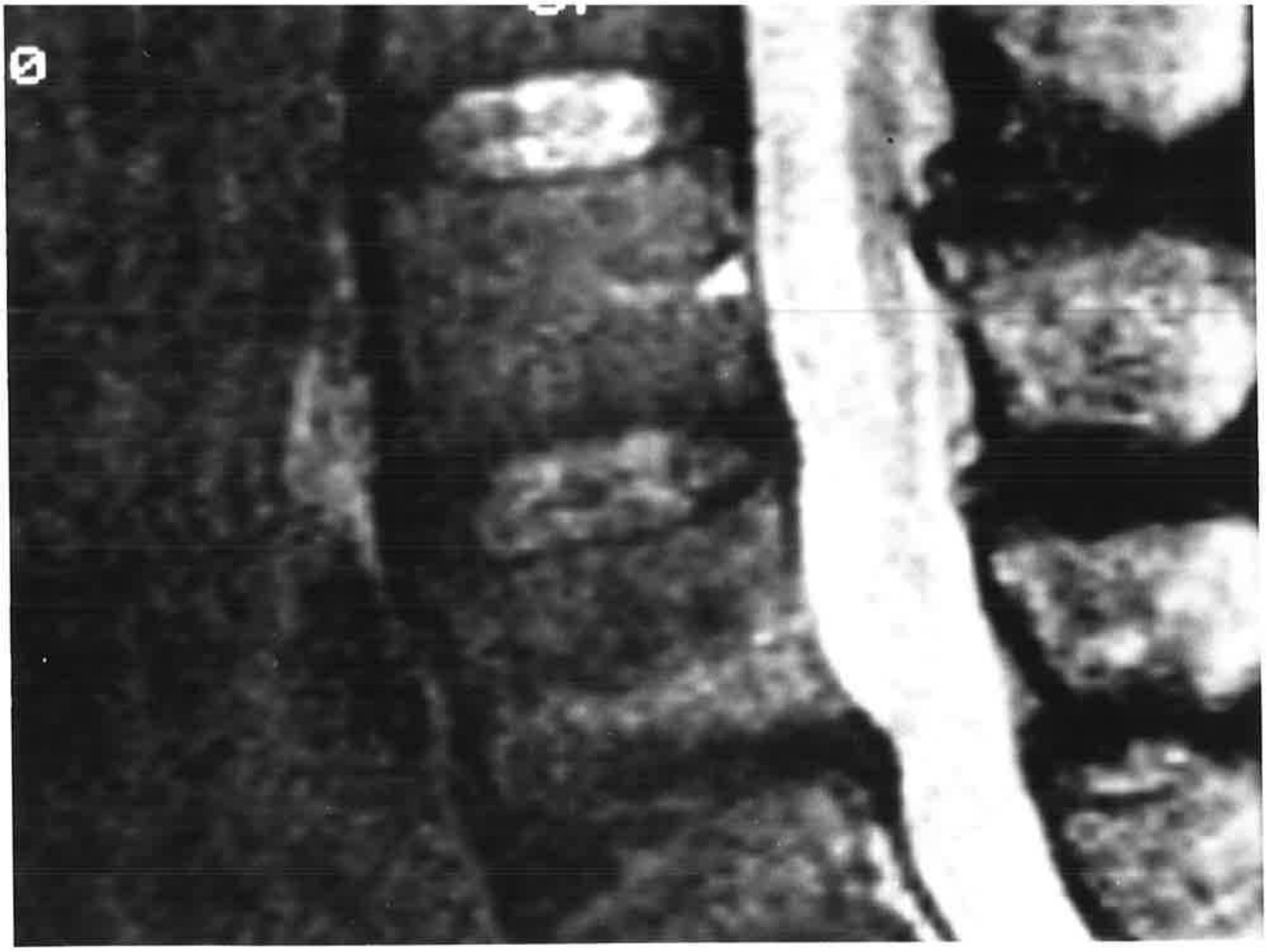
A prospective study was undertaken of patients being investigated for low back pain between May 1987 and December 1988. Patients with spinal osteomyelitis, discitis or other infectious diseases involving the spine were not included in the study. In addition, patients suffering from spinal trauma or tumour were also excluded. There were 33 patients included in the series with a total number of 114 lumbar intervertebral discs. The age of the patients ranged from 24 to 64 years (average : 35). MRI was performed using a Siemens Magnetom 1.0 Tesla. $T_2$ weighted spin echo sagittal images of 5mm thickness were obtained using a TR of 2.5 seconds and a TE of 90 milliseconds. The images from the intervertebral discs were classified according to the signal intensity from the central zone of the disc. Signal intensity was classified as normal, reduced or absent when no difference could be observed between central and peripheral zones as seen in central sagittal slabs. (fig.18A). An attempt was made to classify the shape of the central signal into normal and enlarged anteriorly and/or posteriorly(fig.18B).

Figure 18A

(Top) MRI scan of human lumbar spine. Sagittal $T_2$ weighted image (TR 2.0: TE 90). Example of grading system of signal intensity from the disc: the top disc is graded as normal; the central disc as reduced and the bottom disc as absent (see text).

Figure 18B

(Bottom) MRI scan of human lumbar spine. Sagittal $T_2$ weighted image. The shape of the central signal of all discs seen in the picture appear enlarged. In the upper disc the enlargement is mainly towards the anterior annulus whilst in the bottom disc an area of increased signal intensity is seen in the posterior annulus fibrosus, possibly related to a radiating tear.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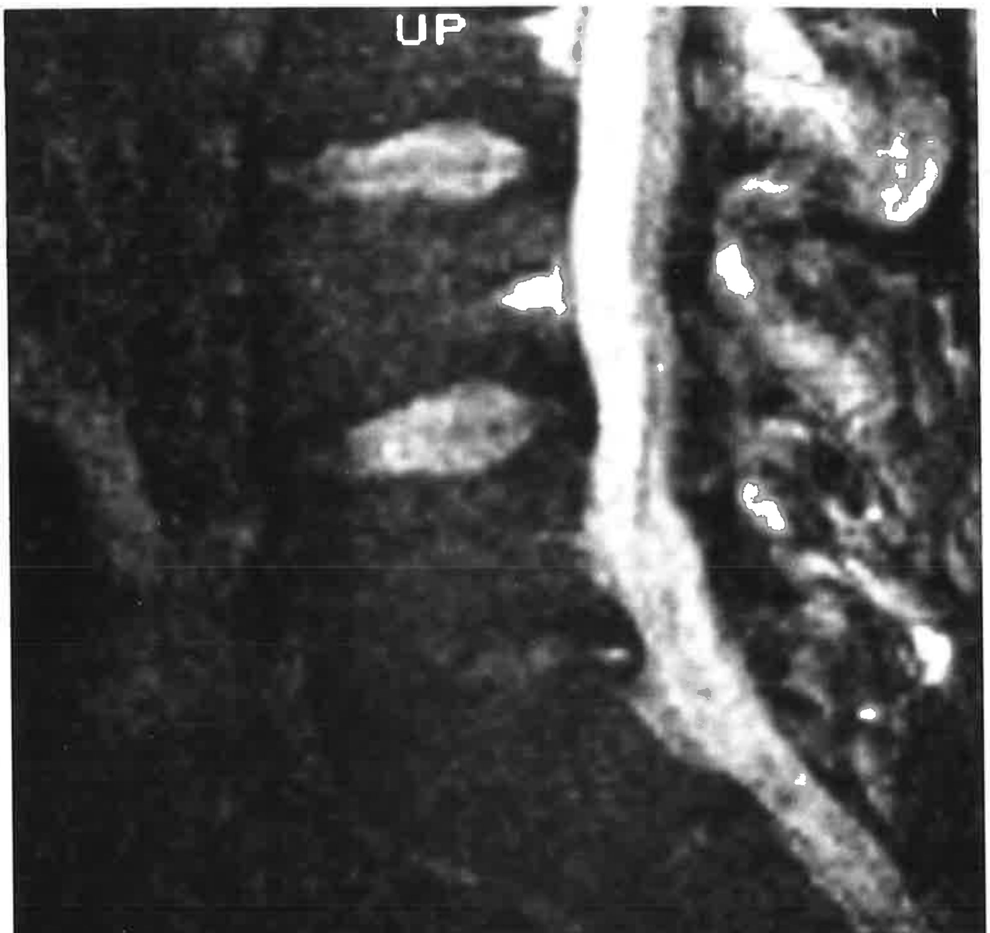
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It was found, however, that distinction between normal and enlarged contour was, in most instances, extremely difficult to define. It was decided therefore to limit the MRI classification of disc signal to intensity only.

Following MRI, discography was performed using the lateral approach described by McCulloch and Waddell [128] and a two needle stiletted technique. All procedures were carried out with the radiographic contrast material mixed with antibiotic as a prophylactic measure to prevent discitis[153]. Care was taken to record pain response to injection and the pressure employed. The discographic pattern was classified into four different categories: 1, (normal) where the nucleus was well outlined by the contrast in a roundish, regular, simple or bilobulated pattern and no annular tears could be seen; 2, where the nucleus was well outlined by the contrast but an anterior or posterior tear could be seen extending to the inner or outer annulus with no leak of contrast into the epidural space (fig.19); 3, where the nucleus was again well outlined by the contrast but one or more tears could be seen extending to the outer annulus anteriorly or posteriorly with leakage of contrast into the epidural space or along the anterior longitudinal ligament; 4, where the nucleus proper could not be outlined by the contrast and the latter extended within the intervertebral disc with or without leakage into the epidural space. (fig.20) Pain reproduction was classified as absent, atypical (where the injection caused discomfort or pain but this was clearly different from the usual symptoms of the patient) and typical - when the injection of contrast reproduced the patient's usual back symptoms. Pressure used for the injection of contrast was recorded as high, medium or low.

Figure 19A

[Top] Discography of L4-5 and L5-S1 human intervertebral disc. The appearance of the contrast in the L4-5 disc is consistent with a normal pattern.

Figure 19B

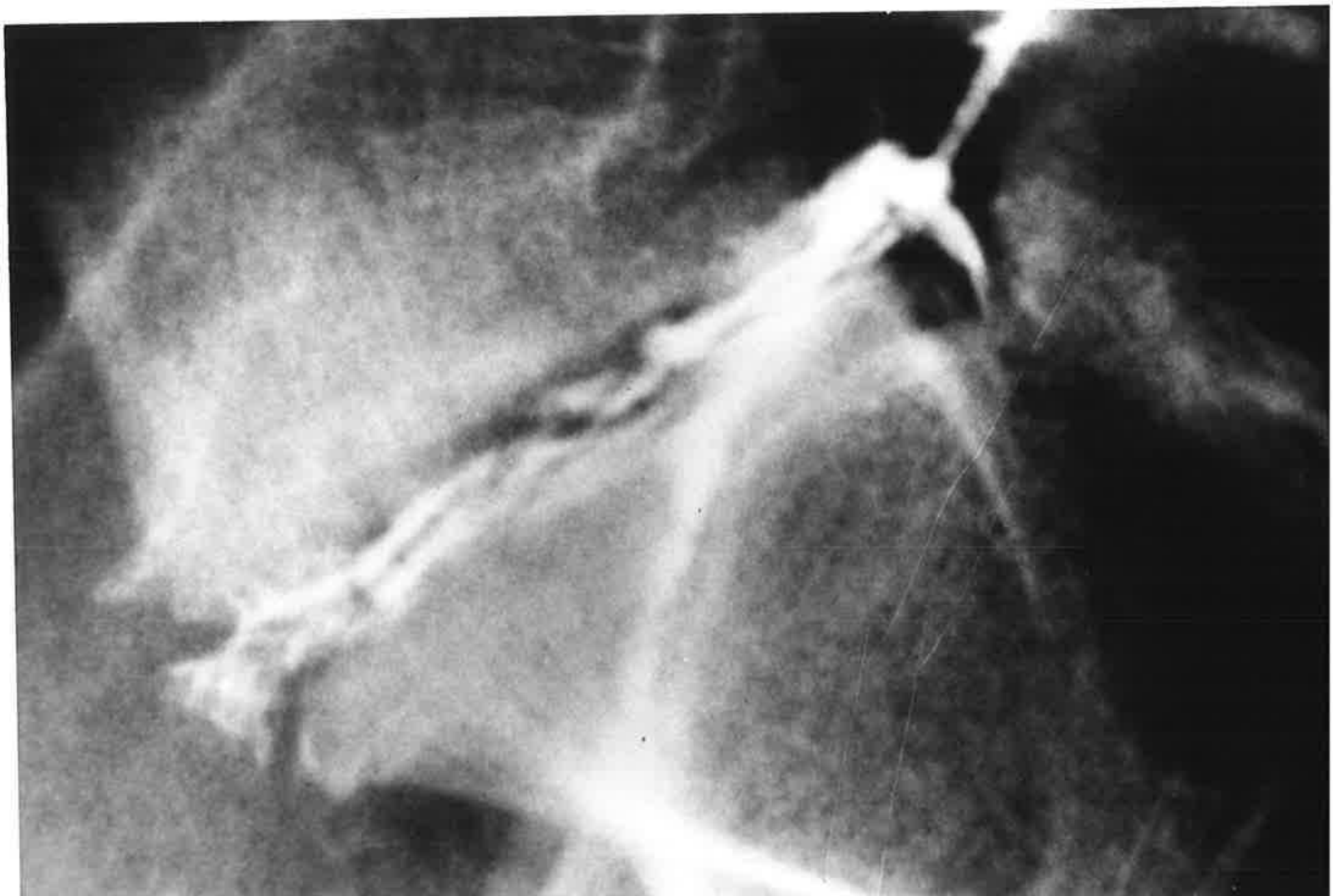
[Bottom] L3-4 discogram. Although there is a physiological pooling in the nuclear zone of the intervertebral disc the contrast is outlining the inner and mid fibres of the anterior annulus. A cleft is also seen outlined by the contrast towards the upper end plate of the lower vertebra. The contrast appearance is consistent with a type II pattern [see text].

gure 20A

(Top) L4-5 discogram showing extravasation of contrast posteriorly along the perispinal ligaments and into the epidural space. The appearance of the contrast is consistent with a type III pattern (see text).

Figure 20B

(Bottom) L5-S1 discogram. No pooling of contrast is seen in the central zone of the intervertebral disc. Multiple tears are highlighted by the contrast anteriorly and posteriorly. This is consistent with a type IV pattern (see text).



Whilst pain reproduction and pressure recordings were graded by one of three radiologists performing the procedure the classification of the MRI signal pattern and discography was carried out by the principal investigator.

### 4.3.2 Results

These are summarised in table IV and V.

None of the abnormal discs identified by MRI as having decreased or absent signal showed normal morphological pattern at discography (fig.21).

Eighteen of the sixty discs with normal signal intensity at MRI, however, showed marked degenerative changes at discography. In addition, a further 15 discs had annulus tears in the presence of a well outlined nucleus pulposus (fig.22).

None of the fifteen markedly degenerated discs at MRI had high levels of intradiscal pressure, whilst only six of the sixty discs classified normal at MRI had low degrees of pressure.

Of the thirty-nine discs with typical pain reproduction at discography twenty-seven had abnormal signal at MRI. All symptomatic discs had degenerative contrast pattern at discography.

Conversely of the thirty-nine asymptomatic discs thirty-three had normal MRI signal and twenty-four normal contrast pattern at discography. Only six of the forty-six degenerated discs at MRI were asymptomatic at discography.

Table IV (see text)

Table V (see text)

## CORRELATION BETWEEN MRI AND DISCOGRAPHY

| MRI SIGNAL INTENSITY FROM CENTRAL ZONE OF DISC | NO. OF DISCS | PATTERNS AT DISCOGRAPHY (DISCS) | | | |
|---|---|---|---|---|---|
| | | NORMAL | INNER TEAR | OUTER TEAR | MARKED DEG. |
| NORMAL | 60 | 27 | 15 | 12 | 6 |
| DECREASED | 39 | — | 9 | 15 | 15 |
| ABSENT | 15 | — | — | 6 | 9 |
| TOTAL | 114 | 27 | 24 | 33 | 30 |

## CORRELATION BETWEEN MRI AND DISCOGRAPHY

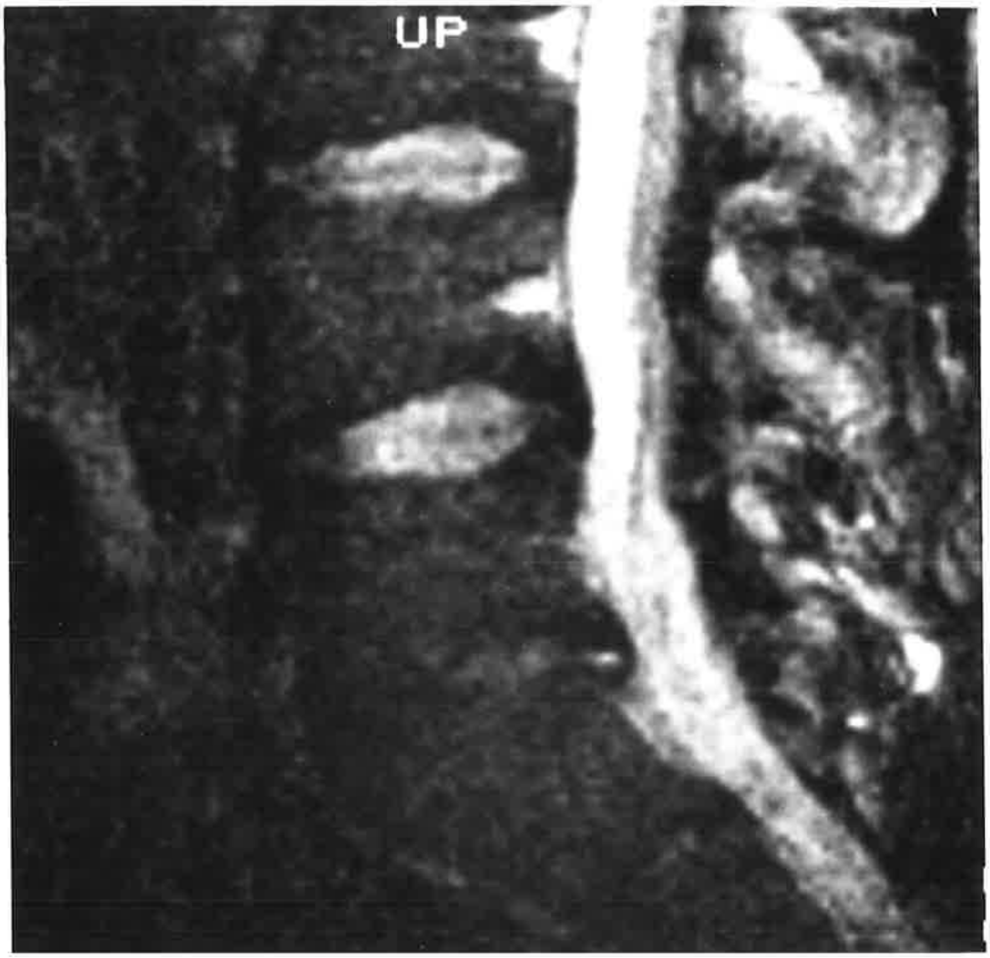
| PAIN REPRODUCTION AT DISCOGRAPHY | DISCS NO. | PATTERNS AT DISCOGRAPHY | | | | MRI SIGNAL INTENSITY | | |
|---|---|---|---|---|---|---|---|---|
| | | NORMAL | INNER | OUTER | MARKED DEGEN. | NORMAL | DECREASED | ABSENT |
| ABSENT | 39 | 24 | 6 | — | 9 | 33 | 3 | 3 |
| ATYPICAL | 36 | 3 | 15 | 6 | 12 | 15 | 18 | 3 |
| TYPICAL | 39 | — | 3 | 27 | 9 | 12 | 18 | 9 |
| TOTAL | 114 | 27 | 24 | 33 | 30 | 60 | 39 | 15 |

gure 21A

[Top] L4-5 and L5-S1 discogram. Same case of figure 18B. There is marked extravasation of contrast anteriorly and posteriorly at the L4-5 level. Contrast is seen posteriorly in the epidural space and anteriorly highlighting the very peripheral layers of the annulus fibrosus. The contrast appearance at the L5-S1 level is consistent with a type IV pattern.

'igure 21B

[Bottom] MRI scan of case of figure 18B and 21A. Although the corresponding L4-5 level at MRI shows some enlargement of the central signal the visualisation of the intradiscal pathology is significantly less impressive than the one seen at discography. The L5-S1 disc which shows absence of signal with the exception of a small area in the posterior annulus is consistent with the type IV pattern seen at discography.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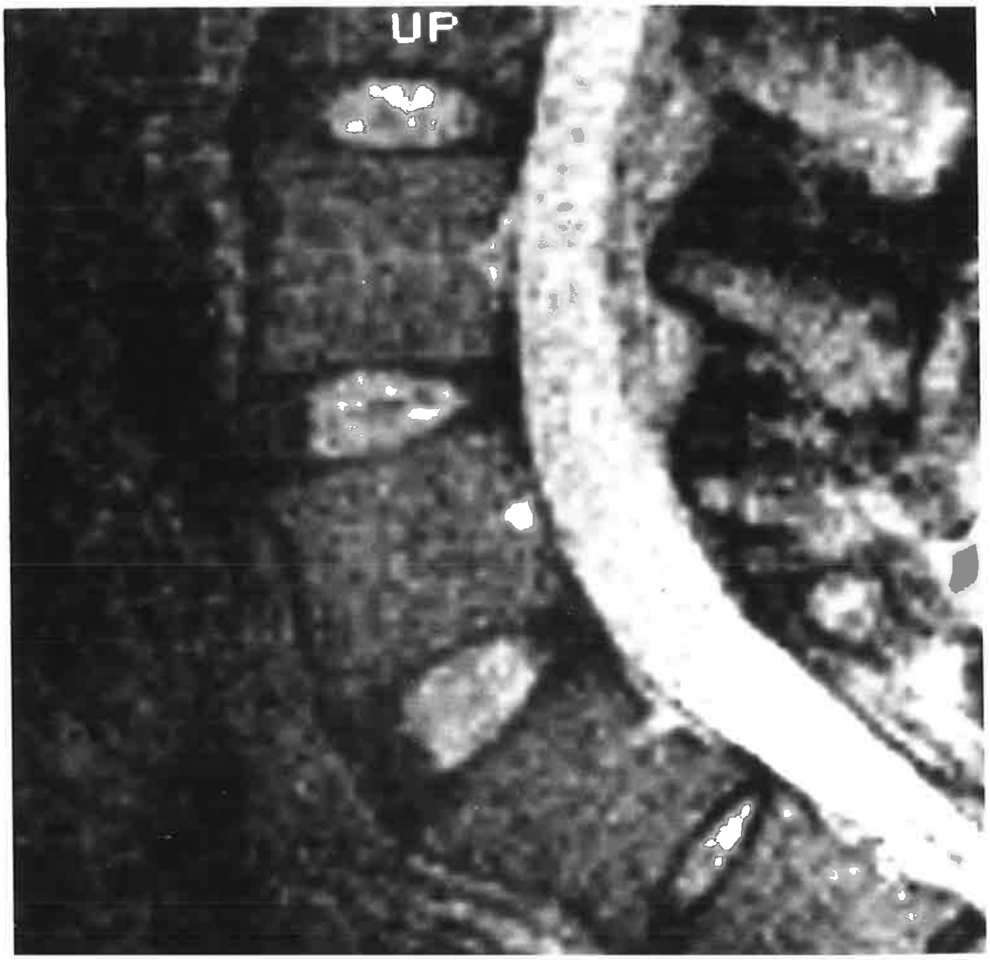
'igure 22A

(Top) L3-4, L4-5 and L5-S1 discogram. The L3-4 and L4-5 contrast appearance are consistent with normal pattern. The L5-S1 discogram shows extravasation of contrast in the posterior annulus fibrosus possibly related to a postero-lateral tear.

Figure 22B

(Bottom) MRI scan of the same patient of figure 22A. There is no obvious abnormality in signal intensity or shape at any of the three levels.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### 4.3.3 Discussion

Cloward in 1952 commented on the diagnostic value of discography in "ruptured lumbar intervertebral disc" in view of the potential for highlighting tears and disorganisation of the fibres of the annulus fibrosus.

In 1965 Nachemson observed the relationship between intradiscal pressure as measured by the injection of fluid into the nucleus pulposus and morphological patterns of discography. He concluded that significant differences could be found between discs classified normal at discography and those labelled moderately, or severely degenerated. Moderate degeneration was associated with an increase of about 100% of vertical load on the annulus fibrosus and a 50% decrease of tensile stress when compared to normal discs.

Brodsky and Binder in 1979 proposed as a way of explaining the phenomenon of pain reproduction following intradiscal injection that transmission of pressure through a "torn or weakened annulus" would produce pain by stretching the annulus fibres. Park et al in the same year reported on 14 patients between 16 and 40 years of age with discographic evidence of posterior annular fissures extending backwards from an otherwise normally outlined nucleus. The authors postulated that radiating fissures of the annulus fibrosus could represent early evidence of disc injury. Two patients in the series failed to show pain reproduction by discography. In both cases the pattern of contrast demonstrated complete radial rupture of the annulus with rapid extravasation of contrast into the epidural space. The authors explained this on the basis that pain provocation at discography would be the result of "tranference of increased nucleus tension to the outer annulus" which would not occur if a complete radial rupture was present.

In 1986 Adams, Dolan and Hutton correlated the pattern obtained by the contrast at discography with various stages of the degeneration as seen in cadaver material. Five different types were identified. The authors stated that discs with radiating clefts extending to the outer edge of the annulus but with no leakage of contrast material into the epidural space would be commonly found in patients with back pain and would most often be associated with symptomatic pain reproduced by the injection.

In our study thirty-six of the thirty-nine discs with typical pain reproduction at discography had tears extending to the outer annulus. Moreover, none of the twenty-seven discograms with normal morphological pattern reproduced the patient's typical pain, with twenty-four failing to cause any discomfort.

Attempts at correlating intradiscal pressure values and pain did not demonstrate a definite relationship. Whilst most of the normal discs at discography had high value of intradiscal pressure and no pain reproduction three of the nine discograms with low value of intradiscal pressure reproduced the patient's typical pain.

In the last few years magnetic resonance imaging (MRI) of the spine has been used as an alternative method of obtaining information on the state of the intervertebral disc.

In 1987 Schneiderman et al reported on the correlation between MRI and discography in 101 discs from thirty-six patients. They concluded that in 100 of the 101 levels, MRI was accurate in predicting whether disc morphology would be normal or abnormal at discography, but stated that based on signal intensity alone the exact pattern of disc morphology seen at discography could not be determined by MRI. The authors added that in patients with early acute symptoms due to acute herniation or tears in the annulus fibrosus, MRI may not show changes in signal intensity.

The results of our study suggest that with current standard techniques MRI would fail to demonstrate some of the structural changes in the annulus fibrosus which would be outlined by the contrast at discography. It seems incorrect, therefore, to assume that normal MRI signal intensity from the central zone of the disc is proof of normal disc morphology.

Our results are in keeping with the report of Zucherman et al of 18 patients with normal MRI and grossly abnormal intervertebral discs at discography.

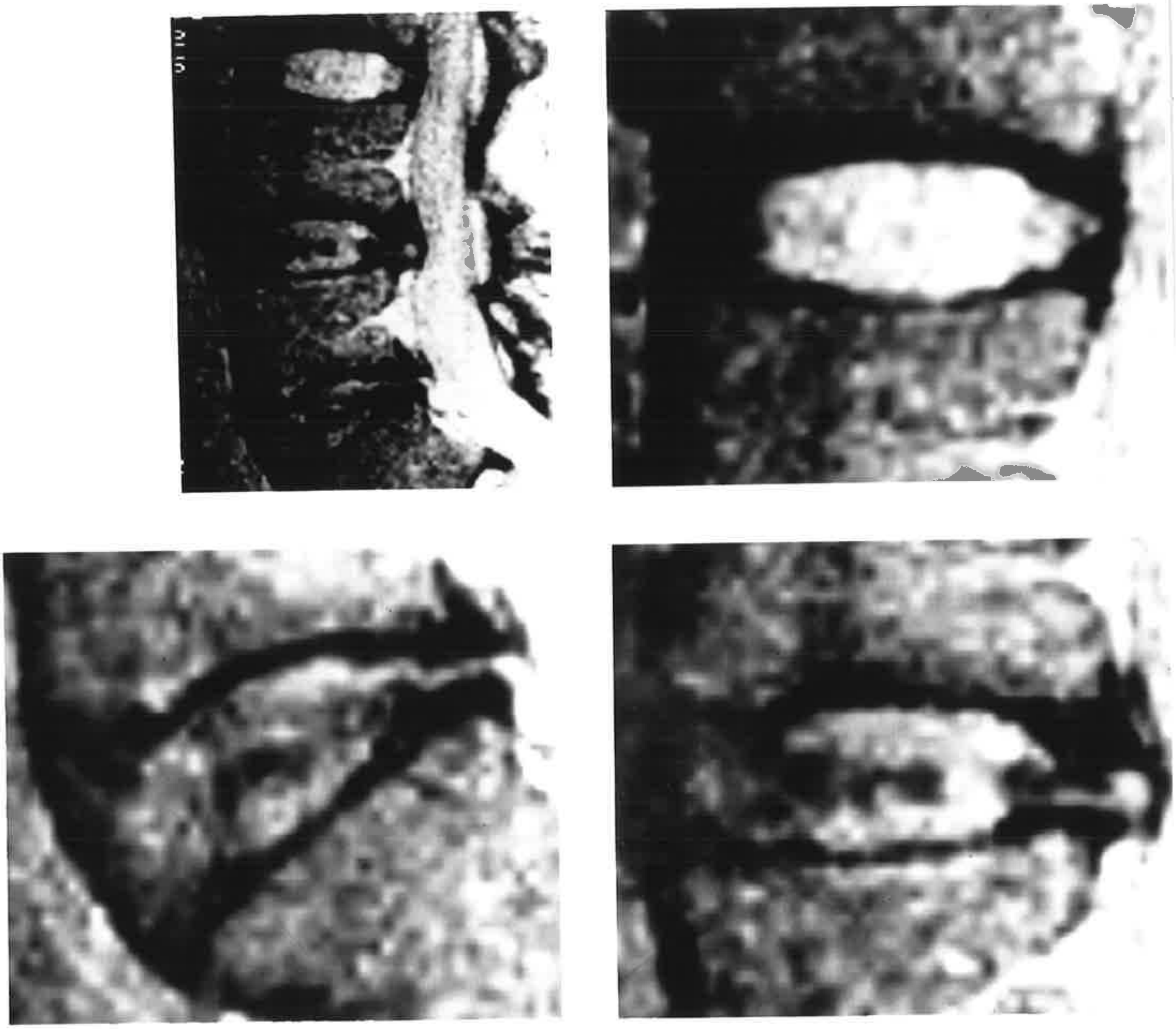
In our series in fact of the 60 discs with normal signal intensity at MRI, 18 had marked annular pathology as outlined by the radiographic contrast.

A recent in vitro study[206] comparing anatomical specimens and MRI of human intervertebral discs concluded that with $T_2$ weighted image sequences, different types of tears within the annulus fibrosus could be demonstrated. Although this study was based on cadaver material only it is likely that with continous technological improvements and the experimentation of new enhancing contrast agents the quality of the MRI signal from the annulus fibrosus will be improved in the near future. This may allow visualisation of annular tears in absence of major degenerative changes of the nucleus pulposus (fig.23).

Based on our results discography seems to represent at present a more accurate investigation for the documentation of annular tears which may be relevant in the production of low back pain. The relationship between signal intensity at MRI and biochemical composition of the intervertebral disc in an animal model of intervertebral disc degeneration is analysed in detail in Chapter 5.4.1.

Figure 23

MRI scan (proton density sequence) of human lumbosacral spine. Clockwise from top left: Overall view of lumbosacral spine; normal appearance of the L3-4 disc; the L4-5 disc signal appears decreased in intensity; an area of increased signal intensity is seen in the posterior annulus extending to the very outer layer. This appearance may be related to a radiating cleft associated with posterior displacement of nuclear material; L5-S1 disc: signal intensity is slightly reduced in relation to the L3-4 level. An area of increased signal is seen in the posterior annulus. This may reflect the presence of a radiating cleft to the outer layers of the posterior annular lamellae.



# 5. EXPERIMENTAL STUDIES OF INTERVERTEBRAL DISC DEGENERATION

## 5.1 Summary

In this chapter, following the review of the literature, a new animal model of intervertebral disc degeneration using the sheep is presented. This is based on the hypothesis that discrete peripheral tears of the annular lamellae could precede and influence the structural changes of the intervertebral disc commonly associated with degeneration.

In this model a cut was made parallel and adjacent to one of the end plates in the anterolateral quadrant of the outer annulus fibrosus in randomly selected lumbar intervertebral discs. The inner annulus and the nucleus pulposus were left intact.

This cut simulated the outer annulus tear described by Schmorl[171], Vernon-Roberts[198], and Hilton and Ball[90,91].

## 5.2 History

The first report in the English literature of experimental intervertebral disc degeneration induced in animals is that of Key and Ford[108] of 1948. Two previous reports are quoted in that article, one from Germany and one from Italy. In the first one by Lob (1933) surgical insult to the annulus fibrosus of rabbits was found to produce similar changes to those seen in human spondylosis deformans. In the second paper by Filippi (1935) within three months of surgical division of the anterior annulus fibrosus in rabbits regeneration of the annulus lamellae was seen with reconstitution of normal structure on examination. The findings by Filippi were contradicted by Key and Ford's work employing a dog model. In each of the fourteen animals entered into the study an incision was made transversely in the posterior longitudinal ligament and annulus fibrosus of lumbar intervertebral discs on the left side, through a laminectomy approach. The incision caused immediate prolapse of nuclear material. The disc was not curetted. The authors noted that at sacrifice the defect in the annulus fibrosus had tended to heal only at the surface whilst the operative defect in the deeper layers had remained open. The authors proposed, based on the result of their experiment, that the primary lesion leading to protrusion of the intervertebral disc in men may be a weakening of the posterior portion of the annulus fibrosus due to degenerative changes or to injury. Degenerative changes in the nucleus pulposus, according to Key and Ford, appeared to be secondary.

Smith and Walmsley[177] in 1951 reported on the result of their investigation using a rabbit model. They incised the annulus fibrosus transversely and deep into the nucleus pulposus obtaining immediate nuclear prolapse in the majority of rabbits. They analysed fifty-five surviving animals at intervals varying from one day to two years after the operation. They concluded that in keeping with the experimental findings of Key and Ford, healing occured in the superficial lamellae of the annulus in association with a fibroblastic reaction typical of tissue healing in general. The failure of healing of the deep part of the wound was attributed to the "recognized avascularity of the deeper annular fibres". The authors postulated that the presence of the displaced nucleus pulposus separating the annular lamellae would have been an additional factor acting against the bridging of the defect. They observed extensive ossification of the ventral annulus in the animals sacrificed at longer time intervals after the operation. Smith and Walmsley postulated that in humans an increase in pressure of the nucleus pulposus in association with movement of the vertebral column and in the presence of initial degenerative changes may rupture the deepest lamellae of the disc. This would allow the nucleus to prolapse "between the torn ends" of the annulus fibres. The rupture would extend to the more superficial layers of the annulus fibrosus to produce "a progressing tracking of the nucleus to the periphery of the disc".

In 1981 Lipson and Muir[120,121] reported on the biochemical changes induced with degeneration using the same rabbit model described by Smith and Walmsley. In all animals acute nucleus herniation was produced which was followed by secondary degenerative changes (metaplasia of the intervertebral disc into fibrocartilage and osteophyte formation at the site of the lesion). The water content of the disc after an immediate loss was rapidly restored two days after the operation. Progressive dehydration of the disc occurred however in the long-term in association with changes in the total uronic acid content. The proportion of aggregated proteoglycans increased rapidly during the first two days but a progressing loss was noticed six to seven weeks after the operation. The authors suggested that loss of confined fluid mechanics of the disc would appear to initiate the chemical changes seen in degeneration. They postulated that when a communication occurs between radial fissures and circumferential annular tears a "concealed herniation would take place" which would then lead to irreversible mechanical damage and progressive degeneration.

## 5.3 THE SHEEP MODEL

### 5.3.1 THE CHOICE OF THE SHEEP AS AN ANIMAL MODEL

Previous experience employing a sheep model had suggested it to be a particularly suitable animal for spinal research. The sheep was found to be economical, consistent, reliable and easy to handle. In addition based on the comparative studies of Butler[29,30,31], the embryology and chemical composition of the sheep disc seemed to be significantly closer to the human than that of other animal species used in previous studies (dogs and rabbits).

All sheep entered into the study were of the same breed (Merino) sex (wethers) and age at the time of surgery. The age, as identified by the front teeth, was in all animals between 18 and 24 months which corresponded to a young adult at completion of skeletal development.

## 5.3.2 MATERIAL AND METHODS

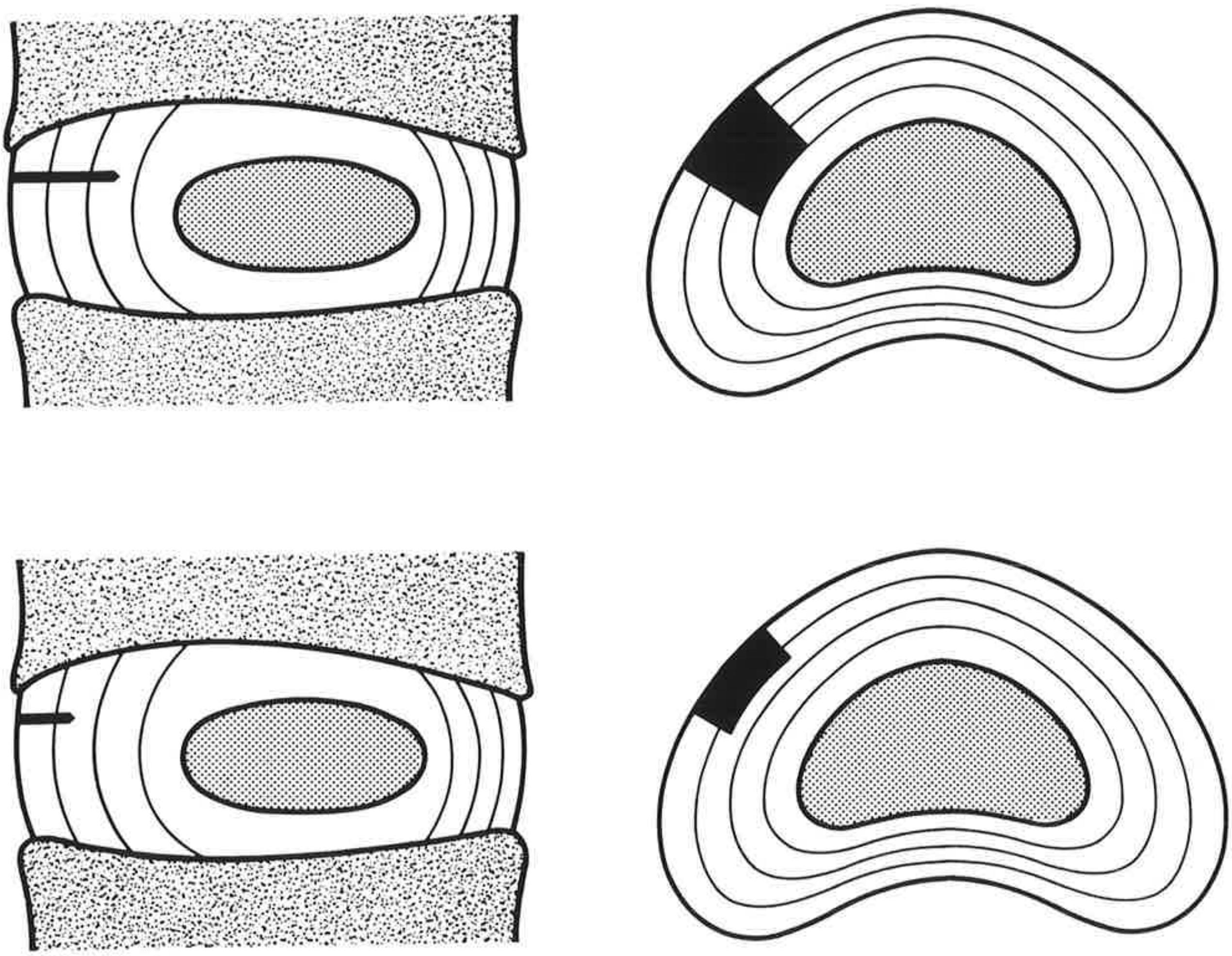
### 5.3.2.1 Surgical Technique

In all animals the anterior lumbar spine was exposed through a left sided retroperitoneal approach performed under general anaesthetic. Induction was obtained with intravenous barbiturates. The sheep was then intubated using a 9mm endotracheal tube and anaesthesia maintained with halothane, oxygen and nitrous oxide. The skin incision was vertical and anterior to the transverse processes, extending from the last rib proximally to the iliac crest distally. The peritoneum was retracted anteriorly and five lumbar intervertebral discs with the exclusion of the lumbosacral level were exposed between psoas major and minor. In one group of sheep, three randomly selected lumbar discs were exposed and a cut of 5mm width and 5mm depth was made in the left anterolateral annulus fibrosus. A guard was used on the knife to ensure the exact depth of the cut. This was parallel and adjacent to the lower endplate of the upper vertebra. In the remaining sheep, four randomly selected lumbar discs, with the exclusion of the lumbosacral level, were exposed and a cut was made of 5mm width and 2.5mm depth in the left anterolateral annulus fibrosus. The cut, as for the other animals, was parallel and adjacent to the lower endplate of the upper vertebra (fig.24).

The total depth of the anterior annulus fibrosus in the midsagittal plane in the lumbar spine of the sheep varies from a minimum of 7mm to a maximum of 9mm. A 5mm deep cut, therefore, would involve the outer and mid third of the annulus fibrosus. A 2.5mm deep cut would involve the outer third of the annulus only.

Figure 24A (Top) Diagram of 5mm deep cut.

Figure 24B (Bottom) Diagram of 2.5mm deep cut (see text).



Following the procedure discography was performed using a 27.5 gauge needle and 0.10 ml of Conray 280. Lateral radiographs were then taken and the wound was closed in layers with absorbable suture material. No antibiotics were used. The sheep were allowed to ambulate immediately after surgery and 3 days later sent to a sheep station where they were allowed to move freely in the open with no limitations.

5.3.2.2 Follow Up

The animals were randomly allocated to 8 groups in relation to the time interval between operation and sacrifice, the interval varying from one month for group one to twenty-four months for group eight. Of the twenty five sheep with a 5mm deep cut, two were allocated to group one (one month), two to group two (two months), four to group three (four months), two to group four (six months), three to group five (eight months), and four respectively to groups six (twelve months), seven (eighteen months) and eight (two years).

Of the ten sheep with a 2.5mm deep cut two each were allocated to group three (four months), five (eight months), six (twelve months), seven (eighteen months) and eight (twenty-four months).

For the purpose of the comparative analysis of the results all sheep in the study were divided into three main groups: early phase (with a follow up of one and two months); intermediate phase (follow up of four, six, eight and twelve months); late phase (follow up of eighteen and twenty-four months).

All sheep were sacrificed using an intravenous overdose of barbiturate. The lumbar spine was then transected at D10 above and in the mid sacrum below and removed en bloc.

5.3.2.3 Radiological Investigations

MRI was performed on the fresh specimen using a Siemens Magnetom 1.0 Tesla. Parasagittal slices of 4mm thickness were obtained using $T_2$ weighted images (TR 2.0-2.5, TE 60-90). Axial views were obtained of selected discs using $T_1$ weighted images (TR 0.55; TE15). Following MRI, discography was performed using a 27.5 gauge needle and Conray 280 as contrast medium to a maximum volume per disc of 0.15 ml. MRI signal was graded according to intensity and shape based on mid sagittal $T_2$ weighted images. Intensity was graded as normal if the signal from the central area of the disc had a bright intensity similar to the one obtained from the cerebral spinal fluid in the spinal canal; reduced, when the signal intensity from the central area of the disc appeared reduced in relation to the control levels and absent when no difference was observed between central and peripheral areas of the intervertebral disc. Shape of the signal from central zone of the disc was classified as: 1) normal, when it occupied the central two thirds of the intervertebral disc with no clear extension into the peripheral layers of the annulus fibrosus both anteriorly and posteriorly; and 2) enlarged, when extension of the area of increased signal intensity was seen towards the outer layers of the annulus anteriorly and posteriorly with or without bulging of the outer boundary of the intervertebral disc into the spinal canal posteriorly.

In the case of lack of signal from the disc the signal shape was classified as absent (3).

Discography was classified into four groups according to contrast pattern. Normal, when contrast was seen pooling in the central or nuclear zone of the disc with a roundish or bilobulated configuration. Pattern two when the central pooling in the nuclear zone was again observed but contrast could be seen extending to the inner or outer layers of the annulus fibrosus without extravasation of contrast outside of the physiological boundaries of the disc. Group three, when central nuclear pattern was still visible but contrast would be seen extending to the outer layers of the annulus fibrosus with extravasation into the epidural space posteriorly or along the anterior longitudinal ligament anteriorly. Group four, when the central nuclear pooling of contrast would not be visible and multiple tears could be observed outlined by the contrast in the anterior and posterior annulus. This pattern was considered consistent with marked disc degeneration.

5.3.2.4 Morphological Analysis and Conventional Histology

After discography the lumbar spines were fixed in formal saline. Following fixation the specimens were cut using a bandsaw in individual joint units, (comprising the intervertebral disc with the adjacent half of vertebral bodies and both apophyseal joints) and decalcified. Decalcification was checked with daily radiographs and when completed the intervertebral disc with the adjacent end plates was cut into six parasagittal slabs of uniform thickness. Each of the slabs was observed using a dissecting microscope and the morphological characteristics recorded using a standard form and photographed. Measurements were made of the height of the intervertebral disc and, when present, of the length of the annular lesion.

The macroscopic appearance was recorded with special reference to the following features:

1: Inner annular failure as indicated by nuclear displacement.

2: Nuclear degeneration as shown by:

   a) loss of definition between nucleus and inner annulus;

   b) the state of dehydration as indicated by the turgidity and bulging of the cut surfaces of the nucleus;

   c) the presence of clefts and discoloration.

(Based on these characteristics nucleus degeneration was graded as absent, mild to moderate and marked.)

3: Narrowing of the intervertebral disc space;

4: Presence of osteophytes.

Two of the six parasagittal disc slabs (the one containing the annular lesion and a contralateral - generally the 2nd and 5th from the left) were embedded in wax and processed for histology. Five micrometre sections were obtained and stained routinely with Hematoxylin and Eosin. Additional histo-chemical stains (P.A.S., Alcian blue at various Ph, and Masson-Trichromic stain for collagen) were obtained from selected discs.

The analysis of histological sections was made following a standardised form. In addition a three grade system (absent, mild-moderate, marked) was used to classify the extent of:

a) vascularisation of the annulus fibrosus;

b) secondary changes of the end plates (chondrosis and ossification);

c) nuclear degeneration.

### 5.3.2.5 Quantitative Histology

An experiment was designed to analyse in a quantitative fashion the effect of the peripheral lesion of the annulus on the vascular channels present in the cartilage end plates. 31 sheep were entered into the study. As for the previously described experiment in all animals the lumbar spine was exposed under general anaesthetic using a retroperitoneal approach.

In three randomly selected lumbar discs a cut was made parallel and adjacent to the cranial end plate. The cut had a controlled depth of 5mm. Plain x-rays were taken at the time of surgery to record the levels operated.

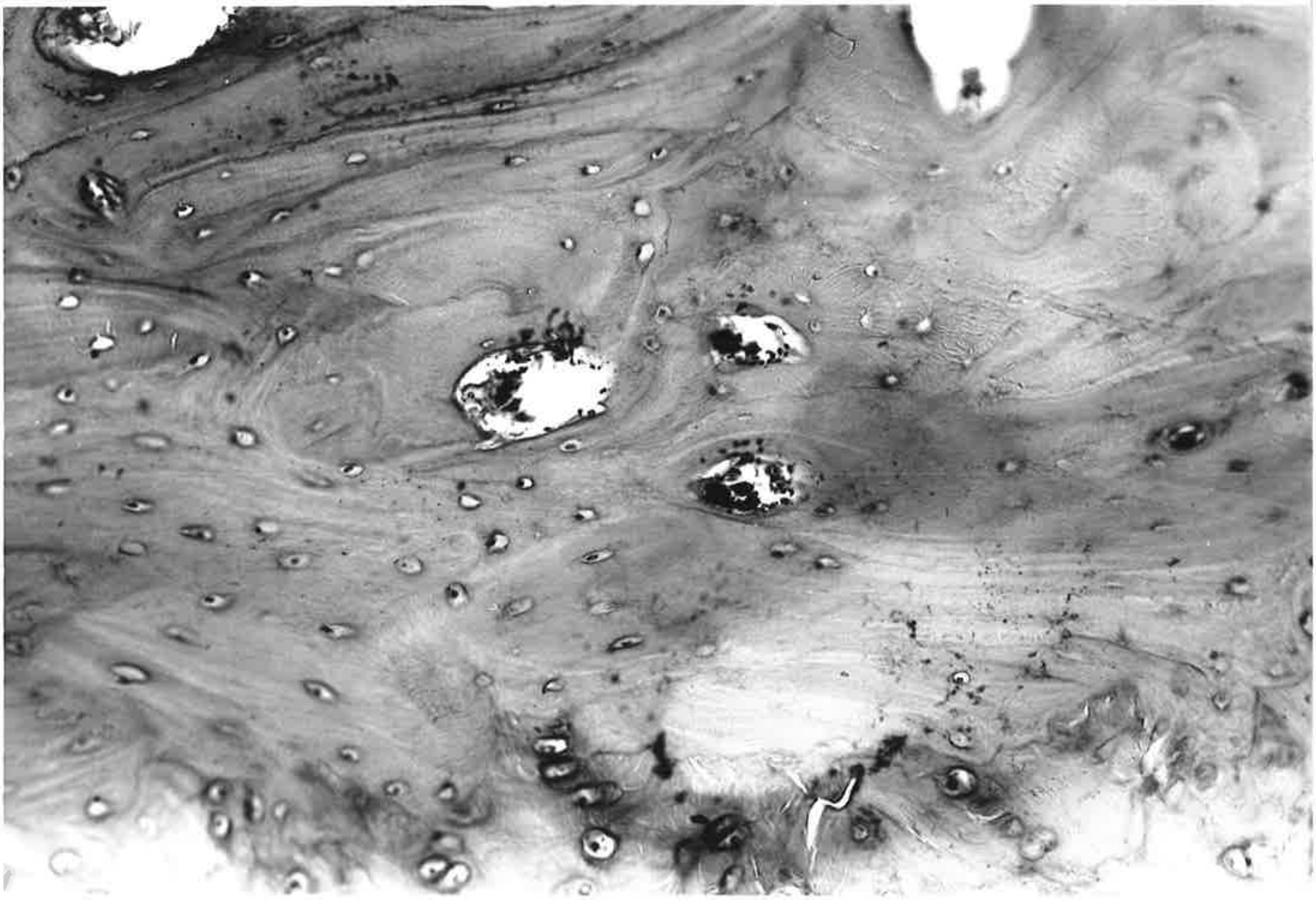
Sheep were sacrificed at various intervals after operation ranging from two weeks to two years. Following sacrifice with an overdose of barbituate the lumbar spine was removed en bloc from D10 to the sacrum below. After fixation in formal saline individual joint units comprising the intervertebral disc and adjacent end plate together with the relative apophyseal joints were decalcified. At completion of decalcification all discs were cut into six parasagittal slabs of uniform thickness. Each slab was then embedded in wax and processed for histology. 5 micron thick sections were cut and stained with Haematoxylin and Eosin.

Eight additional sheep without annular lesion were entered into the study to serve as control and processed according to the protocol described above.

An ocular-mounted Weibel II grid was used to count the number of vascular channels present in the cartilage end plate and adjacent subchondral bone using a manual point counting method(fig 25). Each end plate was divided into three areas: 1) anterior, in relation to the anterior annulus fibrosus; 2) central, related to nucleus pulposus and 3) posterior, in relation to the posterior annulus. The superior and inferior end plates were analysed separately. All quantitative assessments were performed at a magnification of 400.

Figure 25

High power micrograph of the end plate of sheep intervertebral disc. Three vascular channels are seen in the centre of the section. Subchondral bone is seen at the top. (H & E; x 50-original magnification)



### 5.3.2.6 Biochemistry

#### 5.3.2.6.1 Material and Methods

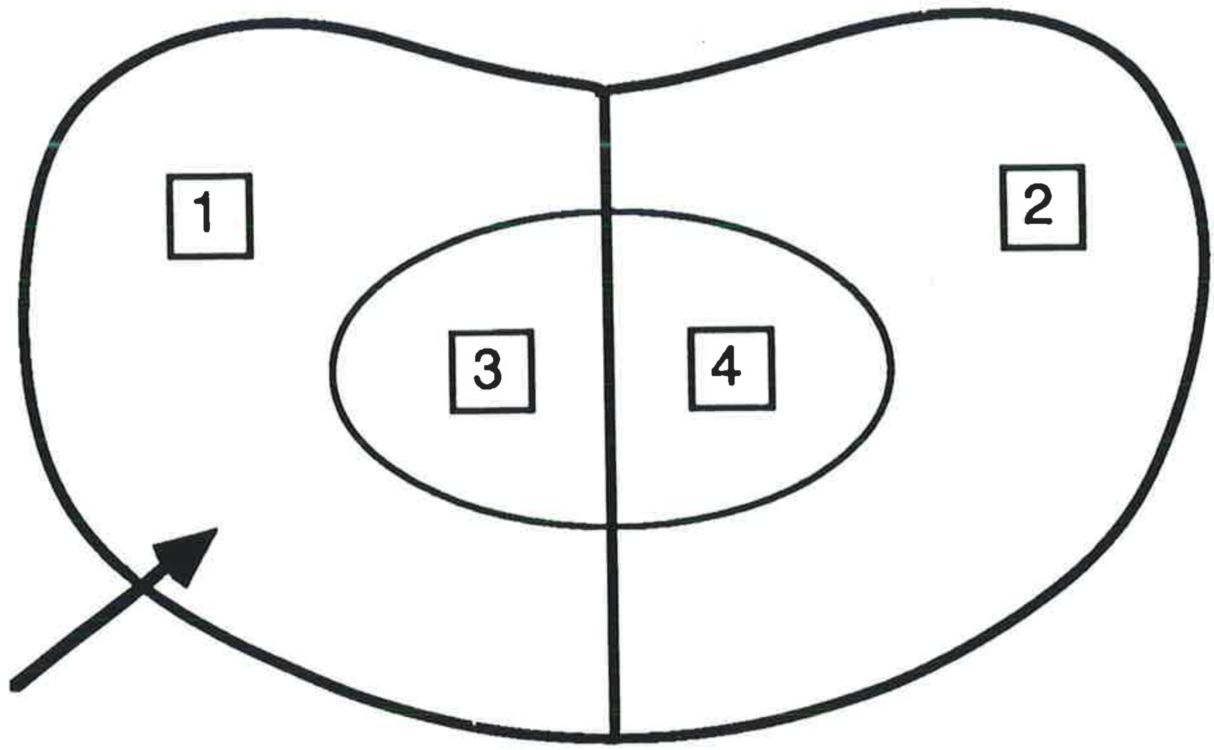
21 sheep were entered into this experiment. In all sheep the lumbar spine was exposed under general anaesthetic and using the technique described previously a cut was made in the anterolateral annulus fibrosus of a randomly selected lumbar disc. In 15 of the sheep the cut had a depth of 5mm; in the remaining six animals the depth was 2.5mm. The sheep were sacrificed at various intervals ranging from two months to 18 months for the group of 15 animals with 5mm lesions and between four and 12 months for the six sheep with the 2.5mm deep lesion. At sacrifice the treated disc with adjacent end plates together with one control and the lumbosacral disc were frozen in liquid nitrogen at -70$^{o}$. Partially thawed discs were initially bisected by a transverse incision across the mid substance of the intervertebral disc. The morphological appearance of the two halves so exposed was recorded with colour photographs. The intervertebral disc was then dissected from the cartilage end plate and divided into four zones (fig 26): zone one and three: left annulus and nucleus pulposus (the side of the lesion): and zone two and four: right annulus and nucleus pulposus respectively. The wet weight of each zone was determined prior to processing. Representative portions of each of the four disc zones, of known weight (circa 50-80mg) were freeze dried to constant weight, the difference in weight between the wet and the dry tissue representing its moisture content.

Figure 26A

(Top) Diagram of sample dissection for biochemical analysis. Zone 1 and 3: left annulus and nucleus pulposus (the side of the lesion); zone 2 and 4: right annulus and nucleus pulposus.

Figure 26B

(Bottom) Colour photograph of sheep intervertebral disc following transverse incision across the mid substance. The area of the original lesion is clearly identifiable by the disorganisation of the lamellar structure of the annulus and by the hyperpigmentations of the annulus and the adjacent half of the nucleus pulposus. The expansion of the outer margin of the disc circumference related to the lesion is due to early osteophyte formation.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4

Triplicate portions (3-5mg of dry weight) of each of the disc zones were digested with papain (1mg per ml) in 50 mM of Tris HCl at pH 7.0 containing 25mM of EDTA and 10mM of Cysteine (1ml) at 60$^{o}$ for 12 to 16 hours. Aliquotes of supernatant solution from the digest were assayed for uronic acid by the method described by Blumenkrantz and Asboe-Hansen using D-glucuronolactone.

Triplicate portions of each of the four disc zones (1-2mg of dry weight) were hydrolised in 6N in CHl at 110$^{o}$C for 16 hours in sealed glass ampules. Collagen contents were determined from the hydroxyproline values of neutralised hydrolysates by the method described by Stegeman and Stalder using a multiplication factor of 7.4.

The remainder of each of the disc zones left after compositional analysis was finely diced over ice and extracted with 4M GuHCl buffered with 50mM of Tris HCl at pH 7.4 containing the following proteinase inhibitors: EDTA (25mM) 6-aminohexanoic acid (25mM), NEM (10mM) benzamidine (1mM) for 48 hours at 4$^{o}$C with end over end mixing. The extract was recovered by centrifugation (2000g per 15 minutes) and residual tissue washed with a further five volumes of the same extraction buffer at 4$^{o}$C for two hours. The washings were again recovered by centrifugation and the washing and extract combined. Residual extracted tissue was thoroughly washed with distilled water and freeze dried. Proteoglycan extractability expressed in percentage was determined by measuring the hexuronate content of the 4M GuHCl extract as a proportion of the total hexuronate content of the tissue. (Hexuronate content of the papain digested residue plus 4M GuHCl extract).

Aliquots of extracts from each of the four disc zones containing approximately 500 micrograms of hexuronic acid were mixed with high molecular weight purified HA (HEALON 4% of PG hexuronate) and the GuHCl content of the extract were reduced to 0.4M by dialysis against nine volumes of 50mM of Tris at pH 7.4 containing the same proteneise inhibitors as in the extraction buffer, for 72 hours at 4$^{o}$C. Solid CsCl was then added to the samples to provide a starting density of 1.50g/ml and the PG samples were subjected to associative ultracentrifugation using a Sorval OTD65B ultracentrifuge and TV865, 8 per 5.0ml titanium vertical rotor at 50,000 rpm, 208,00 Gav for 24 hours at 4$^{o}$C. Ultracentrifuge samples were then frationated manually into 6 fractions each of 0.7ml of volume, and aliquots used to monitor hexuronic acid and protein by specified methods, and density by weighting an aliquot of known volume. The most dense fraction of density more than 1.55g/ml containing more than 80% of the hexuronic acid of the sample was used as a source of A1 purified proteoglycans. The percentage aggregation of A1 purified proteoglycan was determined by gel chromatography on a calibrated column of Sepharose CL2B (1.0 per 29.5cm) of an aliquot (approximately 100 micrograms hexuronic acid) of the PG sample. Elution was with 0.5M sodium acetate buffer pH 6.8 at a flow rate of 4.0ml per hour. Fractions of 0.25ml were collected and aliquots assayed for the presence of sulphated GAG. PG aggregation was assessed from the area of the excluded PG aggregate peak (of Kav = 0) as a proportion of the total area of the chromatogram.

5.3.2.6.2 Analytical Methods

Protein contents of 4M GuHCl extracts and ultracentrifugation fractions were determined by the Bicinchoninic acid method using BSA as standard, and 96 well microtitre plates.

Hexuronic acid was determined by the method of Blumenkrantz and Asboe-Hansen using D-glucuronolactone as standard.

Sulphated GAG was used to monitor PG separations by Sepharose CL2B gel chromatography using the dye 1,9-dimethylmethylene blue and a modification of the method of Farndale et al using 96 well micrititre plates and automatic absorbance measurement employing a Titertek microtitre plate reader (Flow Laboratories, Sydney).

Table VI (see text)

Table VII (see text)

## MRI AND DISCOGRAPHY
sheep with 5mm cut (NO. = 25)

| SHEEP (NO.) | DISCS TREATED (NO.) | M. R. I. SIGNAL 1 | 2 | 3 | SHAPE 1 | 2 | 3 | DISCOGRAPHY 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EARLY CHANGES (1-2 MONTHS) | | | | | | | | | | | |
| 4 | 12 | 12 | - | - | 10 | 2 | - | 10 | 2 | - | - |
| INTERMEDIATE CHANGES (4-12 MONTHS) | | | | | | | | | | | |
| 13 | 39 | 36 | 3 | - | 33 | 6 | - | 5 | 18 | 16 | - |
| LATE CHANGES (18-24 MONTHS) | | | | | | | | | | | |
| 8 | 24 | 10 | 12 | 2 | 18 | 4 | 2 | - | 6 | 7 | 11 |
| TOTAL | | | | | | | | | | | |
| 25 | 75 | 58 | 15 | 2 | 61 | 12 | 2 | 15 | 26 | 23 | 11 |

## MRI AND DISCOGRAPHY
sheep with 2.5mm cut

| SHEEP (NO.) | DISCS TREATED (NO.) | M. R. I. SIGNAL AND SHAPE 1 | 2 | 3 | 1 | 2 | 3 | DISCOGRAPHY 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| INTERMEDIATE CHANGES (4-12 MONTHS) | | | | | | | | | | | |
| 6 | 24 | 24 | - | - | 20 | 4 | - | 17 | 7 | - | - |
| LATE CHANGES (18-24 MONTHS) | | | | | | | | | | | |
| 4 | 16 | 9 | 6 | 1 | 10. | 5 | 1 | 5 | 5 | 5 | 1 |
| **TOTAL** | | | | | | | | | | | |
| **10** | **40** | **33** | **6** | **1** | **30** | **9** | **1** | **22** | **12** | **5** | **1** |

## 5.4 RESULTS

### 5.4.1 RADIOLOGICAL INVESTIGATION

The results of MRI and discography in 25 sheep with the 5mm deep cut and in 10 sheep with the 2.5mm deep cut are summarised in table VI and VII respectively.

Sheep were divided for the analysis of the results into three groups according to the time interval between the annulus lesion and sacrifice. The first group (early phase), with a follow up of one and two months; the second group (intermediate phase), with a follow up between four and 12 months and the third group (late phase), with a follow up of 18 and 24 months.

#### 5.4.1.1 Early Phase

Of the 12 treated discs from the four sheep with a 5mm deep cut none showed any reduction in the intensity of the signal at MRI. Signal shape was graded as normal in ten and as slightly enlarged in the remaining two. There was good correlation with discography as the only two abnormal discograms corresponded to the two MRI with enlarged signal from the central area of the disc (fig.27).

#### 5.4.1.2 Intermediate Phase

Of the 39 treated discs from 13 sheep with 5mm deep annular lesions MRI signal intensity was graded normal in 36 and reduced in the remaining three. Signal shape was considered normal in 33 and moderately enlarged in the remaining six.

Discography, however, was classified as normal in only five of the 39 treated discs. In 18 the contrast showed some extravasation into the inner or outer annulus [fig.28] and in 16 the contrast was seen leaking anteriorly along the anterior longitudinal ligament, highlighting the presence of a radiating tear connecting the original outer cut with the nucleus pulposus.

Of the 24 discs with the 2.5mm annular cut none had decreased intensity of signal at MRI. Discography, however, was graded as showing some extravasation of contrast towards the periphery of the annulus in seven of the 24 discs. The remaing 17 discograms were graded as normal [fig.30].

#### 5.4.1.3 Late Phase

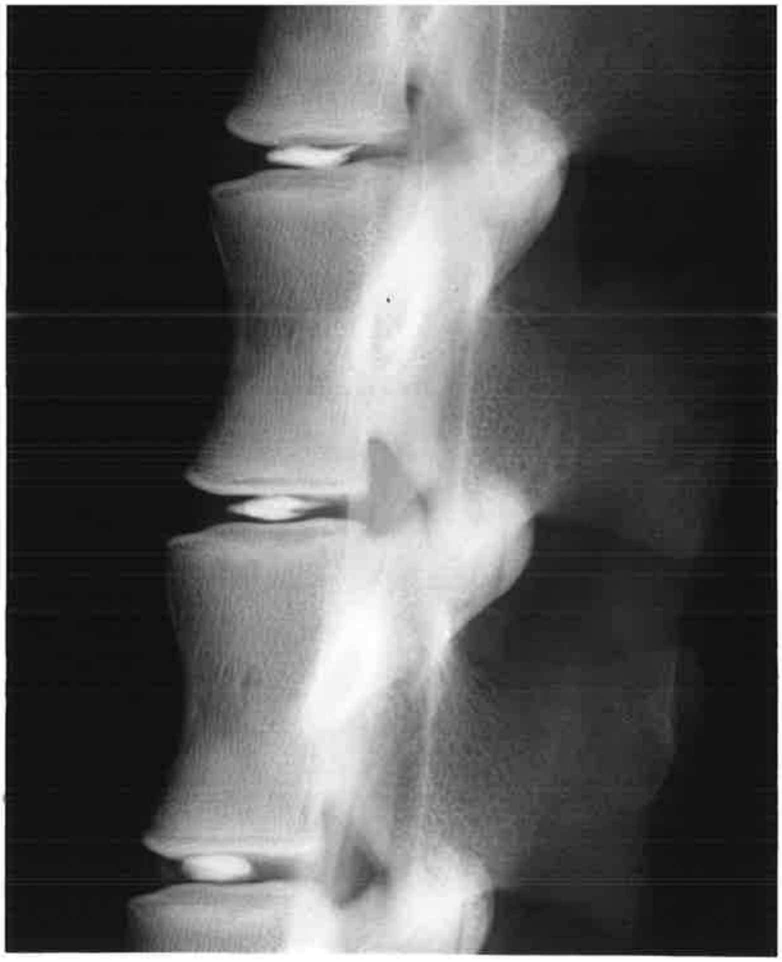
Of the 24 treated discs from the eight sheep with 5mm deep cuts, signal intensity at MRI was graded normal in ten and reduced in 12. In the remaining two because of severe dehydration of the disc and subsequent loss of signal no difference was seen between the central zone and the periphery of the disc. Of the 22 discs with central signal present at sagittal $T_2$ weighted images, signal shape was graded normal in 18 and enlarged in four. In comparison, discography demonstrated a greater sensitivity in highlighting abnormal disc morphology. None of the discograms in this group in fact were normal and in 18 marked degeneration as highlighted by the contrast was observed [fig. 31 and 32].

Figure 27A

(Top) Three level discogram in sheep with 5mm deep lesion sacrificed at 2 months. The upper level is a control, the bottom two levels have been treated with 5mm annular cuts. An inner annulus tear is seen highlighted by the contrast in the bottom disc.

Figure 27B

(Bottom) $T_2$ weighted MRI of same specimen seen in figure 27A. Intensity of signal is normal at all three levels. There is some enlargement of the shape of the signal at the bottom level which is in keeping with the discographic appearance seen above.

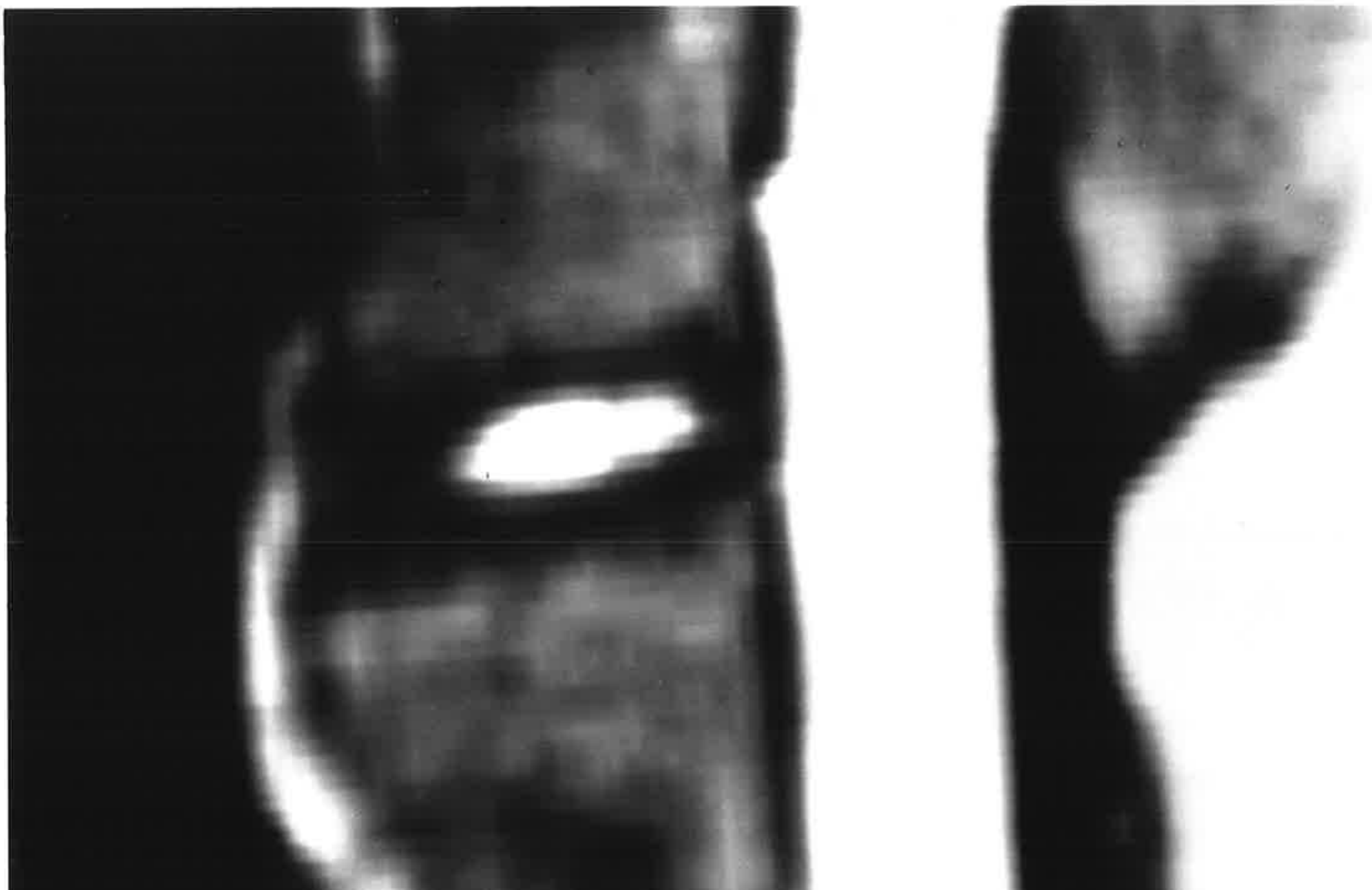


igure 28A

(Top) Discogram of sheep with 5mm deep lesion sacrificed at 12 months. A radiating tear is seen in the anterior annulus extending to the site of the original outer cut. There is no extravasation of contrast outside of the disc boundary.

Figure 28B

(Bottom) $T_2$ weighted MRI of same disc seen in figure 28A. The intensity and the shape of the signal from the central part of the disc is normal.



igure 29A

(Top) Discogram of sheep with 2.5mm deep lesion sacrificed at 18 months. An anterior inner annular tear is seen with extension of the contrast to the mid portion of the annular lamellae.

'igure 29B

(Centre) Discogram of sheep with 2.5mm deep lesion sacrificed at 24 months. A radiating tear is seen extending from the nucleus pulposus to the anterior annulus. There is normal nuclear pattern in the centre part of the disc.

'igure 29C

(Bottom) Discogram of sheep with 2.5mm deep lesion sacrificed at 18 months. No evidence of radiating tears is seen. Some remodelling of the anterior corners of the bony end plate is seen which may suggest early osteophyte formation.



Figure 30

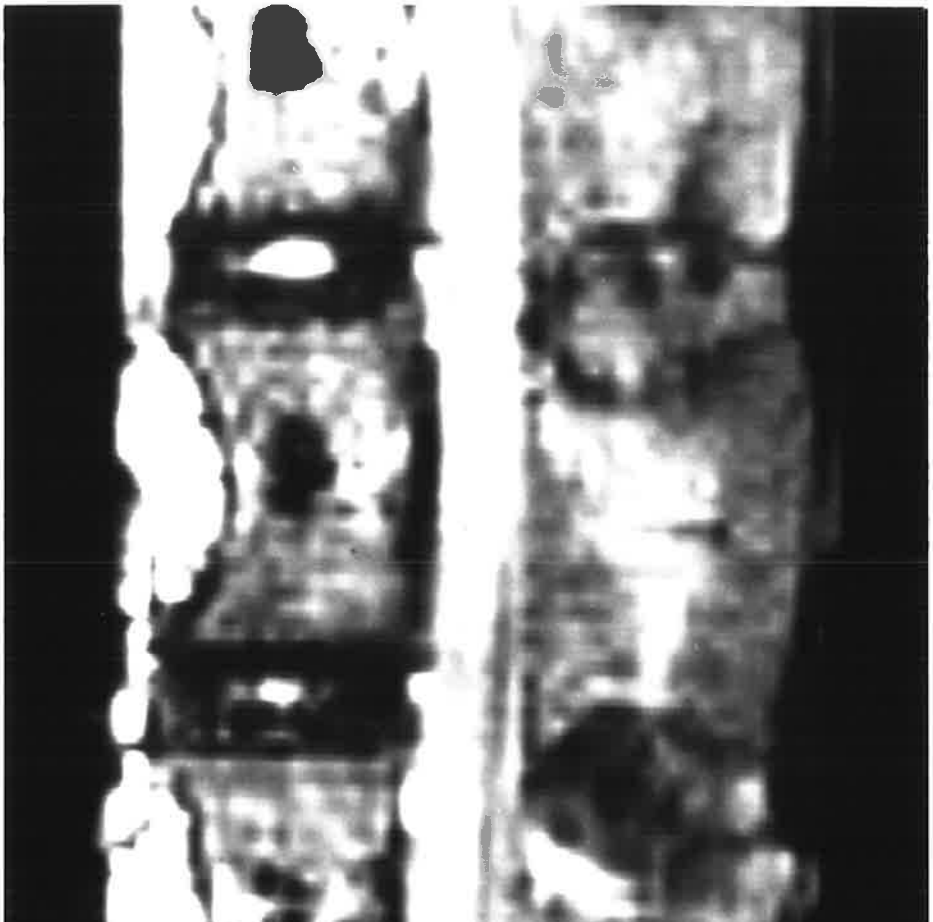
Five level discography in sheep with 2.5mm deep lesion. The metallic clip is adjacent to the bottom level (L5-6). All discograms have normal appearance.

'igure 31A

(Top) Two level discography in sheep with 5mm deep lesion sacrificed at 18 months. The top level is a control. The bottom level shows contrast pattern consistent with marked degeneration.

Figure 31B

(Bottom) $T_2$ weighted MRI of same specimen seen in figure 31A. There is marked reduction in intensity of the signal from the bottom disc which is in keeping with the discographic appearance.

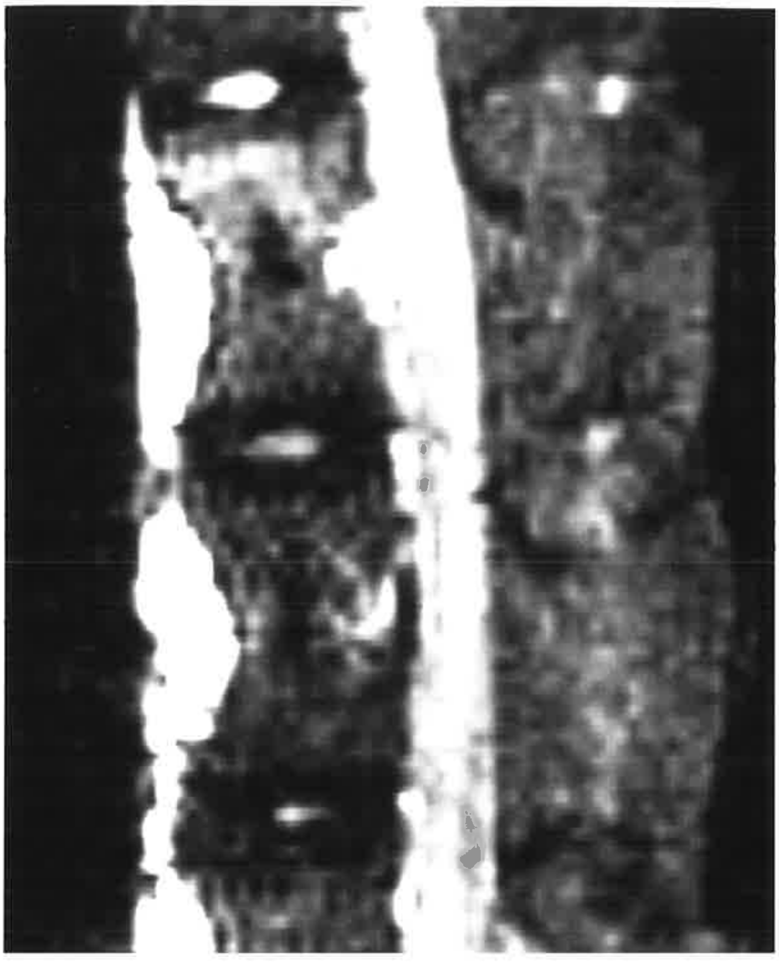


gure 32A

(Top) Three level discogram in sheep with 5mm deep lesion sacrificed at 18 months. The top level is a control. The central and lower level show contrast pattern consistent with marked disc degeneration.

'igure 32B

(Bottom) $T_2$ weighted MRI of same specimen seen in figure 32A. There is marked reduction of signal intensity in both central and bottom levels in keeping with the discographic appearance.



Of the the 16 discs treated with the shallower cut of 2.5mm, nine had normal signal intensity at MRI, six had reduced signal intensity from the central zone of the disc and in one signal was absent. Discography was considered normal in five discs (fig.29). Six discograms showed marked degeneration with extravasation of contrast anteriorly along the longitudinal ligament.

## 5.4.2 MORPHOLOGICAL ANALYSIS AND CONVENTIONAL HISTOLOGY

### 5.4.2.1 Macroscopic Appearance at Operation

At the time of surgery following the annulus cut no prolapse of nucleus material through the lesion was seen in any of the 75 discs treated with a 5mm deep cut or in the 40 discs treated by 2.5mm deep cut. All animals made an uneventful post-operative recovery and were able to mobilise freely with no limitations within three days of operation.

### 5.4.2.2 Early Phase (one and two month post-operation)

12 discs with 5mm deep cut (figs. 33, 34, 35, 36, 37 and 38).

#### 5.4.2.2.1 Macroscopic Appearance

The macroscopic appearance is summarised in Table VIII.

Failure of the inner annulus was observed in relation to the annulus cut in only one of the twelve operated discs included in this group. This was accompanied by nuclear displacement through the defect to the outer annulus(fig.33). Early nuclear degeneration, as assessed by the lack of bulging of the cut surfaces, presence of clefts, and the early loss of definition between outer nucleus and inner annulus, was observed in five of the twelve operated discs. Moderate narrowing of the intervertebral disc space measured at the centre of the disc in the mid sagittal slab was seen in two of the twelve operated discs. However, in both instances the decrease in height represented less than 10 percent of the same measurement in the control discs. None of the operated discs showed macroscopic evidence of anterior osteophyte formation. Nuclear pigmentation was not observed in any of the discs.

Table VIII (see text)

MACROSCOPIC APPEARANCE OF SHEEP INTERVERTEBRAL DISCS
5mm deep lesion

TIME INTERVAL BETWEEN OPERATION & SACRIFICE (MONTHS)

| | | 1/12 | 2/12 | 4/12 | 6/12 | 8/12 | 12/12 | 18/12 | 24/12 |
|---|---|---|---|---|---|---|---|---|---|
| TOTAL NUMBER OF TREATED DISCS | | 6 | 6 | 6 | 9 | 12 | 12 | 12 | 12 |
| INNER ANNULUS FAILURE | | 1 | - | 4 | 8 | 12 | 12 | 12 | 12 |
| NUCLEAR DEGENERATION | - ABSENT | 4 | 3 | 3 | 1 | 1 | - | - | - |
| | - MODERATE | 2 | 3 | 3 | 7 | 10 | 2 | 2 | 4 |
| | - MARKED | - | - | - | 1 | 1 | 10 | 10 | 8 |
| INTERVERTEBRAL DISC NARROWING | - ABSENT | 5 | 5 | 4 | 4 | 1 | — | — | — |
| | - MODERATE | 1 | 1 | 2 | 5 | 11 | 10 | 2 | 6 |
| | - MARKED | - | - | - | - | — | 2 | 10 | 6 |
| OSTEOPHYTES | | - | - | 2 | 7 | 12 | 12 | 12 | 12 |

.gure 33A

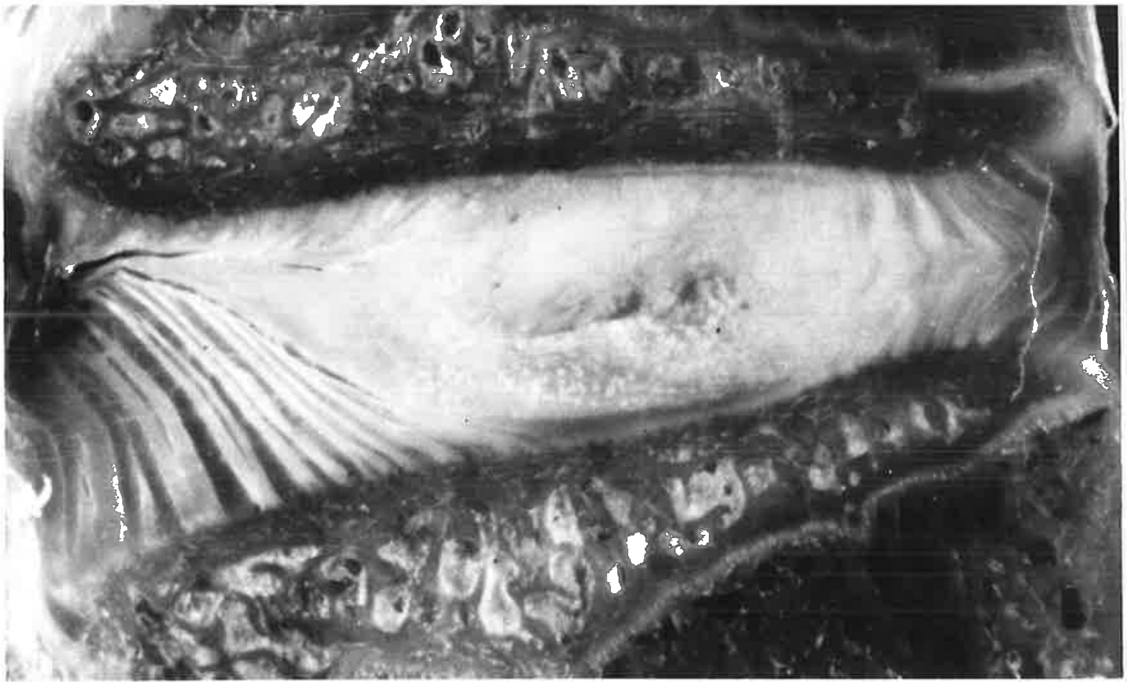
(Top) Macro photograph of sheep intervertebral disc treated by a 5mm deep cut at 2 month follow up. Failure of the inner annulus is seen in relation to the original peripheral tear with nuclear displacement through the defect to the outer annulus. There is anterior infolding of the inner lamellae of the posterior annulus.

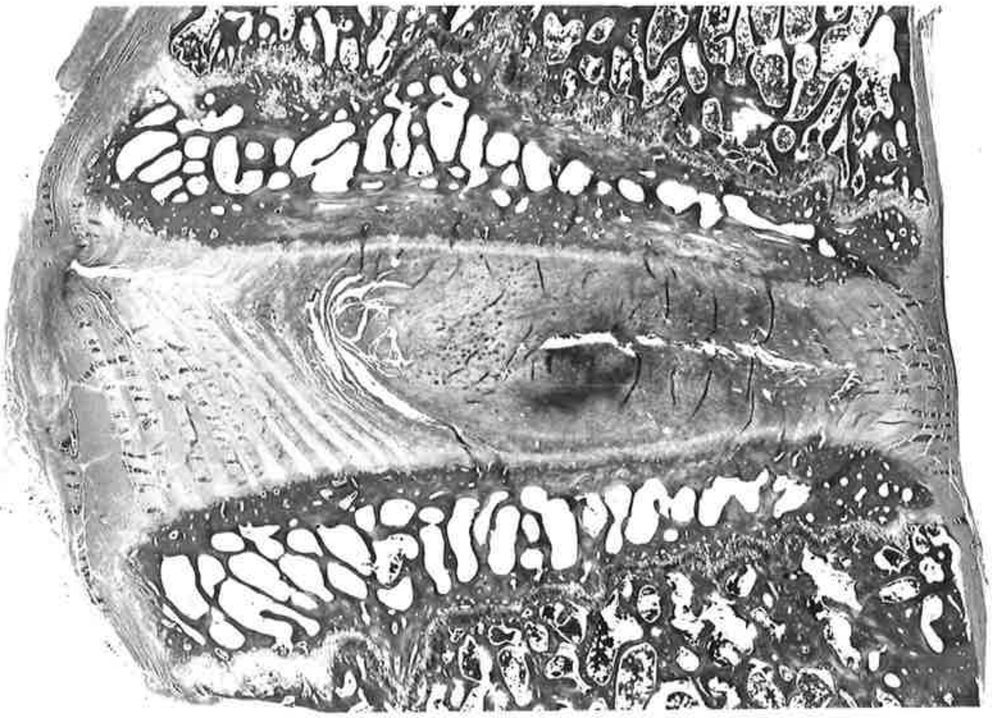
'igure 33B

(Centre) Low power micrograph of same disc of figure 33A. The disorganisation of orientation of the lamellae of the inner anterior annulus is seen accompanied by marked clefting of the area between annulus and nucleus pulposus.

'igure 33C

(Bottom) High power micrograph of same disc of figg. 33A and B. Vascularised granulation tissue is seen in the outer annulus fibrosus. Whilst healing of the outer part of the tear is seen no attempt at bridging the original defect is observed in the mid and inner portion of the original defect. Multiple concentric clefts are seen extending from the transverse cut along the annular lamellae.



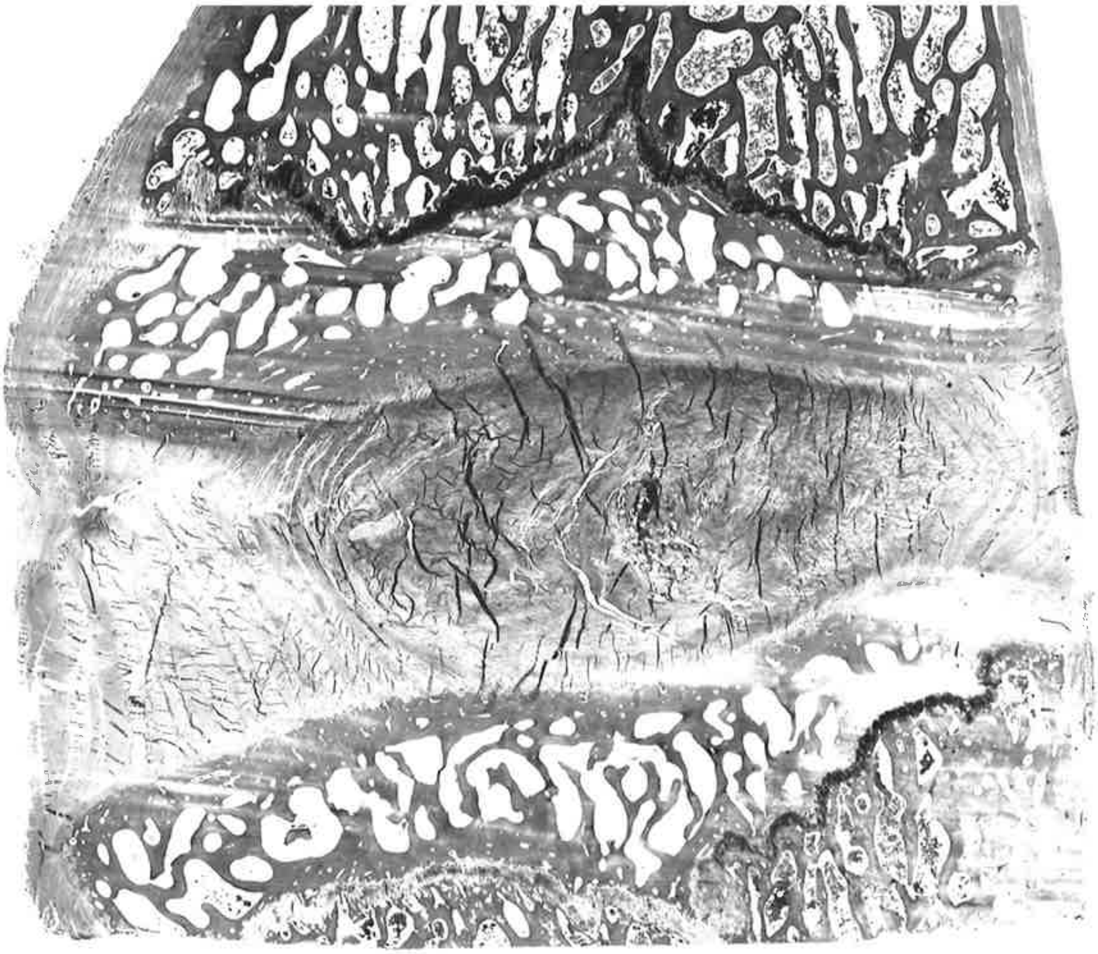


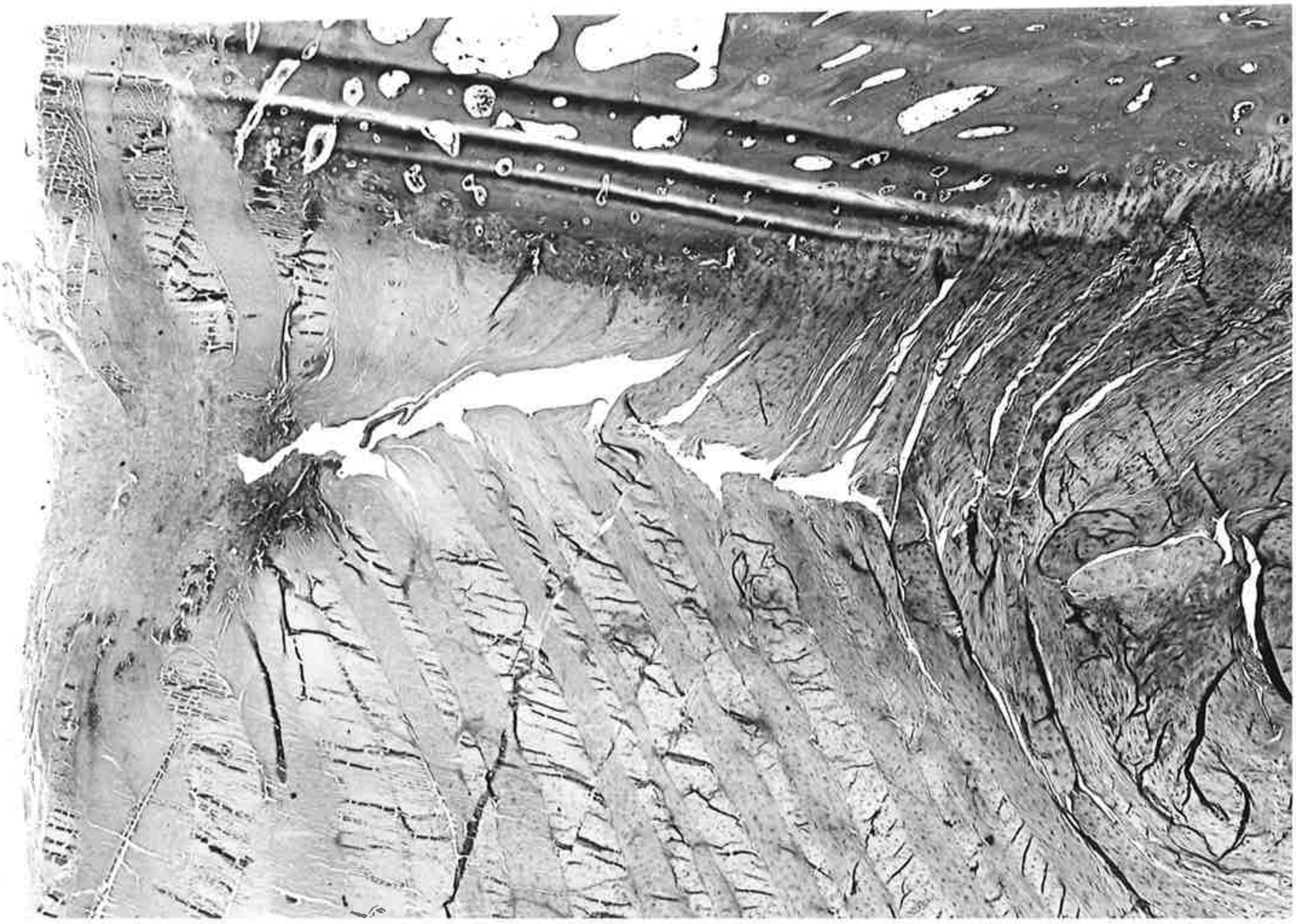
igure 34A

(Top) Low power micrograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 2 months. The original cut is seen parallel and adjacent to the upper end plate with granulation tissue obliterating the outer part of the original lesion. Disc height is preserved and the overall appearance of the nucleus pulposus is within normal limits.

igure 34B

(Bottom) High power micrograph of same disc shown in figure 34A. The extent of vascularised granulation tissue is seen in the outer portion of the annulus fibrosus. Spontaneous failure of the inner annulus is seen in relation to the original cut with multiple concentric clefts radiating towards the upper end plate. Vascularised tissue is seen extending from the outer boundary of the annulus fibrosus to approximately a third of the depth of the anterior annulus.





igure 35A

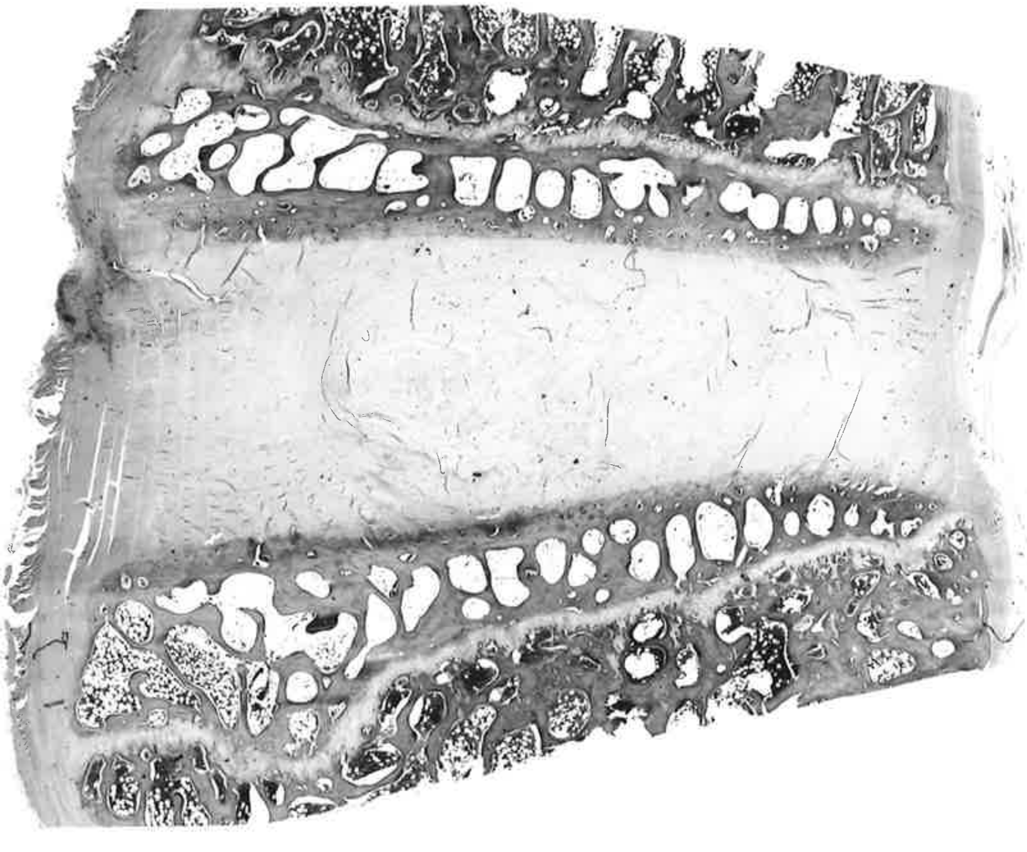
(Top) Macro photograph of sheep intervertebral disc with 5mm deep cut sacrificed at 1 month. Highly vascular immature granulation tissue is seen obliterating the outer third of the original cut. Deformation of the annular lamellae along the course of the cut is seen in the mid annulus. The appearance of the nucleus pulposus is within physiological limits.

igure 35B

(Centre) Low power micrograph of same disc of figure 35A. There is no apparent failure or distortion of the inner annulus fibrosus.

igure 35C

(Bottom Left) High power micrograph of anterior annulus of disc of figure 35A and B. The extent of the granulation tissue which has reached the outer part of the original cut is seen. Note the loss of vascularity in the inner portion of the lesion.



'igure 36A

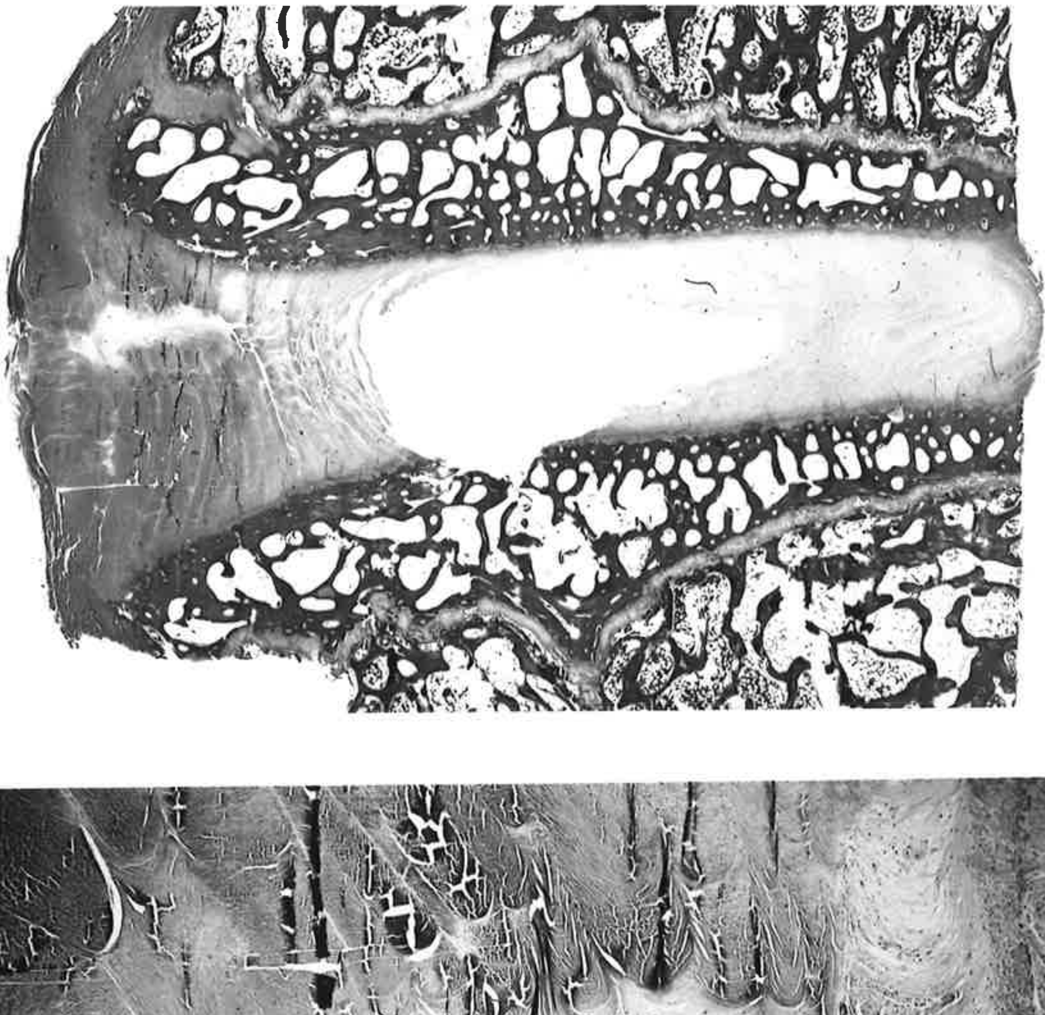
(Top) Macro photograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 2 months. The inner annulus is intact and the original cut is seen in the area of the anterior rim.

Figure 36B

(Centre) Low power micrograph of same disc seen in figure 36A. The absence of nuclear material from the specimen is due to artefact.

Figure 36C

(Bottom) High power micrograph of rim lesion of the disc seen in figg. 36A and B. Vascular granulation tissue is seen in the region of the outer annulus obliterating the original defect. Cellular lining of the cut is seen extending to the inner third of the lesion and disorganisation of the inner annulus is observed with presence of concentric clefting.



igure 37A

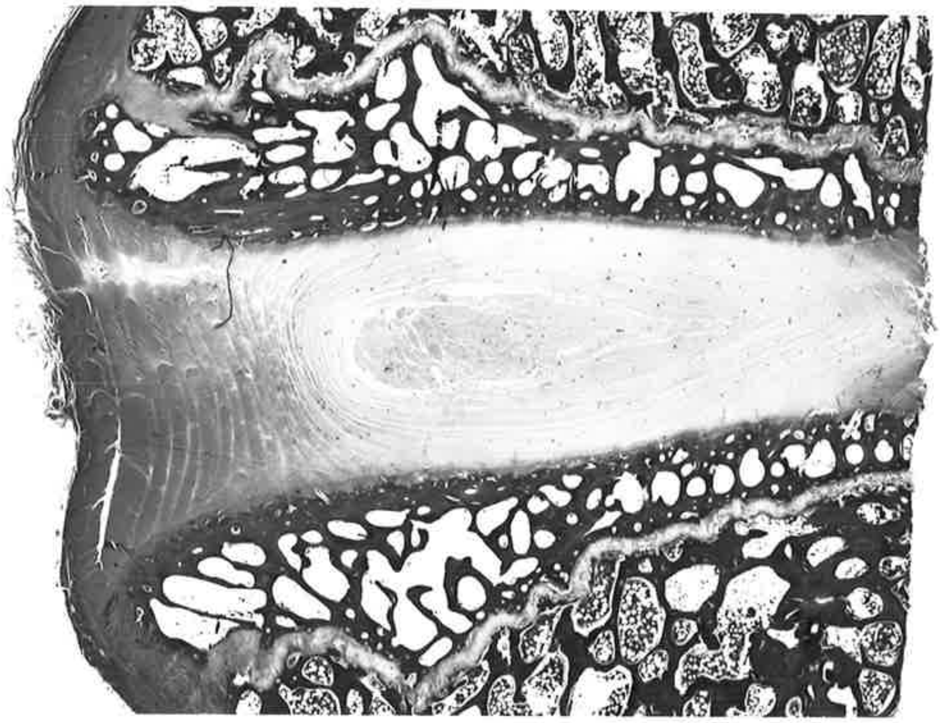
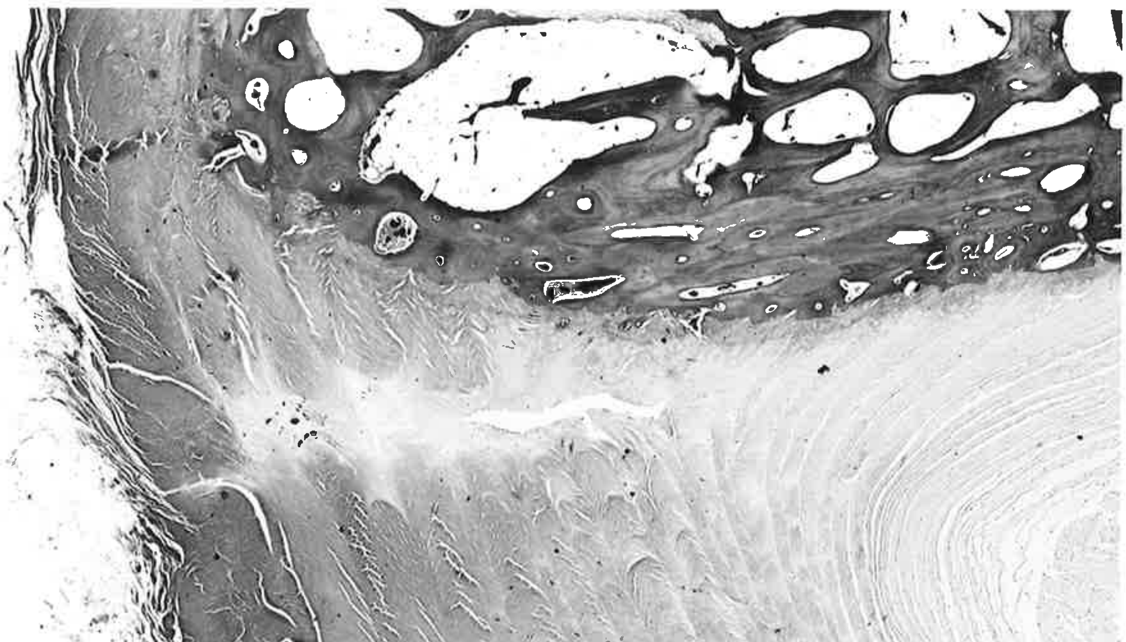
[Top] Photograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 2 months. Note the similarity with the appearance of the disc of figure 36A.

'igure 37B

[Centre] Low power micrograph of disc seen in figure 37A.

Figure 37C

[Bottom] High power micrograph of lesion of disc seen in figure 37A and B. The outer half of the lesion is obliterated by granulation tissue. The bony rim appears normal with no evidence of osteophyte formation.

igure 38A

[Top] Photograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 2 months. The original cut is seen parallel to the antero-superior end plate. Although some disorganisation may be seen in the inner annulus the remaining disc appears grossly intact.

igure 38B

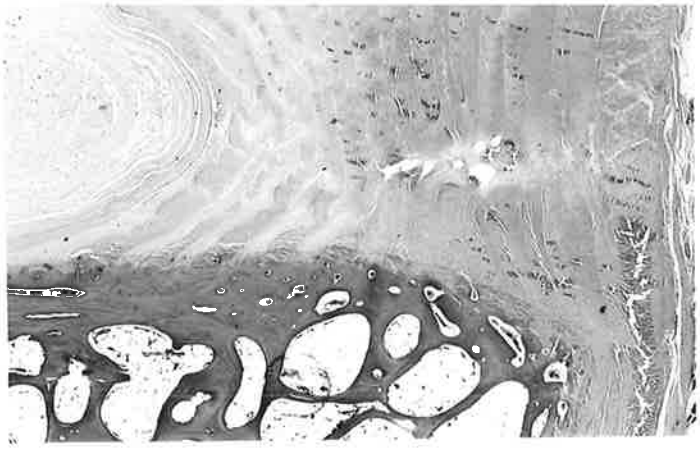
[Centre] Low power micrograph of same disc of figure 38A.

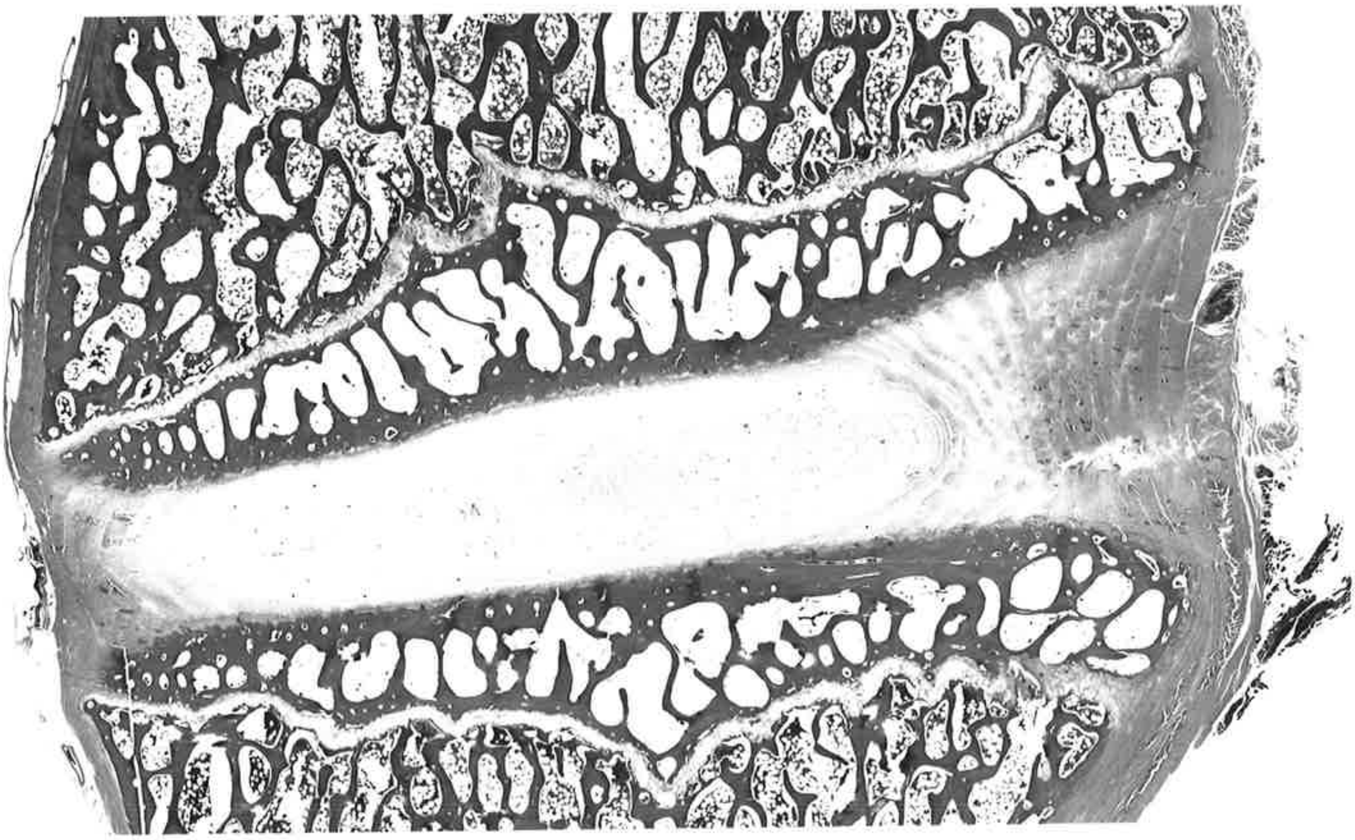
Figure 38C

[Bottom Left] High power micrograph of disc seen in figg. 38A and B. Cystic cavitations are seen due to vascularisation of the granulation tissue produced by the lesion. The inner annulus appears intact.

Figure 38D

[Bottom Right] Magnification of typical cystic lesion seen as a result of the highly vascular granulation tissue caused by the annulus defect.





5.4.2.3.2 Microscopic Features

In all of the operated discs granulation tissue was present, in the peripheral annulus obliterating the outer third of the cut, and this was markedly vascularised in ten of the twelve treated discs. Whilst vessel proliferation was seen extending along the margins of the cut into the mid annulus, the defect was never bridged in its middle and inner portions. Multiple concentric clefts radiating vertically from the transverse cut towards both end plates and separating the annular layers were seen, with larger circumferential clefts extending towards the inner annulus. Plump fibroblast type cells formed the margins of the outer half of the lesion. Ingrowth of vascularised tissue was generally observed to end abruptly at the junction between the outer and middle third of the annulus fibrosus. Cystic cavitations related to highly vascular granulation tissue in the peripheral annulus were observed in two operated discs(fig. 38). With the use of polarised light, early disorganisation and irregularity of the collagen lamellae of the inner annulus was detected in close relation to the inner portion of the transverse cut. Initial nuclear degeneration was observed in five of the twelve operated discs consisting of multiple clefts within the nuclear substance, areas of amorphous granular material devoid of cells, and clustering of nuclear cells suggesting early chondroid metaplasia. In the disc where nuclear displacement had occurred due to failure of the inner annulus, degenerative changes were more marked and were accompanied by infolding of the contralateral inner annulus toward the site of displacement.

### 5.4.2.3 Intermediate Phase (four to twelve months post-operation)

39 nine discs with 5mm deep lesions and 18 discs with 2.5mm deep lesions(fig. 39, 40 and 41).

#### 5.4.2.3.1 Macroscopic Appearance

The macroscopic features are summarised in table VIII and IX.

36 of the 39 operated discs from the 13 sheep with 5mm deep lesion included in this group showed failure of the inner annulus lamellae in proximity to the transverse annular cut. This was accompanied by secondary nuclear displacement along the cut towards the outer annulus and limited by only the healed outer annulus lamellae (fig. 39). Conversely, of the 18 discs in this group with 2.5mm deep lesion only one showed evidence of inner annular failure.

In 10 of the 12 operated discs from the four sheep sacrificed at 12 months with 5mm deep lesion, marked disc degeneration was evidenced by significant cleft formation, pigmentation and fraying. None of the 18 discs with 2.5mm deep lesions in this group had marked nuclear degeneration.

Table IX (see text)

## MACROSCOPIC APPEARANCE OF SHEEP INTERVERTEBRAL DISCS
2.5mm deep lesion

TIME INTERVAL BETWEEN OPERATION & SACRIFICE (MONTHS)

| | | 4/12 | 8/12 | 12/12 | 18/12 | 24/12 |
|---|---|---|---|---|---|---|
| TOTAL NUMBER OF TREATED DISCS | | 6 | 6 | 6 | 6 | 6 |
| INNER ANNULUS FAILURE | | - | 1 | - | - | 4 |
| NUCLEAR DEGENERATION | - ABSENT | 6 | 1 | 4 | 1 | - |
| | - MODERATE | - | 5 | 2 | 5 | 6 |
| | - MARKED | - | - | - | - | - |
| INTERVERTEBRAL DISC NARROWING | - ABSENT | 6 | 2 | 5 | 4 | 1 |
| | - MODERATE | - | 4 | 1 | 2 | 3 |
| | - MARKED | - | - | - | - | 2 |
| OSTEOPHYTES | | - | 1 | - | 3 | 4 |

'igure 39A

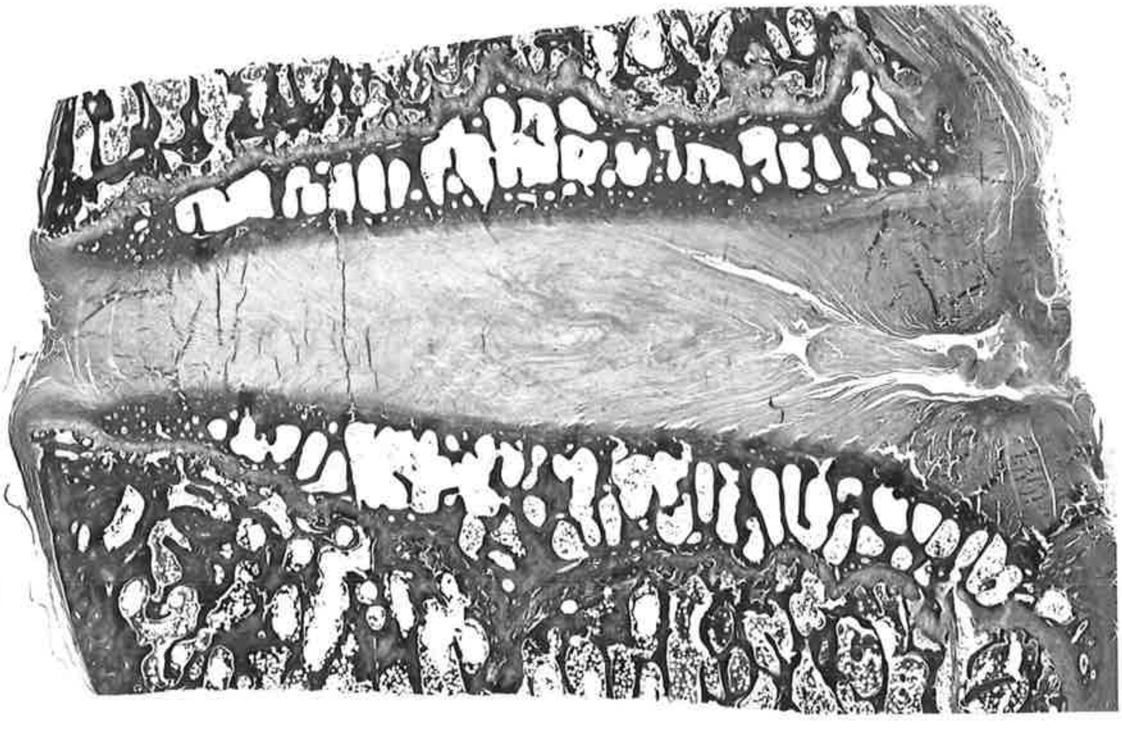
(Top) Photograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 12 months. An obvious radiating cleft is seen parallel to the upper end plate. There is displaced nuclear tissue contained within the defect. Gross deformation of the annular lamellae is seen with displacement towards the site of the lesion.

'igure 39B

(Centre) Low power micrograph of disc seen in figure 39A. There is moderate narrowing of the intervertebral disc space and nuclear tissue contained within the annulus defect is seen extending to the periphery of the intervertebral disc.

Figure 39C

(Bottom) High power micrograph of annular defect seen in figure 39A and B. Vascular granulation tissue is seen at the upper margin of the outer third of the annulus defect. Metaplastic nuclear material is contained within the cleft. This is contained within the intervertebral disc by newly synthesised collagen bridging the outer defect.

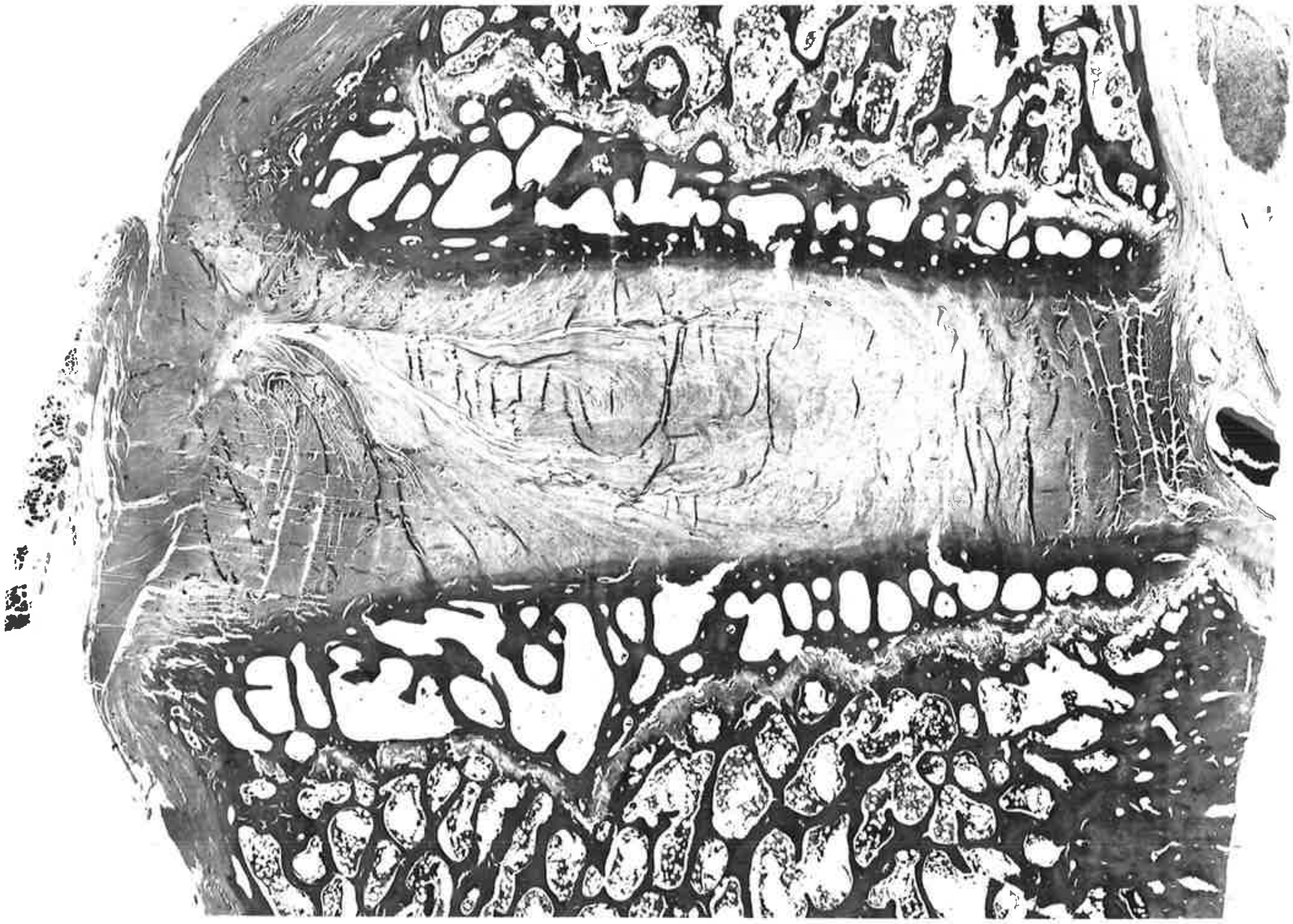


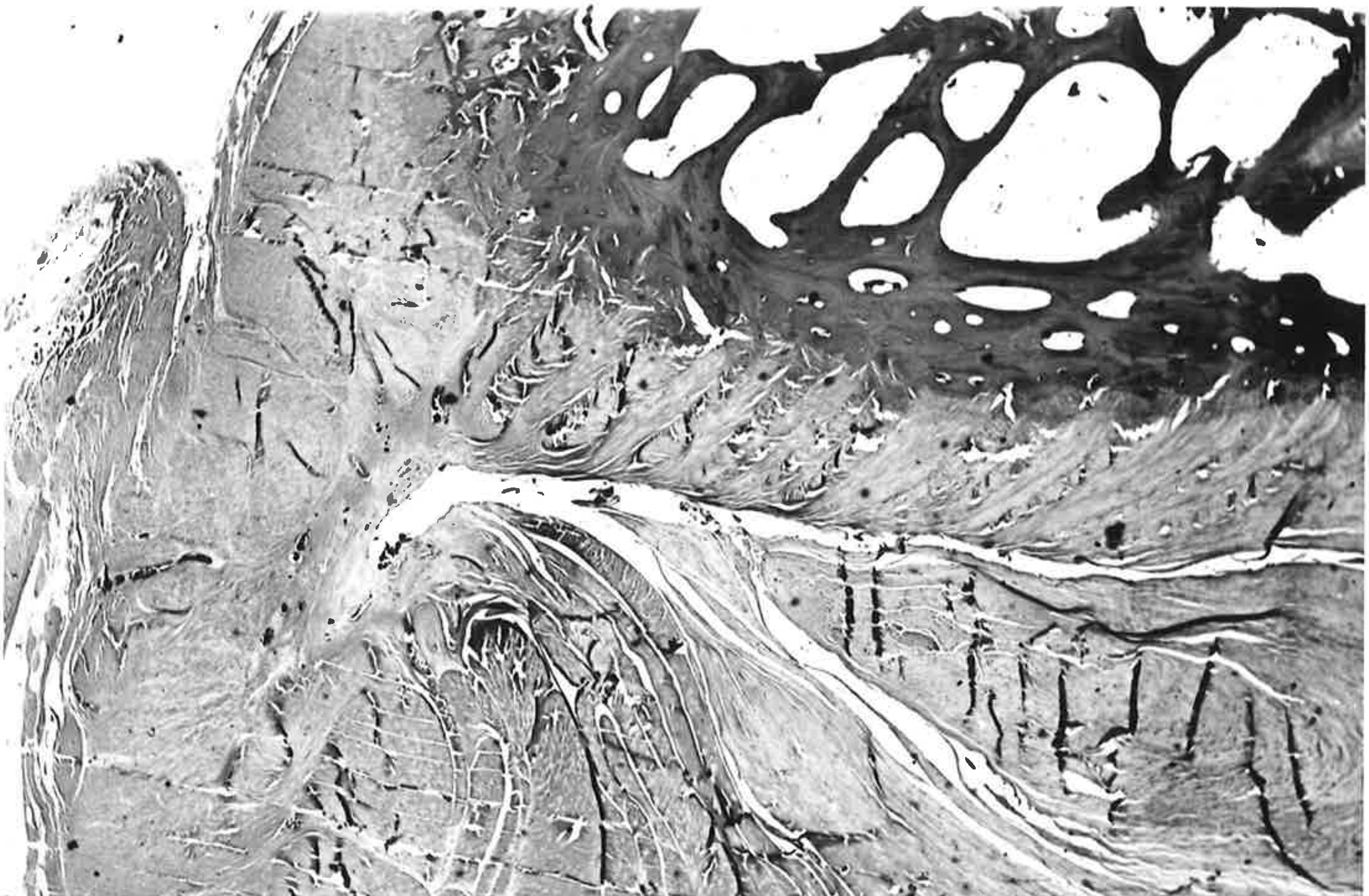
'igure 40A

[Top] Low power micrograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 12 months. Although the overall height is only moderately reduced nuclear material is displaced towards the site of the original lesion with clefts extending from the initial cuts towards the central portion of the disc. The outer annulus has healed but the collagen bundles of the outer annulus appear distorted in relation to the cut.

Figure 40B

[Bottom] High power micrograph of disc seen in figure 40A. Mature granulation tissue in the outer annulus is filling the original defect and folding of the annular bundles is observed towards the periphery in relation to the cut. Displaced nuclear material undergoing chondrometaplasia is seen at the junction between mid and outer annulus.





igure 41A

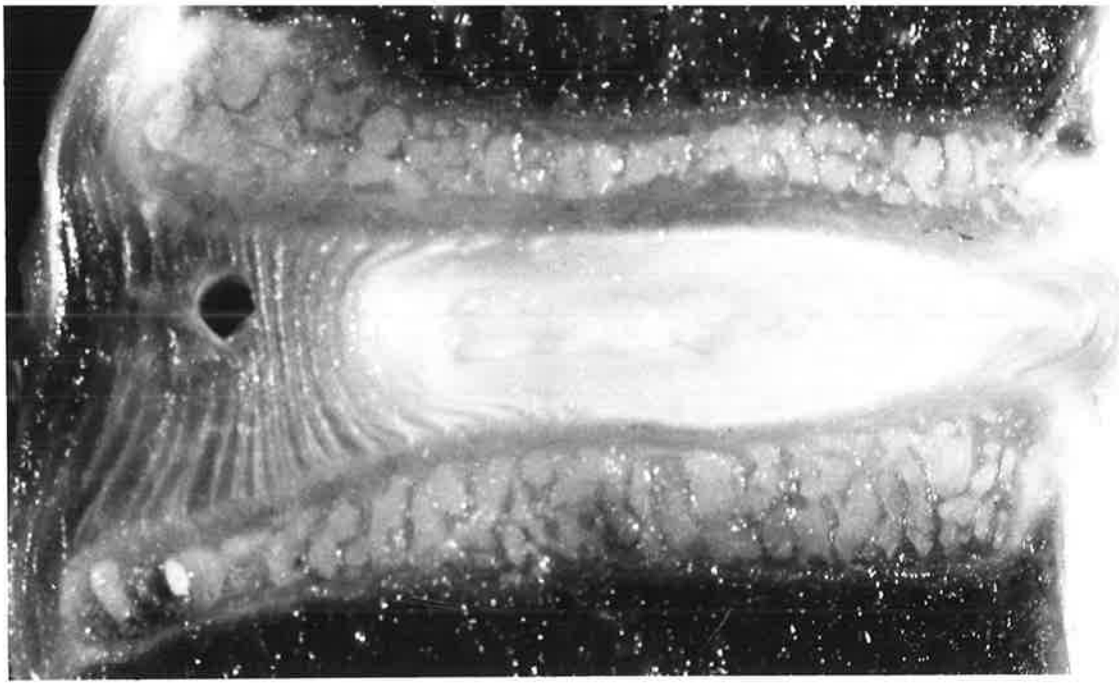
[Top] Photograph of sheep intervertebral disc with 2.5mm deep lesion sacrificed at 4 months. A large cystic defect is seen at the junction between mid and outer third of the anterior annulus. The inner annulus is intact.

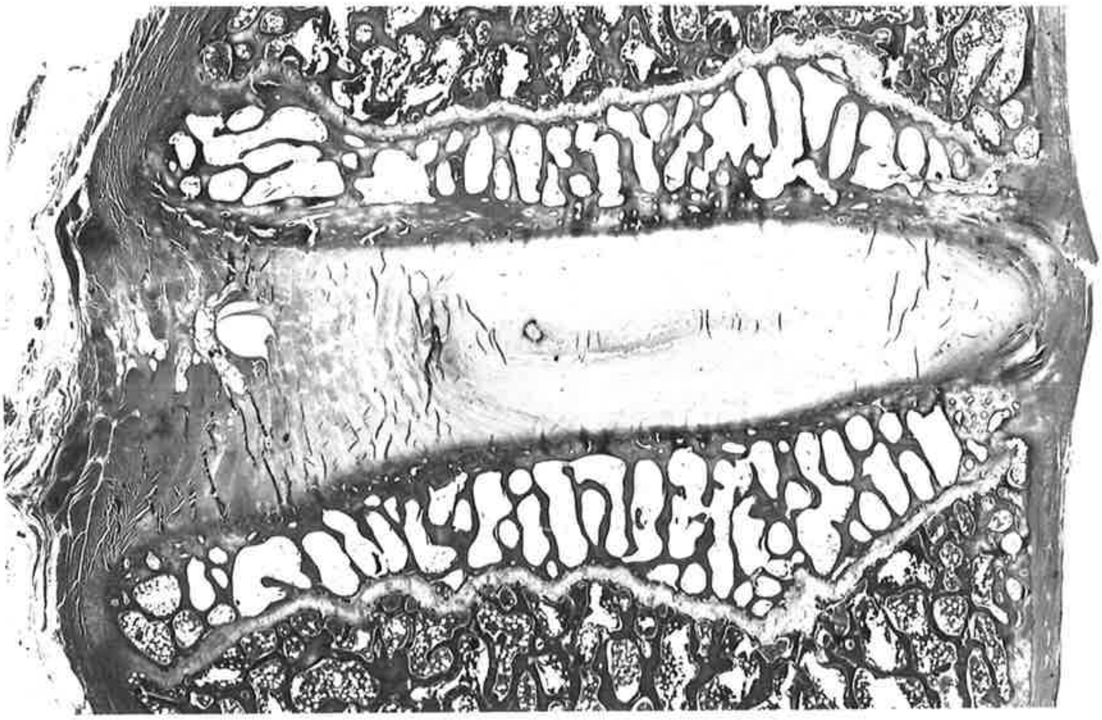
'igure 41B

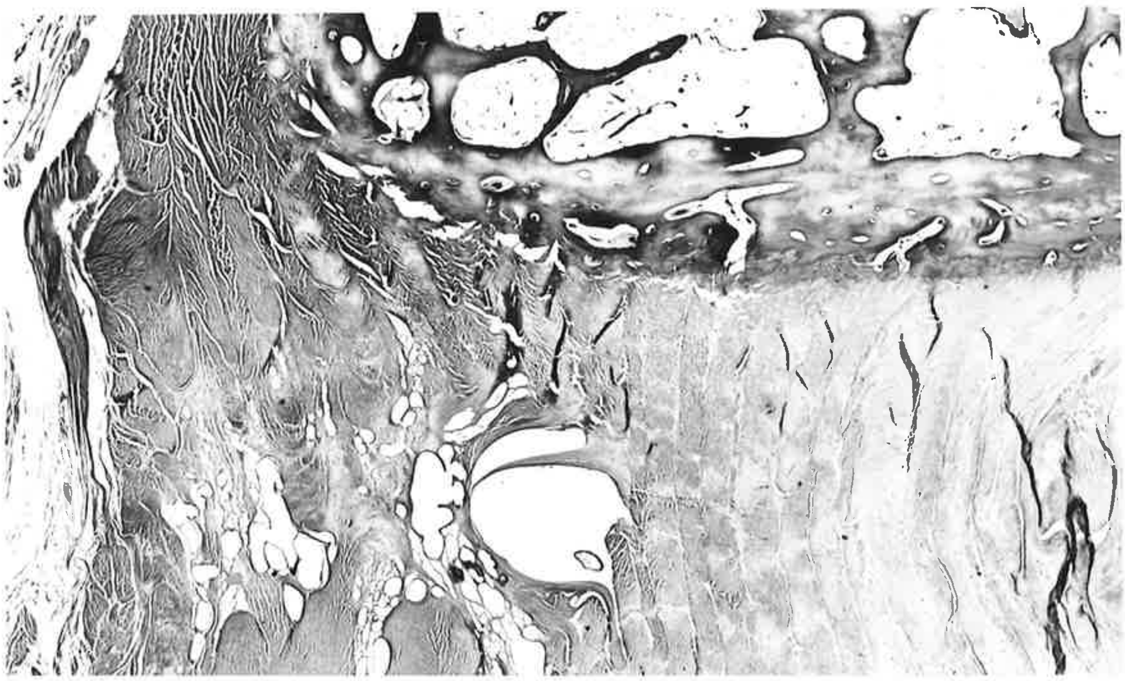
[Centre] Low power micrograph of disc seen in figure 41A. Extended cystic degeneration of the outer and mid anterior annulus is seen at the site of the original annulus defect. There is no apparent involvement of the inner annulus.

Figure 41C

[Bottom] High power micrograph of defect seen in figure 41A and 41B. The extent of the degenerated tissue with characteristic cystic appearance can be observed. This is the result of the vascularisation of the granulation tissue formed in response to the original annular defect.







Moderate narrowing of the intervertebral disc space ranging from 5 to 25 percent of the height of the non-operated controlled discs was observed in seven of the 15 operated discs from the five sheep with 5mm deep lesion sacrificed between four and six months. All but one of the remaining 24 treated discs from the eight sheep with 5mm deep lesion sacrificed between eight and 12 months demonstrated moderate intervertebral disc narrowing. Intervertebral disc narrowing was observed in five of the 18 discs in this group with 2.5mm deep lesion. In the remaing 13 intervertebral discs space height was classified as normal.

Early osteophyte formation was observed in nine of the 15 discs from the sheep with 5mm deep lesions sacrificed at four and six months. Ostoephytes were visualised with the aid of a dissecting microscope in all of the remaining treated discs from the sheep with 5mm deep lesions sacrificed at eight and 12 months. Of the 18 discs with 2.5mm deep lesion however, only one demonstrated initial osteophyte formation.

None of the control discs demonstrated intervertebral disc narrowing, evidence of nuclear degeneration or osteophyte formation.

#### 5.4.3.2.2 Microscopic Features

In all operated discs granulation tissue of varying stages of maturity obliterated the outer third of the original cut (fig.40).

A characteristic feature observed in the six discs with 2.5mm deep lesion from the two sheep sacrificed at four months was the cystic appearance of the area of the original cut due to highly vascular granulation tissue(fig.41). The appearance of the defect in the outer and mid annulus in these discs had striking similarities to morphological features of cystic degeneration of the annulus seen in human lumbar discs.

In the sheep sacrificed at eight and twelve months, collagenized scar tissue bridged the outer region of the defect. However, healing did not occur in the middle and inner third of the original transverse cut in any operated disc. In this group formation of radiating and circumferential clefts had become more extensive. In some instances, clefts along the annular lamellae extended through the centre of the disc into the posterior annulus fibrosus. The annulus lamellae along the margins of the cut showed characteristic deformation and folding towards the site of the lesion. Marked features of degeneration were seen in the nucleus pulposus, and included areas of chondroid metaplasia which were most marked within the displaced tissue. Occasional sequestration of central portions of nuclear tissue due to marked cleft formation was seen, with separation from the cartilage end plate. Vascularisation of the nucleus was not seen in any of the operated discs examined. Chondrosis and initial ossification of the end plates was noticed in fifteen of the twenty nine treated discs. These changes did not appear to be localised to the area of the annulus cut, but occurred along the whole length of the end plate. Endochondral ossification along the anterior margin of the vertebral body with initial osteophyte formation was evident in the majority of operated discs and no significant difference in new bone formation could be observed between the end plate adjacent to the annulus and the contralateral. New bone formation seemed to be more marked where the nuclear displacement had been accompanied by intervertebral disc narrowing.

5.4.2.4 Late Phase (eighteen to twenty-four months post-operation)

24 treated discs with 5mm deep lesion, 12 treated discs with 2.5mm deep lesion (figs. 42, 43, 44, 45, 46 and 47).

5.4.2.4.1 Macroscopic appearance

The macroscopic features observed are summarised in table VIII and IX.

Inner annulus failure with displacement of nuclear material towards the site of the original annulus cut was observed in all 24 discs with 5mm deep lesions. In the twelve discs with 2.5mm deep lesions, however, inner annulus failure was seen in only four cases.

Nuclear degeneration was graded as marked in 18 of the 24 discs with 5mm deep lesion. None of the eight remaining discs in this group had normal nuclear appearance. In contrast, of the 12 discs with 2.5mm deep lesion sacrificed at the 18 and 24 month follow up, one had no evidence of macroscopic nuclear degeneration and in the remaining 11 nuclear degeneration was graded only as moderate.

Marked intervertebral disc narrowing (more than 50%) was seen in 16 of the 24 discs with 5mm deep lesions. Moderate narrowing was seen in the remaining eight. Of the twelve discs with 2.5mm deep lesions five had normal disc height, five moderate narrowing and only two marked reduction in intervertebral disc space.

Osteophytes were seen in all operated discs with 5mm deep lesions with new bone formation present symmetrically on both end plates.

This was in contrast with the observation from the 12 discs with 2.5mm deep lesions where osteophytes were seen in only four cases.

As with the intermediate phase group, osteophyte formation was more marked in the discs with pronounced intervertebral disc narrowing and peripheral nuclear displacement.

5.4.2.4.2 Microscopic feature

Vascularisation of the outer annulus fibrosus related to the original annulus cut was seen to a varying degree in all operated discs (fig.42). This was classified as marked in one of the 24 operated discs with 5mm deep lesion moderate in 18 discs with 5mm deep lesion, and in all 12 discs with 2.5mm deep lesion and mild in the remaining five discs with 5mm deep lesion.

Figure 42

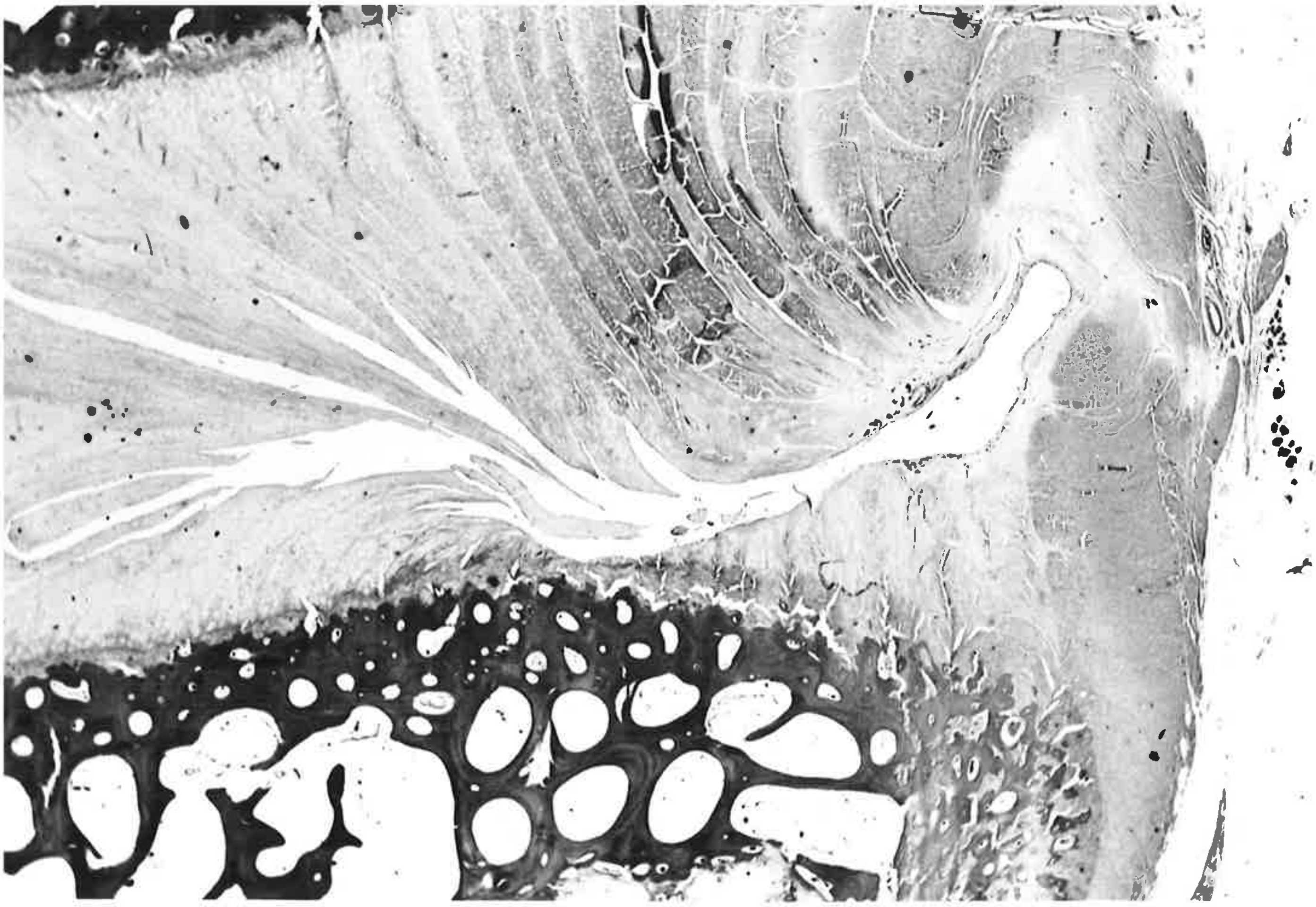
Low power micrograph of sheep intervertebral disc with 5mm deep lesion at 18 months. The overall height appears reduced with marked clefting extending from the original lesion into the mid portion of the intervertebral disc. The well defined boundary between inner annulus and nucleus has been lost. New bone formed at the anterior rim of the lower vertebra is seen. Infolding of the inner portion of the posterior annulus is seen towards the site of the lesion.

Figure 43A

[Top] Low power micrograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 18 months. Moderate narrowing of the disc space is seen. There is advanced osteophyte formation at both the upper and lower corner of the anterior bony end plate. Multiple clefts extend from the original lesion towards the centre of the intervertebral disc.

Figure 43B

[Bottom] High power micrograph of annular defect seen in figure 43A. Mature granulation tissue is seen bridging the defect in the very outer annular layers. Vascularised tissue is seen adjacent to the outer third of the lesion. Marked distortion of the annular lamellae can be observed with multiple clefting extending towards the central zone of the disc.



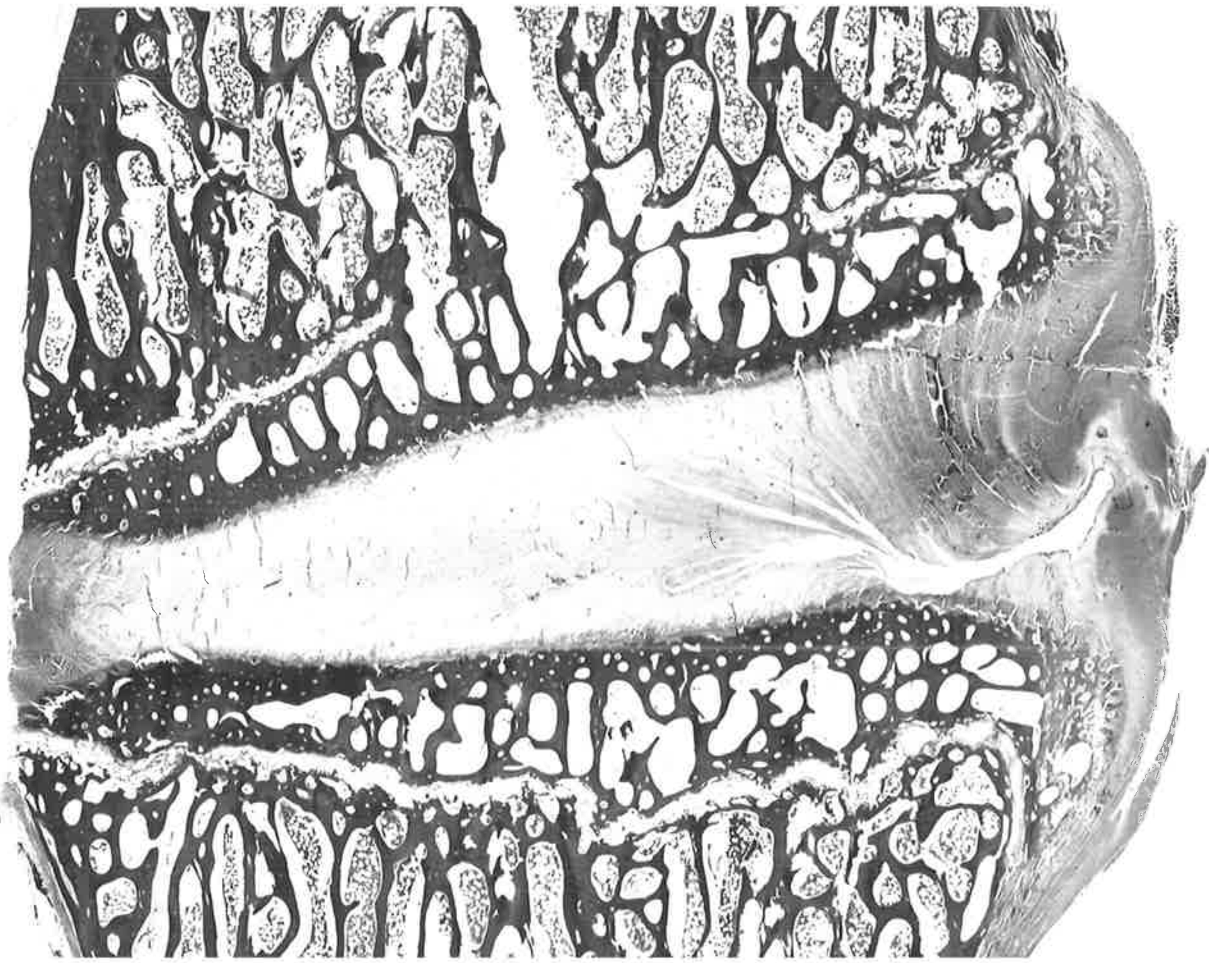
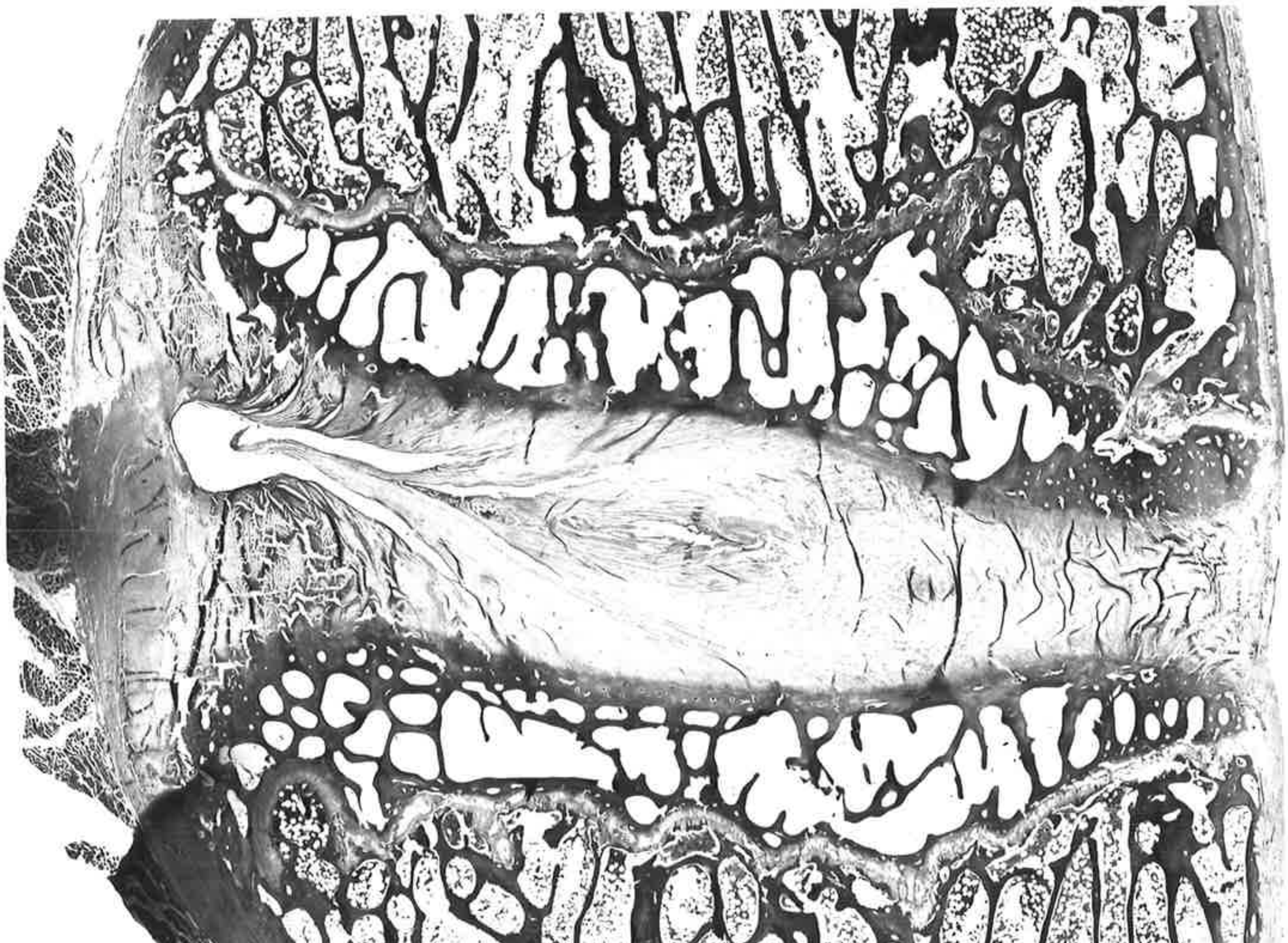


Figure 44 Low power micrograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 18 months. There is an obvious large radiating cleft in the anterior annulus fibrosus containing displaced nuclear material undergoing chondroid metaplasia.



igure 45A

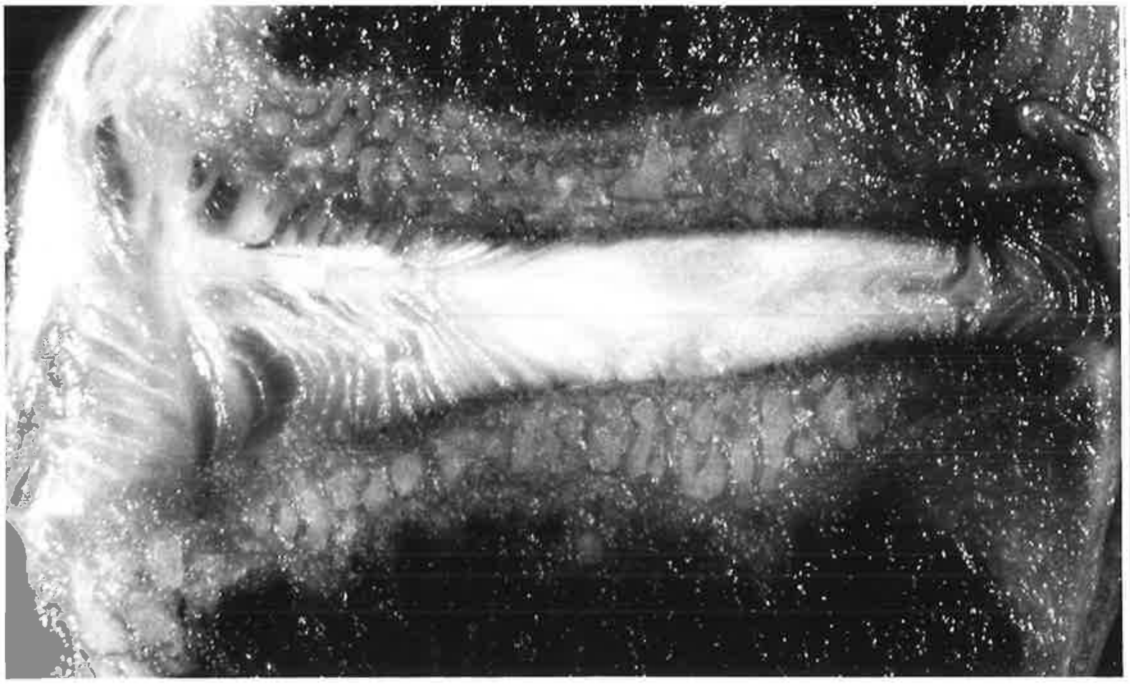
(Top) Photograph of sheep intervertebral disc with 5mm deep lesion sacrificed at 24 months. There is marked narrowing of disc space and characteristic anterior infolding of the posterior annulus lamellae.

'igure 45B

(Centre) Low power micrograph of disc seen in figure 44A. A large radiating cleft is seen adjacent to the upper anterior end plate. Multiple clefts extending from the original lesion may be observed in the central and posterior zones of the disc. There is osteophyte formation evident in the anterior corner of the upper end plate.

'igure 45C

(Bottom) High power micrograph of annular defect seen in figure 45A and B. Mature granulation tissue is obliterating the original outer third of the annular cut. Multiple concentric clefts radiate from the rim lesion along the course of the annulus lamellae.



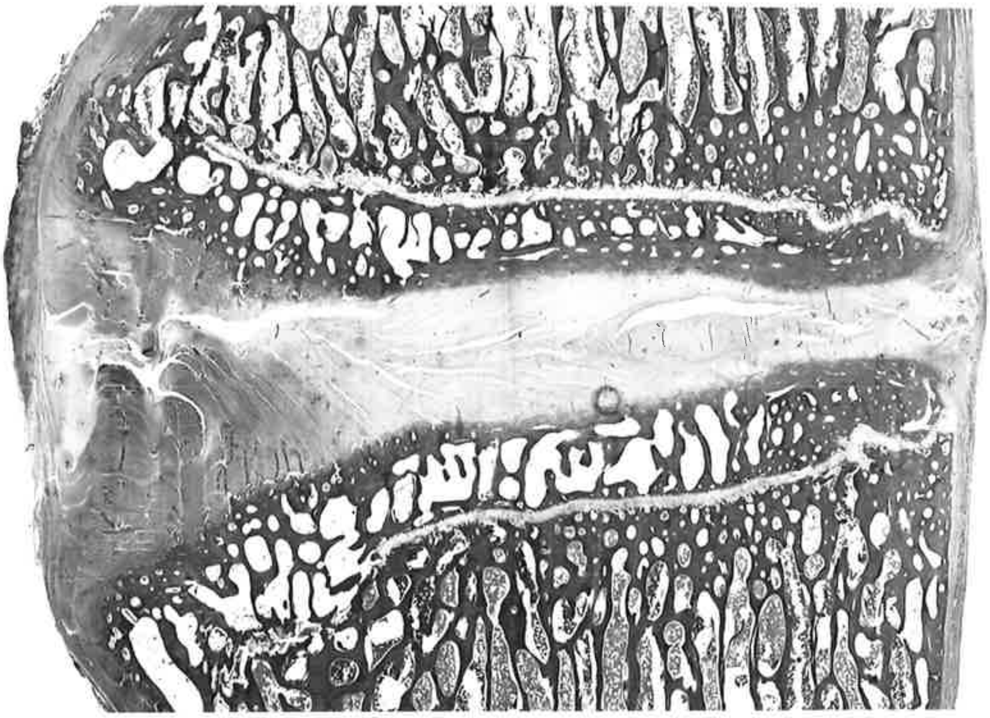
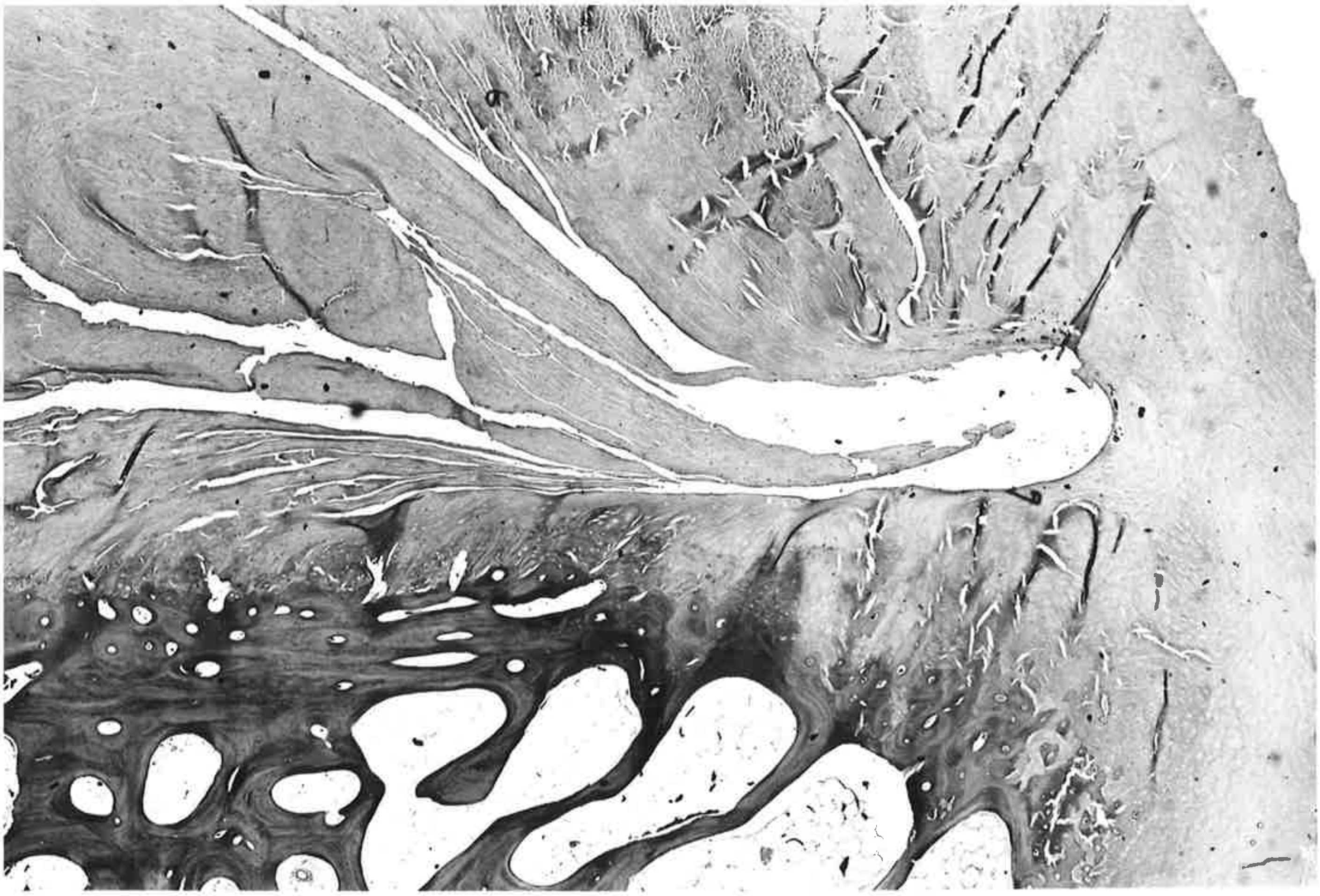


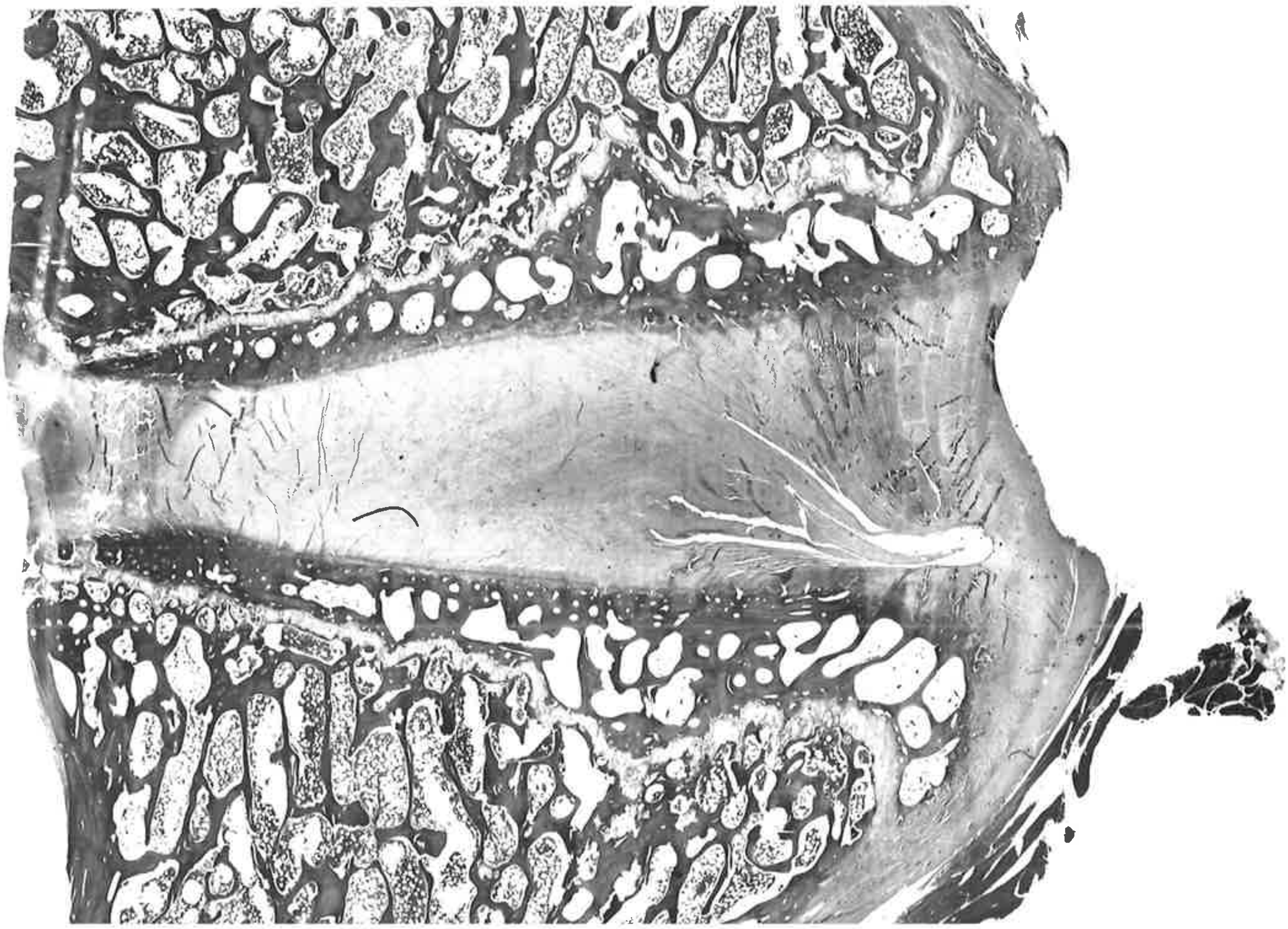
Figure 46A

(Top) Low power micrograph of sheep intervertebral disc with 2.5mm deep lesion sacrificed at 18 months. There is moderate narrowing of the intervertebral disc space with nuclear displacement towards the site of the lesion into the original annulus defect.

Figure 46B

(Bottom) High power micrograph of annular tear seen in figure 46A. Mature granulation tissue has obliterated the original defect in the outer annulus. There is new bone formation in the outer corner of the upper bony end plate.





igure 47A

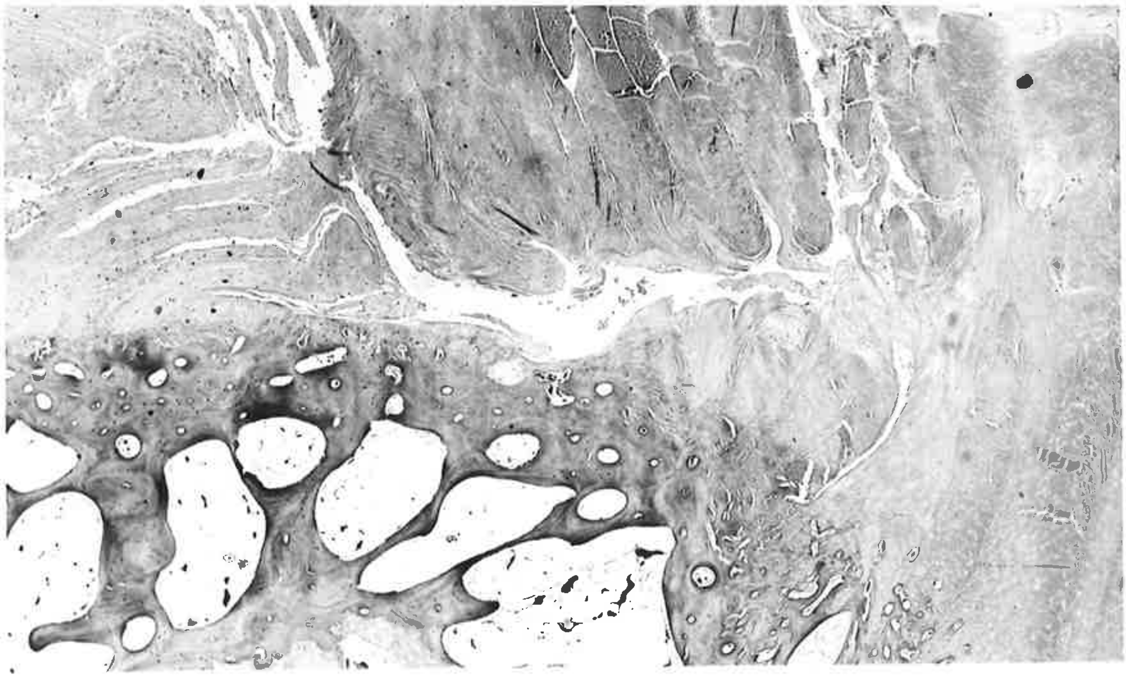
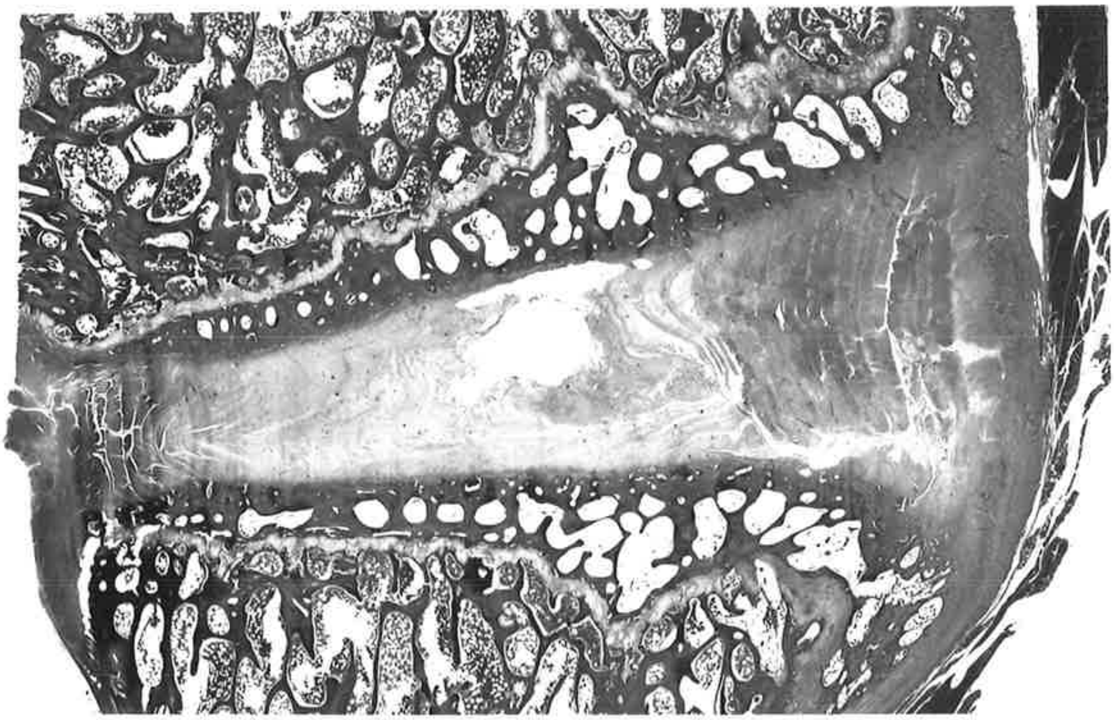
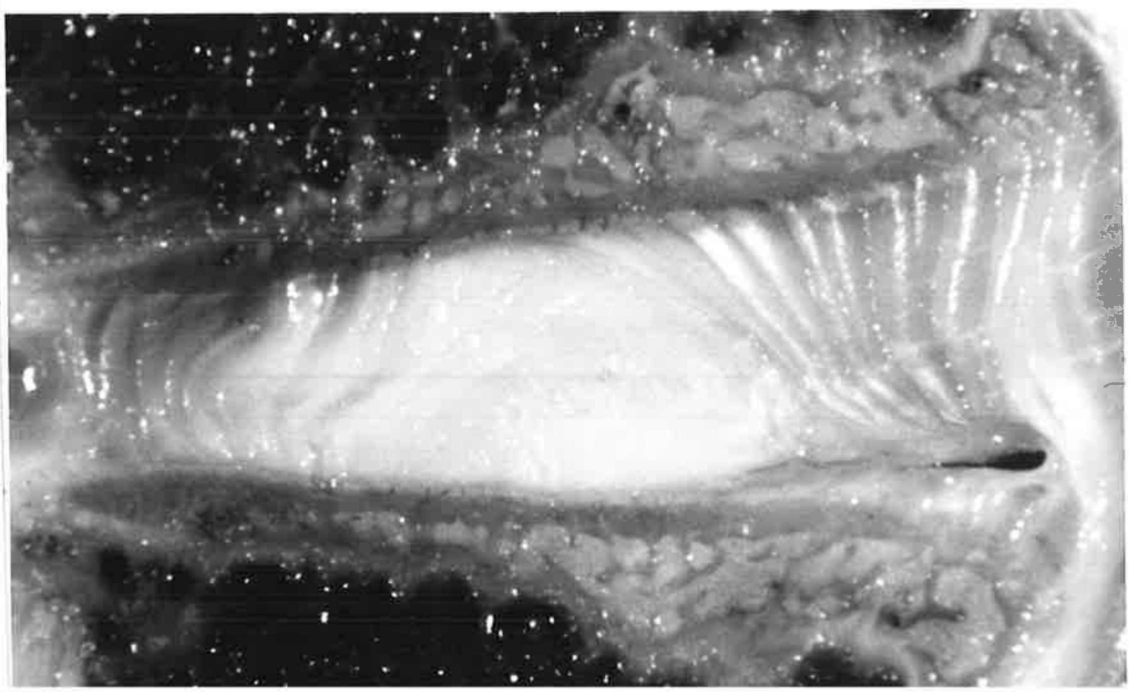
[Top] Photograph of sheep intervertebral disc with 2.5mm deep lesion sacrificed at 18 months. An obvious defect in the anterior annulus is seen parallel to the upper bony end plate. There is failure of the inner annulus with initial displacement of nuclear material towards the site of the lesion.

'igure 47B

[Centre] Low power micrograph of disc seen in figure 47A. Osteophyte formation is seen in the outer margin of the upper end plate. Multiple clefts may be observed radiating from the original defect along the course of the annulus lamellae.

Figure 47C

[Bottom] High power micrograph of annulus defect seen in figure 47A and B. Mature granulation tissue has obliterated the outer third of the lesion. There is marked disorganisation of the orientation of the collagen in the mid and inner third of the annulus fibrosus.

As with the early and intermediate phases, vascular ingrowth did not extend beyond the mid annulus. The reorganization of the collagen lamellae in the healed outer annulus was revealed by polarised light microscopy but no bridging of the original defect had occurred across the mid and inner portion of the cut. There was marked nuclear degeneration in the majority of the discs treated by 5mm deep cut and moderate degeneration in all of the 12 discs with 2.5mm deep cuts. This was characterised by loss of the original definition between inner annulus and nucleus together with extensive replacement of the original nuclear material by chondroid tissue (similar to the less differentiated fibro-cartilage seen in degenerate human intervertebral disc). Extensive clefting was observed with fissures extending towards the periphery.

Marked end plate chondrosis was seen in ten of the 24 discs, with 5mm deep lesions with areas of ossification affecting the hyaline cartilage on both sides of the end plate.

### 5.4.4. QUANTITIVE HISTOLOGY

#### 5.4.4.1 Results

These are summarised in tables X and XI.

A consistently significant difference was seen in the estimate of blood vessels along the length of the cranial and caudal end plates between the left side of the disc, directly related to the original annular cut, and the specular contralateral section. The difference was consistent throughout irrespective of the depth of the lesion (5 or 2.5mm cut) or the length of follow up (1-24 months).

A tendency was seen for the relative number of vascular channels in the end plates to decrease in parallel with the increase in the interval between operation and sacrifice, from original mean values of 9.9 percent in the early phase to approximately 7 percent for the late phase. The difference between the two groups was statistically significant ($P < 0.05$). If the area of end plate directly related to the lesion, that is the superior anterior zone, was analysed separately the overall data showed similar tendencies (table XI). A significant difference was again demonstrated between the left side and the contralateral and this difference was maintained in the three groups with different follow up. The phenomenon of relatively high numbers of vascular channels in the early phases following the annular lesion was again seen with approximately 10% of vascularised end plate in the one and two month follow up group against 7% for the 18 and 24 month follow up.

Table X (see text)

Table XI (see text)

COMPARISON OF THE VESSELS COUNTS IN THE END-PLATES BETWEEN OPERATED (O) AND CONTROLATERAL (C) SIDE OF DISC

| TOTAL NO OF TREATED DISCS | END-PLATE | POST-OP. TIME | SIDE | n | MEAN | (SD) | p |
|---|---|---|---|---|---|---|---|
| 9 | SUPERIOR | EARLY | O | 21 | 9.94 | (4.34) | |
| | | 1-2 MONTHS | C | 15 | 5.37 | (2.13) | 0.0050 |
| | INFERIOR | | O | 21 | 9.39 | (4.34) | |
| | | | C | 15 | 5.53 | (2.01) | 0.0137 |
| 48 | SUPERIOR | INTERMEDIATE | O | 102 | 7.92 | (3.11 | |
| | | 4-12 MONTHS | C | 93 | 6.63 | (2.84) | 0.0010 |
| | INFERIOR | | O | 102 | 7.28 | (3.26) | |
| | | | C | 93 | 6.71 | (2.66) | N.S. |
| 36 | SUPERIOR | LATE | O | 93 | 7.48 | (3.18) | |
| | | 18-24 MONTHS | C | 96 | 6.21 | (2.96) | 0.0002 |
| | INFERIOR | | O | 93 | 7.13 | (2.95) | |
| | | | C | 96 | 6.15 | (2.49) | 0.0002 |

COMPARISON OF THE VESSELS COUNTS IN THE SUPERIOR ANTERIOR END-PLATE REGION BETWEEN OPERATED (O) AND CONTROLATERAL (C) SIDE OF DISC

| | SIDE | n | MEAN | (SD) | p |
|---|---|---|---|---|---|
| EARLY | O | 7 | 9.89 | (3.41) | |
| 1-2 MONTHS | C | 5 | 4.76 | (1.24) | 0.0137 |
| INTERMEDIATE | O | 34 | 7.82 | (2.54) | |
| 4-12 MONTHS | C | 31 | 6.01 | (2.06) | 0.0027 |
| LATE | O | 31 | 6.95 | (2.34) | |
| 18-24 MONTHS | C | 32 | 5.17 | (1.07) | 0.0001 |

No significant difference in the control discs between cranial and caudal end plates and left and right side of the disc was observed. The annular lesion, therefore, had an effect, which was measurable in quantitative terms, on the structure of the cartilage and bony end plate.

## 5.4.5 BIOCHEMISTRY

### 5.4.5.1 Results

The results of the biochemical analysis are summarised in table XII, XIII, XIV, XV and XVI. (see also appendix)

#### 5.4.5.1.1 Collagen Levels (Table XII)

Slight increase in collagen levels as expressed as a percentage of dried weight was seen in the annulus sample related to the side of the lesion. Conversely collagen levels from nuclear samples decreased in a relatively regular pattern following the original lesion.

#### 5.4.5.1.2 Hexuronic Acid Levels (Table XIII)

Hexuronic acid values from annular samples did not show significant variations at the various intervals after surgery. Hexuronic acid levels from the nucleus pulposus however, following moderate increase in the early phases after surgery showed a significant tendency towards markedly decreased levels in the intermediate and late follow up.

#### 5.4.5.1.3 Moisture Content (Table XIV)

This initially did not show significant variation between the treated discs and the control. At later follow up however, an appreciable decrease in moisture content was seen, especially in the samples from nucleus pulposus. This tendency was in keeping with the hexuronic value from the same areas of disc tissue.

5.4.5.1.4 Proteoglycan Extractability

Values of proteoglycan extractability of the samples from the nucleus pulposus did not vary significantly for discs with either a 5mm deep lesion or a 2.5mm deep lesion. Annular sample, however, showed a marked decrease in extractability at the four month follow up which is in keeping with the relative increase in the collagen content from the same sample as a result of the attempt at healing.

5.4.5.1.5 Extractable Proteins (Table XV)

A significant difference in values for the levels of proteins extractable with 4M GuHCl was seen between the operated and the control discs. As a rule extractable protein values were higher in the samples from the nucleus pulposus if compared with the annulus samples. The marked increase in extractable protein values was seen for the discs treated by 5mm deep cuts at the eight and 12 month follow up. The sheep treated by 2.5mm deep cut showed a similar trend but with less marked variation.

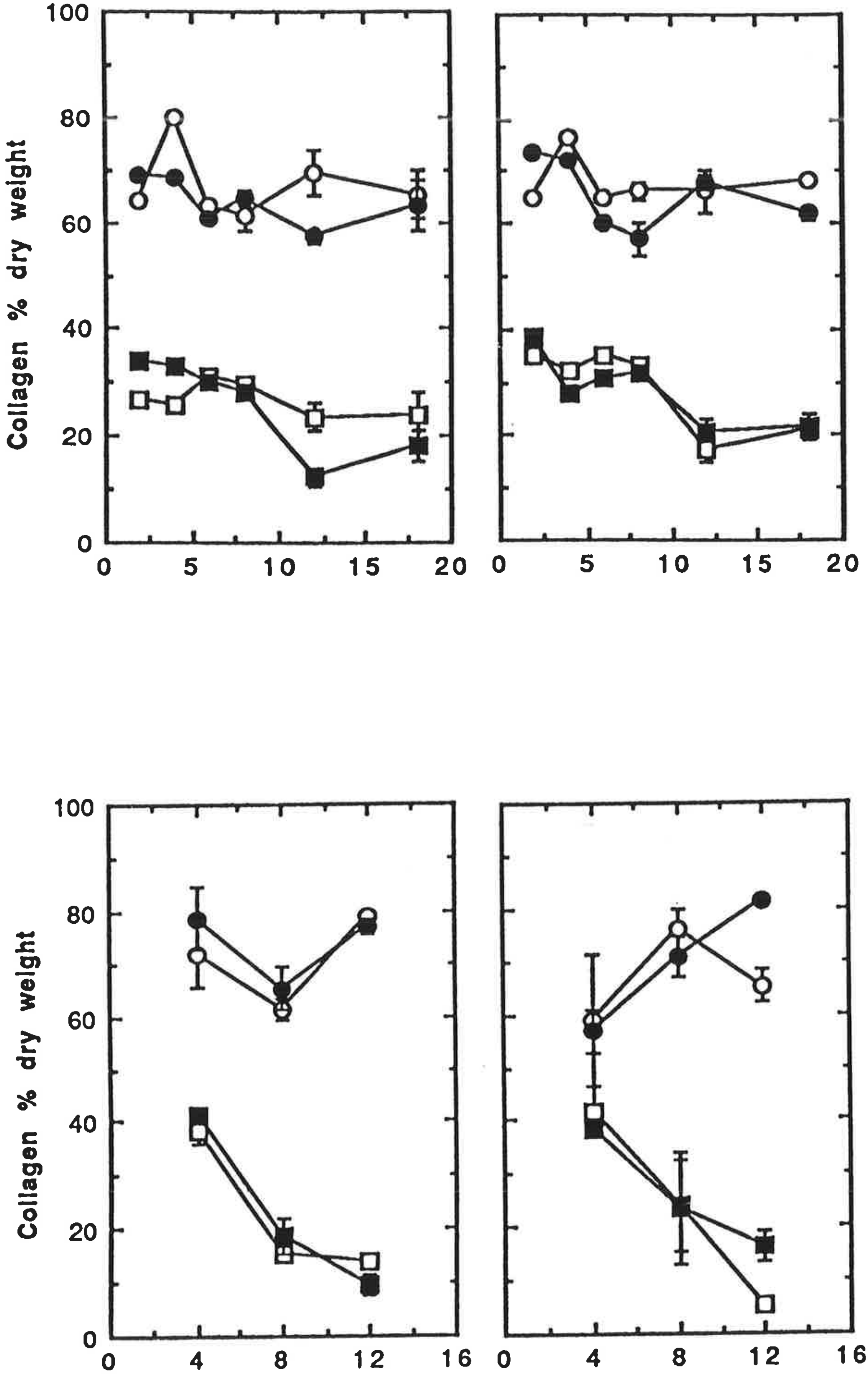
Because of the parallel decrease in hexuronic acid and collagen content from nucleus pulposus sample it is likely that the increase in extractable protein represented non-collagenous proteins (NCP) synthesised by disc cells as part of the reparative process.

5.4.5.1.6 Proteoglycan Aggregation (Table XVI)

Proteoglycan aggragation levels showed a significant decrease in the intermediate phase in the sheep with discs treated by 5mm deep cuts. This was more evident in the annular samples with aggregatability falling to 12 to 20 percent in comparision to the initial 40 percent value.

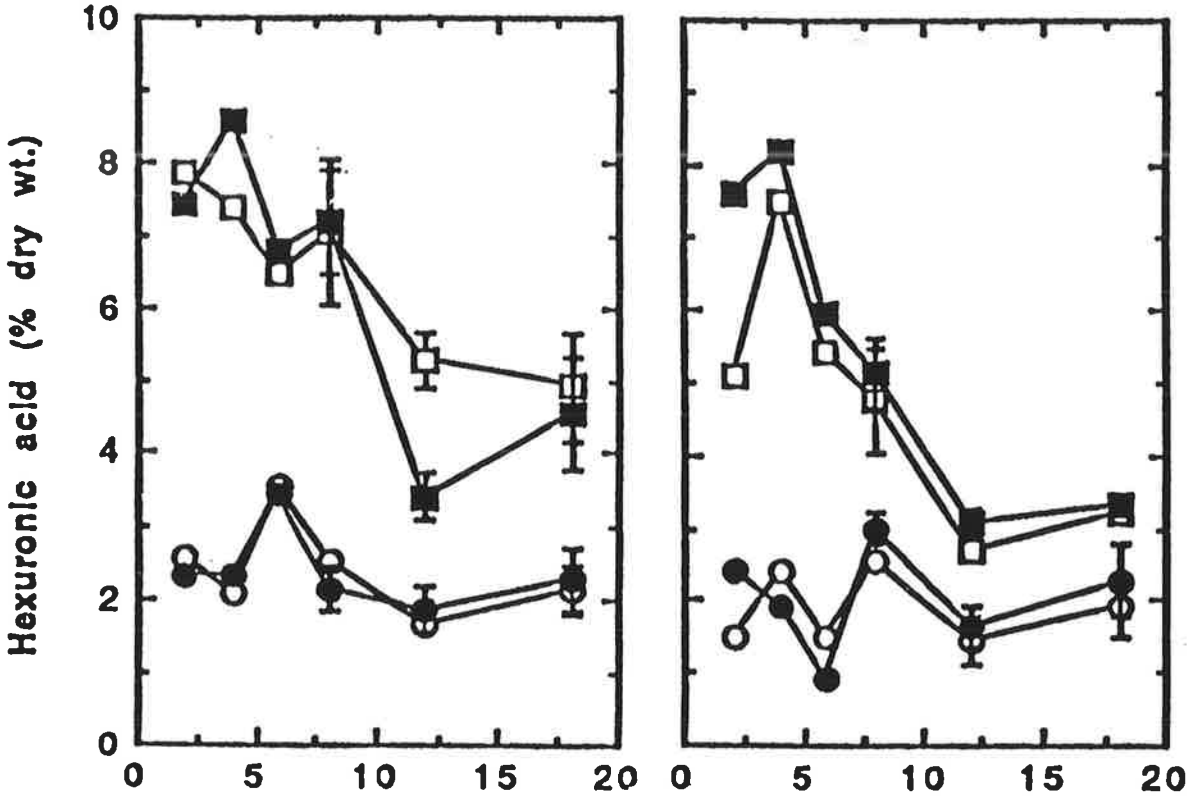
TABLE XII A (Top) Variation with time of mean collagen levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (left-hand graph); 5mm defect operated discs (right hand graph).

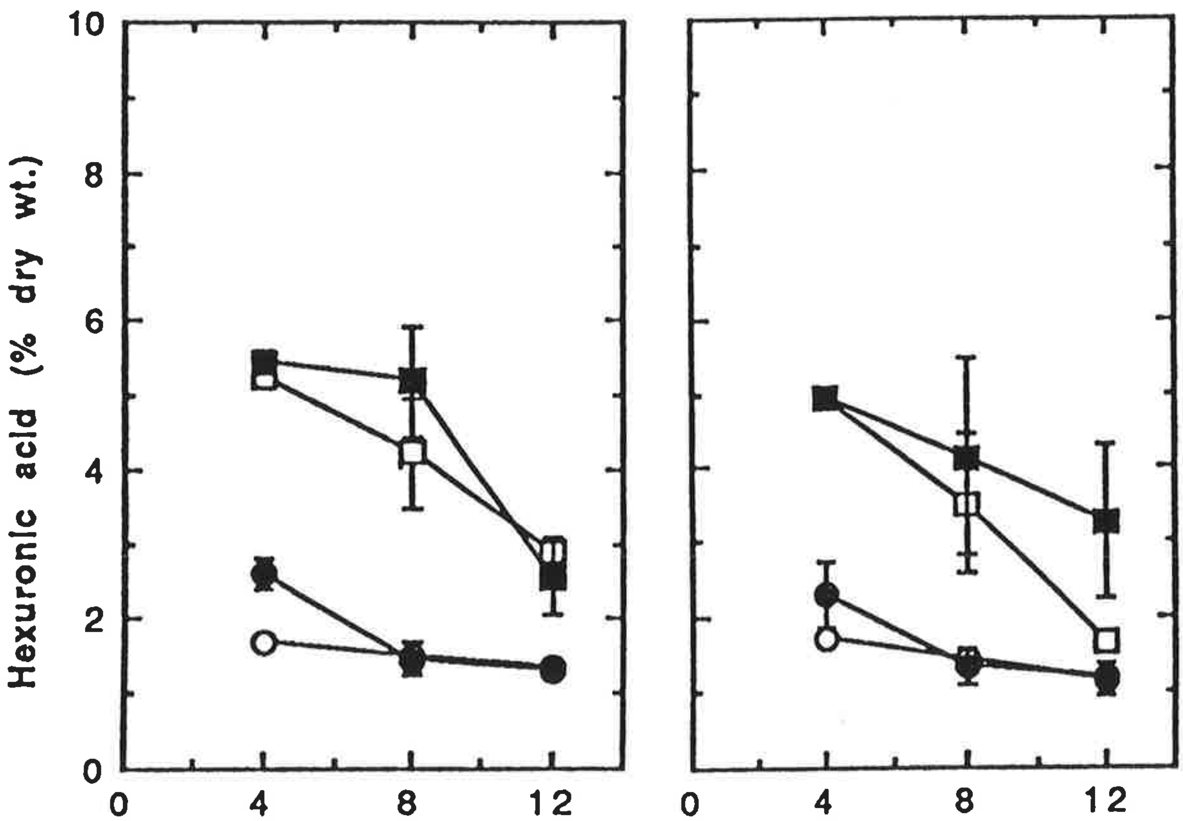
TABLE XII B (Bottom) Variation with time of mean collagen levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (left-hand graph); 2.5mm defect operated discs (right hand graph).


Collagen % dry weight
100
80
60
40
20
0
0
5
10
15
20
Collagen % dry weight
100
80
60
40
20
0
0
4
8
12
16

ABLE XIII A(Top) Variation with time of mean hexuronic acid levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (left-hand graph); 5mm defect operated discs (right hand graph).

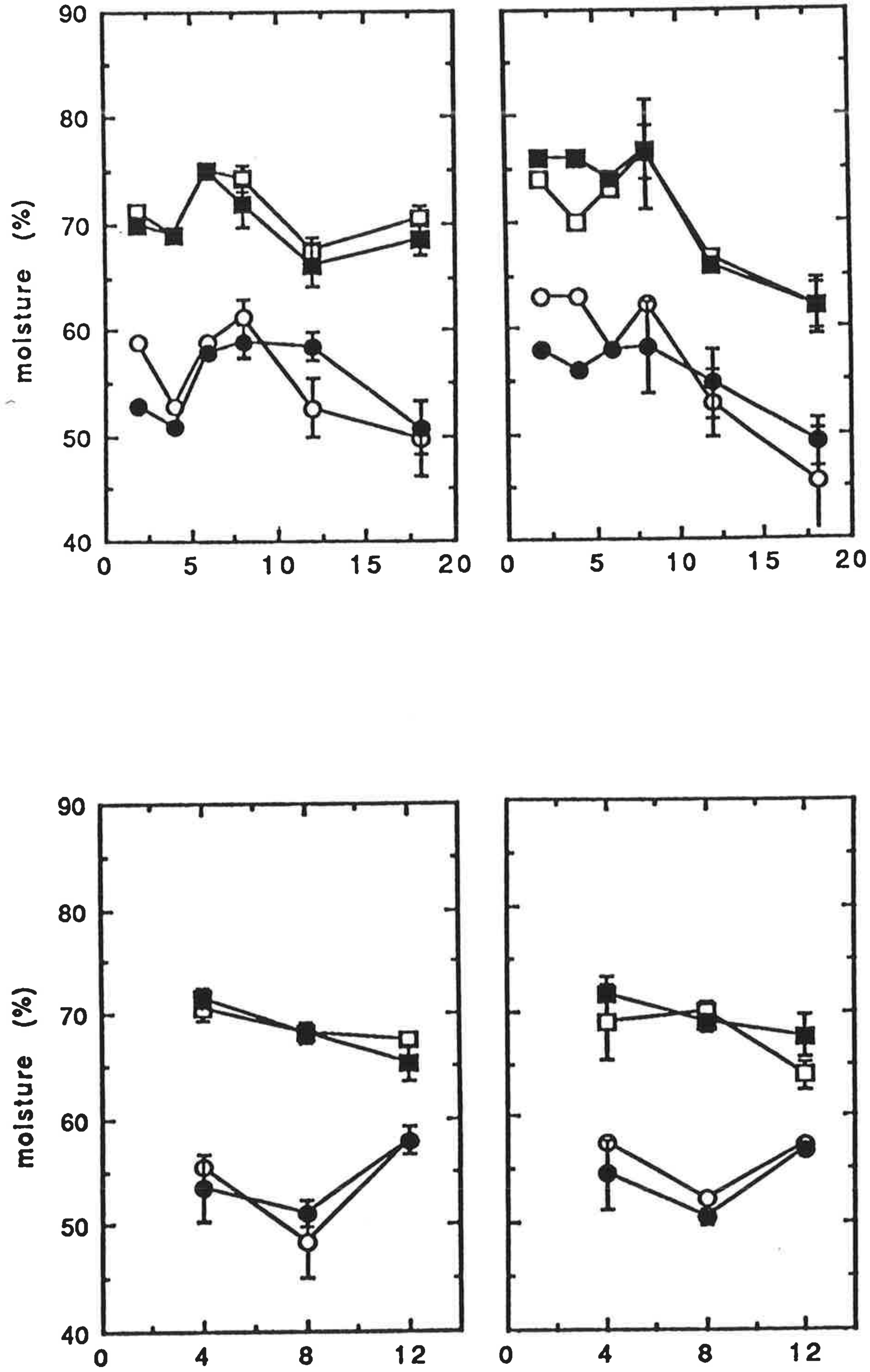
TABLE XIII B (Bottom) Variation with time of mean hexuronic acid levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (left-hand graph); 2.5mm defect operated discs (right hand graph).


Hexuronic acid (% dry wt.)
0
2
4
6
8
10
0
5
10
15
20
0
5
10
15
20

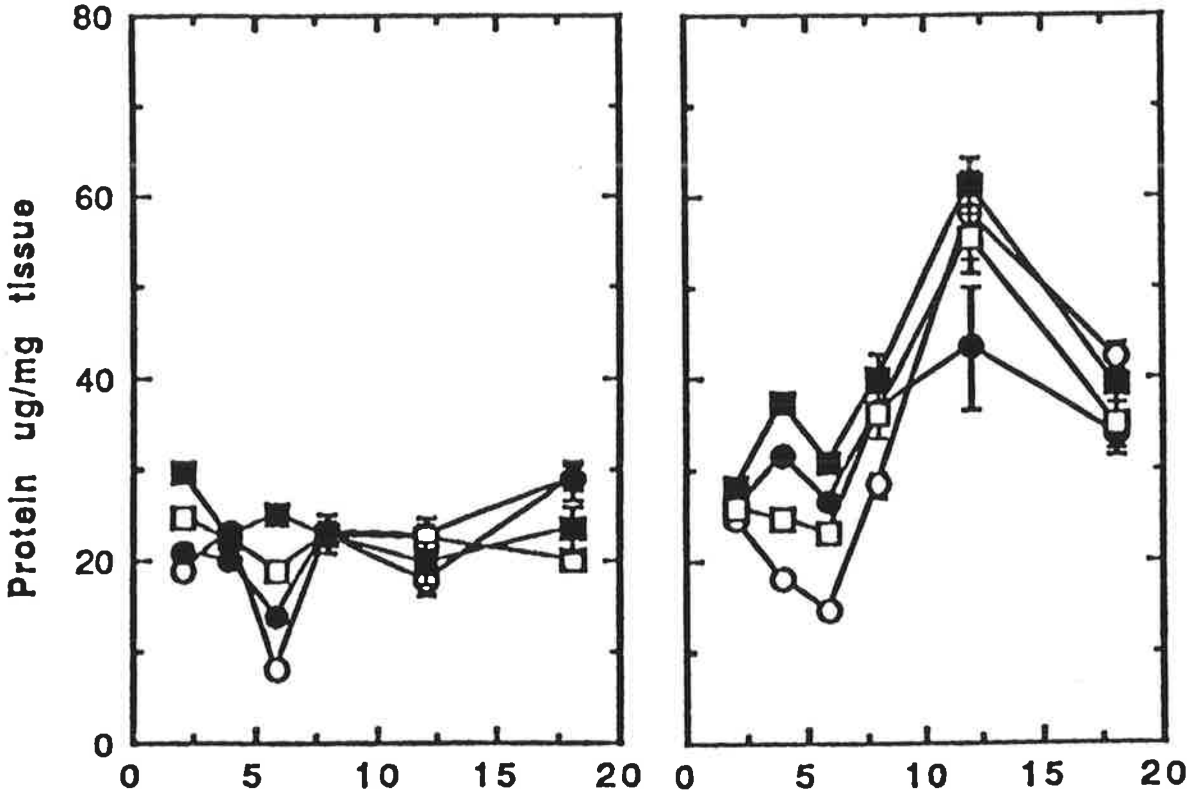

Hexuronic acid (% dry wt.)
0
2
4
6
8
10
0
4
8
12
0
4
8
12

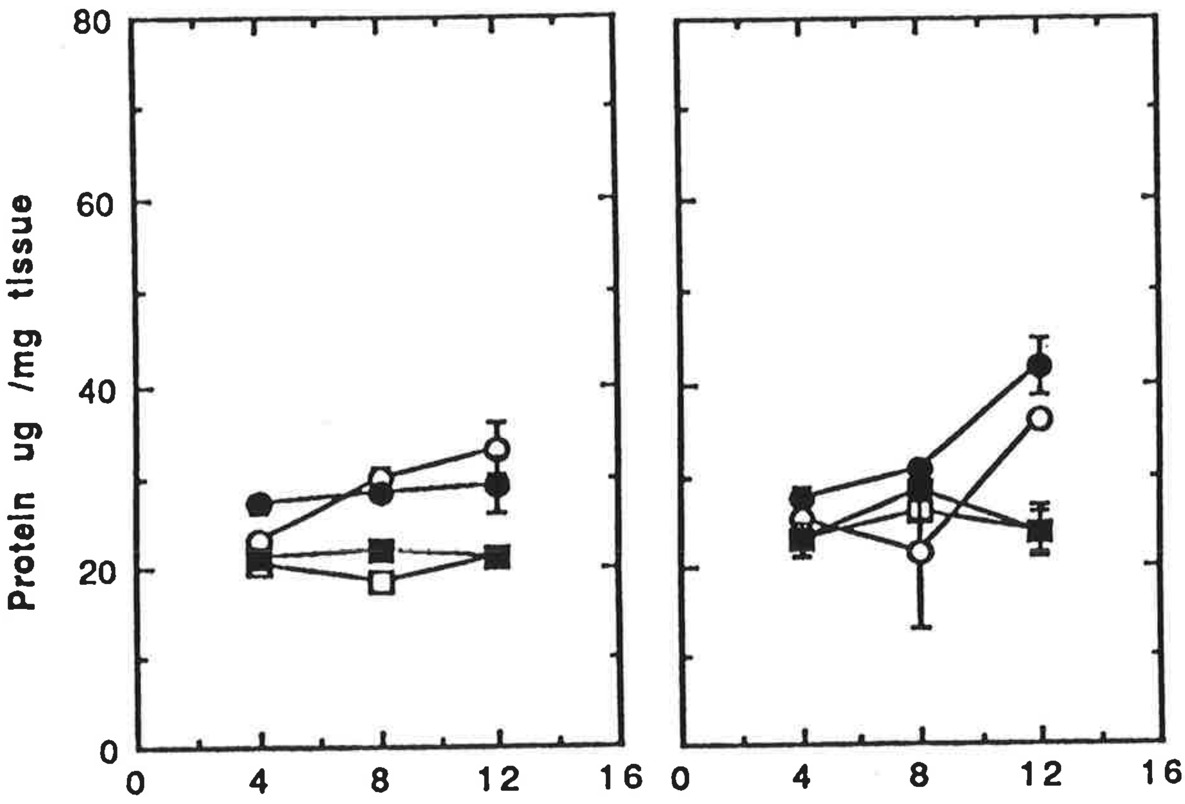
ABLE XIV A (Top) Variation with time of mean moisture levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (right hand graph); 5mm defect operated discs (central graph).

[ABLE XIV B (Bottom) Variation with time of mean moisture levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (left-hand graph); 2.5mm defect operated discs (right hand graph).


moisture (%)
90
80
70
60
50
40
0
5
10
15
20
moisture (%)
4
8
12

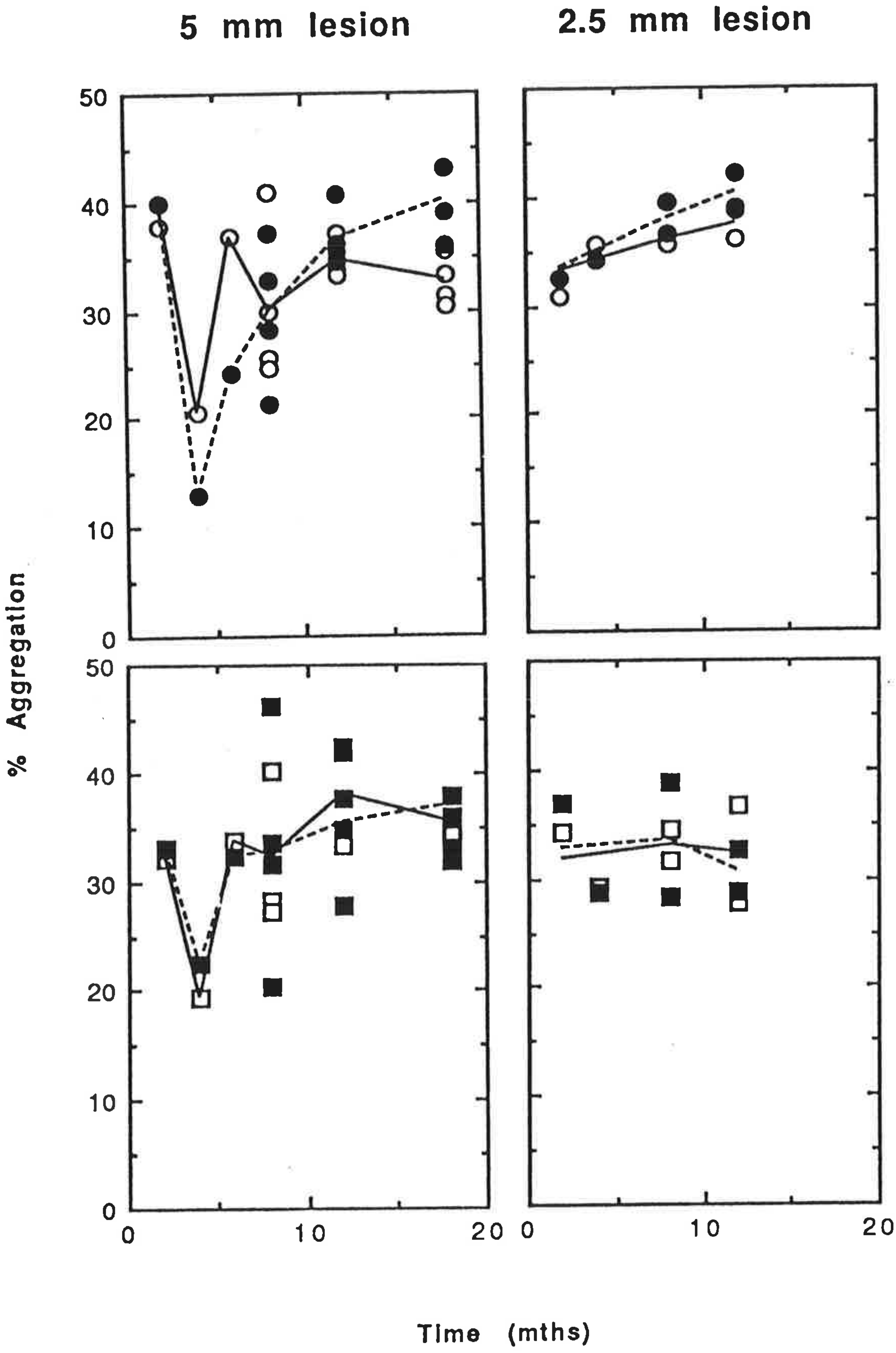
| | |
|---|---|
| TABLE XV A (Top) | Variation with time of 4M GuHCl extractable protein levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (left-hand graph); 5mm defect operated discs (right hand graph). |
| TABLE XV B (Bottom) | Variation with time of 4M GuHCl extractable protein levels ± SEM of sheep disc AF zones 1 (o) and 2 (●) and NP zones 3 (□) and 4 (■) of lumbar control discs (left-hand graph); 2.5mm defect operated discs (right hand graph). |


Protein ug/mg tissue
80
60
40
20
0
0
5
10
15
20


Protein ug /mg tissue
80
60
40
20
0
0
4
8
12
16

[ABLE XVI

Scattergram demonstrating the variation with time of proteoglycan aggregation levels with excess hyaluronic acid of proteoglycans purified by associative ultracentrifugation and subjected to Sepharose CL 2B gel permeation chromatography, PG's from AF zones 1 (o) and 2 (●)and NP zones 3 (□) and 4 (■) from 5 and 2.5mm defect operated discs.



5 mm lesion
2.5 mm lesion
% Aggregation
Time (mths)

Proteoglycan aggregation values from annular samples however, returned to normal at the 12 month mark.

Proteoglycan aggregation level from the nucleus pulposus samples showed a similar trend at the four month mark with values of approximately 20% compared to the initial 30% value. Proteoglycan aggregation levels from these samples increased significantly in the intermediate phase with absolute higher levels in comparison to the initial data at 12 months. Because of the relative depletion of proteoglycan from the nucleus pulposus tissue it is likely that the increase in aggregatability of the total proteoglycan population would be consistent with selective loss of non-aggregatable proteoglycans. This is in keeping with the hypothesis that aggregatable proteoglycans are provided with protection from proteolysis due to steric exclusive effect. If the hydrodynamic size of the non-aggregating proteoglycans is analysed, in the early and intermediate phases following a 5mm deep lesion a decrease in size was observed at the four month period with a return to a normal size in the proteoglycan monomers at the eight and 12 month follow up. This data could be interpreted as a result of increased proteolysis in the four month proteoglycan samples and is consistent with the significant decrease in proteoglycan aggregatibility at the same time.

Overall changes seen in the disc treated by the 2.5mm deep lesion were less marked but a similar trend was observed.

## 5.5 DISCUSSION

### 5.5.1 THE BASIS OF THE SHEEP MODEL

The concept of inducing experimental intervertebral disc degeneration in animals by damaging the annulus fibrosus is not new. As illustrated in detail in the history of experimental studies of disc degeneration, since 1948 various experiments have been published where animals have been subjected to surgical lesions of the annulus fibrosus in order to obtain immediate prolapse of nuclear material in the attempt of simulating pathological changes seen in human discs.

Our model using the sheep differs from those previously described since the annular lesion induced closely resembles the peripheral tears seen in young and otherwise normal human discs. Great care was taken to ensure that the inner annulus was left intact in all animals. Despite this, progressive failure of the inner annulus was seen in all sheep and occurred in a majority of discs with the 5mm deep lesion between the fourth and the twelfth month after the operation. In the group with 2.5mm deep lesion spontaneous inner annulus failure was seen after 18 months.

The choice of localisation of the tear in our model in the anterolateral annulus fibrosus is based on the original description of rim lesion by Schmorl and more recently by Vernon-Roberts and Hilton and Ball.

Based on extensive analysis of human lumbar intervertebral discs it is possible to conclude that whilst radiating ruptures extending from the central layers of the annulus fibrosus towards the periphery are a constant feature of advanced degeneration and are commonly seen in aging discs, especially posteriorly, discrete failure of the outer annulus which is at the base of the sheep model seems to be a feature commonly seen in young and otherwise healthy discs.

Although no attempt was made in our experiments to analyse the results of the peripheral tear in the posterior annulus, based on the analysis of autopsy material it is postulated that discrete lesions of the peripheral annulus can occur in the posterior annulus despite the relative protection offered by the facet joints in the lumbar spine.

The degree of similarity in the changes observed following the annulus cut in the two groups of sheep with deeper and shallower annulus lesions confirms the reproducibility of our model.

The choice of the sheep may require some further elucidation.

A series of considerations have to be taken into account when designing an animal model. The first and most important is the reproducibility of the model and potential for comparison with the human.

Although this may be considered at present a controversial issue it is felt based on the review of the literature that the sheep disc is biochemically closer to the human than that of other animals used in the past for similar experiments (rabbits, cats, chondrodystrophoid and non chondrodystrophoid dogs). In addition it is possible in Australia to purchase with ease sheep of consistent age and breed which offer a highly reproducible base for the experiment.

The second main consideration which, is at least in Australia of high priority in designing an animal experiment, is ethical. The use of animals for medical research which are commonly identified by the public as pets exposes investigators to a very high probability of opposition by antivivisection activists in the first instance and by personnel involved in the handling of the animals afterwards. Furthermore, in the case of dogs for instance it would appear a complex task to ensure constant characteristics of breed.

The ease in handling the animals during and after the experiments is of great relevance especially when large numbers are employed.

Based on personal experience of more than 150 sheep used for basic spinal research it would seem difficult to find a similarly reliable and pleasant subject amongst other species.

Another important aspect in the practical planning of major experimental studies is the cost involved in the purchase and housing of the animals.

The sheep used in our experiment were bought at market prices and kept after the first three days of post-operative recovery in open air stations with minimal supervision. None of the animals included in this study developed any complication or illness as a result of the procedure or from natural causes.

The fact that the sheep does not assume the erect posture represents a main theoretical draw-back for research related to spinal disorders.

It is argued that our experiments did not try to analyse biomechanical aspects of intervertebral disc degeneration. The supposedly primary mechanical lesion of the annulus was obtained by surgical intervention. The sheep model was used to observe the effects of the initial annular injury to the morphology, biochemistry and imaging characteristics of discs.

For the purpose of the present study it was felt that the sheep offered a valid and practical alternative.

### 5.6.2 THE EFFECTS OF THE ANNULAR DEFECT: A HYPOTHESIS OF POSSIBLE SEQUENCE OF EVENTS

In morphological terms the sequence of events observed in the sheep model following a discrete lesion of the outer annulus was the progressive failure of the inner annulus in relation to the site of the original annulus cut.

Despite the relatively good healing potential of the very outer annulus, in the presence of a highly hydrated nucleus pulposus the increased tensile strain on the inner annulus fibrosus related to the damaged outer layer led initially to deformation and bulging of the collagen bundles and eventually to extension of the tear and complete failure.

The theoretical explanation for the spontaneous deformation and failure of the inner annulus is two-fold.

On one side it is conceivable that the presence of high levels of hydration of an otherwise healthy nucleus pulposus would result in increased stress on the collagen bundles adjacent to those damaged by the original cut.

Values of moisture content from the various areas of the discs treated by the annulus cut suggest that inner annulus failure preceeds the secondary dehydration of the nucleus pulposus. Whilst inner annulus failure in the model with a 5mm deep cut consistently occurred between the fourth and the twelfth month after the operation, marked nucleus degeneration as expressed by loss of water and proteoglycans biochemically and by relative decrease in signal intensity at MRI was seen only in the late
phase at 18 and 24 months.

It is likely, however, that the progressive degeneration of the intervertebral joint complex seen as a result of the discrete lesions of the outer annulus fibrosus is not only related to a relative inability of the remaining annulus fibres to cope with the sustained tensile stress.

The quantitative analysis of the vascular changes seen in the end plate region and the marked variation in levels of non-collagenous proteins from the biochemical analysis of the discs prior to the stage of inner annulus failure suggest that this could be related to the disc cell response to the injury and its subsequent reparative processes.

As witnessed by the abundant highly vascular granulation tissue in the early phases after surgery the disc cells would react vigorously to discrete lesions of the outer annulus lamellae.

The first direct consequence of tissue repair mechanisms in the periphery of the intervertebral disc is an effect on the nutritional pathways to the mid and inner annular layers through the outer annulus. Although no extensive studies have been carried out in the sheep disc, based on the results of several studies in the human disc, it is postulated that the most active disc cells in the synthesis of proteoglycans may be found in the mid zone of the annulus, which is the most vulnerable to nutritional insult.

Another possible explanation for the relatively irreversible changes that occur in the sheep disc as a consequence of discrete outer annulus defect may be based on disc cell density.

Although attempts at quantifying alterations in cell numbers in the discs treated by the lesion were frustrated by the extreme variability of disc cell types between the two extremes of chondrocytes and fibroblast cells it was apparent that cell density increased substantially in the areas directly affected by the original lesion. In a recent study on factors influencing the oxygen transport into the disc, Urban et al[194]. postulated that a strong dependance would exist between disc oxygen concentration and cell density. Despite the fact that the disc would obtain only 10 percent of its energy from oxygen it is postulated that disc cells would not be able to survive at very low oxygen concentration. Concentration of oxygen in disc specimen halved when cell density increased by 8 percent.

The analysis of disc cell response to an annulus lesion will be the scope of further research. It is postulated, however, that the combination of unprotected mechanical stress, jeopardised nutrition and altered equilibrium between cell density, oxygen concentration and end plate diffusion may explain why the repair process would not appear to prevent but possibly accelerate the biochemical degradation of the intervertebral disc.

### 5.6.3 CORRELATION BETWEEN MRI, BIOCHEMICAL AND MORPHOLOGICAL CHANGES

The results of MR imaging in our animal model and their correlation with morphological and biochemical changes confirm the experimental data of Lipson and Muir[120,121] of relatively higher hydration of the nucleus pulposus immediately following the original annulus injury. Loss of signal in $T_2$ weighted images which parallel the hydration of the intervertebral disc was seen only after 12 months in the 5mm deep lesion and after 18 months in the sheep treated by the 2.5mm deep lesion.

Our study confirms recent experimental evidence of good correlation between signal intensity at $T_2$ weighted images and water content in disc tissue samples.

In the early phases of degeneration in the presence of substantial annulus damage the nucleus pulposus may retain a relatively normal water content which would be associated with high values of intradiscal pressure. This would apply specifically to stress related injuries to the outer annulus fibrosus.

These findings should not be confused with the more common situation in clinical practice of posterior disc prolapse. This entity in fact is, we believe, an expression of advanced disc degeneration and would be invariably associated with dehydration, fraying and fissuring of the disc and consequent displacement of disc tissue fragments outside its physiological boundaries. It is therefore likely that disc prolapse may show marked alteration of signal intensity at $T_2$ weighted images. If, however, as in the case of the sheep model, annulus failure in the outer layers were present, which may be a feature of early disc degeneration, MRI signal may fail to reveal any abnormality in intensity.

It is only at a later stage that due to the relative changes in the proteoglycan content disc water would decrease and this would be highlighted by reduced or absent signal at MRI.

It is felt that at present discography would offer a more sensitive evaluation of disc morphology and because of added data on pressure values and pain provocation may be of use in the investigation of young symptomatic discs.

Because of inevitable continuous improvement in the techniques available and the possible use of enhancing contrast agents like Gadolinium in the near future MRI may be able to visualise annular defects in the presence of a normally hydrated nucleus pulposus. The possibility of associating the administration of enhancing agents prior to MR imaging is yet to be explored.

### 5.6.4 PAIN PRODUCTION AND ANNULAR DEFECTS

It is beyond the scope of this study to attempt to correlate the morphological features of peripheral annulus damage with the pathogenesis of low back pain.

Based on anatomical studies and on the review of patients with typical pain reproduction at discography it is likely that pain related to the disc would be linked to damage of the outer portion of the annulus fibrosus.

It is more difficult to evaluate the role of internal pressure in the pathogenesis of discogenic pain. It would seem logical to assume that a hypermobile intervertebral joint with sustained values of intradiscal pressure would be more prone to pain referred in the outer annulus layers because of higher tensile strain. The results of our prospective study, however, have failed to confirm a systematical relationship between typical pain reproduction at discography and high values of disc pressure.

It can be concluded that at present the only consistent morphological change present in patients with pain reproduction at discography is the presence of annular defects extending to its outer layers.

Because of the presence of highly vascular granulation tissue in the areas of peripheral annulus damage it seems reasonable to postulate that discrete peripheral defects may be associated with the production of discogenic pain.

## CONCLUSION

In conclusion our experimental studies suggest that new ordered collagen can be synthesised in the outer annulus as a response to injury but that the repair process would not appear to prevent but possibly accelerate the biochemical degradation of the intervertebral disc.

Peripheral tears of the annulus fibrosus may therefore play an important role in the degeneration of the intervertebral joint complex.

# CHAPTER 6

# 6. SUGGESTIONS FOR FURTHER RESEARCH

## 6.1 THE ANALYSIS OF CELL RESPONSE TO ANNULAR DAMAGE

The sheep model offers the opportunity of reproducible and controlled observations on the disc cell response to a discrete peripheral annular defect.

It would be of great interest to analyse in more detail the characterics of disc cells involved in the repair process both adjacent to the original lesion and in the other areas of the disc. Experimental studies could be designed in both an in vivo and in vitro type setting. Quantitative histomorphometric techniques could be used in the asessment of cell density. Ultra structural studies could be carried out on specific cell populations to gather additional information.

It is postulated that because of the likely relevance of outer annular defects in the pathogenesis of intervertebral disc degeneration and their possible role in the production of lumbar back pain these studies could lead to substantial progress in patients treatment modalities.

## 6.2 EFFECTS OF SURGICAL TECHNIQUES ON SEQUENCE OF DISC DEGENERATION

Using the established sheep model of disc degeneration further studies could be designed whereby in randomly selected animals the original defect may be repaired by direct suture or indirect methods using tendons and/or ligament reinforcement technique. It would be of great interest to compare discs treated by surgical repair with control discs in order to evaluate possible therapeutic implications.

The sheep model in addition, could be used for the assessment of drugs which have been investigated in other joints (as knee and shoulder) for their potential in enhancing repair of chondral defects.

## 6.3 ANALYSIS OF ENHANCED IMAGING TECHNIQUES IN EARLY DISC DEGENERATION

The sheep model could be used to assess the effect of enhancing contrast agents such as Gadolinium injected parenterally or in selected areas of the annulus fibrosus for its imaging potential in the presence of annular defects.

Another imaging technique that has not yet been explored is the software elaboration in three dimensional terms of computerised discography. 3-D reconstructions which are commonly used in the assessment of skeletal trauma, especially in the pelvic area and for intra-articular fractures could be of use in the evaluation of annulus defects as highlighted by the intradiscal contrast.

More data is needed on the correlation between symptoms, clinical signs and results of imaging in the area of intervertebral disc pathology. Due to the relatively easier access to MR imaging and its supposed lack of side effects it is postulated that epidemiological studies could be designed where randomly selected and representative samples of asymptomatic individuals could be subjected to imaging of the lumbar spine. In correlation with data on age, employment and lifestyle characteristics and any known history of back symptoms these studies could lead to a more scientifically controlled understanding of the incidence and characteristics of degenerative spinal conditions.

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Sheep disc collagen levels for zones 1-4
5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | Collagen (% dry weight) ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|---|
| Operated levels 5mm lesion | | | | | | |
| 1 | L4-L5 | 2 | 65.0 | 73.5 | 35.0 | 38.5 |
| 2 | L4-L5 | 4 | 76.5 | 72.0 | 32.0 | 28.0 |
| 3 | L4-L5 | 6 | 65.0 | 60.0 | 35.0 | 31.0 |
| 4 | L3-L4 | 8 | 74.0 | 44.5 | 36.0 | 28.5 |
| 5 | L4-L5 | 8 | 67.5 | 67.5 | 29.5 | 29.0 |
| 6 | L3-L4 | 8 | 66.0 | 67.0 | 39.0 | 38.0 |
| 7 | L4-L5 | 8 | 57.5 | 49.0 | 28.0 | 31.0 |
| 8 | L4-L5 | 12 | 80.5 | 76.0 | 26.0 | 29.0 |
| 9 | L4-L5 | 12 | 78.0 | 67.0 | 19.0 | 16.0 |
| 10 | L4-L5 | 12 | 46.0 | 67.0 | 19.0 | 27.0 |
| 11 | L4-L5 | 12 | 60.0O | 61.0 | 5.0 | 11.0 |
| 12 | L4-L5 | 18 | 69.0 | 62.1 | 23.6 | 11.7 |
| 13 | L3-L4 | 18 | 72.4 | 64.5 | 16.8 | 17.8 |
| 14 | L4-L5 | 18 | 65.3 | 55.1 | 28.4 | 27.1 |
| 15 | L5-L6 | 18 | 65.5 | 65.9 | 15.0 | 30.7 |
| Lumbar control levels | | | | | | |
| 1 | L1-L2 | 2 | 64.0 | 69.0 | 27.0 | 34.0 |
| 2 | L1-L2 | 4 | 80.0 | 68.5 | 26.0 | 33.0 |
| 3 | L5-L6 | 6 | 63.0 | 61.0 | 31.0 | 30.0 |
| 4 | L4-L5 | 8 | 62.0 | 68.5 | 31.0 | 27.0 |
| 5 | L1-L2 | 8 | 70.0 | 67.0 | 30.0 | 30.0 |
| 6 | L4-L5 | 8 | 66.5 | 66.0 | 34.0 | 32.0 |
| 7 | L1-L2 | 8 | 46.0 | 56.0 | 24.0 | 24.5 |
| 8 | L5-L6 | 12 | 72.0 | 58.0 | 30.0 | 11.0 |
| 9 | L5-L6 | 12 | 80.0 | 60.0 | 16.0 | 11.0 |
| 10 | L5-L6 | 12 | 82.0 | 50.0 | 35.0 | 21.0 |
| 11 | L5-L6 | 12 | 44.0 | 61.0 | 13.0 | 6.0 |
| 12 | L5-L6 | 18 | 72.2 | 84.8 | 35.2 | 35.8 |
| 13 | L4-L5 | 18 | 48.6 | 71.8 | 47.2 | 12.8 |
| 14 | L2-L3 | 18 | 52.5 | 48.2 | 15.2 | 11.5 |
| 15 | L4-L5 | 18 | 87.7 | 44.5 | 8.9 | 13.0 |

Sheep disc collagen levels for zones 1-4
2.5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | Collagen (% dry weight) ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|---|
| Operated levels 2.5mm lesion | | | | | | |
| 1 | L5-L6 | 4 | 76.5 | 62.5 | 48.5 | 40.0 |
| 2 | L4-L5 | 4 | 41.0 | 51.0 | 35.0 | 37.0 |
| 3 | L5-L6 | 8 | 70.2 | 76.4 | 10.1 | 8.8 |
| 4 | L6-S1 | 8 | 81.5 | 65.6 | 37.6 | 38.3 |
| 5 | L4-L5 | 12 | 69.6 | 81.5 | 6.5 | 20.2 |
| 6 | L5-L6 | 12 | 60.8 | 81.35 | 4.5 | 12.166 |
| Lumbar control levels | | | | | | |
| 1 | L4-L5 | 4 | 81.0 | 70.0 | 33.0 | 40.0 |
| 2 | L5-L6 | 4 | 63.0 | 87.0 | 44.0 | 42.0 |
| 3 | L4-L5 | 8 | 67.7 | 77.5 | 11.0 | 10.1 |
| 4 | L4-L5 | 8 | 57.4 | 64.7 | 17.7 | 16.7 |
| 5 | L5-L6 | 8 | 58.3 | 53.1 | 17.8 | 28.9 |
| 6 | L5-L6 | 12 | 79.7 | 75.0 | 15.6 | 6.6 |
| 7 | L4-L5 | 12 | 78.1 | 79.3 | 12.2 | 12.5 |

Sheep disc hexuronate acid levels for zones 1-4
5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | Hexuronate (% dry weight) ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|---|
| Operated levels 5.0mm lesion | | | | | | |
| 1 | L4-L5 | 2 | 1.50 | 2.40 | 5.10 | 7.60 |
| 2 | L4-L5 | 4 | 2.40 | 1.90 | 7.50 | 8.15 |
| 3 | L4-L5 | 6 | 1.50 | 0.90 | 5.45 | 5.95 |
| 4 | L3-L4 | 8 | 2.60 | 2.40 | 5.70 | 5.10 |
| 5 | L4-L5 | 8 | 2.60 | 3.00 | 5.20 | 6.45 |
| 6 | L3-L4 | 8 | 2.60 | 3.50 | 2.40 | 4.20 |
| 7 | L4-L5 | 8 | 2.30 | 3.10 | 5.70 | 4.80 |
| 8 | L4-L5 | 12 | 1.00 | 1.85 | 3.20 | 3.40 |
| 9 | L4-L5 | 12 | 0.90 | 1.20 | 2.65 | 2.60 |
| 10 | L4-L5 | 12 | 1.60 | 2.40 | 2.50 | 3.40 |
| 11 | L4-L5 | 12 | 2.35 | 1.15 | 2.50 | 2.90 |
| 12 | L4-L5 | 18 | 3.02 | 1.49 | 3.37 | 3.54 |
| 13 | L3-L4 | 18 | 1.49 | 2.98 | 3.11 | 3.48 |
| 14 | L4-L5 | 18 | 2.10 | 3.48 | 3.01 | 3.25 |
| 15 | L5-L6 | 18 | 1.05 | 1.10 | 3.43 | 3.08 |
| Lumbar control levels | | | | | | |
| 1 | L1-L2 | 2 | 2.60 | 2.35 | 7.85 | 7.40 |
| 2 | L1-L2 | 4 | 2.10 | 2.35 | 7.35 | 8.55 |
| 3 | L5-L6 | 6 | 3.55 | 3.45 | 6.45 | 6.80 |
| 4 | L4-L5 | 8 | 3.80 | 3.10 | 8.20 | 8.70 |
| 5 | L1-L2 | 8 | 2.75 | 2.00 | 8.00 | 7.25 |
| 6 | L4-L5 | 8 | 2.30 | 1.90 | 4.05 | 5.30 |
| 7 | L1-L2 | 8 | 2.50 | 1.70 | 7.85 | 7.50 |
| 8 | L5-L6 | 12 | 1.85 | 1.50 | 6.00 | 2.80 |
| 9 | L5-L6 | 12 | 1.55 | 2.50 | 4.20 | 3.05 |
| 10 | L5-L6 | 12 | 1.30 | 2.30 | 5.30 | 3.70 |
| 11 | L5-L6 | 12 | 1.65 | 1.25 | 5.60 | 4.10 |
| 12 | L5-L6 | 18 | 1.69 | 1.42 | 6.98 | 6.68 |
| 13 | L4-L5 | 18 | 1.63 | 3.27 | 5.02 | 4.54 |
| 14 | L2-L3 | 18 | 2.68 | 2.85 | 3.82 | 3.56 |
| 15 | L4-L5 | 18 | 2.69 | 1.57 | 3.80 | 3.38 |

Sheep disc hexuronate acid levels for zones 1-4
2.5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | Hexuronate (% dry weight) | | | |
|---|---|---|---|---|---|---|
| | | | ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
| Operated levels 2.5mm lesion | | | | | | |
| 1 | L5-L6 | 4 | 1.70 | 1.70 | 4.80 | 4.90 |
| 2 | L4-L5 | 4 | 1.70 | 2.90 | 5.10 | 5.00 |
| 3 | L5-L6 | 8 | 2.10 | 0.97 | 2.20 | 2.27 |
| 4 | L6-S1 | 8 | 1.65 | 1.70 | 4.81 | 5.98 |
| 5 | L4-L5 | 12 | 1.47 | 1.10 | 1.45 | 4.70 |
| 6 | L5-L6 | 12 | 0.85 | 1.27 | 1.90 | 1.85 |
| Lumbar control levels | | | | | | |
| 1 | L4-L5 | 4 | 1.70 | 2.30 | 5.40 | 5.60 |
| 2 | L5-L6 | 4 | 1.70 | 2.90 | 5.10 | 5.00 |
| 3 | L4-L5 | 8 | 1.37 | 1.36 | 2.53 | 3.40 |
| 4 | L4-L5 | 8 | 1.08 | 1.46 | 5.17 | 6.29 |
| 5 | L5-L6 | 8 | 1.97 | 1.49 | 4.95 | 5.83 |
| 6 | L5-L6 | 12 | 1.39 | 1.15 | 3.09 | 2.21 |
| 7 | L4-L5 | 12 | 1.33 | 1.40 | 2.67 | 2.92 |

Sheep disc moisture contents for zones 1-4
5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | Moisture (%) ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|---|
| Operated levels 5.0mm lesion | | | | | | |
| 1 | L4-L5 | 2 | 63.0 | 58.0 | 74.0 | 76.0 |
| 2 | L4-L5 | 4 | 63.0 | 56.0 | 70.0 | 76.0 |
| 3 | L4-L5 | 6 | 58.0 | 58.0 | 73.0 | 74.0 |
| 4 | L3-L4 | 8 | 69.0 | 69.0 | 78.0 | 80.0 |
| 5 | L4-L5 | 8 | 62.0 | 59.0 | 67.0 | 70.0 |
| 6 | L3-L4 | 8 | 63.0 | 48.0 | 90.0 | 81.0 |
| 7 | L4-L5 | 8 | 62.0 | 57.0 | 70.0 | 75.0 |
| 8 | L4-L5 | 12 | 61.0 | 59.0 | 58.0 | 67.0 |
| 9 | L4-L5 | 12 | 52.0 | 46.0 | 65.0 | 66.0 |
| 10 | L4-L5 | 12 | 53.0 | 60.0 | 65.0 | 65.0 |
| 11 | L4-L5 | 12 | 46.0 | 54.0 | 68.0 | 65.0 |
| 12 | L4-L5 | 18 | 34.4 | 45.9 | 55.3 | 57.7 |
| 13 | L3-L4 | 18 | 45.1 | 47.8 | 60.0 | 60.8 |
| 14 | L4-L5 | 18 | 58.5 | 56.1 | 66.5 | 67.9 |
| 15 | L5-L6 | 18 | 44.2 | 47.2 | 65.8 | 61.8 |
| Lumbar control levels | | | | | | |
| 1 | L1-L2 | 2 | 59.0 | 53.0 | 71.0 | 70.0 |
| 2 | L1-L2 | 4 | 53.0 | 51.0 | 69.0 | 69.0 |
| 3 | L5-L6 | 6 | 59.0 | 58.0 | 75.0 | 75.0 |
| 4 | L4-L5 | 8 | 63.0 | 62.0 | 78.0 | 78.0 |
| 5 | L1-L2 | 8 | 56.0 | 56.0 | 73.0 | 69.0 |
| 6 | L4-L5 | 8 | 62.0 | 61.0 | 73.0 | 70.0 |
| 7 | L1-L2 | 8 | 64.0 | 57.0 | 73.0 | 70.0 |
| 8 | L5-L6 | 12 | 55.0 | 55.0 | 69.0 | 61.0 |
| 9 | L5-L6 | 12 | 45.0 | 61.0 | 69.0 | 67.0 |
| 10 | L5-L6 | 12 | 51.0 | 58.0 | 65.0 | 65.0 |
| 11 | L5-L6 | 12 | 59.0 | 60.0 | 68.0 | 66.0 |
| 12 | L5-L6 | 18 | 45.4 | 47.3 | 72.6 | 71.6 |
| 13 | L4-L5 | 18 | 41.9 | 49.7 | 68.8 | 69.8 |
| 14 | L2-L3 | 18 | 47.0 | 58.2 | 72.5 | 67.3 |
| 15 | L4-L5 | 18 | 54.8 | 48.0 | 67.3 | 65.3 |

Sheep disc moisture contents for zones 1-4
2.5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | Moisture (%) | | | |
|---|---|---|---|---|---|---|
| | | | ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
| **Operated levels 2.5mm lesion** | | | | | | |
| 1 | L5-L6 | 4 | 58.0 | 50.0 | 64.0 | 69.0 |
| 2 | L4-L5 | 4 | 57.0 | 59.0 | 74.0 | 74.0 |
| 3 | L5-L6 | 8 | 52.0 | 49.5 | 68.5 | 67.6 |
| 4 | L6-S1 | 8 | 52.4 | 51.6 | 71.2 | 70.1 |
| 5 | L4-L5 | 12 | 57.7 | 56.6 | 62.0 | 70.4 |
| 6 | L5-L6 | 12 | 56.6 | 56.7 | 65.7 | 64.8 |
| **Lumbar control levels** | | | | | | |
| 1 | L4-L5 | 4 | 56.0 | 49.0 | 69.0 | 73.0 |
| 2 | L5-L6 | 4 | 55.0 | 58.0 | 72.0 | 70.0 |
| 3 | L4-L5 | 8 | 47.4 | 52.3 | 68.9 | 68.0 |
| 4 | L4-L5 | 8 | 55.3 | 52.8 | 70.2 | 69.5 |
| 5 | L5-L6 | 8 | 42.4 | 48.3 | 65.7 | 67.2 |
| 6 | L5-L6 | 12 | 57.7 | 55.9 | 67.1 | 62.9 |
| 7 | L4-L5 | 12 | 58.0 | 59.9 | 67.1 | 67.5 |

Sheep disc protein levels extractable from zones 1-4 with 4 M GuHCl 5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | ug protein/mg tissue extracted ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|---|
| Operated levels 5.0mm lesion | | | | | | |
| 1 | L4-L5 | 2 | 24.7 | 25.6 | 25.9 | 27.9 |
| 2 | L4-L5 | 4 | 18.2 | 31.4 | 24.7 | 37.2 |
| 3 | L4-L5 | 6 | 14.6 | 26.7 | 23.2 | 30.9 |
| 4 | L3-L4 | 8 | 27.7 | 26.3 | 39.5 | 43.7 |
| 5 | L4-L5 | 8 | 23.7 | 42.6 | 32.8 | 44.5 |
| 6 | L3-L4 | 8 | 26.3 | 26.3 | 24.5 | 23.8 |
| 7 | L4-L5 | 8 | 36.0 | 50.6 | 47.5 | 48.6 |
| 8 | L4-L5 | 12 | 39.0 | 34.3 | 55.1 | 71.4 |
| 9 | L4-L5 | 12 | 51.3 | 42.9 | 74.2 | 71.8 |
| 10 | L4-L5 | 12 | 56.2 | 63.5 | 40.8 | 46.4 |
| 11 | L4-L5 | 12 | 85.3 | 72.1 | 50.7 | 54.7 |
| 12 | L4-L5 | 18 | 50.2 | 37.7 | 44.6 | 44.9 |
| 13 | L3-L4 | 18 | 44.3 | 31.9 | 23.1 | 35.5 |
| 14 | L4-L5 | 18 | 38.7 | 38.9 | 49.7 | 44.4 |
| 15 | L5-L6 | 18 | 35.3 | 26.7 | 22.8 | 31.7 |
| Lumbar control levels | | | | | | |
| 1 | L1-L2 | 2 | 18.6 | 20.7 | 24.5 | 29.6 |
| 2 | L1-L2 | 4 | 23.0 | 19.9 | 22.3 | 22.6 |
| 3 | L5-L6 | 6 | 8.2 | 13.9 | 18.7 | 24.8 |
| 4 | L4-L5 | 8 | 32.2 | 20.3 | 20.7 | 26.4 |
| 5 | L1-L2 | 8 | 26.3 | 20.9 | 19.6 | 16.4 |
| 6 | L4-L5 | 8 | 16.2 | 33.8 | 20.0 | 17.1 |
| 7 | L1-L2 | 8 | 16.2 | 17.6 | 30.7 | 29.7 |
| 8 | L5-L6 | 12 | 13.3 | 17.3 | 25.1 | 25.5 |
| 9 | L5-L6 | 12 | 22.6 | 22.6 | 32.0 | 22.9 |
| 10 | L5-L6 | 12 | 23.2 | 20.8 | 17.9 | 24.0 |
| 11 | L5-L6 | 12 | 11.3 | 29.6 | 14.5 | 21.5 |
| 12 | L5-L6 | 18 | 26.5 | 33.4 | 19.4 | 20.5 |
| 13 | L4-L5 | 18 | 26.67 | 36.5 | 15.9 | 17.9 |
| 14 | L2-L3 | 18 | 32.4 | 17.1 | 24.7 | 37.4 |
| 15 | L4-L5 | 18 | 29.9 | 27.2 | 19.4 | 16.9 |

Sheep disc protein levels extractable from zones 1-4
with 4 M GuHCl 2.5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | ug protein/mg tissue extracted ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|---|
| Operated levels 2.5mm lesion | | | | | | |
| 1 | L5-L6 | 4 | 24.8 | 26.5 | 25.6 | 22.3 |
| 2 | L4-L5 | 4 | 26.1 | 28.9 | 20.3 | 23.5 |
| 3 | L5-L6 | 8 | 30.3 | 30.2 | 27.1 | 32.4 |
| 4 | L6-S1 | 8 | 31.5 | 30.8 | 24.8 | 23.9 |
| 5 | L4-L5 | 12 | 34.9 | 37.4 | 20.5 | 27.8 |
| 6 | L5-L6 | 12 | 36.8 | 46.2 | 27.1 | 20.0 |
| Lumbar control levels | | | | | | |
| 1 | L4-L5 | 4 | 22.3 | 25.7 | 22.0 | 20.6 |
| 2 | L5-L6 | 4 | 24.0 | 28.3 | 18.5 | 21.6 |
| 3 | L4-L5 | 8 | 25.6 | 29.2 | 16.8 | 23.2 |
| 4 | L4-L5 | 8 | 33.1 | 27.9 | 18.7 | 21.0 |
| 5 | L5-L6 | 8 | 32.1 | 27.9 | 19.2 | 20.8 |
| 6 | L5-L6 | 12 | 29.1 | 24.6 | 21.9 | 17.0 |
| 7 | L4-L5 | 12 | 37.0 | 33.3 | 20.3 | 17.2 |

Sheep disc PG extractability levels with 4 M GuHCl for zones 1-4 5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | PG extractability (%) | | | |
|---|---|---|---|---|---|---|
| | | | ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
| Operated levels 2.5mm lesion | | | | | | |
| 1 | L4-L5 | 2 | 83.0 | 79.0 | 92.0 | 96.0 |
| 2 | L4-L5 | 4 | 74.0 | 62.0 | 91.0 | 92.0 |
| 3 | L4-L5 | 6 | 87.0 | 88.0 | 94.0 | 94.0 |
| 4 | L3-L4 | 8 | 83.0 | 85.5 | 95.0 | 95.0 |
| 5 | L4-L5 | 8 | 57.0 | 65.0 | 83.0 | 89.0 |
| 6 | L3-L4 | 8 | 77.0 | n.d. | 92.0 | 94.0 |
| 7 | L4-L5 | 8 | 82.5 | 73.0 | 94.0 | 90.0 |
| 8 | L4-L5 | 12 | 69.0 | 68.0 | 84.0 | 91.0 |
| 9 | L4-L5 | 12 | 70.0 | 83.0 | 90.0 | 92.5 |
| 10 | L4-L5 | 12 | 72.0 | 67.0 | 88.0 | 88.0 |
| 11 | L4-L5 | 12 | 77.0 | 86.0 | 88.0 | 92.0 |
| 12 | L4-L5 | 18 | 76.5 | 76.9 | 82.8 | 90.4 |
| 13 | L3-L4 | 18 | 87.7 | 84.6 | 85.6 | 86.8 |
| 14 | L4-L5 | 18 | 83.4 | 83.0 | 92.2 | 91.5 |
| 15 | L5-L6 | 18 | 71.5 | 67.5 | 94.0 | 92.6 |
| Lumbar control levels | | | | | | |
| 1 | L1-L2 | 2 | 80.0 | 84.5 | 94.0 | 95.0 |
| 2 | L1-L2 | 4 | 67.0 | 68.0 | 92.5 | 93.0 |
| 3 | L5-L6 | 6 | 80.0 | 82.0 | 95.5 | 96.0 |
| 4 | L4-L5 | 8 | 85.5 | 82.5 | 96.0 | 96.0 |
| 5 | L1-L2 | 8 | 71.0 | 70.5 | 93.0 | 93.0 |
| 6 | L4-L5 | 8 | n.d. | n.d. | 90.0 | 88.5 |
| 7 | L1-L2 | 8 | 75.0 | 82.0 | 96.0 | 96.0 |
| 8 | L5-L6 | 12 | 72.0 | 74.5 | 92.0 | 94.0 |
| 9 | L5-L6 | 12 | 68.5 | 74.0 | 96.0 | 95.0 |
| 10 | L5-L6 | 12 | 41.0 | 69.0 | 91.0 | 92.0 |
| 11 | L5-L6 | 12 | 68.0 | 75.0 | 93.0 | 94.0 |
| 12 | L5-L6 | 18 | 85.4 | 90.6 | 95.0 | 92.2 |
| 13 | L4-L5 | 18 | 81.1 | 83.4 | 92.8 | 93.4 |
| 14 | L2-L3 | 18 | 79.3 | 88.3 | 96.5 | 98.2 |
| 15 | L4-L5 | 18 | 83.0 | 75.3 | 95.8 | 92.5 |

Sheep disc PG extractability levels with 4 M GuHCl
for zones 1-4 2.5mm lesion samples

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | PG extractability (%) | | | |
|---|---|---|---|---|---|---|
| | | | ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
| **Operated levels 2.5mm lesion** | | | | | | |
| 1 | L5-L6 | 4 | n.d. | n.d. | 92.0 | 92.0 |
| 2 | L4-L5 | 4 | 81.0 | 80.0 | 95.0 | 94.5 |
| 3 | L5-L6 | 8 | 92.8 | 87.2 | 94.2 | 96.9 |
| 4 | L6-S1 | 8 | 82.9 | 82.7 | 92.0 | 92.1 |
| 5 | L4-L5 | 12 | 79.1 | 83.4 | 97.4 | 96.2 |
| 6 | L5-L6 | 12 | 86.8 | 80.2 | 86.6 | 93.0 |
| **Lumbar control levels** | | | | | | |
| 1 | L4-L5 | 4 | 87.6 | 88.6 | 92.6 | 94.3 |
| 2 | L5-L6 | 4 | 88.9 | 90.1 | 95.6 | 94.8 |
| 3 | L4-L5 | 8 | 76.5 | 81.4 | 95.2 | 96.9 |
| 4 | L4-L5 | 8 | 86.1 | 81.0 | 96.2 | 95.6 |
| 5 | L5-L6 | 8 | 88.1 | 76.8 | 94.0 | 94.4 |
| 6 | L5-L6 | 12 | 86.7 | 83.8 | 95.8 | 95.7 |
| 7 | L4-L5 | 12 | 82.7 | 86.9 | 96.0 | 95.1 |

Changes in non-aggregating Proteoglycan monomer hydrodynamic size by Sepharose CL2B gel chromatography with duration after surgical treatment

5mm deep lesion
early and intermediate phase

| SHEEP | TIME POST-SURGERY MONTHS | KAV. ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|
| 1 | 2 | 0.580 | 0.590 | 0.600 | 0.600 |
| 2 | 4 | 0.595 | 0.610 | 0.660 | 0.670 |
| 3 | 6 | 0.590 | 0.600 | 0.650 | 0.645 |
| 4 | 8 | 0.585 | 0.550 | 0.640 | 0.635 |
| 5 | 8 | 0.595 | 0.600 | 0.625 | 0.650 |
| 6 | 8 | 0.590 | 0.600 | 0.635 | 0.640 |
| 7 | 12 | 0.580 | 0.550 | 0.610 | 0.620 |
| 8 | 12 | 0.590 | 0.590 | 0.630 | 0.640 |
| 9 | 12 | 0.585 | 0.590 | 0.620 | 0.620 |
| 10 | 12 | 0.580 | 0.585 | 0.625 | 0.615 |
| 11 | 12 | 0.580 | 0.580 | 0.630 | 0.625 |

Aggregation levels (%) with 4% v/v HA of A1 purified proteoglycans from disc zones 1-4 determined by Sepharose CL2B gel chromatography

| SHEEP | DISC LEVEL | TIME POST-OPERATION (MONTHS) | ZONE 1 | ZONE 2 | ZONE 3 | ZONE 4 |
|---|---|---|---|---|---|---|
| Operated levels 5.0mm lesion | | | | | | |
| 1 | L4-L5 | 2 | 40.00 | 39.80 | 32.10 | 33.00 |
| 2 | L4-L5 | 4 | 20.60 | 12.80 | 19.40 | 22.40 |
| 3 | L4-L5 | 6 | 36.90 | 24.20 | 33.80 | 32.40 |
| 4 | L3-L4 | 8 | 30.00 | 37.00 | 28.30 | 33.40 |
| 5 | L4-L5 | 8 | 25.20 | 21.30 | 33.50 | 31.50 |
| 6 | L3-L4 | 8 | 40.90 | 32.70 | 27.20 | 20.30 |
| 7 | L4-L5 | 8 | 24.70 | 28.30 | 40.10 | 46.10 |
| 8 | L4-L5 | 12 | 34.70 | 40.60 | 42.40 | 41.90 |
| 9 | L4-L5 | 12 | 37.20 | 35.50 | 42.20 | 34.70 |
| 10 | L4-L5 | 12 | 33.80 | 34.40 | 34.30 | 37.50 |
| 11 | L4-L5 | 12 | 33.30 | 36.20 | 33.30 | 27.70 |
| 12 | L4-L5 | 18 | 33.30 | 43.00 | 42.90 | 43.20 |
| 13 | L3-L4 | 18 | 31.40 | 35.90 | 32.50 | 31.80 |
| 14 | L4-L5 | 18 | 35.50 | 43.10 | 34.30 | 35.80 |
| 15 | L5-L6 | 18 | 30.40 | 38.90 | 32.40 | 37.80 |
| Operated levels 2.5mm lesion | | | | | | |
| 1 | L5-L6 | 4 | 30.80 | 32.50 | 34.20 | 36.90 |
| 2 | L4-L5 | 4 | 35.70 | 34.20 | 29.20 | 28.60 |
| 3 | L5-L6 | 8 | 36.60 | 36.50 | 31.60 | 28.20 |
| 4 | L6-S1 | 8 | 35.60 | 39.40 | 34.40 | 38.80 |
| 5 | L4-L5 | 12 | 36.20 | 42.10 | 36.50 | 28.80 |
| 6 | L5-L6 | 12 | 39.00 | 38.70 | 27.80 | 32.60 |