



Allograft Intervertebral Disc Transplantation In The Sheep

An Assessment of the Potential for Allograft Disc Replacement

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Signed Statement

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1. ABSTRACT

STUDY DESIGN: An allograft intervertebral disc transplantation procedure was carried out in a total of twenty 2-year old Merino wethers.

OBJECTIVE: The aims of this study were to test the viability and integrity of an intervertebral disc following transplantation from one animal to another, and to assess the safety and reliability of the transplant procedure.

SUMMARY OF BACKGROUND DATA: Because of the unpredictable clinical results reported in the literature, the role of spinal fusion for chronic disabling back pain associated with degeneration of the intervertebral disc remains controversial. The challenge for the future will be to develop other forms of spinal stabilisation procedures that may produce better and more reliable results than spinal arthrodesis. Recently, prosthetic intervertebral disc replacement has been developed and trialled in a number of centres but concerns exist regarding the likely long term effects of these implants, particularly those related to loosening, wear particles and infection.

Allograft intervertebral disc transplantation, however, may be able to overcome the inherent limitations of artificial disc replacement and may also be able to better withstand the normal physiological demands and restore the function of the motion segment. If successful, allograft disc replacement may become, in theory, an appealing alternative to fusion surgery.

METHODS: The study was carried out in a total of twenty 2-year old Merino wethers. Donor intervertebral disc units were harvested from an additional eight sheep which were sacrificed within four hours of transplantation. The disc units were harvested using an oscillating saw and comprised the entire intervertebral disc together with a thin plate of adjacent vertebral bone. A single intervertebral disc unit was inserted into the recipient lumbar spine via an antero-lateral approach under a general anaesthetic and with strict aseptic conditions. Attempted stabilisation of the allograft employing small screws inserted obliquely into the adjacent vertebral bodies proved to be unsatisfactory in the first group of sheep. Internal fixation of adjacent vertebrae with a lateral plate and screws was subsequently used in an attempt to prevent ventral displacement of the disc unit.

A second intervertebral disc unit was also implanted free into the psoas muscle of five recipient animals to assess whether diffusion of nutrients from body fluids alone would be sufficient to maintain disc nutrition. Following insertion of the allograft the position was checked by AP and lateral radiographs. In the first group, x-rays were repeated at two weeks under a general anaesthetic and then at six weeks immediately following sacrifice. Following the removal of all plates at three weeks the sheep in a second group were allowed to roam free in a paddock for a total survival time of three months. X-rays were performed immediately post-operation and then following sacrifice of the animal. Mid-sagittal sections of the lumbar spine were processed and a detailed macroscopic and histological examination performed.

RESULTS: All allografts were viable on histological grounds. Seven disc units were found to have partly displaced, all of which had developed end-plate fractures through the thinnest portion of the adjacent harvested bone leading to disc degeneration. A non-union had occurred at the donor-recipient interface on the cranial side in six cases.

The remaining transplanted discs including those implanted free into the psoas muscle showed no evidence of accelerated degenerative change when compared with controls.

CONCLUSION: This study has demonstrated the many technical difficulties and risks associated with attempted allograft transplantation and that allograft intervertebral discs can survive after transplantation. Diffusion of nutrients from body fluids would appear to be sufficient to maintain disc nutrition until blood supply to the end-plate is restored. Accurate placement and prevention of displacement is essential if osteophyte formation and end-plate fractures are to be prevented. Further extensive studies would be required to refine the surgical technique and to determine the health of the transplanted disc in the longer term, before an allograft transfer procedure could be considered in the human.



2. INTRODUCTION

Total joint replacement has revolutionised the treatment of degenerative hip and knee conditions and accordingly the indications for arthrodesis of these joints have become more limited. In the lower back, however, the indications for arthrodesis since the early reports of its use for the management of spinal deformity, have broadened to include chronic disabling back pain associated with degeneration of the intervertebral disc. Unfortunately, the reported clinical results of spinal fusion have been unpredictable and associated with a significant failure rate (Turner et al, 1992; Kostuik and Frymoyer, 1991). Furthermore there is some clinical and experimental evidence that spinal fusion may potentiate the degeneration of levels adjacent to the fusion mass (Lehmann et al, 1987; Leong et al, 1983; Lee, 1988; Lee and Langrana, 1984; Quinell and Stockdale, 1981). The role of spinal fusion for chronic back pain therefore remains controversial.

More recently there has been a move to replace the intervertebral joints using various prostheses with the aim of providing early stabilisation and at the same time maintaining movement (Lee, 1991).

The early clinical results reported are encouraging (David, 1993; Enker et al, 1993) but concerns exist regarding the long term survival of these implants.

The challenge for the future will be to develop other forms of spinal stabilisation procedures that will overcome the inherent limitations of these currently available techniques. Given these limitations, we considered exploring the potential for allograft intervertebral disc transplantation. It was considered that this procedure may be able to re-establish spinal stability and at the same time preserve spinal mobility.

3. AIMS OF THE STUDY

The intervertebral disc is essentially an avascular structure and derives its nutrition largely by diffusion of nutrients across the endplate. We would therefore hypothesize that the viability of the intervertebral disc could be maintained following transplantation from one animal to another. The aims of this research were to test this hypothesis and, more importantly, to assess the safety and reliability of allograft intervertebral disc transplantation using an animal model. This experimental study involved work in two stages:

- (1) A pilot study to develop the surgical technique.

- (2) A more definitive follow-up study to determine the health of the transplanted discs in the longer term.

4. LITERATURE REVIEW

To the best of our knowledge, transplantation of fresh isolated intervertebral discs from one animal to another has not been previously reported in the literature. However, a related experimental investigation in dogs to assess the potential of vertebral column allografts in the treatment of segmental spinal defects was reported in 1991 by Olson et al. The donor allograft unit comprised the entire body of the eighth thoracic vertebra and the two intact adjacent intervertebral discs harvested together with a triangular wedge of bone from the seventh and ninth thoracic bodies. The allografts were frozen to -80°C for at least two weeks prior to the implantation in eleven mongrel hounds. The animals were followed for eighteen months post-operatively with serial radiographs which demonstrated a gradual loss of intervertebral disc height. Following sacrifice a number of investigations were performed including a macroscopic assessment of the intervertebral disc allografts. This demonstrated that the majority of the discs had degenerated severely.

More recently, independent groups of researchers have been exploring the new concept of isolated intervertebral disc grafting. One unpublished study from Chicago, Illinois, explored the feasibility of cryo-preserved osteodiscal allografts in a canine model (Hammerberg and Dewald, 1993). Two of the five dogs suffered neurologic injury as a consequence of graft dislodgement and were therefore sacrificed. The remaining three animals were sacrificed at twelve months. All three demonstrated advanced degenerative changes in the transplanted intervertebral discs on gross morphological and histological grounds. In a similar study reported recently from Japan, thirteen adult mongrel dogs underwent allografting of cryo-preserved intervertebral discs (Katsuura et al, 1994). Histological and radiological evidence of progressive degeneration was demonstrated following the sacrifice of three groups of dogs at three, six and twelve months respectively.

Other unpublished studies have assessed the feasibility of intervertebral disc autografts. A group of investigators from Charlotte, North Carolina, performed disc transfer surgery in ten mature mongrel dogs (Frick et al, 1993). This involved the transposition of an upper lumbar and a lower lumbar intervertebral disc in one surgical setting. The spines were harvested at three months and then studied histologically and biochemically.

Preservation of disc viability was demonstrated but radiological studies at three months showed average disc height narrowing of 20%. This was considered by the authors to be a sign of degeneration.

In a related study from Hong Kong, twelve Rhesus monkeys were used to assess the biochemical and histological changes of an intervertebral disc which was essentially devascularised (Luk & Ruan, 1993). A lumbar disc was completely mobilised en bloc together with a thin plate of adjacent vertebral bone and then re-inserted into its anatomical position. Three groups of four monkeys were sacrificed after two, four and six months respectively.

At six months disc viability was preserved and no evidence of accelerated degeneration was reported on macroscopic examination. Early narrowing of disc height was observed however, which persisted until the final follow-up.

These studies have assessed the potential for both autograft and frozen allograft intervertebral disc replacement, but to date, we are not aware of any study which has explored the feasibility of transplanting isolated fresh allograft intervertebral discs in vivo. This was the principle aim of our study.

5. THE PILOT STUDY

5.1 TECHNICAL CONSIDERATIONS

It was expected that a number of technical aspects would influence the outcome of allograft intervertebral disc replacement. In order to optimise conditions for a successful transplant, we considered the following aspects to be important.

- (i) Allograft thickness
- (ii) Recipient bed preparation
- (iii) Allograft retention
- (iv) Timing of transplanation

5.11 ALLOGRAFT THICKNESS

In order to maintain the structural integrity of the intervertebral disc it was thought necessary to harvest at least a thin plate of adjacent vertebral cancellous bone to protect the underlying bony cartilaginous endplate. This would also improve conditions for a more rapid bony union to the vascular cancellous bony bed of the recipient animal.

We also considered that the viability of the transplanted intervertebral disc may be affected by the bony thickness of the disc unit. The question was "how thick should it be"?

Apart from a small blood supply to the peripheral annulus fibrosis, the intervertebral disc is an avascular structure which derives its nutrition by diffusion of nutrients from blood vessels adjacent to the endplate (Maroudas, 1982). The rate of diffusion is dependent on a number of factors including the diffusion distance. Mathematically, the efficiency of diffusion is inversely proportional to the square of the diffusion distance (Wright, 1978). It would therefore seem necessary to harvest only a very thin plate of adjacent vertebral bone, so that nutrition to the disc could be re-established as soon as possible following transplantation. We thus planned to harvest only a very thin plate of adjacent vertebral bone, together with the donor disc.

5.12 RECIPIENT BED PREPARATION

An equivalent thickness of bone, together with the disc, would be excised to match the thickness of the harvested allograft.

5.13 ALLOGRAFT RETENTION

Because of the natural lordosis and horizontal alignment of the sheep lumbar spine, we anticipated problems relating to allograft retention, with potential instability, especially in a ventral direction.

Coincidentally, other investigators have encountered problems with retention of the transplanted disc unit (Frick, 1993). In one study, two animals suffered spinal cord injury as a consequence of graft dislodgement and were sacrificed (Hammerburg & Dewald, 1993). In order to prevent this from occurring, we planned to preserve the recipient anterior longitudinal ligament or, if this proved unsatisfactory, to use two small screws inserted anterolaterally into the adjacent recipient vertebrae angled away from the graft so that the screw heads would abutt the graft bone. This method of fixation was chosen in the first instance with the aim of causing minimal interference with the structural integrity and functional mobility of the transplanted disc.

5.14 TIMING OF TRANSPLANTATION

Allograft disc units would be transplanted fresh as soon as possible after harvest. The rationale for utilising fresh rather than preserved intervertebral disc allografts was based on a review of the results of studies exploring methods of preservation of osteochondral allografts.

Large osteochondral allografts have been used clinically to replace traumatic joint defects (Gross, 1992). These studies have shown that cartilage harvested without a blood supply is 100% viable within 24 hours of the death of the donor and can be preserved for up to four days at 4°C. Freezing, on the other hand, kills cartilage cells. The viability rates reported following different methods of cryo-preservation vary considerably from 15% to 50% at best. Bone harvested without a blood supply will not remain viable, whether fresh or preserved because of its inability to survive without immediate re-vascularisation. It remains structurally intact, however, and mechanically strong until it is replaced by host bone by creeping substitution.

If these results can be extrapolated to the cartilaginous and bony components of the intervertebral disc, we would expect that the use of fresh specimens would optimize the chances of graft survival *in vivo*. Justification for the use of fresh intervertebral disc allografts is further supported by the results of frozen intervertebral disc allograft replacement in the canine model (Katsuura et al, 1994; Hammerburg & Dewald, 1993; Olson et al, 1991), where advanced degenerative changes were observed at six, twelve and eighteen months respectively.

We planned to explore methods of allograft disc storage prior to transplantation *in vivo*, only if the viability and structural integrity of the transplanted fresh disc could be demonstrated.

5.2 METHODOLOGY

Permission to carry out the study was granted by the Animal Ethics Committee of the Institute of Veterinary and Medical Science in Adelaide, South Australia in early 1993.

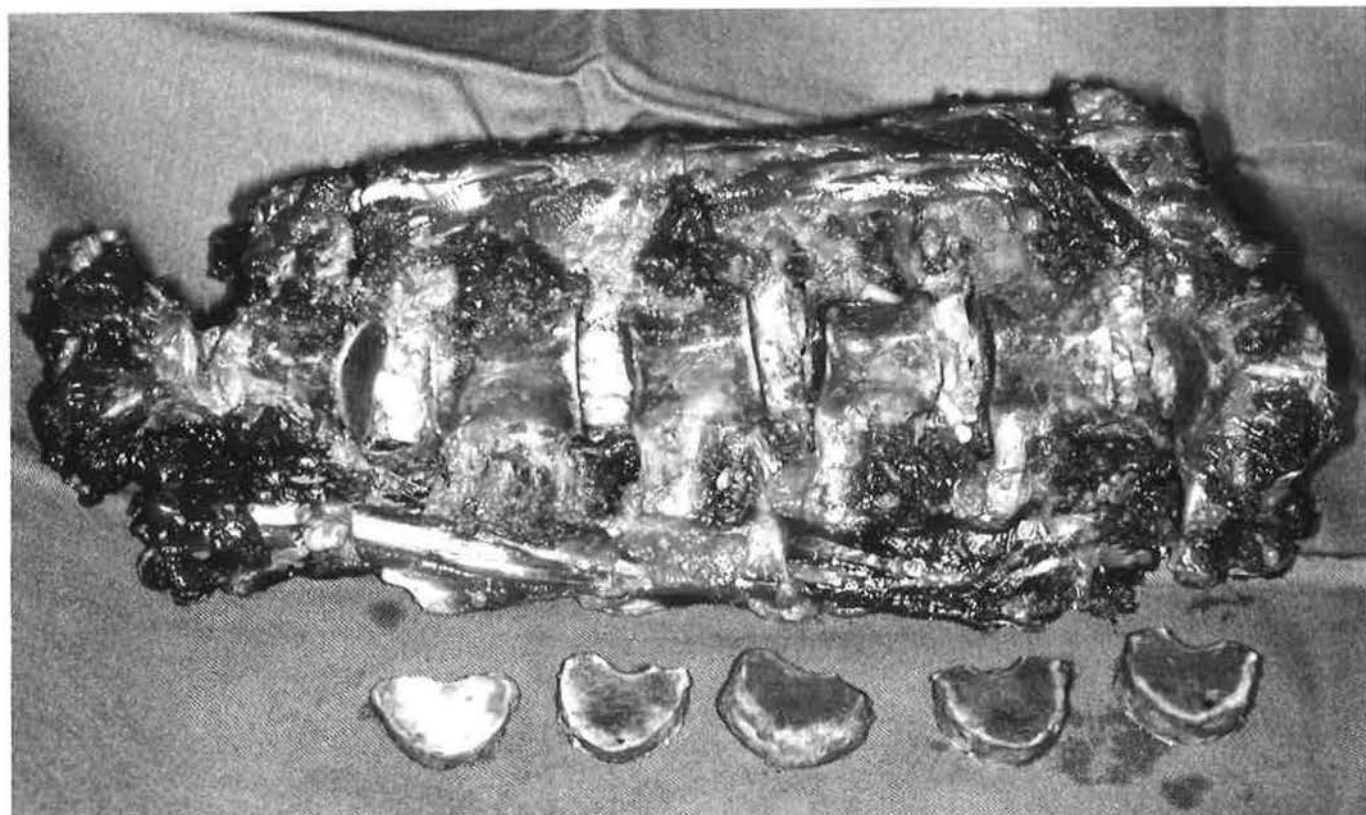
5.21 ALLOGRAFT HARVEST

A total of four two year old Merino wethers acted as donors. The donor animal was sacrificed on the same day and within four hours of the transplantation procedure using a lethal dose of intravenous pentobarbitone sodium (20mls; 325mg per ml) which was injected into the internal jugular vein. The skin was shaved over the lumbar area and under strict aseptic conditions the entire lumbar spine was removed via a posterior mid-line approach.

The psoas muscles and the anterior longitudinal ligament were dissected carefully from the underlying vertebral bodies. Individual donor discs were then harvested using an oscillating saw and comprised the entire intervertebral disc sandwiched between thin plates of adjacent vertebral cancellous bone which ranged in thickness from approximately 1-3mm (Figs. 1;2).

Fig. 1 Following the removal of the entire lumbar spine, donor intervertebral disc units were harvested using an oscillating saw.

Fig 2. The allograft comprised the entire intervertebral disc sandwiched between thin plates of adjacent vertebral bone ranging in thickness from approximately 1-3mm.



The line of attachment of the anterolateral annular fibres to the adjacent vertebral body rim was used as a guide to make parallel cuts in an anteroposterior direction until the spinal canal was entered (Fig 1). This technique unexpectedly resulted in the harvesting of unequal thicknesses of adjacent vertebral bone with a much thinner plate on the cranial side of the disc. It was only then that the precise insertion of the outer annular fibres to the adjacent vertebral body was appreciated. On the caudal side, the fibre attachments encroached onto a greater portion of the adjacent vertebral body rim. In order to completely extricate the disc unit from this side, it was necessary to cut into the most proximal aspect of the pedicles. The macroscopic appearance of the freshly cut disc unit surface varied depending on the thickness of the adjacent vertebral cancellous bone.

Harvesting a thin plate of bone of approximately 2mm or less resulted in a cut made through dense and avascular looking bone producing a pale appearance to the cut surface. This was in contrast to the thicker plate of bone (approximately 3mm) harvested on the caudal side which was softer to saw and was cut through more vascular cancellous bone.

In order to refine this technique, disc units were harvested from all available lumbar levels. The sheep lumbar spine consists of six lumbar vertebrae with six corresponding intervertebral discs. Allograft disc units with the most accurately cut parallel sides were chosen for transplantation. Individual grafts were placed in sterile plastic containers filled with Ringer's lactate solution and were transplanted within four hours of harvest.

5.22 RECIPIENT PREPARATION

A total of 7 recipient sheep were anaesthetized using one gram of sodium thiopentone as the induction agent and then maintained with nitrous oxide and isoflurane with the animals breathing spontaneously. Prophylactic intramuscular antibiotics were administered in a standardised regime consisting of penicillin (1gm) and streptomycin (1gm) injected into the left gluteal region at the time of induction and then repeated at the completion of surgery. The sheep were placed in a left lateral position and the skin over the left flank and lumbo-sacral area was shaved using clippers.

The shaved area was washed with 70% alcohol and prepared with povidone iodine in spirit. Sterile operating drapes were placed across the sheep leaving only the operation site exposed and surgery was performed under strict aseptic conditions using standard sterile techniques (Fig. 3).

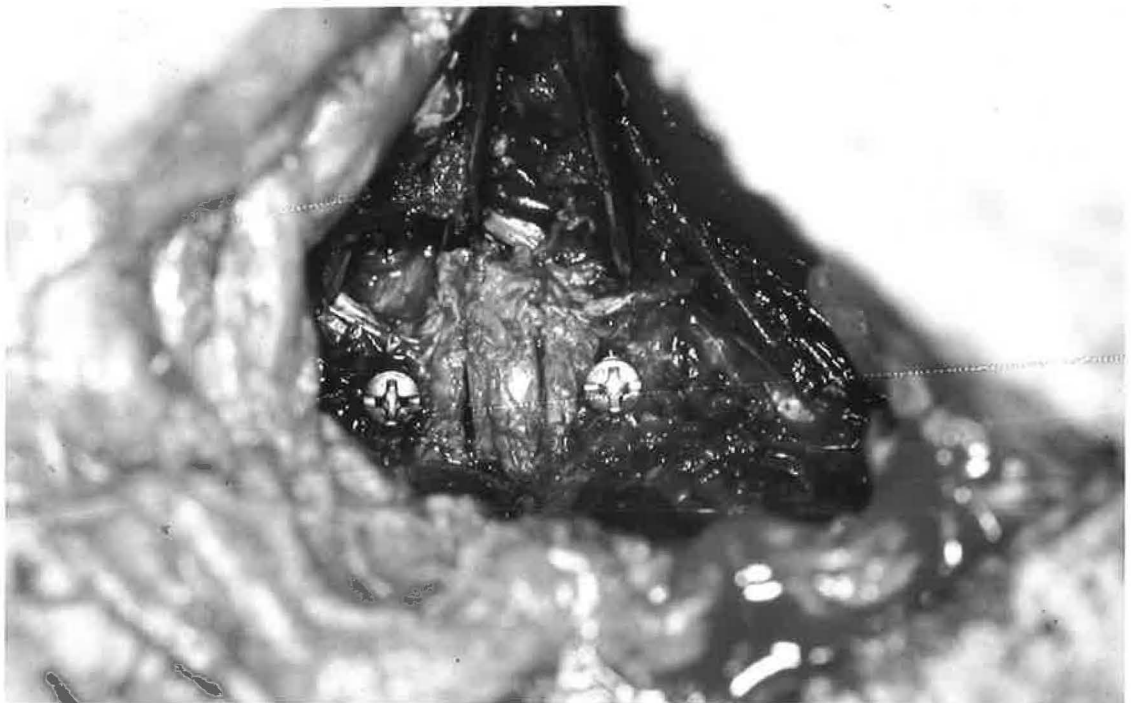
A longitudinal incision was made just anterior to the tips of the palpable transverse processes and through the posterior wall of the abdomen to gain access to the retroperitoneal space (Fraser et al, 1986).

The psoas muscles (minor and major) were then retracted anteriorly to expose the mid-lumbar spine from the left lateral aspect. A thin bladed osteotome was used to make parallel cuts in a fashion similar to the harvesting technique. Starting from the left lateral side and proceeding through to the right, extreme care was employed to avoid penetration of the spinal canal. The mid-lumbar disc was excised in its entirety, together with the adjacent vertebral bone using disc rongeurs, upcutting punches and curettes until the intact posterior longitudinal ligament was exposed (Fig. 4).

Fig. 3 A left anterolateral approach was used to expose the mid-lumbar spine.

Fig. 4 The recipient bed was prepared by excising the entire intervertebral disc, together with a thin plate of adjacent vertebral bone.

Fig. 5 The disc unit was transplanted into the recipient site. In this case a distracting device was used between 2 screws to facilitate insertion of the graft.



Preservation of the anterior longitudinal ligament proved difficult and almost impossible despite careful initial attempts to do so. Excision of the vertebral body bone posteriorly on the caudal side of the recipient site proved to be a tedious and time consuming procedure because of the following three anatomical facts:

- (a) The pedicles in the sheep lumbar spine attach very close to the superior endplates of their respective vertebral bodies.
- (b) The most proximal aspect of the pedicles lie within the outer annular fibre attachments.
- (c) The transverse processes are long and project ventrally over the vertebral column.

Preparation of the caudal recipient bed therefore necessitated excision of the most proximal attachment of the pedicle. A similar problem was encountered during the harvesting process.

In order to gain additional access posteriorly, the long forward-projecting transverse processes were osteotomized at their bases and retracted posteriorly in the last two recipient animals. This improved the exposure considerably and facilitated the preparation of the recipient bed, especially on the caudal aspect.

5.23 ALLOGRAFT INSERTION

The donor disc unit was prepared by rounding off any protruding bone with fine bone nibblers.

In the first four animals, the recipient site was distracted to facilitate the insertion of the graft. A distracting device was used between two screws which were placed through the adjacent vertebral bodies in the coronal plane. The graft was inserted into the recipient bed and the distracting device was then released (Fig 5). Because of the technical difficulties encountered with the harvesting process and preparation of the recipient bed, accurate siting of the disc units was extremely difficult, often necessitating re-siting after excision of more bone from the recipient bed. Some residual ventral displacement of approximately 1-3mm was accepted in most cases.

In the first animal the distracting screws were left in place, but in the subsequent three cases they were removed. This distracting technique was thereafter abandoned because of problems encountered with screw bending and subsequent breakage in one case.

In the last three animals, allograft insertion was facilitated by extending the lumbar spine with a hand placed over the spinous processes. This manoeuvre essentially distracted the recipient site.

5.24 ALLOGRAFT STABILISATION

In an attempt to stabilise the graft, a vicryl sling was placed around the protruding heads of the screws in the first animal. This proved to be unsatisfactory and in the next four animals tiny screws (4 gauge by 10mm self-tapping screws) were placed anterolaterally into the adjacent vertebral bodies as far anteriorly as the exposure would allow. They were angled away from the donor-recipient interface so that the heads of the screws would overlap and abutt the allograft bone (Figs. 17;18). This method of fixation failed to adequately stabilise the graft and so in the last two animals internal fixation of the adjacent vertebral bodies was employed using a four hole cobalt-chrome metal alloy plate (Sherman Plate).

The plate was bridged across the allograft and secured with four stainless steel screws (6 gauge x 25mm self-tapping screws), two above the allograft and two below (Figs. 23-26). The inner two screws were angled away from the donor-recipient interface in order to minimise damage to the recipient bed. The allograft position was checked with AP and lateral radiographs and the wound then closed in layers.

Intramuscular methadone hydrochloride was injected in 25mg boluses as required for post-operative analgesia and the sheep housed individually in pens in the theatre complex for the first two weeks. They were then transferred to larger pens in an animal house for another four weeks. AP and lateral radiographs were repeated at two weeks under a general anaesthetic and immediately following sacrifice at six weeks.

5.23 SPECIMEN COLLECTION

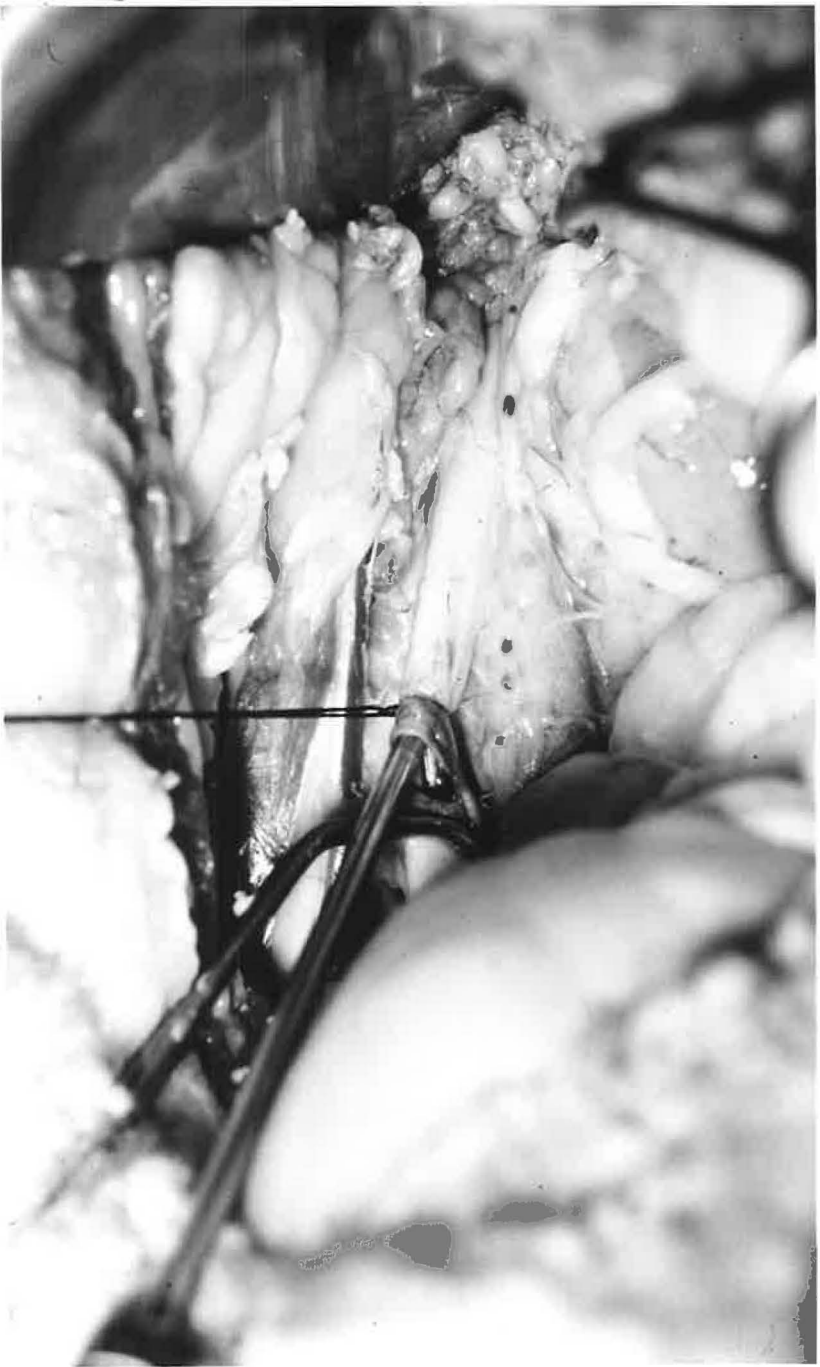
The animals were killed using a lethal dose of intravenous pentobarbitone sodium (325mg per ml) and allowed to exanguinate after cutting the carotid and jugular vessels. In order to assess re-vascularisation of the grafts histologically, dye-perfusion studies were performed prior to the retrieval of the specimen in all animals. The aorta was exposed via a left retroperitoneal approach, clamped proximally just below the renal arteries and distally above the trifurcation and then catheterised (Fig. 6). A solution containing indian ink, gelatine and barium was mixed in warm water and then 50mls injected under pressure using a large-bore syringe (Fig. 7).

Following a 30-minute cooling period to allow the gelatine to set, the entire lumbar spine was removed en bloc via a posterior mid-line approach. After carefully clearing the soft tissues a mid-sagittal cut was made through the entire specimen using a band saw.

Following close macroscopic examination of the transplanted unit, all specimens were photographed.

Fig. 6 Following sacrifice of the recipient animals, dye-perfusion studies were performed after cannulating the abdominal aorta.

Fig. 7 A solution containing Indian ink, gelatine and barium was injected under pressure into the abdominal aorta.



The specimens were fixed in 10% neutral buffered formalin and de-calcified in 9.5% nitric acid and 1% EDTA in solution. Para-saggital specimens of the transplanted disc unit including the adjacent vertebral bodies were embedded into paraffin wax and then sectioned. The sections for light microscopy were cut into five micro-metre slices, stained with haematoxylin and eosin and a detailed histological examination performed.

5.24 PLAIN RADIOGRAPHS

AP and lateral radiographs were performed immediately post surgery, at two weeks under a general anaesthetic and immediately following sacrifice at six weeks. A serial x-ray analysis was performed and the following features recorded:

- (a) The position of the transplanted disc unit immediately post-surgery. Residual displacement was recorded as a percentage of the maximum AP diameter of the disc unit measured on the lateral plain radiograph.
- (b) Any displacement at two and six weeks.
- (c) Narrowing of the disc space. This was measured on the lateral radiographs. In order to eliminate errors due to different magnifications, the height of the allograft disc, as well as the disc space below (calculated as the distance between the middle of the upper and lower end-plates) were measured and expressed as a ratio on both initial and final radiographs. In this way, any loss of disc height could be determined.
- (d) Presence of osteophytes.

5.25 MACROSCOPIC EXAMINATION

An assessment was made of the following features:

- (1) The anatomical position of the allograft.
- (2) The extent of union at the donor-recipient interface.
- (3) The structural integrity of the endplates.
- (4) The state of the transplanted disc with particular reference to signs of nuclear degeneration including
 - (a) Narrowing of the intervertebral disc space.
 - (b) Loss of turgidity and bulging of nuclear material from the freshly cut specimen indicating dehydration.
 - (c) Loss of definition between the inner annulus and nucleus pulposus.
 - (d) Osteophyte formation.

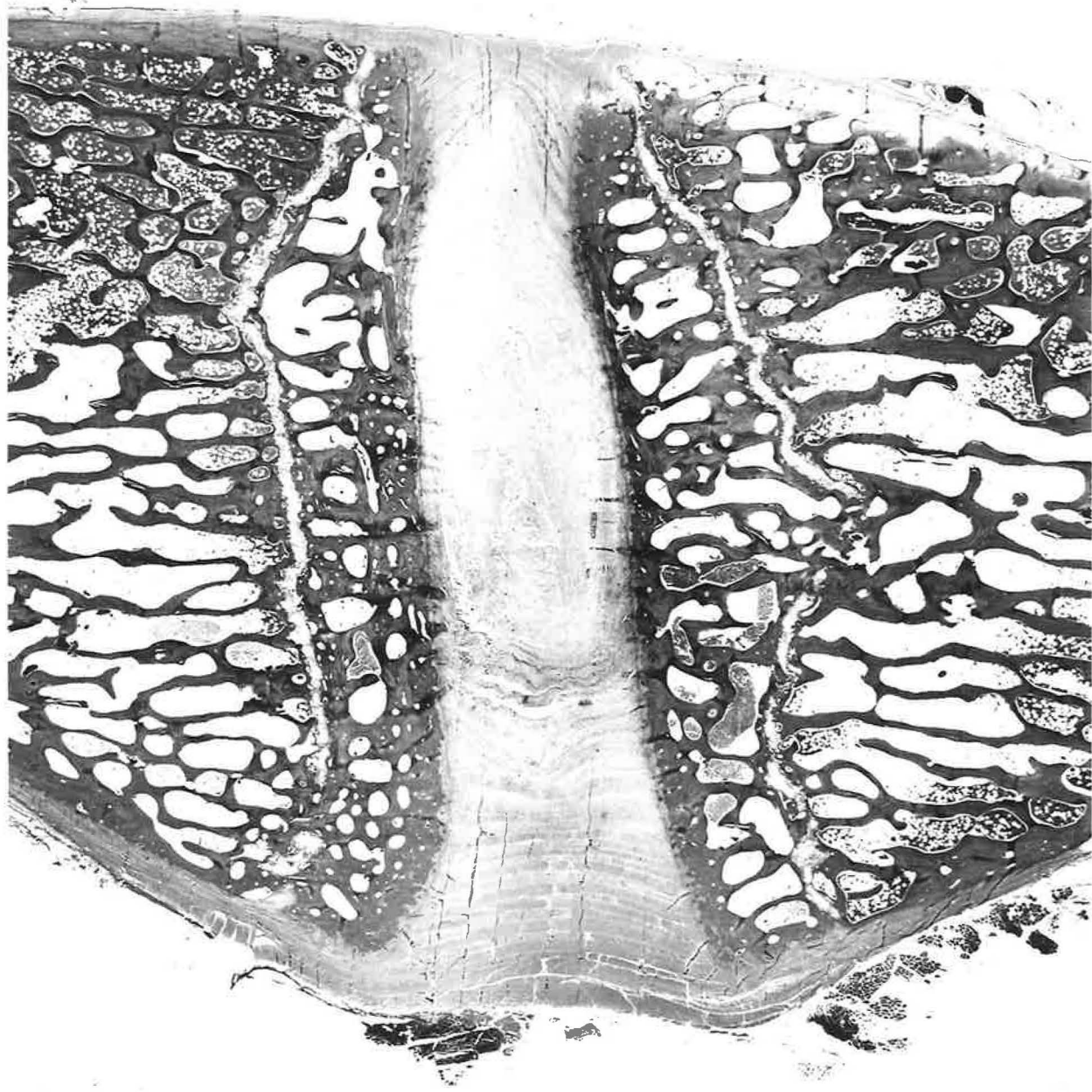
5.26 HISTOLOGICAL EXAMINATION

An assessment was made of the following parameters:

- (1) The extent of union at the donor-recipient interface.
- (2) The viability of transplanted chondrocytes and other connective tissue cells within the cartilaginous end-plate, nucleus pulposus and annulus fibrosus.
- (3) The structural integrity of the endplates.
- (4) Evidence of degeneration.

The latter three parameters were compared with control intervertebral lumbar discs harvested and processed in a similar fashion from two year old Merino wethers (Fig 8). The adult sheep lumbar disc differs from the human with persistence of epiphyseal growth plate cartilage. A zone exists between the growth plate and the disc consisting of bone which is less vascular than the rest of the vertebral body.

Fig. 8 Low power micrograph of a control intervertebral lumbar disc showing persistence of epiphyseal growth plate cartilage.



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5

5.3 COMPLICATIONS

A small leak of cerebrospinal fluid during the preparation of the recipient bed complicated one procedure. A dural tear could not be visualised and the leak resolved spontaneously. Profuse arterial bleeding which could not be controlled using bipolar diathermy was encountered in two cases. The wounds were packed with swabs for approximately five minutes and haemostasis was achieved in both cases.

Post-operative recovery was uneventful in all animals.

5.4 RESULTS

SHEEP NO. 1

RADIOGRAPHS

Post-operative radiographs showed that accurate siting of the allograft had been achieved (Fig. 9). The plate of bone harvested on the cranial side of the disc was very thin when compared with the caudal side. Lucencies were obvious at the recipient-donor interface on both sides indicating incongruity of the cut surfaces. The vicryl sling which was secured around the protruding heads of two distracting screws failed to contain the allograft. Significant forward displacement of the disc unit had occurred at two weeks (Fig. 10). The intervertebral disc height was maintained and the lucencies at the recipient-donor interface were no longer evident. At six weeks no further displacement was obvious, but the disc height was clearly diminished.

Fig. 9 Post-operative radiograph of a transplanted disc unit. In this case, only a very thin plate of bone was harvested adjacent to the donor disc on the cranial side.

Fig. 10 At two weeks, significant forward displacement of the disc unit had occurred.



MACROSCOPIC APPEARANCE

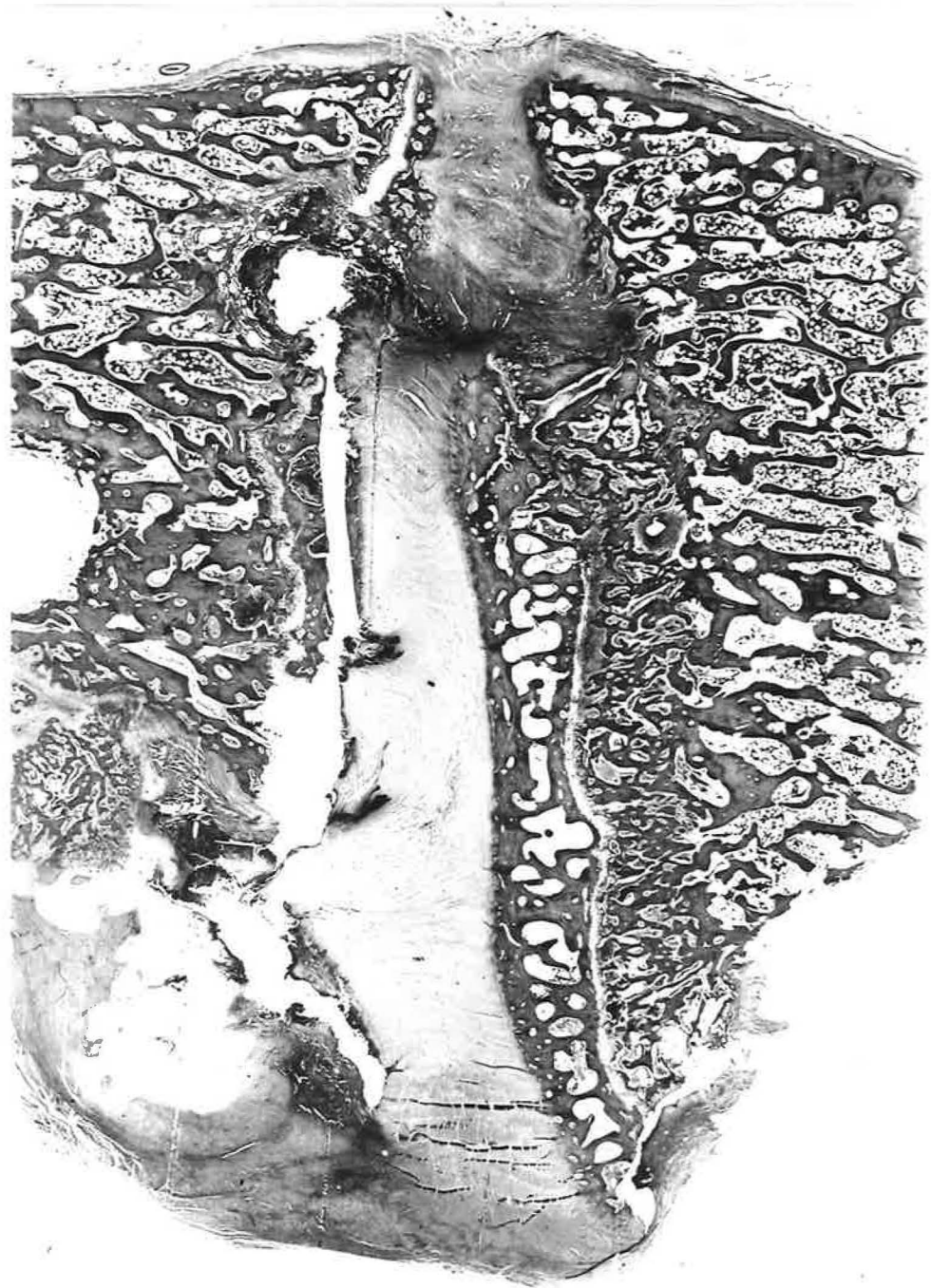
The macroscopic specimen cut through the mid-sagittal plane showed that a pseudarthrosis had developed on the cranial side and that the disc was severely degenerate (Fig. 11). When compared with an adjacent healthy disc the gross appearance of the allograft nucleus pulposus was clearly abnormal. The turgid and glistening nuclear material which normally pouts from the cut surface of the specimen was absent with concomitant loss of disc height. Obvious forward displacement of the transplanted disc unit had occurred and a marked soft tissue reaction surrounding the subluxed allograft was present anteriorly. Osteophyte formation was observed within this soft tissue on the cranial side.

HISTOLOGICAL EXAMINATION

Histological examination of the mid-sagittal section confirmed the presence of a complete non-union at the cranial end (Fig. 12). On this side, the allograft had been harvested immediately adjacent to the bony endplate of the intervertebral disc. Retention of recipient epiphyseal growth plate cartilage was also present.

Fig. 11 Photograph of the macroscopic specimen showing a severely degenerate disc with a complete pseudarthrosis on the cranial side.

Fig. 12 Low power micrograph of the specimen shown in Fig. 11. Retention of recipient epiphyseal growth plate cartilage was evident on the cranial side.



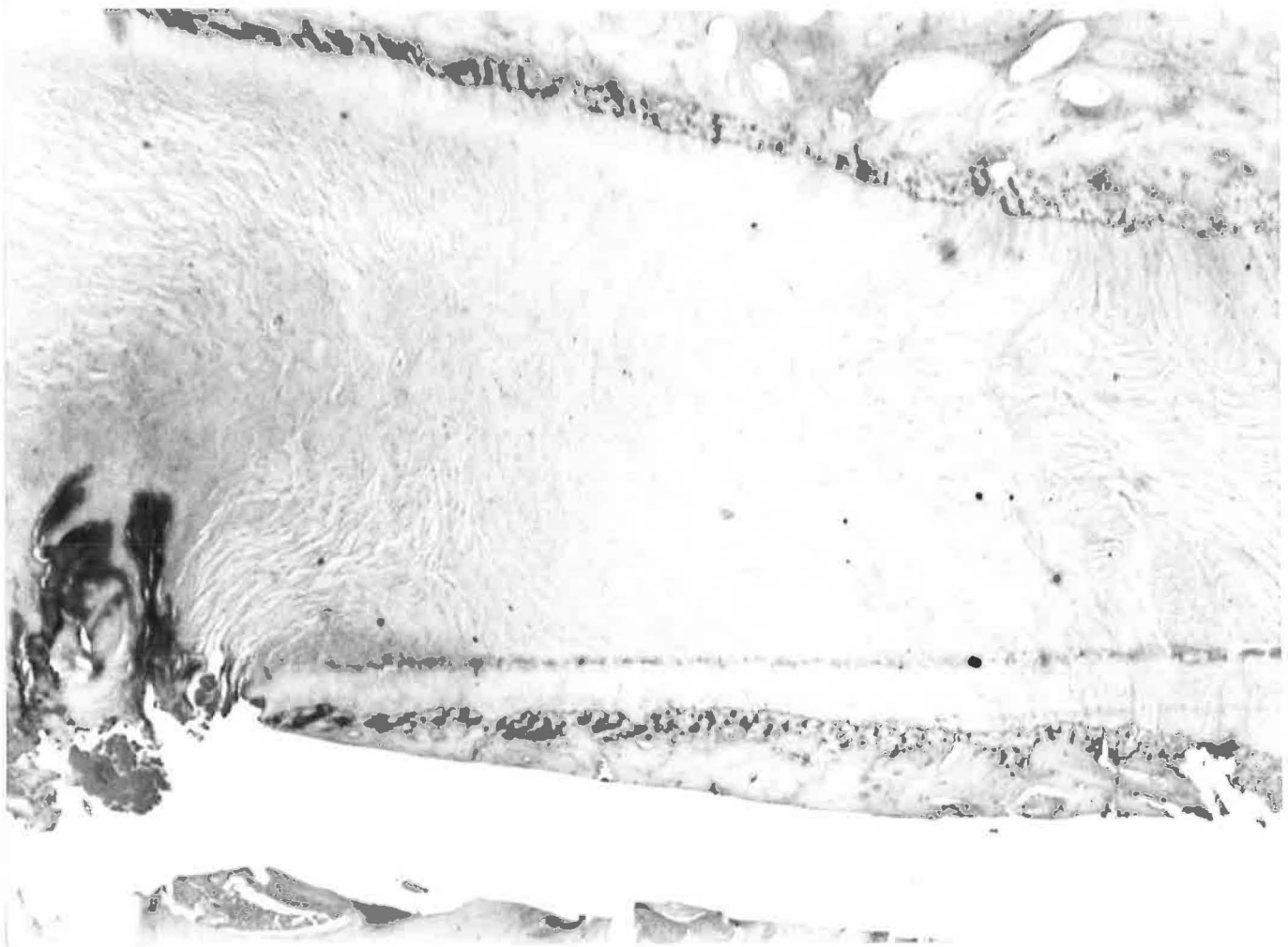
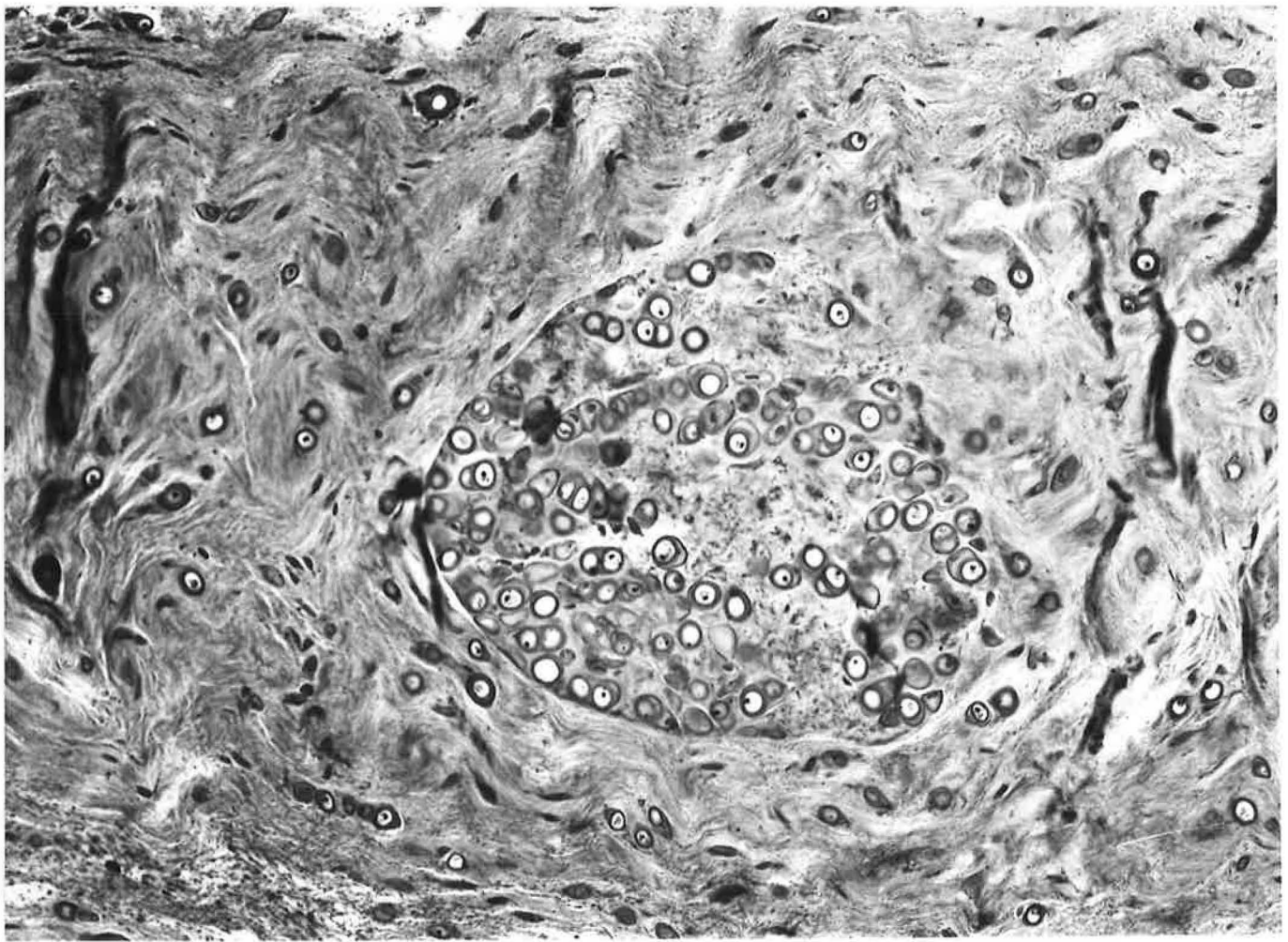
In contrast, union was complete at the caudal end. On this side, the allograft had incorporated the entire growth plate, together with adjacent vertebral cancellous bone. All of the recipient epiphyseal growth plate cartilage had been excised.

The structural integrity of the transplanted bone was maintained despite the obvious and expected death of the allograft osteocytes. Polarised light microscopy revealed abundant formation of woven bone at the union site. Creeping substitution of the allograft bone remnant was still in progress.

A more detailed examination revealed that segmental fractures had occurred through the thinnest portion of the cranial endplate with herniation of nuclear material into the pseudarthrosis (Fig. 12). On the high power view infolding of the inner annular fibres was evident with loss of definition between the inner annulus and nucleus pulposus (Fig. 13). On closer microscopic examination clumps of chondrocytes were observed in the nucleus pulposus (Fig. 14). Also known as chondrones, these clusters of proliferated chondrocytes are commonly found in the nucleus pulposus of the degenerate human disc (Vernon-Roberts, 1988).

Fig. 13 Higher power micrograph of the specimen shown in Fig. 12. Infolding of the inner annular fibres was present as well as loss of definition between the inner annulus and nucleus pulposus.

Fig. 14 High power micrograph of a chondrone in the nucleus pulposus of the transplanted disc.



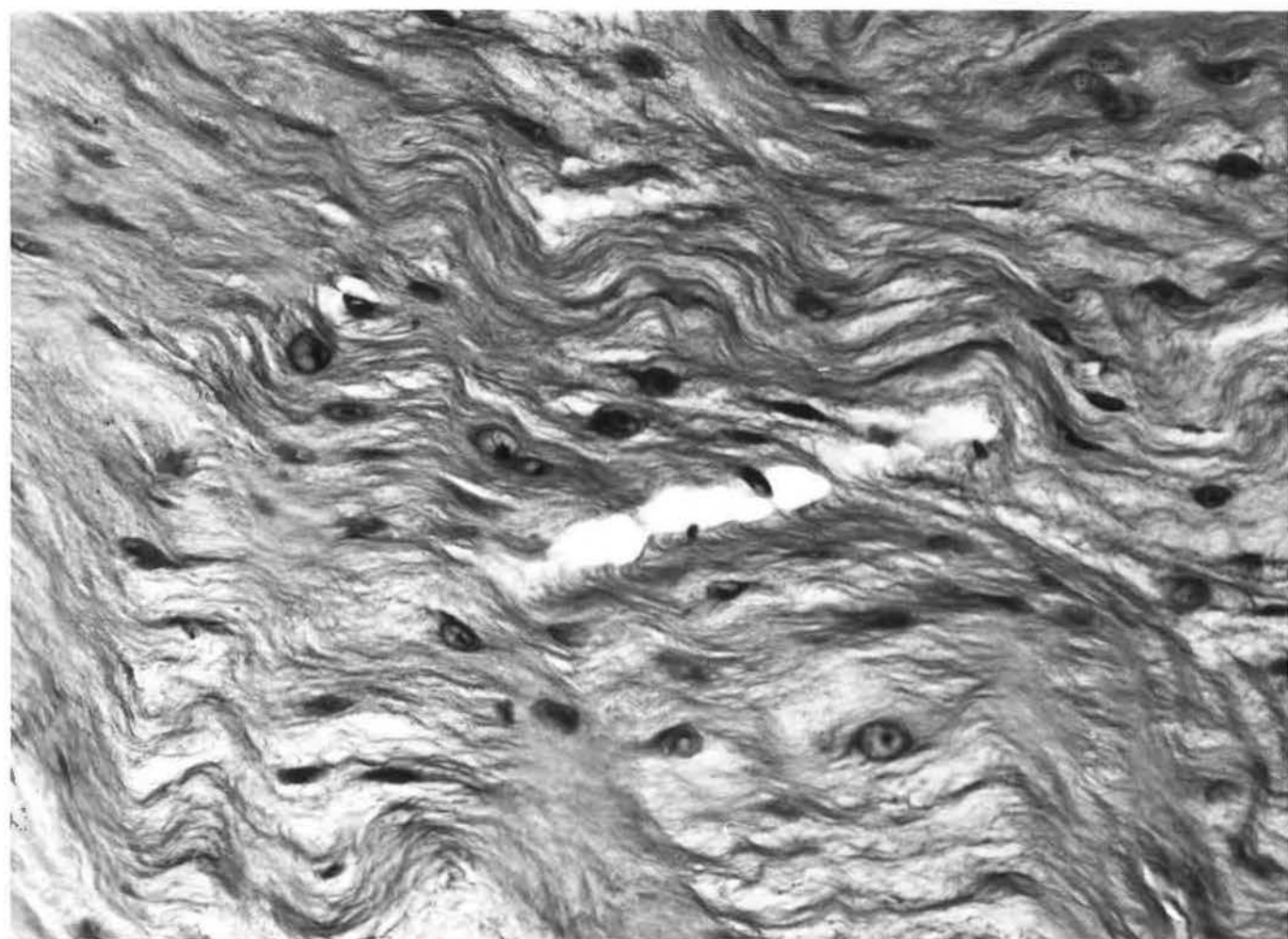
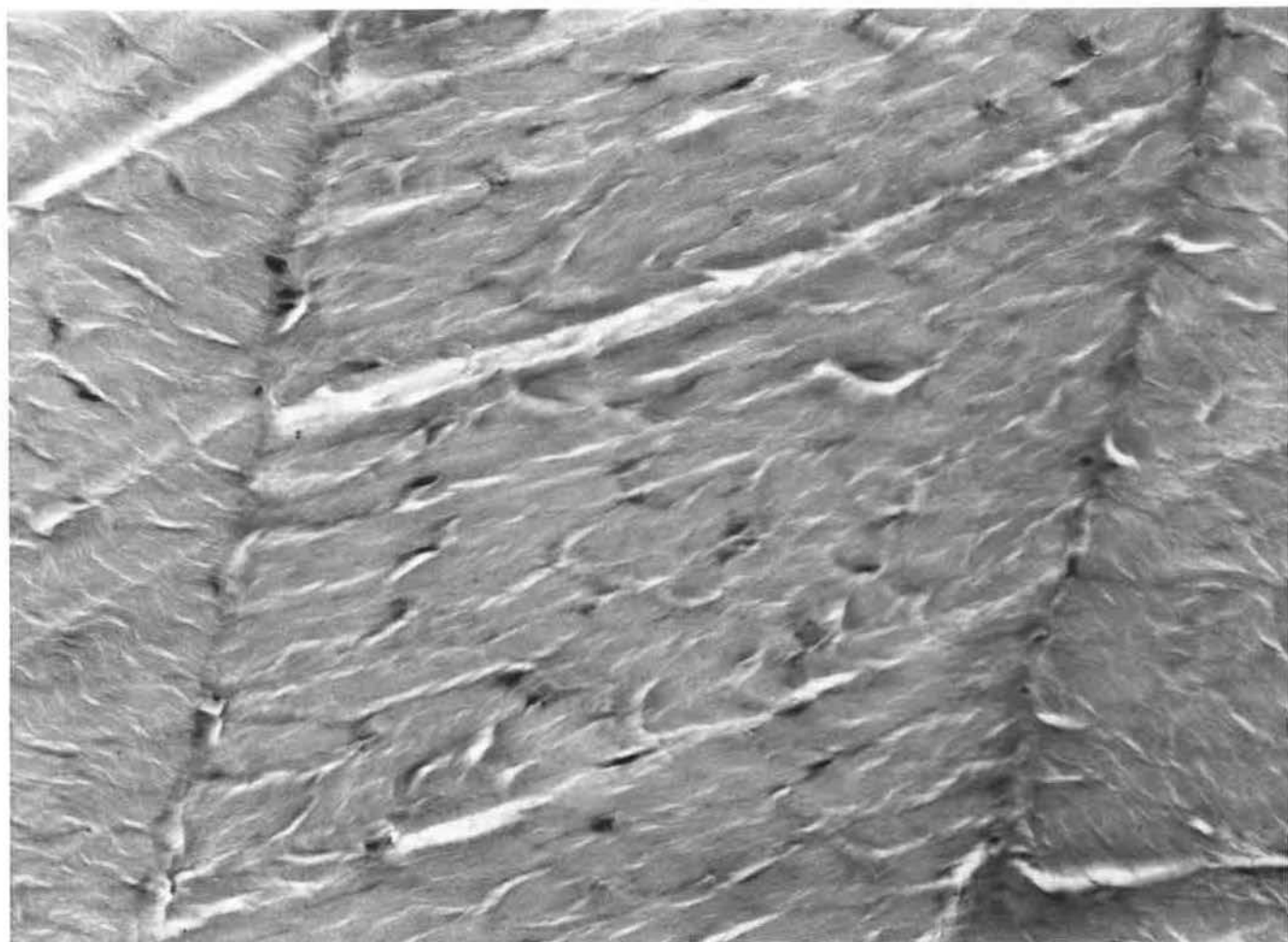
Similarly, chondrone formation has been observed in established degeneration of human articular cartilage.

Although degenerate, the disc was viable. There was no histological evidence of connective tissue cell death. The cells within the annulus fibrosus and nucleus pulposus were plump and their nuclei stained normally (Figs. 15;16).

There was no evidence of inflammatory cell infiltration to suggest a host-versus-graft immune response.

Fig. 15 High power micrograph showing viable annulus fibrosus with preservation of normal architecture.

Fig. 16 The cells of the nucleus pulposus were plump and their nuclei stained normally.





SHEEP NOS. 2 to 5

RADIOGRAPHS

In an attempt to prevent ventral displacement, small screws were placed obliquely into the vertebral bodies with the heads overlapping the bony endplates (Figs. 17;18). Some residual ventral displacement of between 10% to 20% was noted on post-operative x-rays in three of the four animals. At two weeks, x-rays demonstrated further ventral displacement of up to 50% in all three cases (Fig. 19). No further displacement was obvious at six weeks (Fig. 20). Mild disc space narrowing was noted in three of the four cases at six weeks, and in two cases fractures through the endplates on the cranial aspect were detected. Anterior osteophytes were present in all cases.

MACROSCOPIC APPEARANCE

Macroscopic examination revealed the ventral displacement evident on x-rays (Fig. 21). Obvious non-union was present at the cranial end in three of the four cases and a fracture through the endplate on the same side was detected in two.

Fig. 17 Post-operative radiograph showing residual ventral displacement of the transplanted disc unit.

Fig. 18 Antero-posterior radiograph showing the position of small screws used in an attempt to prevent ventral displacement.



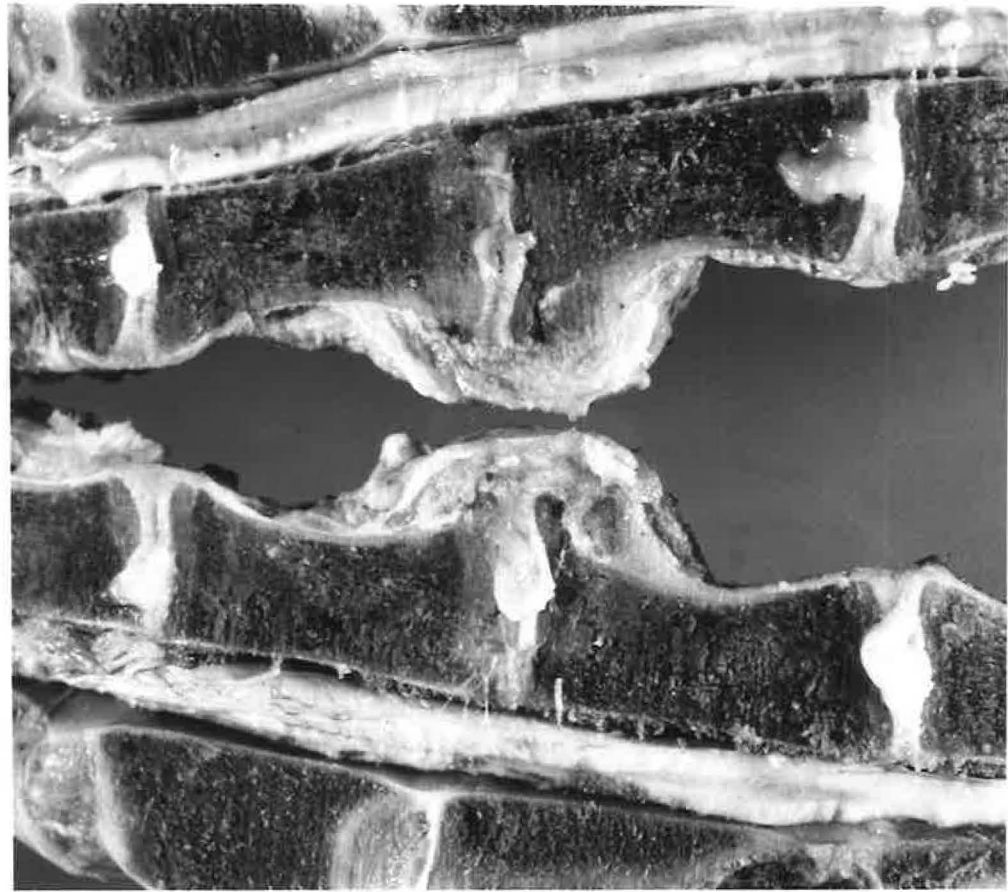
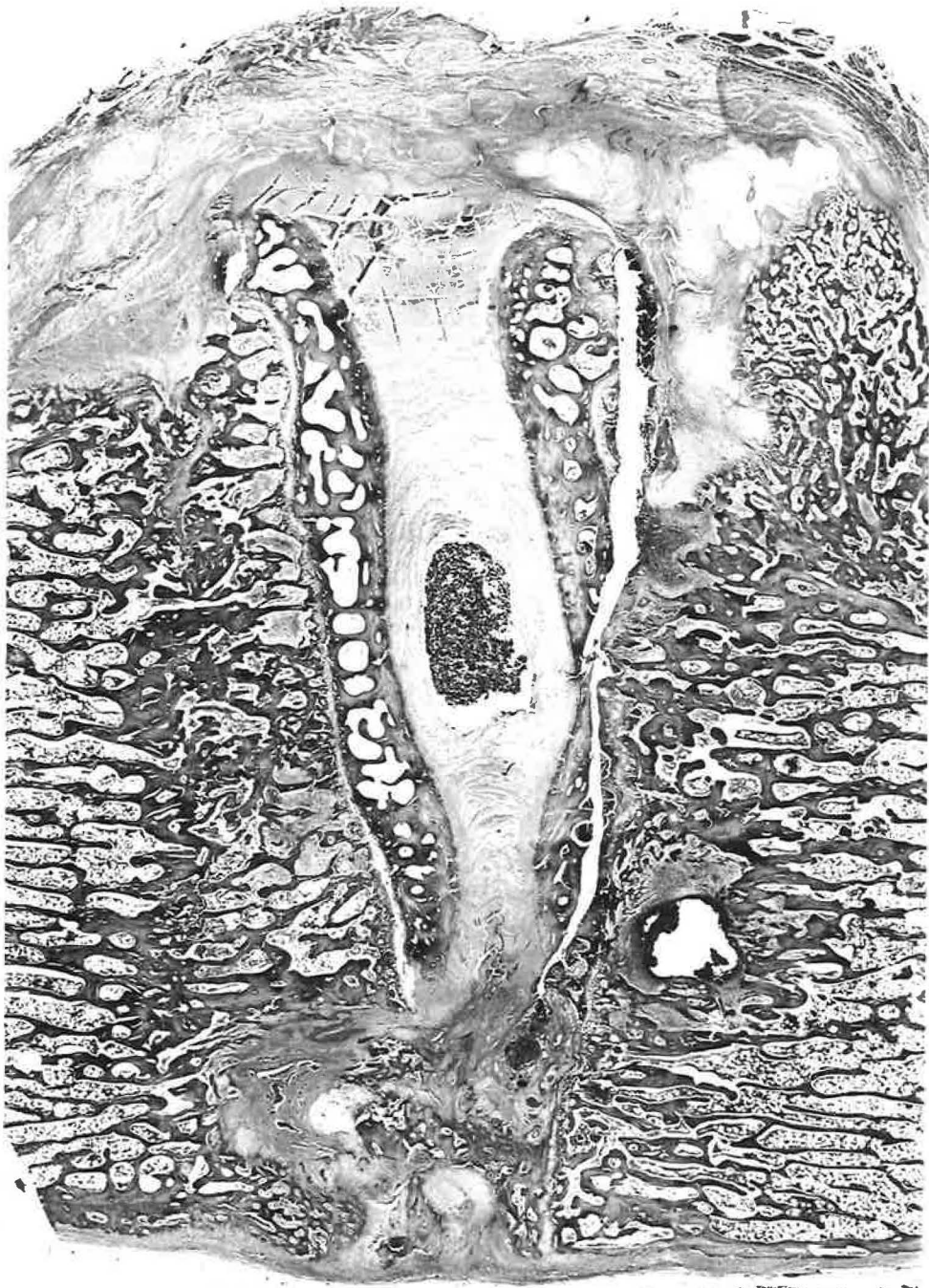
Fig. 19 Post-operative radiograph of the case shown in Fig. 17. Further ventral displacement has occurred.

Fig. 20 At six weeks, no further displacement was evident.



Fig. 21 Macroscopic examination of the case shown in Fig. 20 revealed an abnormal disc with loss of nuclear turgidity.

Fig. 22 Low power micrograph of the specimen in Fig. 21 showing a non-union on the cranial side as well as anterior osteophyte formation. A fracture through the thinnest portion of the harvested bone was present. A cystic area filled with cellular debris can be seen in the central nucleus pulposus.



All of the transplanted discs were abnormal. Narrowing of the intervertebral disc spaces and loss of nuclear turgidity were present in all cases. The definition between the inner annulus and nucleus pulposus was less distinct and a marked soft tissue response was prominent anterolaterally. Osteophyte formation was a prominent feature in the three allografts which had displaced ventrally.

HISTOLOGICAL EXAMINATION

As well as the non-unions and fractures detected macroscopically, histological examination revealed a microscopic non-union and endplate fractures in the remaining cases at the cranial end (Fig. 22). As in the first case, the allografts had been harvested either through the epiphyseal growth plate cartilage or through the zone between the growth plate and bony endplate. All fractures had occurred through the thinnest portions of harvested bone and retention of recipient epiphyseal growth plate cartilage was again observed.

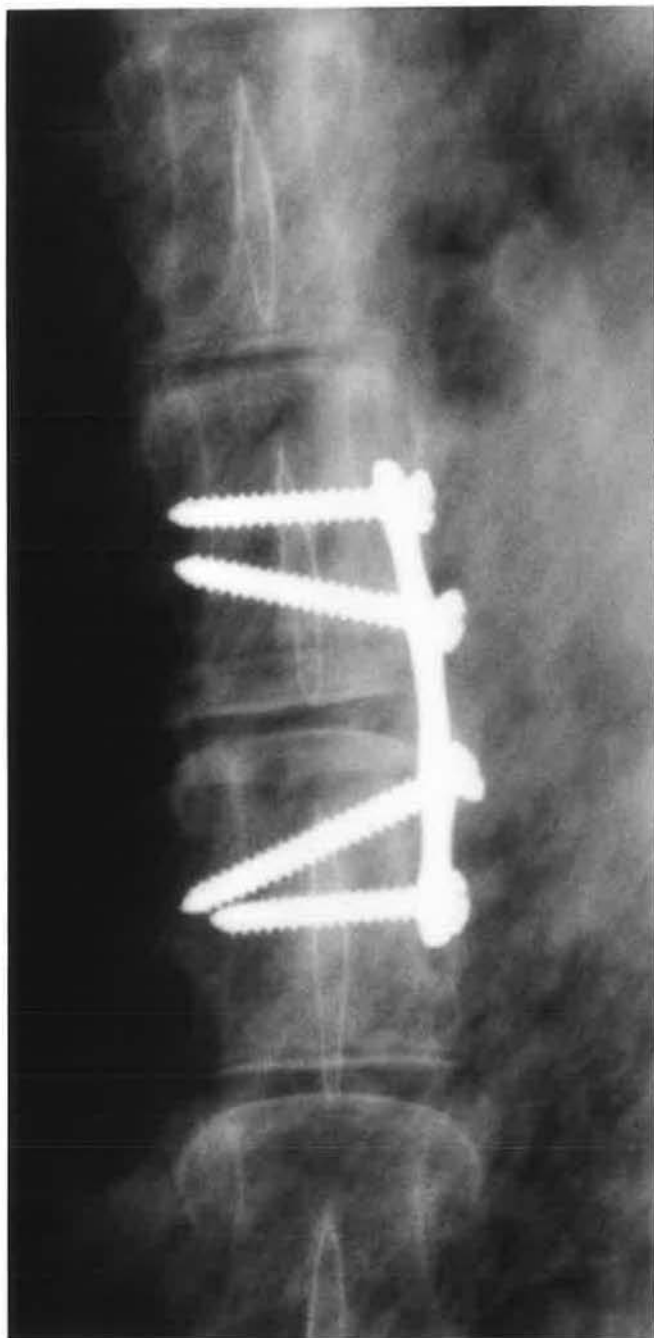
In contrast, a successful union was achieved on the caudal side where harvesting incorporated the entire epiphyseal growth plate cartilage and where all of the recipient epiphyseal growth plate had been excised.

All four specimens displayed histological evidence of degeneration. Infolding of the inner annular fibres, loss of distinction between the inner annulus and nucleus and chondrone formation featured prominently. A cystic area filled with cellular debris was present in the centre of the nucleus in one case (Fig. 22). Large osteophytes were present anteriorly and the disc units were surrounded by abundant vascular granulation tissue. The viability of the transplanted chondrocytes and connective tissue cells, however, were well preserved.

SHEEP NOS. 6 & 7

Following an analysis of these initial results, the surgical technique was modified in order to prevent the complications observed. These changes included the harvesting of a thicker plate of vertebral bone on the cranial side of the allograft so that it would incorporate the entire epiphyseal growth plate cartilage. Similarly, more bone was excised from the recipient bed on the cranial side to ensure complete excision of the epiphyseal growth plate. The long forward-projecting transverse processes were again osteotomised at their bases to improve the exposure. This facilitated preparation of the posterior recipient bed, so that a more accurate siting of the allograft could be achieved. Finally, in an attempt to prevent displacement of the disc unit, internal fixation of adjacent vertebrae was performed. A four hole cobalt-chrome metal alloy plate (Sherman Plate) was bridged across the allograft and secured with four stainless steel screws (6 gauge x 25mm self-tapping screws). The two inner screws were angled away from the recipient bed (Figs. 23;24).

Figs. 23;24 Lateral and antero-posterior radiographs showing internal fixation of adjacent vertebrae.



RADIOGRAPHS

Initial radiographs demonstrated inadequate clearance of bone from the posterior recipient bed with residual ventral displacement of the allografts (Figs. 23;24). Repeat X-rays at two weeks and at six weeks showed no further displacement of the transplanted disc units (Figs. 25;26). Intervertebral disc heights were preserved at six weeks.

MACROSCOPIC APPEARANCE

Both macroscopic and microscopic examinations reconfirmed the presence of residual host bone in the posterior recipient bed (Figs. 27-30). Concomitant ventral displacement of approximately 15% was present in both cases. Turgid nuclear material pouted normally from the freshly cut specimens. A complete macroscopic non-union which was not apparent on X-rays was present in one case (Fig. 29). The disc units were structurally intact and the intervertebral disc heights were preserved. One specimen was markedly discoloured by ink from the dye-perfusion study (Fig. 27).

Figs. 25;26 Lateral and antero-posterior radiographs of the case shown in Figs. 23;24 at six weeks. The transplanted disc unit was undisplaced.



MICROSCOPIC APPEARANCE

Microscopic examination revealed that the allografts had been harvested to incorporate the entire epiphyseal growth plate on both sides of the disc and that the recipient epiphyseal growth plate cartilage had been completely excised (Figs. 28;30). Despite this, a complete non-union was present on one side in one case (Fig. 30) and a partial microscopic non-union was obvious in the other specimen (Fig. 28). Both discs were viable and showed no evidence of degenerative change when compared with controls. Abundant indian ink was observed within the vascular channels of the allograft bone indicating that revascularisation had occurred at six weeks (Figs. 31;32).

Fig. 27 Macroscopic examination of the specimen shown in Figs. 23-26 revealed turgid nuclear material which pouted normally from the freshly cut surface. This specimen was markedly discoloured by ink from the dye-perfusion study.

Fig. 28 Low power micrograph of the specimen shown in Fig. 27. A partial microscopic non-union was present on one side.



Fig. 29 Macroscopic specimen showing a complete non-union on the cranial side.

Fig. 30 Low power micrograph of the specimen shown in Fig. 29. The transplanted disc showed no evidence of degenerative change when compared with controls.

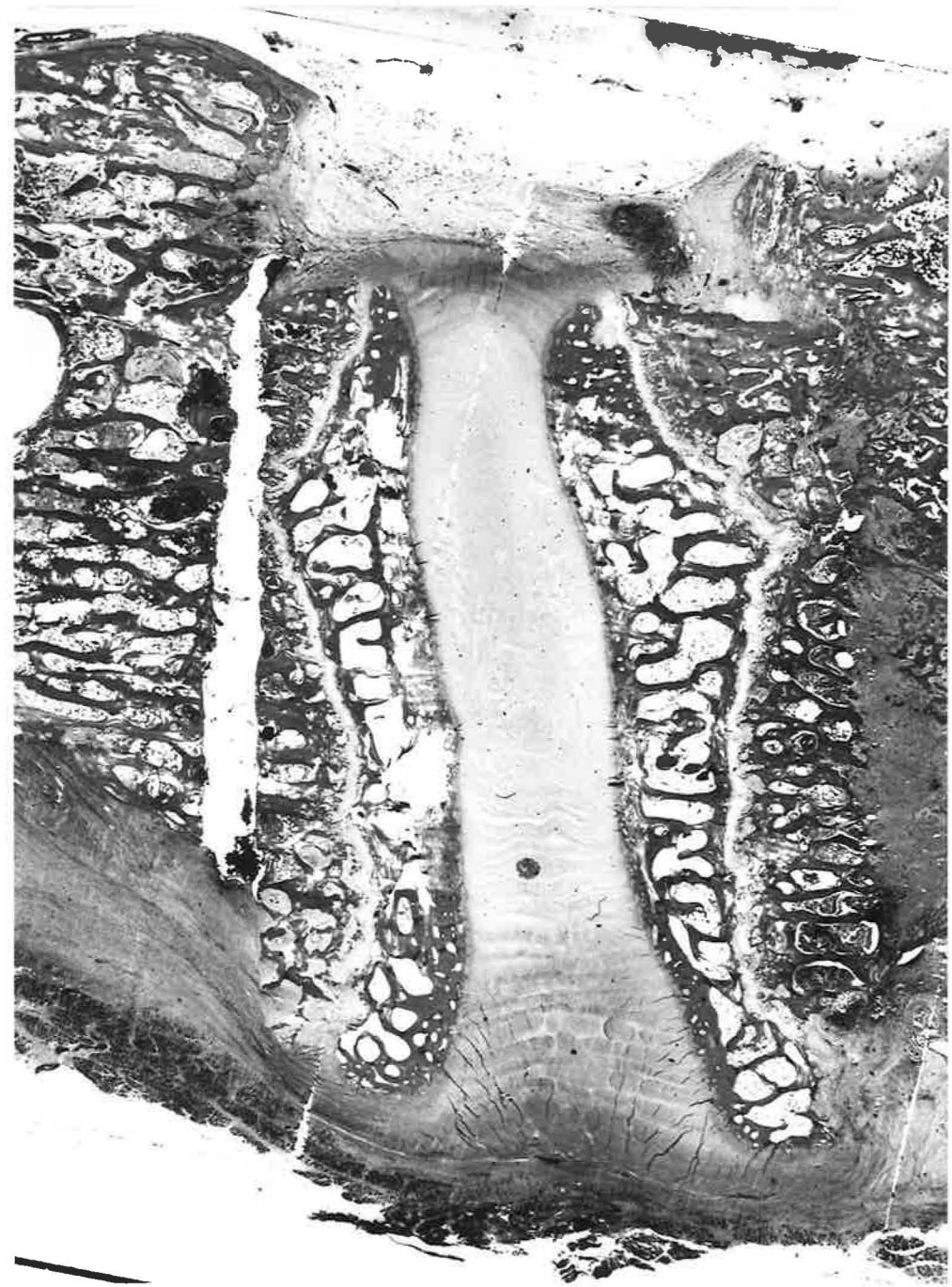
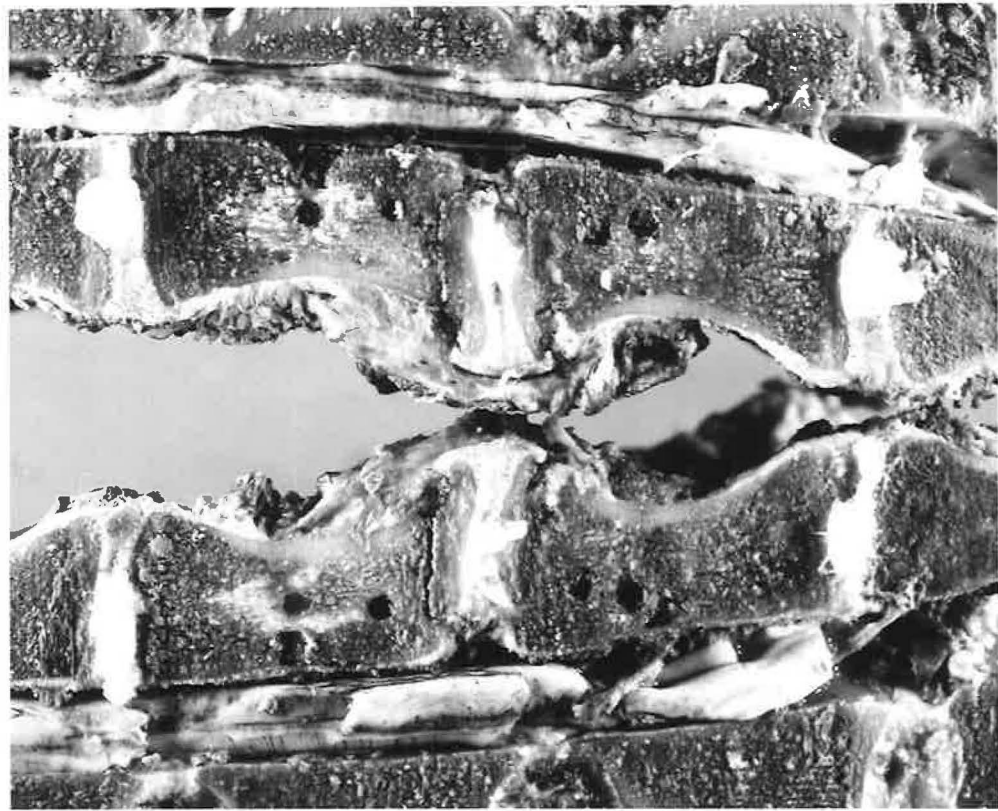
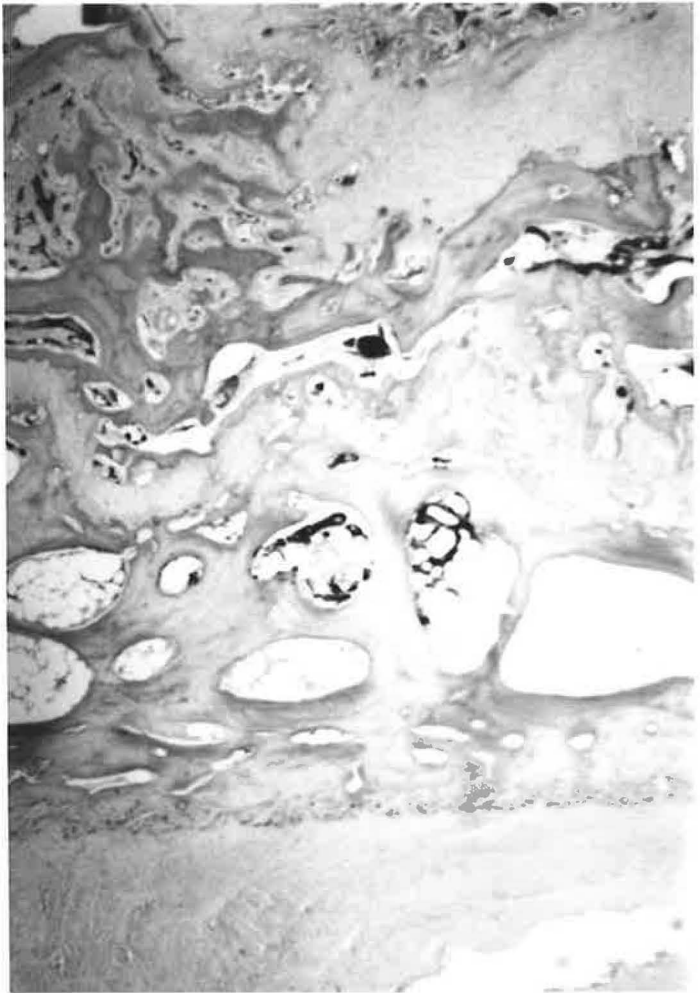
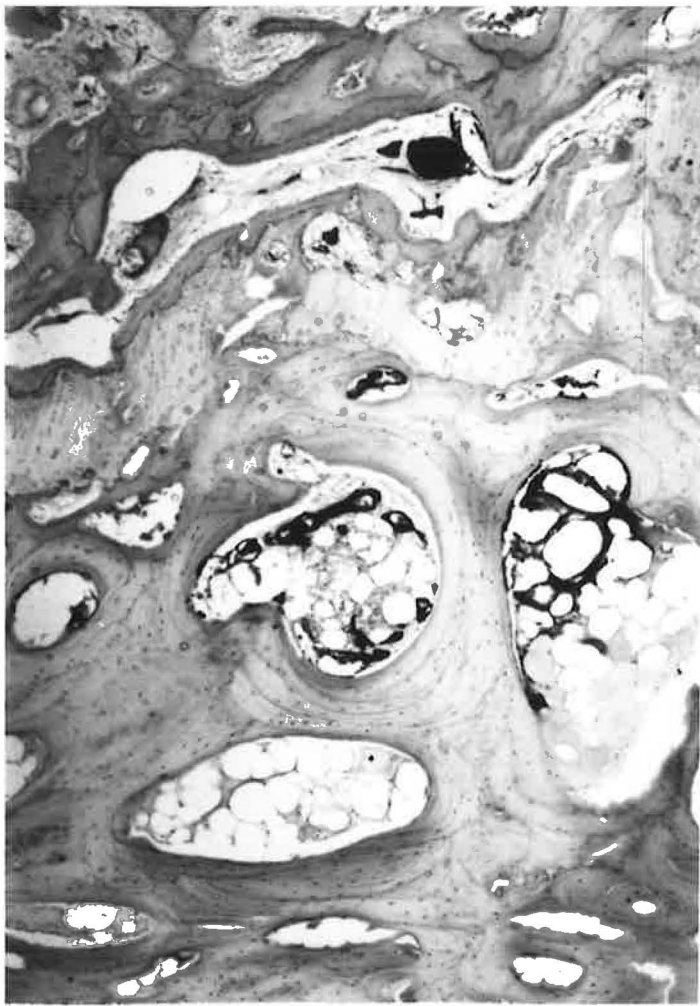


Fig. 31 Low power micrograph showing Indian ink in bone between the intervertebral disc and epiphyseal growth plate of the transplanted unit.

Fig. 32 High power view of Fig. 31 showing ink within the vascular channels of the allograft bone.



5.5 SUMMARY AND DISCUSSION

Of the five transplants where a screw method of fixation was used, four displaced ventrally. All were associated with endplate fractures and a non-union at the cranial end. Retention of epiphyseal growth plate cartilage was identified as a cause for non-union. The discs were clearly degenerate at six weeks. On the other hand, the two allografts which were supported internally by plate fixation of adjacent vertebrae did not displace. They were harvested to incorporate the epiphyseal growth plate cartilage on both sides and were not associated with endplate fractures or degeneration. One specimen exhibited a non-union despite bone to bone apposition. The reason for this was not readily apparent but lack of compression across the donor-recipient interface as a consequence of rigid plate fixation may have been a contributing factor. Numerous technical difficulties encountered with the accurate harvesting and siting of the disc units, together with inadequate stabilisation of the graft resulted in anterior subluxation, endplate fractures and eventual degeneration of the transplanted intervertebral disc.

These complications were overcome by harvesting a thicker plate of allograft bone and by plating of the adjacent vertebrae.

This study has demonstrated that allograft discs can survive after transplantation even in the presence of a complete non-union. Diffusion of nutrients from body fluids would appear to be sufficient to maintain disc nutrition until the blood supply to the end-plate is restored.

Accurate placement and prevention of displacement is essential if osteophyte formation and end-plate fractures are to be prevented.

A follow-up study was subsequently carried out to refine the surgical technique and to determine the health of the transplanted disc at three months.

6. THE FOLLOW-UP STUDY

6.1 MODIFICATION OF THE SURGICAL TECHNIQUE

In a more definitive study the surgical technique was modified as follows:

- (1) The epiphyseal growth plates were included in the harvesting procedure.
- (2) Smaller donor sheep were selected to improve allograft fit.
- (3) The recipient transverse processes were osteotomised at their bases to improve the exposure and to facilitate preparation of the recipient bed.
- (4) An attempt was made to excise more bone from the posterior recipient bed so that more accurate siting of the allograft could be achieved.
- (5) Adjacent vertebrae were internally fixed with a 4-hole plate.

- (6) All plates were removed at three weeks under a general anaesthetic and using strict aseptic conditions. As an additional prophylaxis against infection an intramuscular injection of penicillin and streptomycin was administered at the time of induction.

Disc units were harvested from four donor sheep and transplanted into eleven recipient animals. All sheep were allowed to roam free in a paddock for a total survival time of three months.

In seven sheep, an additional disc unit was transplanted free in the interval between the psoas muscles and secured with an intramuscular stitch. This additional study was undertaken to assess whether diffusion of nutrients from body fluids alone would be sufficient to maintain disc nutrition. A detailed macroscopic and histological analysis of the free allograft and also of the facet joints at the recipient level were performed in this study.

6.2 COMPLICATIONS

Three of the recipient sheep were sacrificed prematurely.

A paraplegia which failed to recover was noted on the first operative day in the first sheep of the study. A postmortem examination revealed the presence of a marked kyphotic deformity at the transplant site with complete dislodgement of the allograft anteriorly. The left-sided pedicle was fractured and the right facet joint completely dislocated. Both the plate and screws were bent and there was obvious compression of the cord at this level. A review of the X-rays taken in the immediate post-operative period showed accurate placement of the transplanted disc unit without residual displacement. To obtain this position, it was necessary to excise more bone from the root of the pedicle at its proximal aspect. Over-zealous clearance of bone and use of the osteotome during the preparation of the recipient bed may have weakened the pedicle sufficiently to cause a fracture and subsequent failure of the transplant procedure.

In the second sheep, the post-operative recovery was complicated by a monoplegia on the right side. The sheep was unable to support its own weight and was nursed in a standing position with the aid of a ventral body sling. There was no evidence of recovery and the sheep was sacrificed on the fifth post-operative day. Post-operative radiographs showed some residual displacement of the allograft of approximately 10%. The cause for the monoplegia could not be identified on the macroscopic examination. There was no compromise of the spinal canal and all nerve roots on the right side were intact.

The third sheep failed to regain consciousness following the general anaesthetic to remove the Sherman plate and screws at three weeks and was therefore sacrificed. An intracerebral event during the surgery was the presumed cause.

The remaining eight sheep were sacrificed at three months.

6.3 RESULTS

RADIOGRAPHS

Radiographs taken in the immediate post-operative period demonstrated residual ventral displacement of approximately 30% of the AP diameter of the transplanted disc unit in five of eight surviving cases (Fig. 33). Displacement of approximately 20% was present in one case (Fig. 35) and approximately 10% in the remaining two sheep.

Further displacement of approximately 10% was evident in two cases on follow-up X-rays at three months (Fig. 34). A fracture through the cranial plate of the transplanted disc unit was obvious in these two cases, both of which were displaced approximately 30% in the immediate post-operative period.

The allograft intervertebral disc heights, however, were preserved at three months in all cases (Fig. 36).

Fig. 33 Lateral radiograph showing residual ventral displacement of the transplanted disc unit.

Fig. 34 Lateral radiograph of the case shown in Fig. 33. Further displacement had occurred at three months. A fracture through the cranial plate of the transplanted disc unit is also evident.



Fig. 35 Lateral radiograph taken in the immediate post-operative period.

Fig. 36 Lateral radiograph at three months of the specimen seen in Fig. 35. The intervertebral disc height was preserved.



MACROSCOPIC APPEARANCE

All allografts were found to have united to the adjacent recipient vertebral bodies. Apart from the two fractures identified on the plain radiographs, the structural integrity of the disc units were intact. In these two cases, there was a noticeable loss of nuclear turgidity. Otherwise nuclear material bulged normally from the cut surface of the remaining specimens.

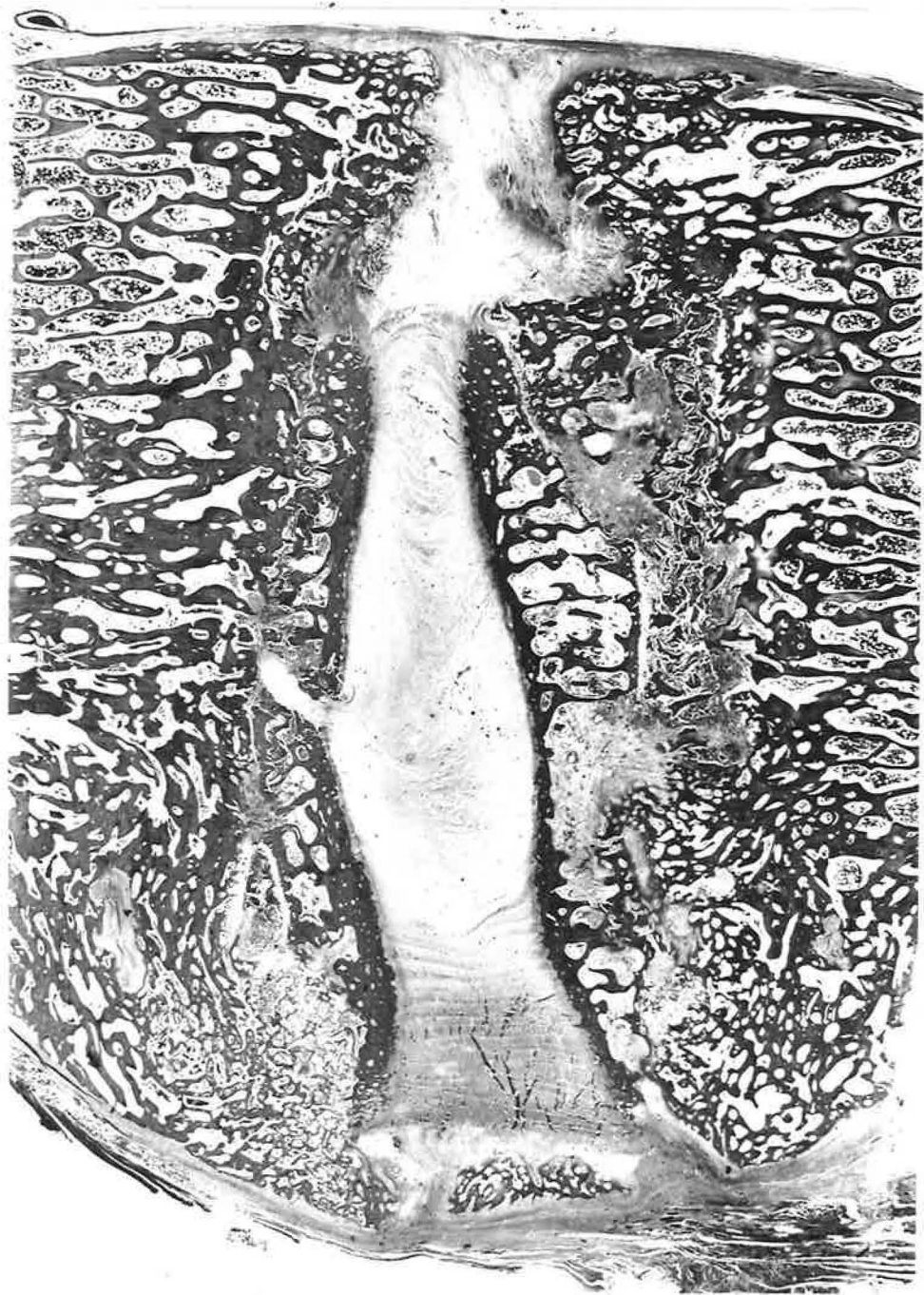
MICROSCOPIC APPEARANCE

Abundant woven bone was evident on polarised light microscopy at the donor-recipient interface and fusion was complete in all specimens. The viability of the transplanted chondrocytes and connective tissue cells was preserved and excluding the two cases where a fracture was present, the allograft nucleus pulposus showed no evidence of accelerated degenerative change when compared with controls.

In six of the eight cases where displacement of greater than 20% was demonstrated, osteophytes had formed anterolaterally and were proportional in size to the degree of residual displacement (Figs. 37;38).

Fig. 37 Low power micrograph of the case shown in Fig. 34.

Fig. 38 Low power micrograph of a transplanted disc unit showing marked ventral displacement, end-plate disruption and osteophyte formation.



In one case, a bridging osteophyte had formed anteriorly with a concomitant ankylosis (Fig. 37). Smaller osteophytes were also present posteriorly between the transplanted disc unit and the posterior longitudinal ligament in these cases. Osteophytes were not evident in the two cases where less than 10% of residual displacement was present (Figs. 39;40).

Microscopic examination of both facet joints at the level of the transplant procedure revealed unexpected chondral damage in six of the eight sheep. This ranged in severity from a full thickness fissure (Fig. 41) to complete destruction of the joint architecture associated with a fibrous ankylosis in 2 cases (Fig. 42).

Fig. 39 Low power micrograph of an undisplaced specimen. Osteophyte formation was absent.

Fig. 40 Low power micrograph of a second specimen where less than 10% of residual displacement was present. Histological examination showed no evidence of degenerative change when compared with controls.

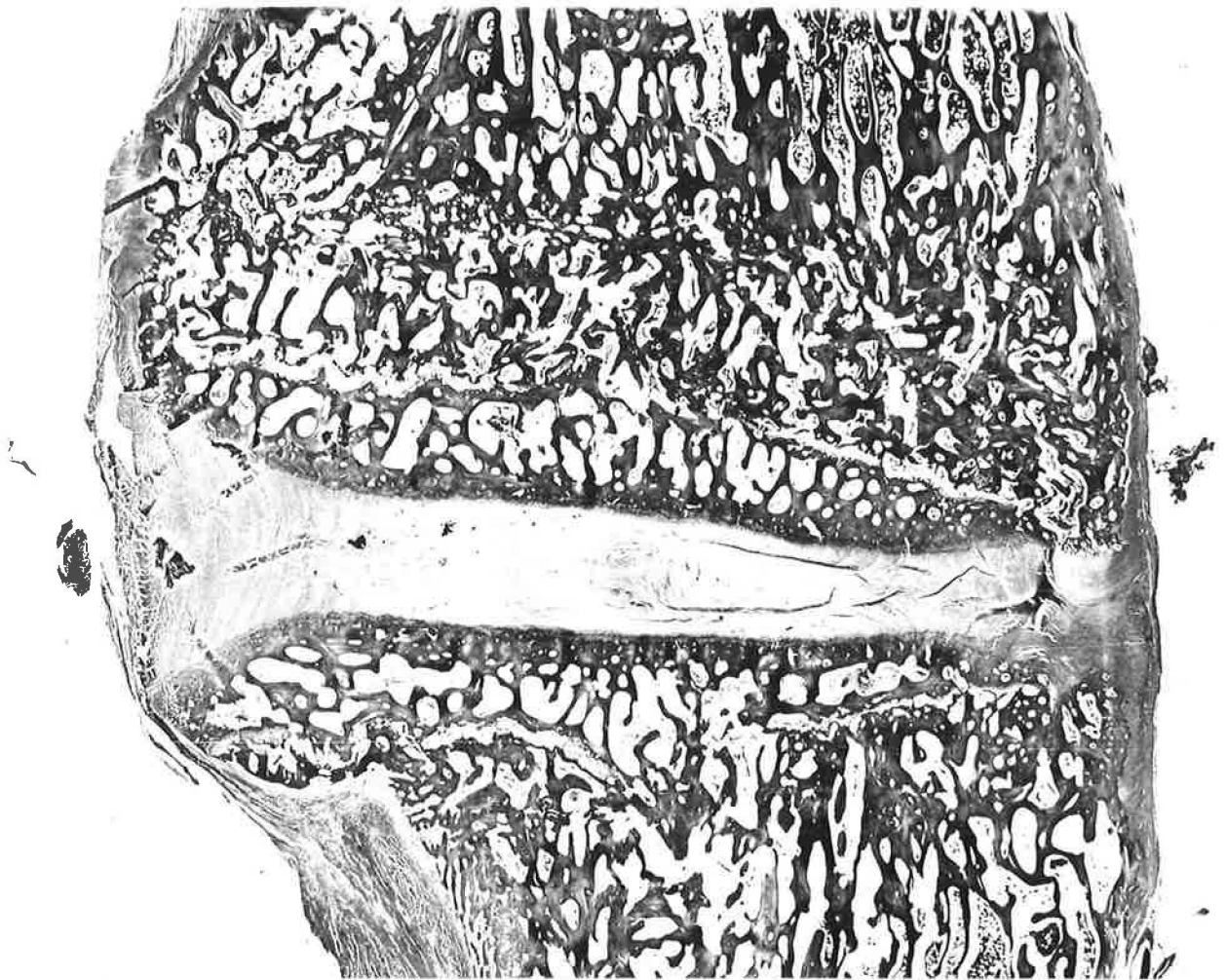
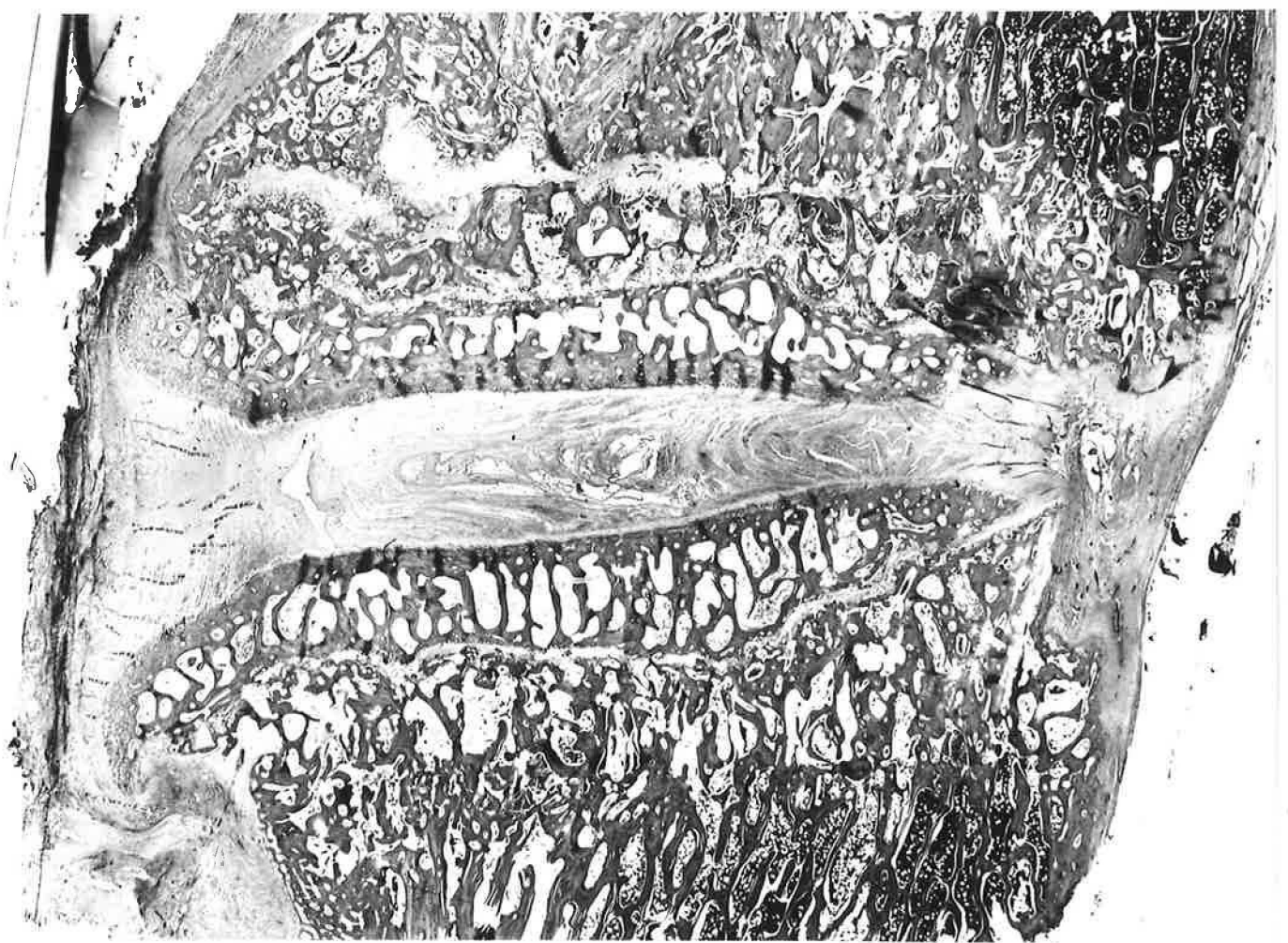
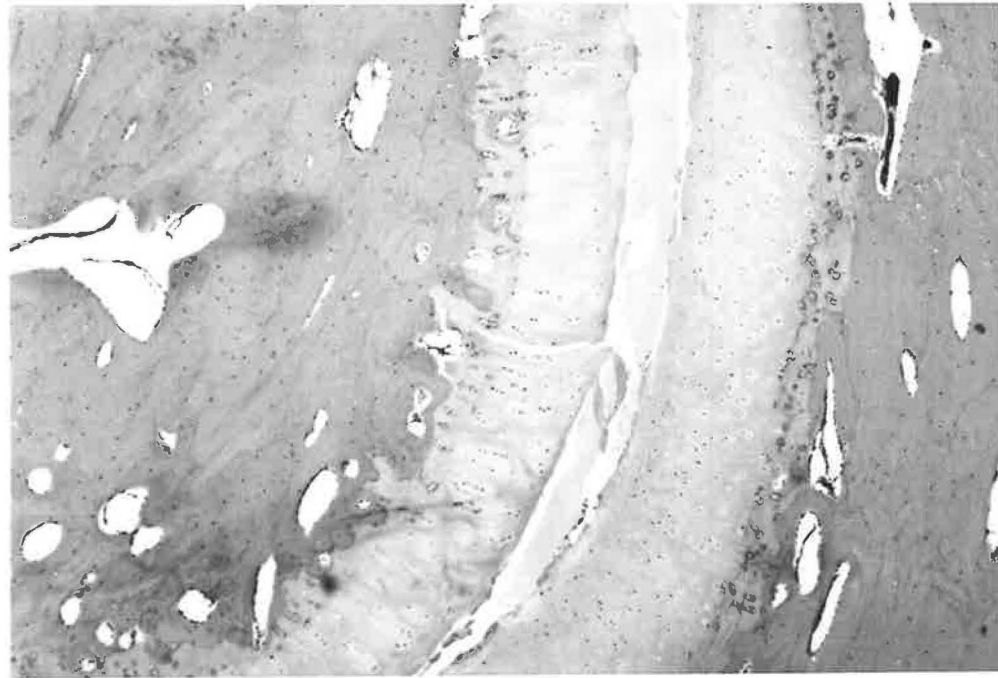
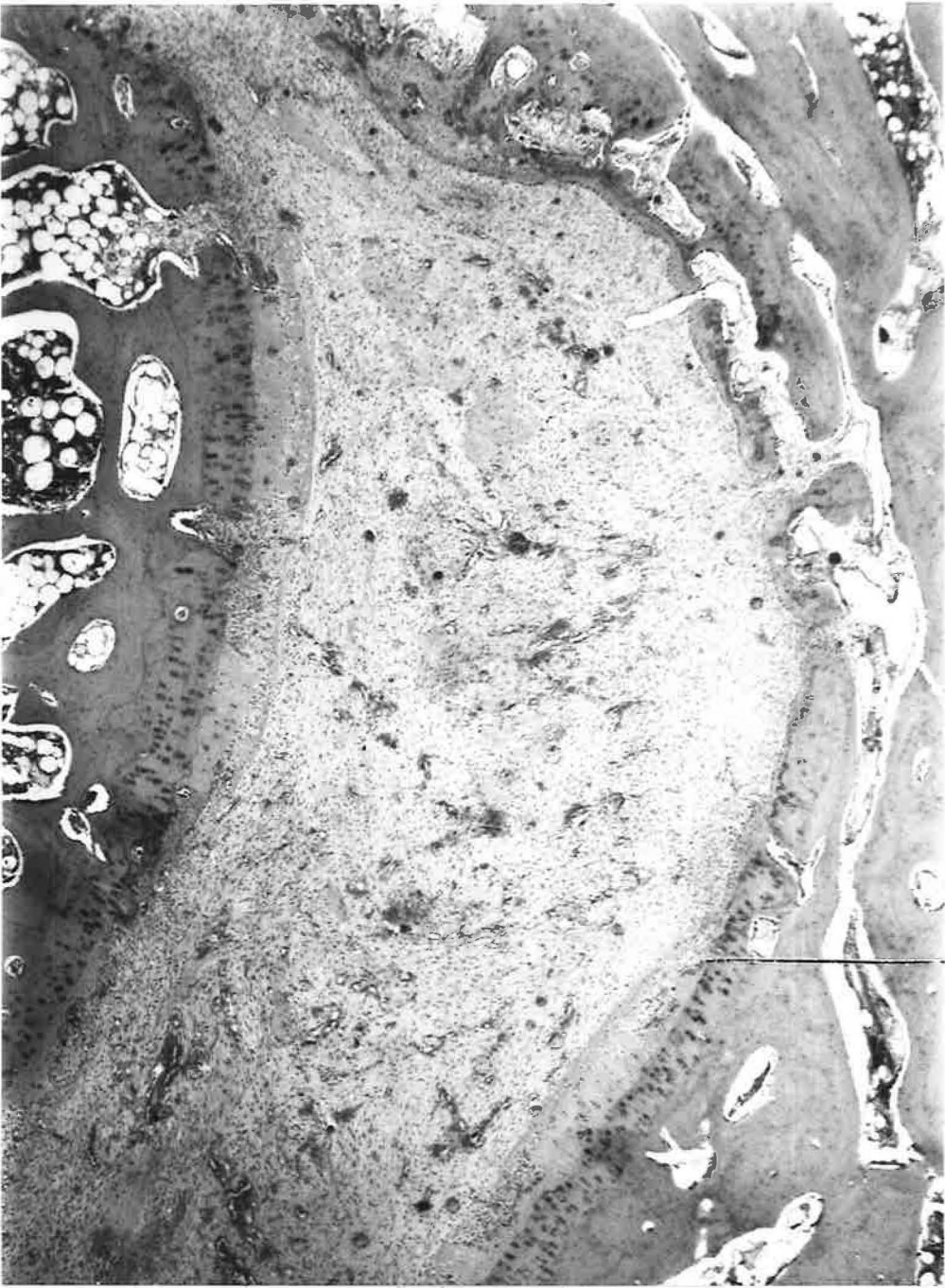


Fig. 41 Low power micrograph of a facet joint at the level of the transplant procedure revealing a full thickness fissure of the articular cartilage and a joint effusion.

Fig 42. Low power micrograph of a facet joint showing complete destruction of the joint architecture associated with a fibrous ankylosis.



FREE ALLOGRAFT DISC UNITS

A second allograft disc unit was implanted free between the psoas muscles in seven cases, but two sheep were sacrificed prematurely. Following retrieval of the five remaining allografts, the surrounding soft tissue was cleared and the specimens were cut through the mid-sagittal plane.

MACROSCOPIC APPEARANCE

The disc units were encapsulated within abundant scar tissue. Nuclear material pouted normally from the freshly cut surfaces and the structural integrity of the allografts was intact (Fig. 43).

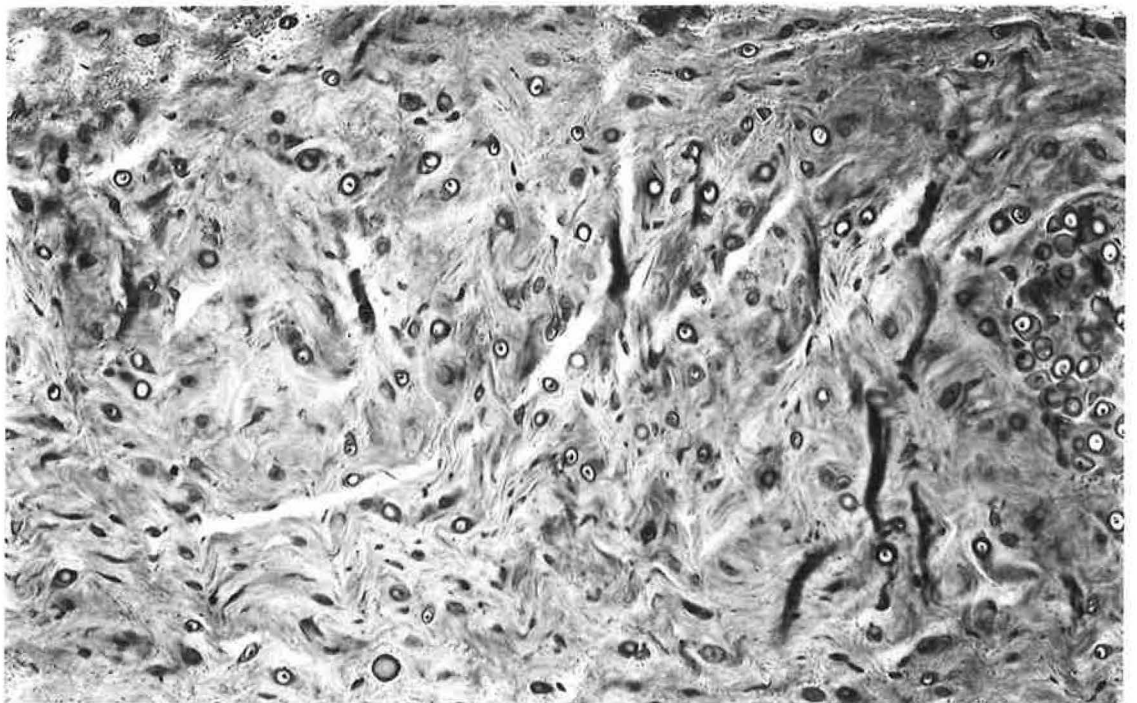
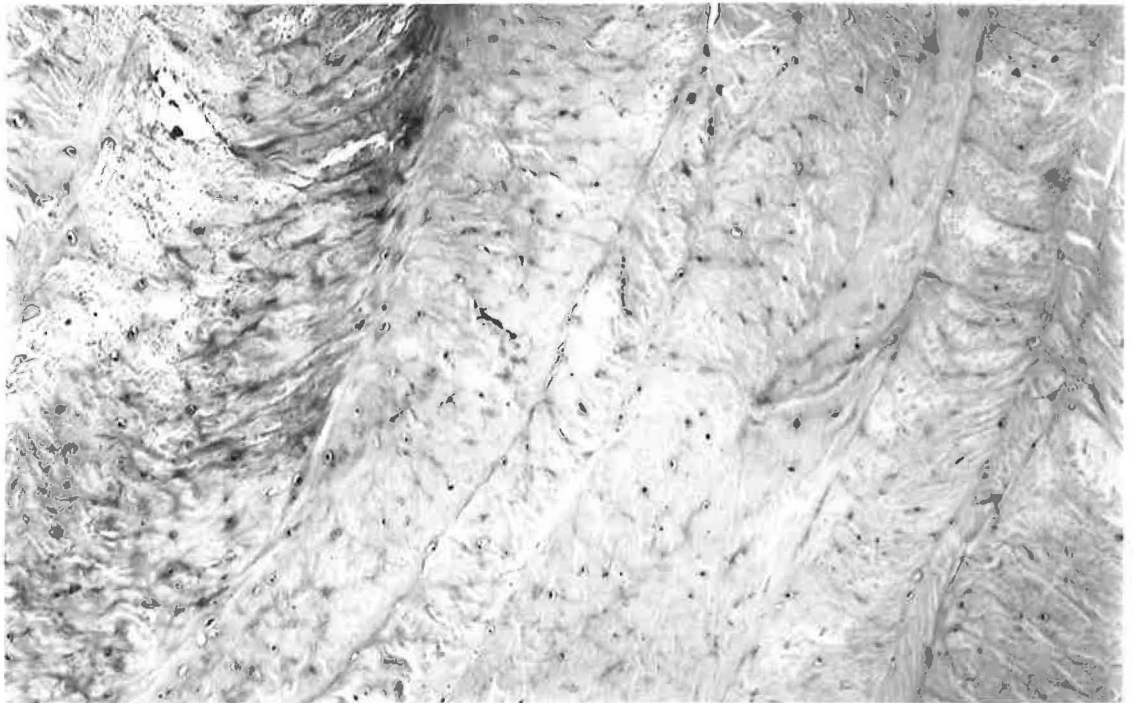
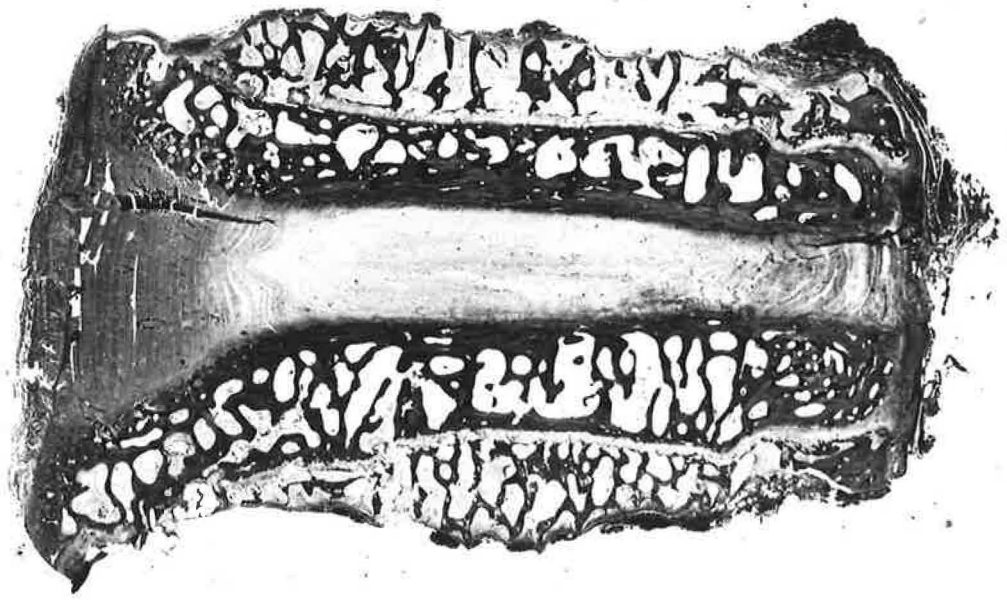
MICROSCOPIC APPEARANCE

Microscopic examination revealed preservation of chondrocyte and connective tissue cell viability in all cases (Figs. 44;45). There was no evidence of degeneration when compared with control discs.

Fig. 43 Low power micrograph of a disc unit which had been implanted free between the psoas muscles for three months.

Fig. 44 High power view of the annulus fibrosus in Fig. 43 showing preservation of normal architecture and connective tissue cell viability.

Fig. 45 High power view of the nucleus pulposus in Fig. 43.



6.4 DISCUSSION

Major difficulties were encountered with the siting of the allograft disc units.

In the first animal, accurate anatomical placement of the disc unit was achieved at the expense of weakening the posterior elements. This resulted in a fracture-dislocation of the spine causing a complete paraplegia.

In order to prevent this disastrous complication from recurring, clearance of bone from the pedicle was avoided and residual ventral displacement was accepted in the remaining cases. Osteophyte formation was associated in all six cases with a residual displacement of greater than 20% and did not feature in the two cases where less than 10% of displacement was present.

Although internal fixation of adjacent vertebrae was generally successful in the prevention of postoperative displacement, further displacement of approximately 10% did occur in two cases. Both of these disc units were associated with a fracture through one endplate.

Degenerative changes were noted in 75% of both facet joints examined at the level of the transplant procedure. The force required with use of the osteotome during preparation of the graft bed, as well as the distraction procedure employed to site the disc units may have irreversibly damaged the facet joints in the intra-operative period.

In addition, altered biomechanics as a result of a mismatch in the size of the allograft transplanted may have produced abnormal stresses across the facet joints, contributing to and further accelerating the degeneration observed.

The results of this follow-up study demonstrated again that accurate placement and prevention of displacement is essential if osteophyte formation and endplate fractures are to be prevented.

The five allografts implanted free between the psoas muscles showed no evidence of accelerated degenerative change when compared with controls. It would appear that diffusion of nutrients from body fluids alone is sufficient to maintain disc nutrition for at least three months.

6. METHODS OF DISC STORAGE

Additional studies to assess the viability of allograft disc transplantation at increasing intervals following various methods of storage would be required before an allograft transfer procedure could be considered in the human.

Two methods of storage were considered during the pilot study.

(1) CRYO-PRESERVATION

Two allograft disc units were frozen to -70°C in a conventional freezer for seven days and then thawed at room temperature before transplantation into separate recipient animals. The screw method of fixation was used in an attempt to stabilise the graft. The sheep were allowed to survive in pens for a total of six weeks.

Radiographs at six weeks demonstrated a loss of intervertebral disc height of greater than 50% in both cases. One disc unit had displaced anteriorly by 50% of its AP diameter and a fracture through the mid portion of its cranial plate was present.

Macroscopic examination revealed a markedly abnormal disc. The normally white and glistening nuclear material with a consistency similar to toothpaste was replaced by non-turgid brown-coloured fluid. Microscopic examination revealed necrotic annular and nuclear tissue devoid of nuclei.

(2) SHORT TERM CORNEAL-PRESERVING MEDIUM

Two disc units were stored in a solution which has been successfully used by eye banks to preserve corneas for up to seven days (Schimmelpfennig, 1986). The M-K medium (McCarey & Kaufman, 1974) consists of 5% Dextran (to minimise stromal swelling), N-2-hydroxyethylpiperazine-N-ethane-sulfonic acid (a synthetic pH-stabilising substance), gentamycin (200mcg/ml) and polymixin (50mcg/ml).

The disc units were removed from the storage solution at seven days and then de-calcified. Histological examination of mid-saggital sections was normal in both cases (Figs 46-48).

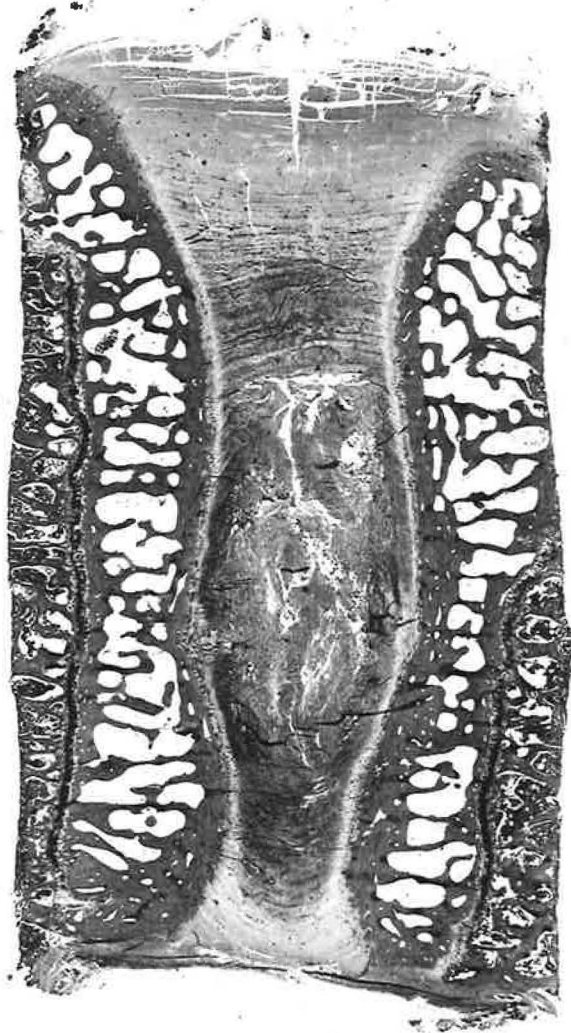
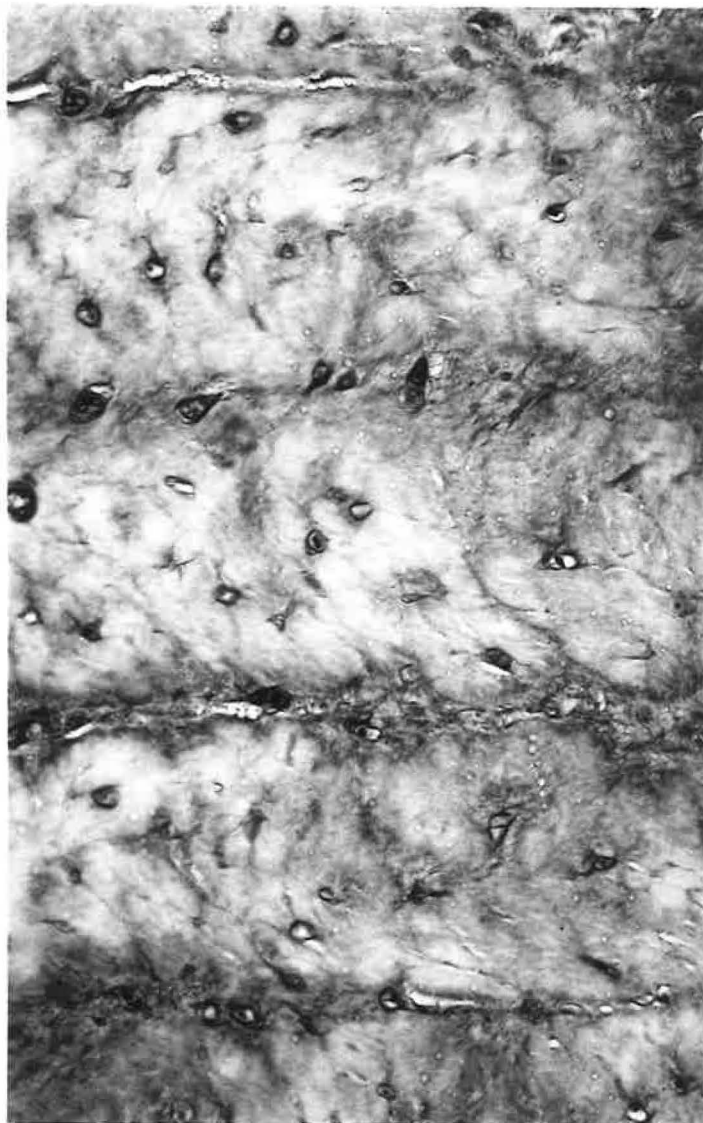
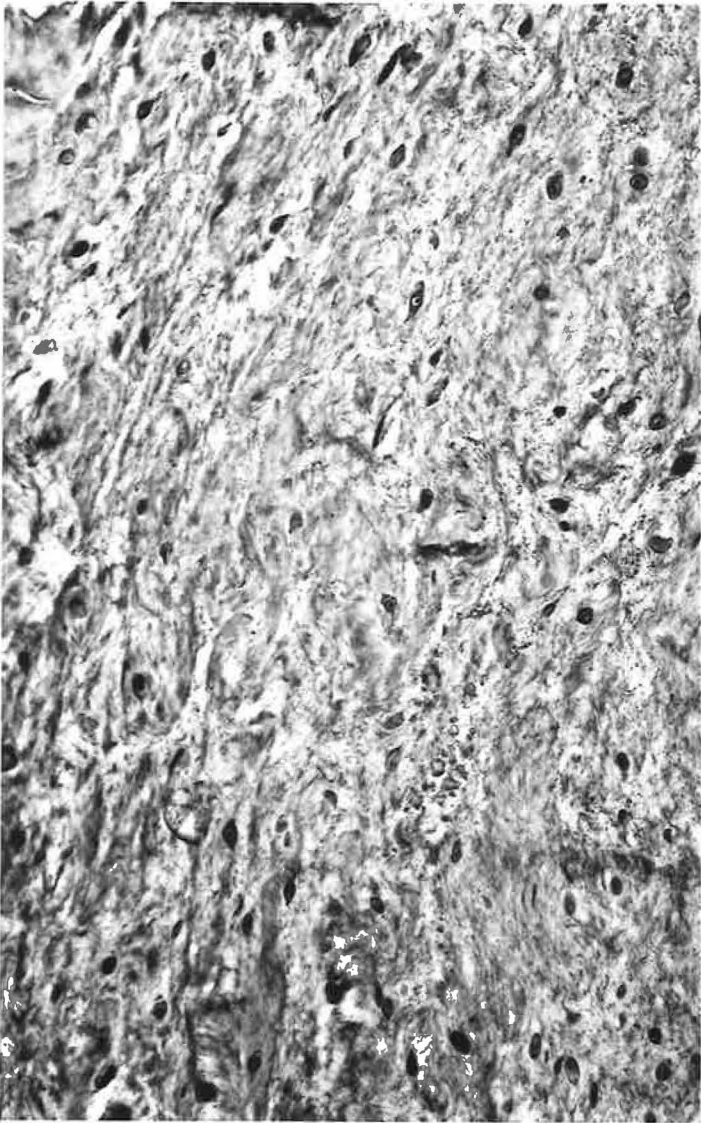
It was decided not to proceed with a definitive study using the M-K storage medium until the technical difficulties encountered with the harvesting and accurate siting of the fresh disc units were resolved.

In addition, the sheep vertebral bone was extremely hard - much more than that of the human vertebral body. This made recipient preparation even more difficult and because of the forces required with osteotomes to excise the bone, the facet joints were severely damaged in the majority of cases. The use of more sophisticated cutting equipment (eg. high speed burrs) may be required in future studies to reduce the risk of facet joint damage.

Fig. 46 Low power micrograph of a disc unit which had been in M-K medium storage for seven days.

Fig. 47 High power view of the annulus fibrosus in Fig. 46.

Fig. 48 High power view of the nucleus pulposus in Fig. 46.



7. CONCLUSION

To be successfully used in clinical practice allograft disc replacement surgery should be able to maintain the viability and integrity of the transplanted intervertebral disc. In addition, the operative procedure itself must be shown to be safe and reliable. The aims of this study were to address these two main pre-requisites for the transplant of an allograft intervertebral disc by using an animal model.

The results of this study have demonstrated that allograft intervertebral discs can survive after transplantation from one animal to another. The integrity of the disc unit could be preserved at three months providing accurate placement was achieved.

The surgical procedure, however, was not safe and could not reliably reproduce a satisfactory result. In particular, difficulties encountered in the preparation of the recipient bed were associated with major neurological complications in two cases. Furthermore, irreversible damage to the facet joints at the operated levels occurred in 75% of cases.

In addition, residual ventral displacement was associated with the early development of osteophytes. Further ventral displacement, which could not be prevented in two cases despite internal fixation of adjacent vertebrae for three weeks, was complicated by end-plate fractures and inevitable degeneration of the intervertebral disc.

Although previous experience employing a sheep model has found it to be suitable for spinal research, the presence of the growth plate as well as the horizontal alignment of the sheep lumbar spine may not make it a particularly suitable animal for assessing the potential for allograft intervertebral disc replacement.

The successful transplantation of an intervertebral disc capable of withstanding the normal physiological demands and restoring the function of the motion segment after disc derangement is, in theory, an appealing alternative to fusion surgery.

This study has demonstrated the many technical difficulties and risks associated with attempted allograft transplantation, but the results suggest that allograft transplantation of disc units may be feasible in the future.

However, further extensive studies would be required to demonstrate that these can be safely and reliably overcome before an allograft transfer procedure of this type could be considered in the human.

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Appendix

(1) Length of Follow-Up

An important limitation of this study with regard to assessment of allograft disc degeneration was the short length of follow-up. However, the other major aim of this study was to assess the safety and reliability of the allograft transfer procedure itself. Many technical difficulties were encountered and major risks were associated with attempted allograft transplantation. The procedure was not safe and could not reliably reproduce a satisfactory result. In particular, two sheep in the follow-up study suffered major neurological complications in the immediate post-operative period and were therefore sacrificed. Residual ventral displacement in six of the eight cases was associated with the development of osteophyte formation. Further displacement in two cases, which could not be prevented despite internal fixation of adjacent vertebrae for three weeks, was complicated by endplate fractures and inevitable degeneration of the disc. Furthermore, 75% of cases demonstrated extensive damage to the facet joints at the operated level. The sheep vertebral bone was found to be extremely hard which made recipient preparation exceedingly difficult. The forces required with osteotomes to excise vertebral bone may have damaged the facet joints irreversibly. The use of more sophisticated cutting equipment (eg. high speed burrs) may be required in future studies to reduce the risk of facet joint damage. In addition to this, the presence of the growth plate as well as the horizontal alignment of the lumbar spine may not make the sheep a suitable animal for assessing the potential for allograft disc transplantation. For these reasons, follow-up in this sheep-model was limited to three months and it was decided not to proceed with a longer term study.

(2) Analysis of Disc Height

Disc space narrowing occurred in four of seven cases in the pilot study and was associated with major fractures of the endplate. In the follow-up study, however, loss of disc height was not demonstrated. The reasons for this are speculative, and are probably multi-factorial. The length of follow-up may have been too short to detect narrowing of the disc space. The initial period of fixation of adjacent vertebrae may have contributed to maintenance of initial disc height. The number of animals in the study was small and intra-observer errors in measurement of small distances, as well as variations in centering of the spine on the radiographs (errors of parallax) may have produced significant inaccuracies to obscure subtle changes in disc height.

(3) Facet Joint Degeneration

The reasons for facet joint degeneration are speculative. This was blamed both on the trauma of the procedure, as well as the altered biomechanics during survival.

The forces required with use of the osteotome during preparation of the graft bed and also the distraction procedure employed to facilitate insertion of the disc units may have damaged the facet joints during the intra-operative period. In addition, altered biomechanics as a result of a mis-match in the size of the allograft transplanted may have produced abnormal stresses across the facet joints, contributing to the degenerative changes observed.

Given the finding that accurate placement and prevention of displacement was essential to prevent osteophyte formation and fractures of the endplate, a more adequate form of fixation may have produced better results.

Appendix

| SHEEP NO. | DISC HEIGHT RATIO | |
|-----------|-------------------|-------|
| | INITIAL | FINAL |
| 1 | 1 | 0.44 |
| 2 | 1.03 | 1 |
| 3 | 1.03 | 1.03 |
| 4 | 0.94 | 0.92 |
| 5 | 0.93 | 0.89 |
| 6 | 0.97 | 0.97 |
| 7 | 0.88 | 0.88 |
| 8 | 0.93 | 0.95 |
| 9 | 0.61 | 0.63 |
| 10 | 0.8 | 0.81 |
| 11 | 0.71 | 0.71 |
| 12 | 0.94 | 0.96 |
| 13 | 1 | 1.02 |
| 14 | 0.92 | 0.95 |
| 15 | 1.2 | 1.1 |
| Frozen 1 | 1.03 | 0.48 |
| Frozen 2 | 1.02 | 0.5 |

Narrowing of the disc space was measured on the lateral radiographs using a digital caliper (Mitutoyo Corporation, Japan). In order to eliminate errors due to different magnifications, the height of the allograft disc, as well as the disc space below, were measured to the nearest 0.1mm and expressed as a ratio on both initial and final radiographs. In this way any loss of disc height could be determined.