VASCULARIZATION OF THE INTERVERTEBRAL DISC
IN PATHOLOGICAL CONDITIONS

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Thesis submitted for the degree of

Doctor of Philosophy

in

The University of Adelaide

(Faculty of Medicine)

February 1995

TABLE OF CONTENTS

ABSTRACT

The intervertebral disc is a remarkable structure. One of its most unusual characteristics is that it becomes almost entirely avascular after maturity, obliging the cells in the matrix to derive their nutrition by diffusion from external sources.

With advancing age, and in association with escalating pathology, significant morphological changes are found in all components of the disc. Notable amongst these changes, is the appearance of small blood vessels in the previously avascular end plates and annulus. This study has investigated the process of neovascularization of disc components, with ageing and in disease.

The aims of this study were:

(i)   to develop a perfusion technique that would identify small blood vessels in the end plate of sheep discs;

(ii)   to develop a reliable method for histoquantitation of these blood vessels;

(iii)   to describe the development of morphological changes in human lumbar discs, with particular reference to those changes that may be involved in the sequestration of fragments;

(iv)   and, to determine if vascularization of sequestrated fragments is related to symptoms of sciatica and duration of pain.

The extent of end plate vascularization in the lumbar discs of young normal sheep was established. Since prolonged acid decalcification of motion segments resulted in poor histological detail, a vascular perfusion technique using India ink was used to identify the small blood vessels in tissue sections. Perfusion revealed the patency of these vessels in the end plate. The reproducibility of the manual histoquantitation method was also established, justifying its use in subsequent studies. Finally, the extent of end plate vascularization was shown to diminish with advancing age in normal sheep.

Previous work with a sheep model showed that a small cut in the peripheral annulus resulted in progressive disc degeneration, despite healing of the outer annular fibres. In the present study, end plate vascularization increased significantly in a short time after surgery, presumably as a repair mechanism, but it appeared not to have a beneficial effect on the disc matrix. It was hypothesized that failure of repair and subsequent degeneration resulted from excessive movement of the affected motion segment. Fixation with a metal plate, however, failed to allow complete repair or arrest the degenerative process.

The development of disc lesions with advancing age was described, after histological examination of a large number of autopsy cases. Special attention was given to those changes that may contribute to the extrusion of sequestrated fragments. Vascularization of the outer annular fibres was noted, suggesting a possible role for pain production. The extent of end plate vascularization in discs with rim lesions was also determined, and compared with the sheep model.

The extent of neovascularization was assessed in a large series of extruded lumbar disc fragments, in an attempt to correlate this histological feature with clinical symptoms. Immunohistochemical localization of neurofilament protein adjacent blood vessels in some of the disc fragments suggested that they may be associated with pain symptoms.

The physical deterioration of the disc with age may be due, in part, to its inherent lack of vascularity. This study, however, has demonstrated that some components of the disc undergo neovascularization in response to physical trauma, possibly as part of a tissue repair response, although increased vascularization of the end plate did not prevent disc degeneration in the sheep model.

This study has demonstrated that vascular repair may be accompanied by ingrowth of neural tissue, providing evidence that it may be involved with

acutely painful rim lesions or with the pain that often accompanies disc herniations.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Robert J Moore

22 August 1995

ACKNOWLEDGEMENTS

DEDICATION

To Sarah

PUBLICATIONS ARISING

xii

Moore RJ; Osti OL; Vernon-Roberts B; Fraser RD.

Changes in endplate vascularity after an outer anulus tear in the sheep.

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AWARDS

Original work contained in this thesis was awarded the *Smith and Nephew Richards Spinal Research Award* at the 1993 Annual Meeting of the Spine Society of Australia.

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

INTRODUCTION

1.1 NUTRITION OF THE INTERVERTEBRAL DISC

The mechanism by which the intervertebral disc is nourished has been the subject of investigation for many years, with considerable attention in more recent years to the cartilage end plate. However, many of the early studies on disc nutrition were extrapolated from work on the articular cartilage of diarthrodial joints, with which researchers were more familiar. As long ago as the 18th Century, it was recognized that articular cartilage lacked an independent vascular supply (Hunter, 1743, cited in McKibbin, 1973). However, since Hunter's findings were limited by the resolution achievable from the microscopes of the day, it was not possible to identify the small diameter blood vessels present in the vicinity of the cartilage-bone interface. Nevertheless, Toynbee (1841) (cited in McKibbin, 1973) supported the view that small blood vessels did exist in this region, but added that the subchondral bone plate represented an impenetrable physical barrier that prevented the advance of these blood vessels beyond the medullary cavity through to the cartilage. Interest in the subject of cartilage nutrition was revived over a century later by Collins (1949) who believed that, if the dense subchondral bone plate were physically breached by blood vessels, then a pathological state was presumed to exist. The concept of a dense impenetrable barrier theory was maintained by other workers. At the time, it was believed that the vitality of articular cartilage depended solely on diffusion of dissolved nutrients from the synovial fluid that bathed the tissue constantly, and this remained a popular view for several decades (Strangeways, 1920; Brower et al. 1962; Mankin, 1963; Stockwell and Barnett, 1964; Maroudas, 1968; Maroudas et al. 1968; McKibbin and Holdsworth, 1968).

The concept of an exclusive function of synovial fluid in the provision of nutritive solutes to articular cartilage was challenged by the histological studies of Holmdahl and Ingelmark (1950), who demonstrated that there were direct microscopic vascular contacts between the medullary cavity of the bone

2

and the cartilage of diarthrodial joints in rabbits. These workers identified two types of blood vessels that were morphologically distinct and that varied in frequency between the different joints studied: a feature, it was reasoned, that indicated variations in the mechanical forces experienced by the joints. Trueta and Harrison (1953) subsequently demonstrated a complex network of blood vessels that originated in the medullary cavity of the hip joint and passed directly through the subchondral bone plate, and that eventually terminated at the deep surface of the calcified cartilage. In subsequent experiments, blood vessels were labelled with a variety of tracers to aid in their visualization. For example, Ekholm (1955) injected radio-labelled gold into the subchondral circulation of live animals and found evidence of tracer penetration into the adjacent cartilage, confirming the existence of a subchondral supply route. In similar experiments, Brodin (1955) and Hansen (1959) examined the distribution of fluorescent dye injected into adolescent rabbits, and proposed that one of the principal nutritional pathways in the intervertebral disc was through the bone directly beneath the cartilage end plate. It was also thought that the annulus might possibly be involved, although to a lesser extent than the cartilage end plate. Fluorescent dye injections were used by Greenwald and Haynes (1969) to demonstrate the existence of similar nutrient pathways in excised human femoral heads, although the same study purported to show that they were absent from the acetabulum, creating some uncertainty about their function.

While all of these studies provided new information on the basic mechanisms of general cartilage nutrition, they also raised doubts about some aspects of the experimental procedures that had been used to relate findings from animal models to the human situation. For instance, some of these early experiments had been performed *in vitro* utilizing injection pressures that were far in excess of normal physiological conditions. In addition, they used tracers that were not considered to be physiologically inert. More importantly, some of

3

these experiments were conducted using small immature animals that were thought by many to be inappropriate models of the adult human. As a result, the use of experimental animal models for this purpose was, at times, viewed with a degree of scepticism, and this led to greater stringency in subsequent experiments in order to provide more relevance to the human.

Despite having a similar biochemical and structural composition, the cartilage of the end plate of the intervertebral disc is fundamentally different from that of the diarthrodial joints, the most obvious difference being the absence of contact with synovial fluid. Apart from Brodin's (1955) study, very little investigation had been undertaken on the mechanism of disc nutrition, prior to the early 1970's. Thereafter, study in this field has gained considerable momentum.

In one of the earliest qualitative studies of disc metabolism, Nachemson et al. (1970) noted that the central zone of the cartilage end plate and the entire annulus fibrosus were permeable to diffusion of small dye molecules. In contrast, the lateral regions of the end plate near the vertebral rim were relatively impermeable. It was concluded that this permeability was related to the small vascular buds that were present in the medullary space immediately beneath the end plate. Although this work was similar to some of the earlier studies, it related the phenomenon of permeability to molecules which were known to diffuse readily through articular cartilage. Maroudas et al. (1975) observed in autopsy spines that there were direct marrow contacts with the matrix of human discs and, using quantitative methods, showed that the central part of the disc in the vicinity of the nucleus pulposus was relatively more vascularized than the periphery. This confirmed the earlier qualitative studies.

In the first of a series of valuable anatomical studies, using beautifully prepared injection techniques, Crock and Yoshizawa (1976) demonstrated a network of arteries and veins that concentrated within the central one-third of the vertebral bodies and, in turn, on the corresponding end plates. The function

4

of this vascular network, specifically in relation to the nutrition and metabolism of the constituent cell population of the disc matrix, was investigated in subsequent *in vivo* and *in vitro* experiments by Urban et al. (1977) using a canine model. These studies showed that diffusion of dissolved molecules from these subchondral vessels was the main mechanism for small solute transport into the discs. There were, however, other aspects which were shown to affect the movement of solutes through the disc matrix. For example, the studies of Urban et al. (1977, 1978) showed that, although solutes were theoretically free to diffuse into the disc by either the annulus or the end plate route, their actual movement was directly dependent on the size of the molecule as well as its ionic charge. By virtue of the high proteoglycan concentration the normal disc has an overall negative charge. This means that small uncharged solutes, such as glucose and oxygen, are able to diffuse relatively freely into the disc matrix. Molecules such as sulphate and chloride ions, which are negatively charged, are able to cross the end plate relatively easily, but have difficulty passing through the nucleus pulposus. Cations, such as sodium and calcium, exchange freely with the nucleus. Larger uncharged solutes, such as immunoglobulins and macromolecules including enzymes, tend to be totally excluded from the normal disc simply due to size restrictions. It became apparent, therefore, that there were two important factors influencing diffusion of solutes into the disc: the first, was the proportion of vascular contacts at the end plate; and second, the steric properties of the solutes involved.

The permeability of the disc itself was shown to vary across the different regions of the disc from almost free transfer through the annulus to virtually nil through the rim at the point where the annular fibres are embedded in the end plate. The central end plate route was also shown to be freely permeable, although not to the same extent as the annulus. The relative importance of these annular and end plate routes was established further by Ogata and Whiteside (1981) using a hydrogen washout technique, and by Holm

5

et al. (1981) using microscopic examination following fluorescent dye injections. Crock and Goldwasser (1984) used a combination of radiological and histological techniques on decalcified tissue sections to characterize the vascular terminations at the vertebral end plate following infusion of a contrast medium into the spinal circulation of greyhounds. These studies reinforced the increasingly widely held belief that the central region of the end plate was vital for the metabolic processes of the disc matrix which is otherwise essentially avascular.

1.2 DEGENERATION AND AGE-RELATED CHANGES TO THE DISC

Disc tissue is richly endowed with blood vessels as it develops. In the early neonatal period, the peripheral annulus has an abundant vascular supply and there is a complex network of vertebral vessels which penetrates the cartilage end plate adjacent to the inner annulus and the nucleus. With maturity, the disc becomes avascular, and by the age of about 25 years there is no direct blood supply to the nucleus pulposus (Coventry et al., 1945a; 1945b; 1945c). The lumbar discs are the largest avascular structures in the human body, and the central region of the largest discs in the adult can be almost 10 mm from the nearest direct blood supply (Holm and Urban, 1987).

The distinction between normal ageing and pathological degeneration of the intervertebral discs is unclear. It has been clearly demonstrated that, with age, the discs lose vertical height due to depletion of proteoglycan content and dehydration, but these changes cannot be distinguished from pathological discs which may appear, radiologically and histologically, exactly the same. Nevertheless, the involvement of the few blood vessels that are located in the vicinity of the disc has been researched extensively. Coventry et al. (1945a, 1945b, 1945c) alluded to the fact that, in the human disc, the vascularity which was present in the early developmental years was lost with maturation, and that it was associated with the gradual degenerative change that accompanies

6

ageing. This was confirmed by Nachemson et al. (1970) who correlated increased disc degeneration with decreased permeability of the cartilage end plate to dye. Brown and Tsaltas (1976) also showed, in the rabbit, that the penetration of fluorescent label into the disc diminished with increasing age despite the persistence of a rich subchondral vascular supply. This was attributed to some intrinsic factors such as decreased permeability of the end plate. Similar observations had been made previously by Amato et al. (1959) in the same animal.

Schmorl and Junghanns (1971) attributed aspects of the natural degeneration of discs to "necrotic areas of degeneration" or "ossification pores" that persist in the cartilage end plates following maturity, suggesting that these structurally weak points predisposed the disc to prolapse into the adjacent vertebral body (Schmorl's nodes). These features have been reported in between 35% (Schmorl and Junghanns, 1971) and 76% (Hilton et al., 1976) of adult autopsy spines and are generally seen in association with significant degenerative changes in the discs involved (Vernon-Roberts and Pirie, 1977). Schmorl's nodes have been reported to occur with equal frequency in spines above and below the age of 50 years, suggesting that they are present from an early age and that relatively few form in later life (Hilton et al., 1976). McFadden and Taylor (1989) supported Schmorl's concept that the eventual closure of the primitive end plate vessels with maturation left the legacy of a congenitally weakened structure that was susceptible to fracture and, eventually, the formation of Schmorl's nodes. One of the functions of the normal end plate is to prevent the highly pressurized nucleus from escaping through the annular fibres or into the adjacent vertebral body. In theory, therefore, the end plate should be a continuous structure.

Vascularization of the normal cartilage end plate is rarely seen, as mature hyaline cartilage is well known to be resistant to invasion by blood vessels (Kuettner et al., 1977). Nevertheless, there are a few reports in the

recent literature of such a finding (Yasuma et al., 1988; Oda et al., 1988). Blood vessels in the end plate have been observed in association with Schmorl's nodes and are therefore presumed to be related to the reparative process that is established from within the vertebral body. These two earlier studies alluded to the regenerative capacity of the end plates: for example, it was shown that the end plates of younger, but not older, discs are capable of a limited proliferation of cartilage cells following injury (Yasuma et al., 1988). However, the vascularization of the end plate, which is invariably followed by focal calcification, may be detrimental to the disc in the long term since it has the potential to completely occlude this route of disc nutrition (Oda et al., 1988).

In relation to the evolutionary process, the phenomenon of living tissue, such as intervertebral disc, being relatively avascular would appear to be both unusual, and undesirable, particularly if it contributes to degeneration of the structure at an early stage. This raises the following questions: (1) is the fate of the disc programmed, such that it degenerates with time, even in the absence of compromising incidents such as acute trauma?, and; (2) can this degenerative process be prevented, or at least slowed, by surgical or pharmacological intervention? It may be significant that osteoporosis of the vertebral bodies, in which the amount of mineralized bone per unit volume of tissue is diminished, is usually accompanied by good disc preservation at an advanced age, possibly as a result of the cushioning of impact forces by "soft" bone (Hansson and Roos, 1981; Kurowski and Kubo, 1986).

1.3 ANIMAL MODELS OF DISC DEGENERATION

In addition to investigations of basic disc metabolism, animal models have also been used widely for the study of human disc degeneration. The first report in the English literature of experimental disc degeneration induced in animals was that of Key and Ford (1948). Two earlier reports, written in non-

English journals are quoted in that article. Thus, Lob (1933) made a cut in the annulus fibrosus of rabbits and produced changes similar to those seen in human spondylosis deformans (osteoarthritis of the spine). Filippi (1935) observed regeneration and reconstitution of the normal lamellar structure of the anterior annulus fibrosus in rabbits three months after surgical division. These findings were challenged by Key and Ford (1948) who induced immediate nuclear prolapse in dogs by incising the annulus transversely through the posterior longitudinal ligament. On inspection, the annular defects had healed only at the periphery, while the inner layers remained separated. On the basis of this study, it was postulated that the primary lesion leading to disc prolapse in man was a weakening of the posterior annular fibres by degeneration or traumatic injury. Degenerative changes in the nucleus pulposus were assumed to be secondary.

Smith and Walmsley (1951) reported a similar experimental investigation of disc degeneration, using rabbits that survived from one day to two years after operation. Their conclusions, consistent with those of Key and Ford (1948), were that healing occurred only in the superficial lamellae of the annulus in association with a fibroblastic reaction typical of general histological repair. They attributed the failure of the deeper part of the wound to heal to the avascular nature of the deeper annulus. The presence of displaced nucleus pulposus, separating the fibres, may have prevented successful bridging of the annular defect. Smith and Walmsley (1951) proposed that, in humans, increased 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 allowing 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".

9

Hansen (1952), investigating disc degeneration in dogs, reported that spontaneous prolapse could be recognized in some breeds very early in life. In particular, the "chondrodystrophoid" breeds of dogs, which include the beagle, dachshund and basset hound, all of which show abnormal development of epiphyseal cartilage during endochondral ossification, are convenient for study since they reliably develop a chondroid metamorphosis in the nucleus pulposus within the first few years of life (Hansen, 1959). The notochordal cells and gelatinous nucleus pulposus are replaced by chondrocyte-like cells and the matrix contains more collagen than normal. The gross morphology and composition of the adult chondrodystrophoid canine disc shows many similarities to the human adult disc (Hansen, 1952; Ghosh et al., 1977) and, with advanced degeneration, the canine nucleus pulposus may also undergo calcification in association with disc prolapse (Hansen, 1959).

More recently, the biochemistry of the disc has been systematically analyzed in animal models. Lipson and Muir (1980, 1981) reported the biochemical changes during degeneration using the rabbit model developed by Smith and Walmsley (1951). Acute herniation of the nucleus was produced in all animals and was followed by secondary degenerative changes, including fibrocartilaginous metaplasia of the disc and osteophyte formation at the site of the incision. The water content of the disc, after an immediate loss, was restored by two days after the operation. However, there was later progressive dehydration of the operated discs in association with changes in the total uronic acid content. The proportion of aggregated proteoglycans increased rapidly during the first two days, but a progressive loss was observed six to seven weeks after the operation. The authors suggested that loss of confined fluid mechanics would appear to initiate the chemical changes seen in degeneration of the disc. They postulated that, when a communication occurs between radial fissures and circumferential annular tears, a "concealed herniation would take

10

place" which would then lead to irreversible mechanical damage and progressive degeneration.

Osti et al. (1990) introduced the young adult sheep as a practical animal model for the study of lumbar disc degeneration. It has the advantage of being a relatively large animal, making it easy for surgical procedures, and in Australia is readily available and is relatively cheap to purchase and maintain. In addition, based on the comparative studies of Butler (1988), the embryology and chemical composition of the sheep disc are significantly closer to the human than the discs of dogs and rabbits which had been used in previous studies. In this sheep model, the annular incisions of earlier experiments were modified. Instead of penetrating the full thickness of the annulus, the cut was restricted to the outer one-third of the antero-lateral lumbar disc, thereby avoiding spontaneous nuclear prolapse. This was designed to reproduce the "rim lesion" in the human disc first described by Schmorl and Junghanns (1971) and later by Vernon-Roberts and Pirie (1977) and Hilton and Ball (1984). The animals in these experiments were maintained for periods of between two weeks and two years post-operatively. In the outer-most annular fibres of the operated discs, an ordered sequence of healing was observed, but repair did not take place in the inner annulus. Disc degeneration developed at a slower rate than in smaller animals, but was invariably seen within six months of the operation, and followed progressive failure of the inner annular fibres and displacement of the nucleus towards the site of the initial incision. Radiating annular tears, degeneration of the nuclear cells and matrix, and marginal osteophytes were also observed, similar to changes seen in degenerate human discs. As with the earlier animal models, the inner annular fibres in the operated sheep discs failed to heal, even after two years. This lack of repair of annular tears reproduced the situation in the human discs.

The biochemical changes in the sheep model were characterized in parallel with the histopathology (Melrose et al., 1992). The nucleus pulposus of

11

injured discs showed a significant loss of proteoglycans and collagen eight months post-operatively, but there was a concomitant increase in non-collagenous proteoglycans. In the operated discs, proteoglycan aggregation initially declined but recovered to within control values six to eight months post-operatively. The nucleus pulposus of discs adjacent to the operated discs also showed time-dependent changes in matrix components, including loss of proteoglycans and collagen.

Several other groups have recently published data based on this sheep model of limited outer annular tears. Kääpä et al. (1993, 1995) created outer annular lesions without nuclear herniation in the lower lumbar discs of adolescent pigs to enable analysis of collagen chemistry following disc injury. Three months after surgery, the discs showed the same degenerative changes seen in human discs. Collagen biosynthesis was elevated across the whole disc, and particularly in the nucleus. This result, in conjunction with the observation of osteophyte formation, was interpreted as an attempt by the disc to correct an unstable biomechanical situation.

The same anterior annular injury was made in the porcine model by Kääpä et al. (1992) to study possible mechanisms of pain generation in the injured disc. Immunohistochemical staining was positive for the neuropeptide Substance P and calcitonin gene-related peptide. The presence of other related substances, including neurofilament protein R39, protein gene product and synaptophysin, also indicated that nerves and nerve terminals are present in the outer annulus following disruption of the collagen fibres. This work provides evidence pointing to a relationship between disc injury and discogenic back pain.

The porcine model of outer annular tears has recently been used to study the effect of daily injections of the non-steroidal anti-inflammatory agents tiaprofenic acid (TPA) and indomethacin (INDO) on proteoglycan metabolism (Karppinen et al., 1994). Treatment with TPA resulted in an accumulation of

proteoglycans in the nucleus not seen with INDO, suggesting that it has a chondroprotective role. This has implications for treatment by stimulating the repair process of the injured disc cartilage matrix.

Within the last decade, large animals have become accepted as experimental models of disc degeneration in man. Important practical considerations in the choice of animal model are (1) the availability of the animal; (2) the ease with which it can be handled under experimental conditions, and; (3) its anatomical, biochemical and physiological relevance to man. Although they have a quadrupedal habit, sheep and pigs are considered to be appropriate models because their discs have structural similarities to human discs, exhibiting well defined annular and nuclear demarcation in the young specimen. Importantly, there is also abundant evidence that natural disc degeneration in these animals follows a clearly defined progression of pathological and biochemical changes with age, similar to that seen in the human, and that these processes can be initiated by surgical manipulation to allow researchers to study them within a reasonable time-frame.

1.4 THIS STUDY

The catalyst for this study was the observation of new blood vessels proliferating in the cartilage end plate of sheep discs in a short time period following surgery that created a discrete injury to the peripheral annular fibres. Since the end plate is an important nutrient supply route for the disc it was of interest to determine if the neovascularization conferred an advantage to the disc in terms of preventing subsequent degenerative changes.

In the first part of this study, a method for identifying blood vessels was developed following careful analysis of a number of existing methods. It was deemed important to ascertain whether the histological features in the tissue sections were actually blood vessels, and not simply artefacts of preparation. In addition, it was also necessary to validate the method used to quantify the

13

blood vessels in subsequent studies, and to establish normal patterns of vascularization.

The second part of the study quantified the long-term post-operative vascular changes to lumbar disc end plates following annular surgery in sheep and related these changes to morphological changes to the disc matrix. This study was then extended to determine whether or not a rigid metal plating device fitted directly over the annular tear would have any effect on disc pathology and end plate vascularity.

The final part of the study examined the vascularization of other disc components in autopsy spines and in surgical specimens retrieved from patients with intractable low back pain. In this study, an attempt was made to correlate vascularization of the disc with pain, to determine if they could reliably predict clinical diagnosis of disc disorders and to gain a better understanding of the etiology and the natural history of disc pathology.

CHAPTER TWO

VASCULAR PERFUSION AND QUANTIFICATION OF BLOOD VESSELS IN THE CARTILAGE END PLATE OF SHEEP DISCS

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2.1 AIMS

The aims of this study were:

1. to develop a method of perfusion in a sheep model, that would enhance the visualization of vascular channels within the cartilage end plate, and thereby investigate their role in nutrition of the intervertebral disc;

2. to develop a reliable and reproducible method to quantify these vascular channels, and;

3. to estimate the extent of vascularization in the lumbar intervertebral discs in normal sheep.

2.2 INTRODUCTION

In the preliminary stages of the work reported in this thesis, it seemed both logical and convenient to describe the microscopic pores within the cartilage end plate of the disc, as "blood vessels". A major problem associated with the rigorous techniques that are used to prepare histological sections from boney material, however, is that the morphology of less robust structures such as small vascular channels within and around the bone is not well preserved, particularly when large blocks of tissue, such as whole motion segments, are unavoidably subjected to extended decalcification. Consequently, a more detailed investigation of these structures was considered justified, in order to gain a better appreciation of their nature, with particular reference to their possible role in disc nutrition. In other words, the vessels needed to be marked in a way that identified them unequivocally.

The literature describes numerous technical methods that have been used to demonstrate blood vessels within tissues and whole organs. In one of the earliest studies of this kind, Johnson (1927) used India Ink to demonstrate the vascular supply associated with the long bones of canine limbs. Ink was also used by Danckwardt-Lilliesröm (1969) to observe the vascular repair process of bone fractures in rabbits and dogs. Other techniques, utilizing fluorescent

16

dyes, radioactive tracers and tinctorial stains, have also been used to investigate the pathways involved with nutrition of cartilage in small animals (Brodin, 1955; Hansen and Ullberg, 1961; Brower et al., 1962). Greenwald and Haynes (1969) combined fluorescent and radioactive tracers in human autopsy studies and live animal experiments for similar purposes. The development of synthetic plastics in the early part of the 20th Century resulted in some application of this technology to this field, and there are reports of resins being polymerized in the blood vessels of laboratory animals following perfusion, for the demonstration of normal vasculature (Batson, 1935) as well as the florid vascular response to bone fracture (Wray and Lynch, 1959).

Radiographic imaging of blood vessels in the disc, following injection of a contrast medium, was reported by Hassler (1970) and rapidly adopted by many other workers. One of the most prominent investigators in the field of vascular anatomy was Crock (Crock et al., 1973, Crock and Yoshizawa 1976) who prepared radiographs from thick frozen sections to demonstrate the vascular network within and around cadaveric spines following perfusion with commercial preparations of barium sulphate. These early techniques were developed further by Crock and Goldwasser (1984) with the perfusion of greyhound dogs using a combination of Japanese Ink and barium sulphate. The rationale behind this variation on the earlier methods was that the ink provided histologic contrast in the blood vessels in tissue sections, and the barium provided radiographic detail. As a result, relatively thick slices could be cut, initially for investigation by fine detail radiography, and the sections could be treated subsequently by the Spalteholz method (Culling, 1974), and embedded in celloidin for histological examination. This latter method was also used in anatomical studies, in which intravascular injections of silicone elastomer (Lopez-Curto et al., 1980) and neoprene latex (Whalen et al., 1985) were used to improve the visualization of vascular networks in gross specimens.

17

All of the methods described were used for a variety of valuable anatomical studies, but have never been applied directly to the study of fine blood vessels in conventional thin section histopathology. In this context, the histological method described in this chapter utilizes many of the technical details used in these previous studies, and modifies them so that the fine vascular channels within the cartilage end plate of the sheep intervertebral disc can be observed by conventional light microscopy.

2.3 METHODS

2.3.1 Animals

In the initial study, four mature Merino wethers were used. Each animal was approximately two years old and weighed about 50 kg. The animals were control (non-operated) subjects which were part of a larger experimental study in which aspects of disc degeneration were being investigated. At the completion of this other study, and while under general anaesthesia, the animals were injected intravenously with 30,000U porcine heparin (David Bull Laboratories, Australia) to prevent intravascular coagulation. The femoral, hepatic, renal, and mesenteric arteries were ligated at the points where they branched from the aorta, to prevent perfusate entering their areas of supply. The animals were then killed with an overdose (7 g) of sodium pentobarbitone, injected directly into the external jugular vein, and the lumbar spine was exposed immediately using an antero-lateral approach. The perfusion mixture, which contained 10% (w/v) aqueous gelatine, to which was added 5% (first animal) or 10% (subsequent animals) (v/v) India Ink and 10% (w/v) barium sulphate, was heated initially to 60°C, to dissolve the gelatine, and subsequently cooled to 40°C for the perfusion. A short length of semi-rigid opaque polythene tubing (5 mm internal diameter) was primed with the perfusate, inserted into the aorta at the mid-thoracic level through a small nick

18

in the vessel wall, and secured firmly with an artery clip at the cranial (superior) end, immediately behind the point of insertion. The tubing was tied in position with plain string. A 50 ml syringe was then filled with perfusate, attached to the tubing, and perfusion was performed by gentle manual injection. Approximately 500-600 ml of fluid was injected into each animal within a period of 5-10 minutes. Each time the syringe was removed for refilling, an isolation valve in the line (distal to the insertion point) was closed to prevent air entering the system. The pressure of injection was monitored at all times with a sphygmomanometer and was maintained between 100 and 130 mm Hg for the procedure. There were no leaks apparent in the system.

2.3.2 Preparation of tissue for histological examination

The section that follows describes the technical procedures that are used to prepare stained histological slides from intervertebral discs of sheep. Since these are standard techniques that are relevant to all sections of this thesis where histology is used for the quantitative analysis of end plate vascularization, they are included in this section to avoid repetition in subsequent chapters.

Immediately following death, the lumbar spines were exposed by dissection of muscle and surrounding tissue, and removed *en bloc* by transecting the thoracolumbar junction above and the mid-sacrum below. Following a range of radiographic investigations, which included plain X-ray, discography, and MRI (for the purpose of other studies), individual motion segments were isolated by cutting midway through the adjacent vertebral bodies with an electric band saw. The specimens then were fixed in 10% phosphate-buffered formal saline (pH 7.2) for a minimum of 72 hours before undergoing decalcification in an aqueous solution containing 10% (v/v) nitric acid and 1% (w/v) EDTA (di-sodium salt). The progress of decalcification was monitored daily with radiographs, using a Faxitron X-ray cabinet (Hewlett

19

Packard) and Min-R E film (Eastman Kodak). When completely decalcified (up to five days later), the residual acid in the specimens was neutralized by immersion in a solution of 6% (w/v) anhydrous sodium sulphate for several hours. The specimens were then cut into six parasagittal slices of uniform average thickness of 4.5 mm and processed into paraffin wax on an overnight cycle using an automated tissue processing machine. Histological sections of selected slices were cut at a nominal thickness of 5 μm on a bench top rotary microtome, stained on glass slides with haematoxylin and eosin, and mounted permanently in Depex for microscopic examination. All tissue sections were examined without knowledge of the identity or the surgical treatment of the animals.

2.3.3 Quantitative histomorphometry

The blood vessels within the cartilage end plate were identified as vascular channels that emerged from the trabecular bone of the adjacent vertebra and penetrated the hyaline cartilage, either partially or fully, into the disc matrix. In almost all cases, erythrocytes could be identified easily within the lumen of these vessels, but it was rare to find a true endothelial lining after prolonged decalcification. Using an ocular-mounted Weibel ll graticule in the light microscope at an overall magnification of 400x, the extent of the vascularity in the cartilage end plate, to a depth of 200 μm from the osteochondral junction, was determined by a standard manual point-counting technique.

As part of this procedure, each end plate was systematically divided into three separate and reproducible zones that were discernible in the sagittal plane - namely that part of the end plate that was immediately adjacent the anterior annulus fibrosus; the nucleus pulposus; and the posterior annulus fibrosus (Figure 2.1). The limit of the annular zones was determined by polarized light

FIGURE 2.1

Low power microscopic view of a young, healthy sheep lumbar disc sectioned in the sagittal plane to demonstrate the cranial (upper) and caudal (lower) cartilage end plates separating the disc matrix from the vertebral bone. Counts of blood vessels were made in three regions of the end plates - adjoining the anterior (A) and posterior (P) annulus fibrosus, and the nucleus pulposus (M).

A   M   P
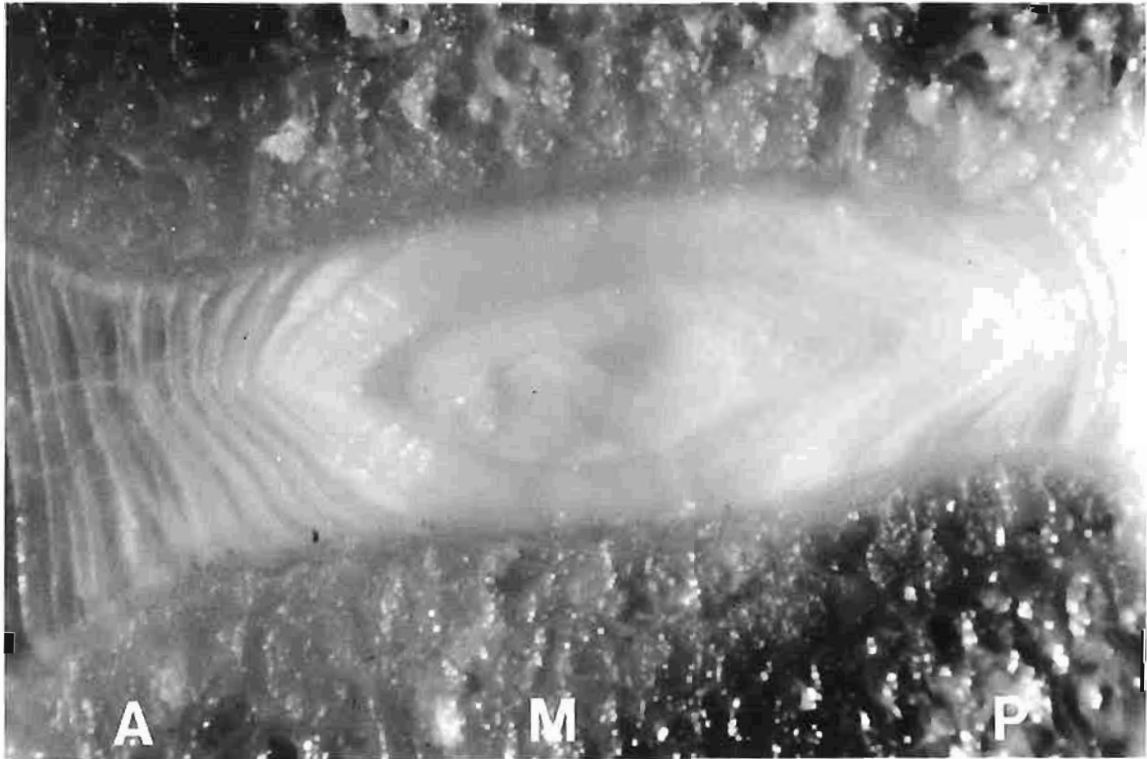
microscopy as each slide was analyzed. The proportion of the total area of cartilage end plate occupied by vascular channels was calculated using a mathematical relationship based on fundamental stereological principles, and expressed as a percentage of the total area measured (Parfitt, 1983). Specific details regarding the application of this quantitative method to individual experiments are described as they arise in each section that follows.

2.3.4 Spalteholz method

Parasagittal slices adjacent to those selected for histology were processed by a modification of the Spalteholz method. In this procedure the tissue blocks were dehydrated gradually through increasing concentrations of ethanol over several days until the clearing agent step was reached. Instead of being transferred to paraffin wax (as is the usual practice in histology), they were retained in methyl benzoate, which rendered the tissue completely transparent and revealed the blood vessels that had been filled with the ink mixture during the perfusion. The samples were observed and photographed under direct fibre-optic illumination using a Wild M400 photomacroscope (Wild Heerbrugg) with Kodak EPY64T and Ilford FP4 Plus or Ilford 100 Delta film. Histological sections were photographed using an Olympus BH-2 photomicroscope and Kodak Tech Pan film.

In the second part of the study, seventeen mature Merino wethers were used. As in the first part, they were approximately two years old and weighed around 50 kg. These animals were part of a separate study in which allograft intervertebral discs were inserted into recipient animals. Heparin was not used as an anticoagulant in these animals, since, with experience gained from the previous group, it was not considered necessary.

Additional changes were also made to the perfusion technique following the earlier experience. In particular, it was considered that the previous study used substantially more perfusate than was necessary. Since, in this second

22

experiment, only the lower lumbar discs were available for study, artery clips were placed across the aorta between the femoral bifurcation and the renal arteries to minimize the area of the spine being perfused and reduce the volume of perfusate required. The perfusion mixture, which contained 10% gelatine, 10% India Ink and 10% barium sulphate in a total volume of 100 ml water, was also allowed to set for up to 2 hours before the spine was removed for examination. Tissue blocks were prepared as previously described (Section 2.3.2).

2.4 RESULTS

2.4.1 General observations

Plain X-ray films of the spines, taken *ex vivo*, immediately after the perfusions confirmed the success of the procedure. In all cases, large blood vessels were clearly localized by the barium in the soft tissue surrounding the spine (Figures 2.2; 2.3).

Sagittal slices of unprocessed discs that had been decalcified but not processed also gave a clear impression of India Ink within blood vessels at the end plate region (Figures 2.4; 2.5; 2.6).

Tissue blocks that had been treated by the modified Spalteholz method provided an equally good impression of vascularity within the tissue, particularly at the cartilage end plate. Discs from the first series of perfusions showed localization of ink within end plate channels, although at times it was difficult to see clearly (Figure 2.7). Visualization was improved subsequently by increasing the concentration of ink in the perfusion mixture, and by limiting the procedure to a smaller portion of the spine (Figures 2.8).

The perfusions revealed capillaries emerging from the vertebral bone and ending blindly in the cartilage end plate (Figures 2.9; 2.10; 2.11; 2.12; 2.13; 2.14).

FIGURE 2.2

Plain X-ray film of a sheep lumbar spine taken immediately after perfusion with a mixture of India ink, gelatine and barium sulphate. The radio-opaque barium sulphate in the perfusion mixture reveals the vasculature within and around the spine.

FIGURE 2.3

Plain X-ray film of a divided motion segment from a sheep lumbar spine perfused with the same mixture as in Figure 2.2. The para-spinal vasculature is clearly visible in this decalcified tissue block.

FIGURE 2.4

The sagittal cut surface of a sheep disc following perfusion. The blood vessels of the cartilage end plate are demonstrated by the black staining of the India Ink from the perfusion mixture. This disc was decalcified but not processed further (x 6).

FIGURE 2.5

Both end plates adjacent the nucleus of the same disc as in Figure 2.4, showing blood vessels stained black with India Ink (x 8).

FIGURE 2.6

The caudal end plate adjacent the anterior annulus of the same disc as Figure 2.4, showing the location of the small blood vessels (x 12).
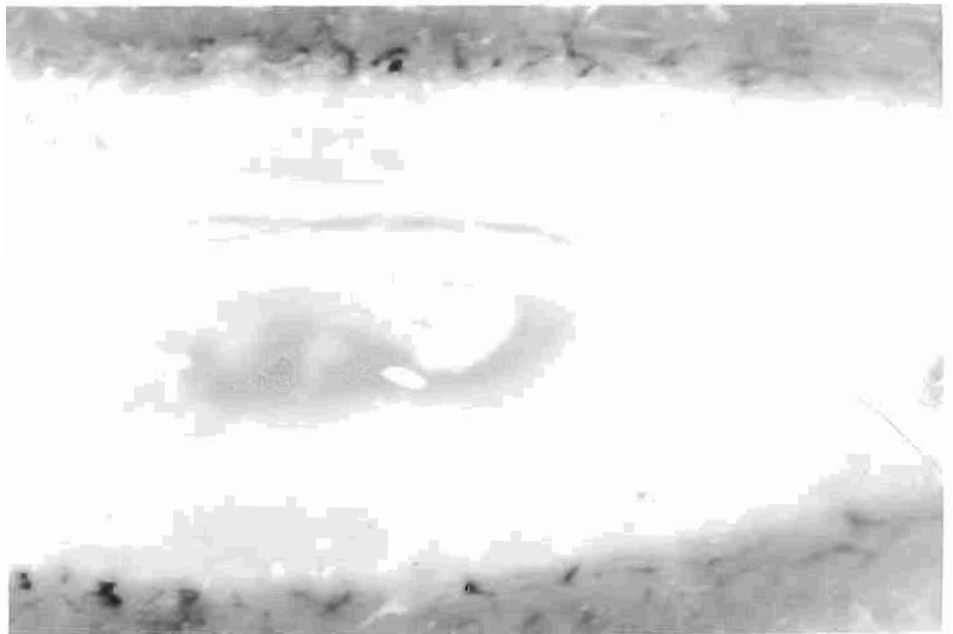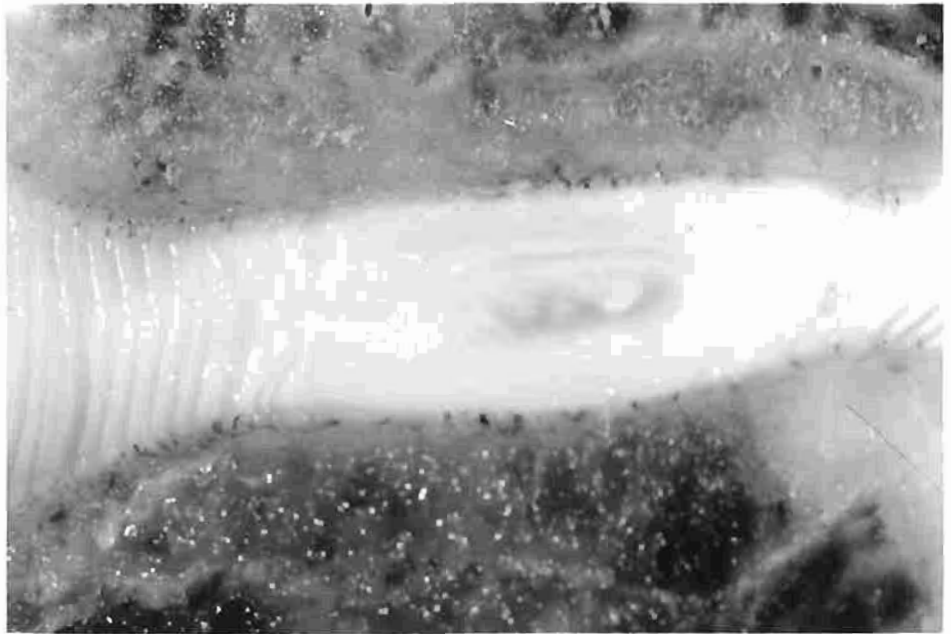
FIGURE 2.7

The anterior annulus fibrosus of a sheep disc prepared by the Spalteholz method, following perfusion with India ink, barium sulphate and gelatine. There is evidence of ink staining in microscopic blood vessels within the cartilage end plate (x 11).

FIGURE 2.8

Improved visualization of blood vessels in the posterior part of a sheep disc after modifications had been made to the perfusion method (Spalteholz method, x 13).

FIGURE 2.9

Low power photomicrograph of blood vessels in the subchondral bone and end plate adjacent the nucleus pulposus of a sheep disc (Spalteholz method, x 12).

FIGURE 2.10

Low power photomicrograph of a sagittal slice of a sheep lumbar disc. Blood vessels filled with India Ink from the perfusion are clearly demonstrated in the cartilage end plate using a modification of the Spalteholz method (x 6).

FIGURE 2.11

Low power photomicrograph showing a major vertebral vessel supplying the end plate region of a sheep lumbar disc (Spalteholz method, x 11).
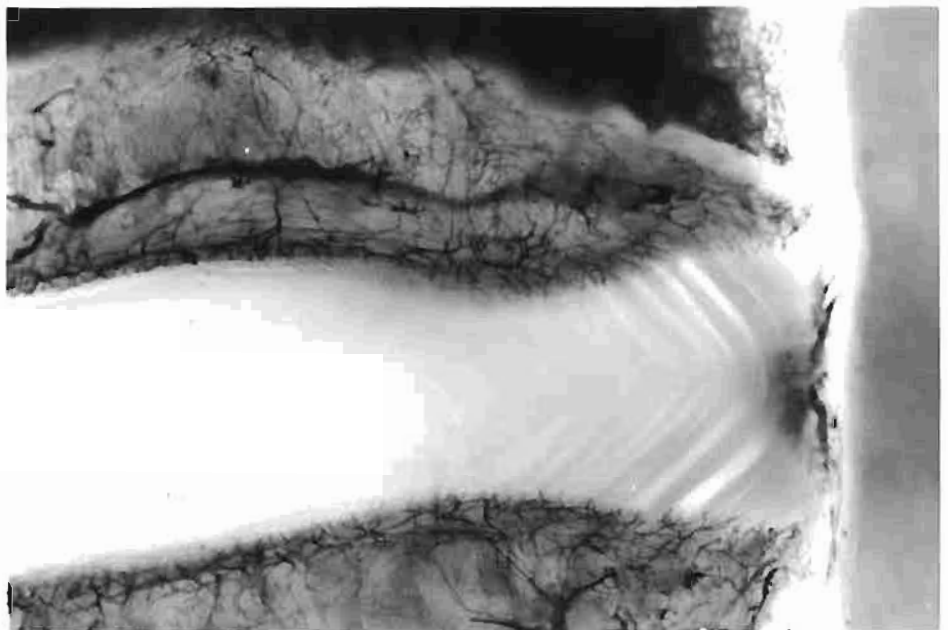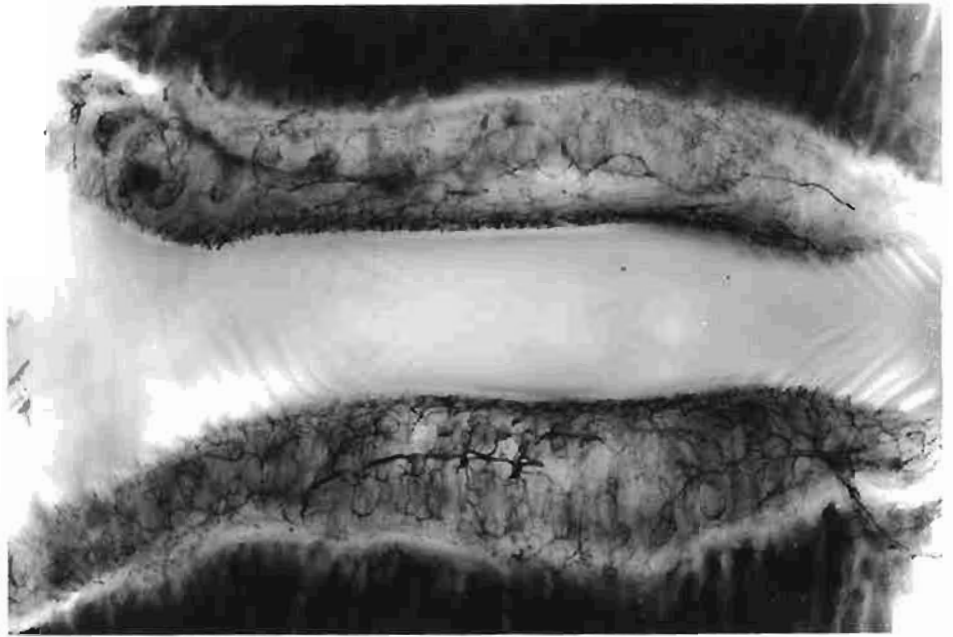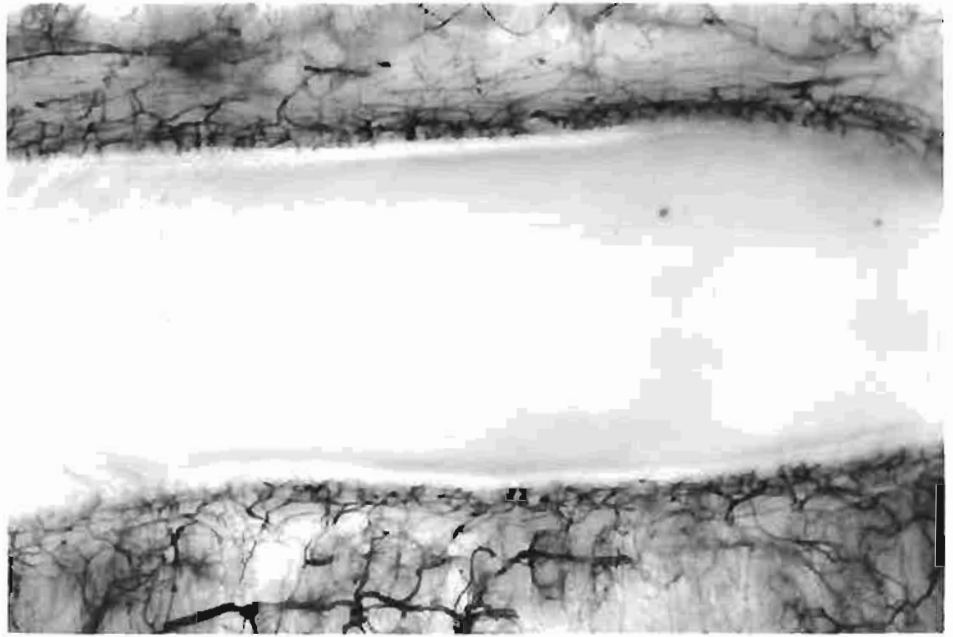
FIGURE 2.12

Low power photomicrograph showing blood vessels penetrating the cartilage end plate adjacent the nucleus of a sheep lumbar disc (Spalteholz method, x 16).

FIGURE 2.13

Photomicrograph of a sheep disc end plate showing the capillary endings of the blood vessels (Spalteholz method, x 18).

FIGURE 2.14

Closer photomicrograph of the same disc shown in the previous figure (Spalteholz method, x 32).
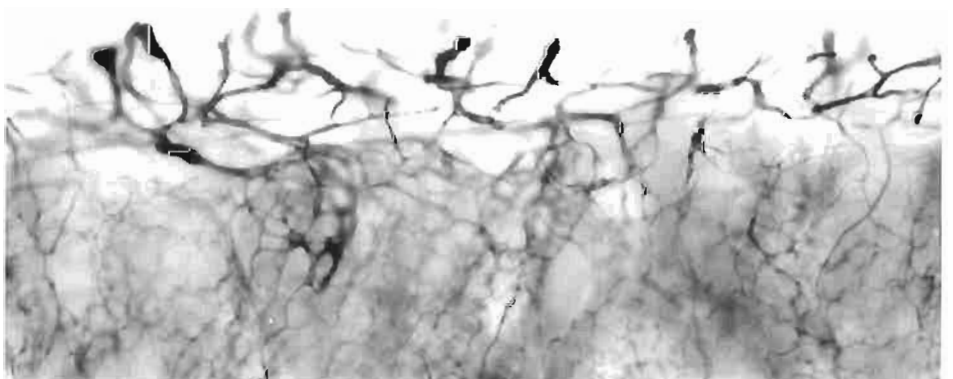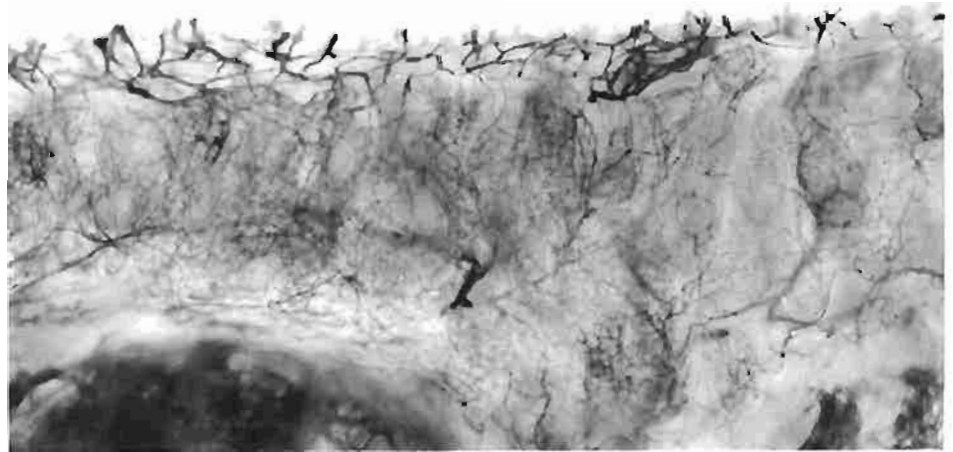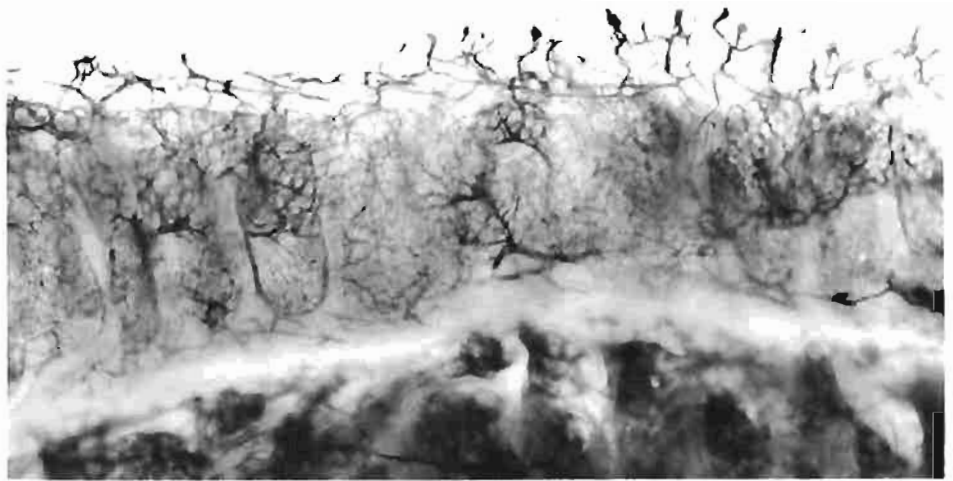
Some blocks with dense vertebral bone required prolonged decalcification before they could be prepared for histological assessment. As vascular channels in these blocks were difficult to identify, the India ink in the perfusion mixture was a valuable marker (Figures 2.15; 2.16).

In addition to the small vessels of the end plates, there was evidence of small blood vessels lying between the peripheral fibres of the anterior and posterior annulus fibrosus (Figures 2.17), and within and around the spinal cord (Figure 2.18).

The stained sections revealed two major types of vascular channels in the end plate region - those communicating directly with the disc matrix, and those ending blindly within the end plate. The proportions of the two types were approximately equal, although this was not specifically investigated in this study. Zones of focal calcification were found extending across the cartilage end plate in association with vascular channels (Figure 2.19).

Sections of skin, kidney and liver failed to show any signs of ink or barium uptake, indicating that ligation of the major efferent vessels from the aorta was effective in preventing perfusion of the peripheral regions of the animals.

2.4.2 Histoquantitation

In this preliminary study, two separate counts were made of the number of vascular channels in the cartilage end plate zone of all discs. Initially, all regions of the end plates that had incorporated ink mixture were counted, while the second count included only those regions that were morphologically identifiable as vascular channels - that is, the small pores within the end plate which often were surrounded by a zone of ossification, occasionally exhibited an endothelial cell lining and, contained red blood cells in the lumen. Across the group, the average area of end plate occupied by vascular channels showing ink staining was 3.1 ± 0.9% (mean ± standard error of mean) and by

morphology alone was 3.9 ± 0.9%. Therefore, the majority of the vascular channels were shown, by perfusion, to be patent.

FIGURE 2.15

High power photomicrograph of a microscopic blood vessel filled with black particles from India ink following perfusion. The blood vessel penetrates the full thickness of the cartilage end plate from the subchondral bone (left side) to the disc matrix (right side) (H&E, x 200).

FIGURE 2.16

High power photomicrograph of a larger blood vessel filled with India ink from the perfusion. As in the previous example, this blood vessel penetrates the cartilage from the subchondral vertebral bone (left side) to the disc matrix (right side) (H&E, x 200).

FIGURE 2.17

Medium power photomicrograph showing small blood vessels interspersed between the peripheral layers of the annulus fibrosus of a sheep lumbar disc. They are filled with India ink following perfusion (H&E, x 100).
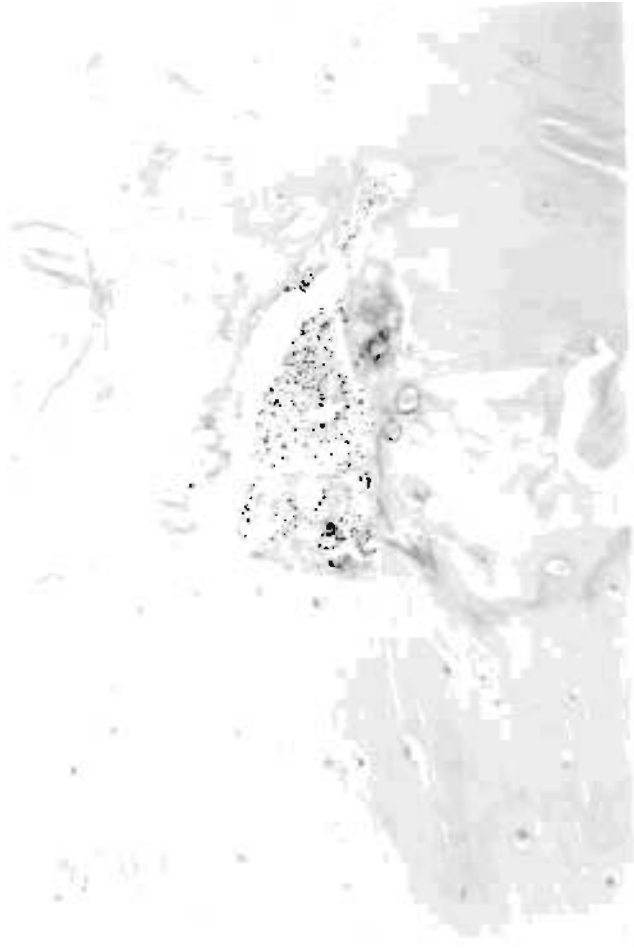
FIGURE 2.18

High power photomicrograph showing India ink in a large blood vessel within the lumbar spinal cord of a sheep following perfusion (H&E, x 200).

FIGURE 2.19

Zone of focal calcification (arrow) surrounding an isolated blood vessel that has penetrated the cartilage end plate of a sheep lumbar disc. The subchondral bone of the vertebral body is at the top and the disc matrix is at the bottom of the photograph (H&E, x 100).
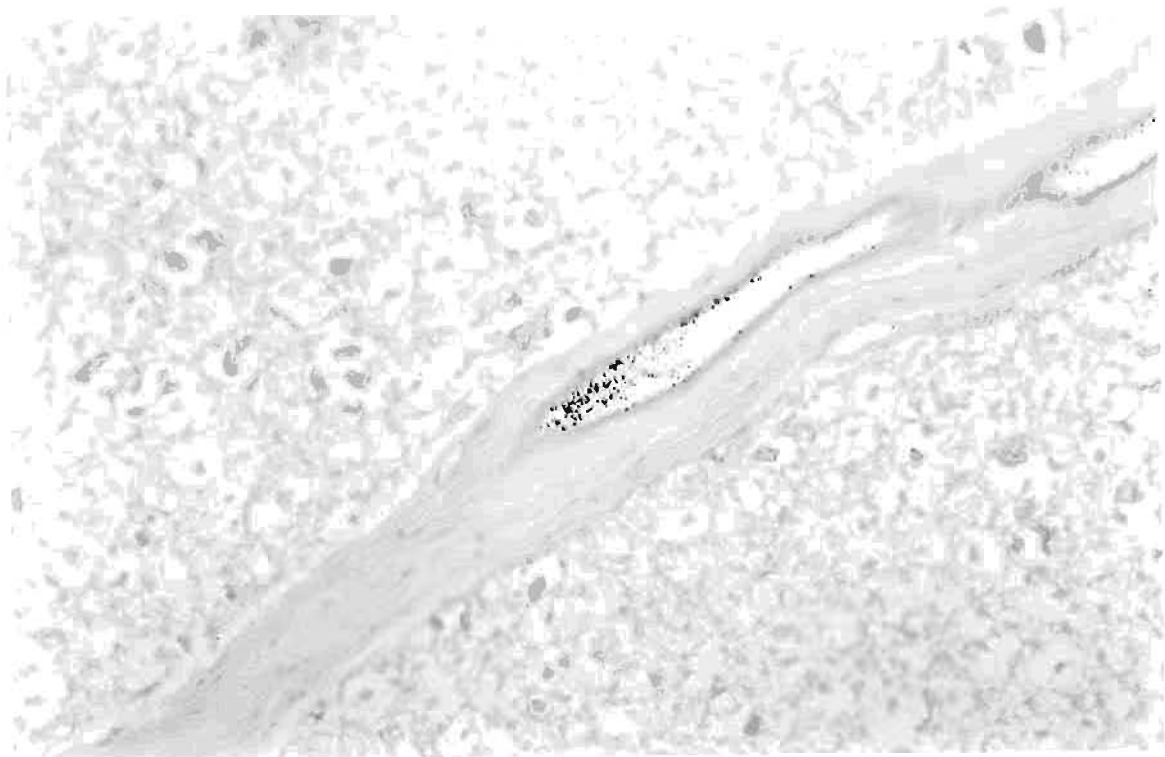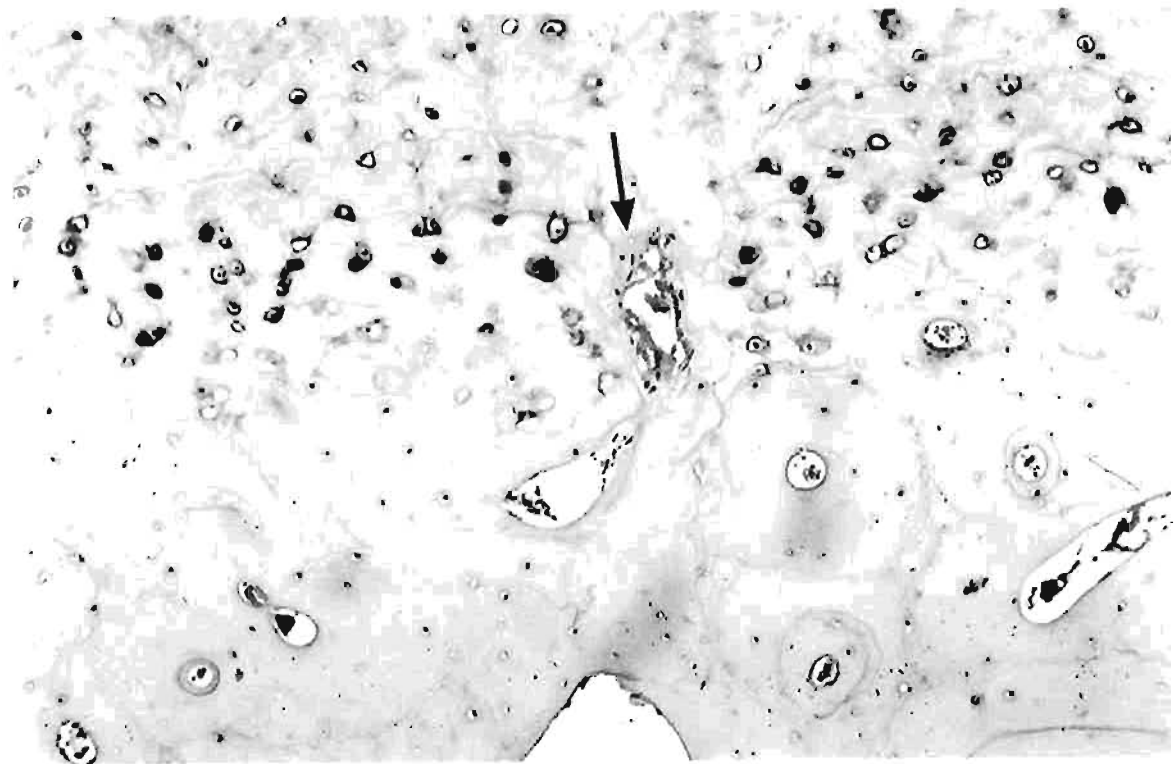
2.5 DISCUSSION

The perfusion technique described in this study is not novel, but has proposed some modifications to the key steps from several other published methods, so that they could be adapted to thin section histology.

Despite having a valid role in certain anatomical investigations, latex and acrylic media were considered inappropriate for use in the present study. The former are more suitable for the demonstration of major vasculature, since they may be too viscous to fill smaller vessels, and infusion techniques with plastics require high pressure which can fill afferent as well as efferent vessels, creating difficulties with interpretation (Rhinelander et al., 1979).

Barium sulphate was not a critical component in the perfusion mixture used in this study, but did provide a clear radiographic impression of the paraspinal vasculature, and the success of the technique was obvious immediately following perfusion. Barium also proved invaluable as a microscopic marker in the stained tissue sections, since in the first attempt, the concentration of India Ink was too low to allow all small vessels to be recognized with certainty.

India Ink was the key component of the perfusate, however, and was essential for identifying blood vessels in the few cases that showed sub-optimal tissue morphology as a result of prolonged decalcification. In the first perfusion, the concentration of ink was obviously too low, and in subsequent attempts it became apparent that a concentration of around 10% was necessary to successfully demonstrate blood vessels. Ink was readily visible in sections stained conventionally by haematoxylin and eosin, but became even more obvious when the eosin counterstain was omitted. Its greatest value, however, was in the Spalteholz method, since the black colour of the ink contrasted clearly against the clear yellow background of the surrounding tissue.

Gelatine was also included in the perfusion mixture to maintain the ink in suspension. Without it, the ink may have drained from the vessels before it

set firmly. As a result, it was necessary to leave the animals for at least 60 minutes following perfusion, to allow the gelatine to cool and set *in situ*. The significant improvement in the second series of perfusions may also have been influenced by the longer time period between perfusion and dissection of the spine, during which time the perfusate may have become relatively more solid.

The results of the histoquantitative component of this study validated the identification of vascular channels in the cartilage end plate on morphological grounds alone, despite the fact that the histological appearance of the tissues following decalcification was often poor. It is clear that the majority of the features that were identified as blood vessels by morphology alone were indeed what they were initially suspected to be. This is significant, since several of the subsequent investigations in this study sought to quantify changes in vascularity of the end plate following surgical manipulation of the disc. To undertake perfusion of all the animals in these experiments would have been extremely demanding on technical time, and the confirmation of the reliability of morphological identification of vessels made it possible to prepare and analyze the tissue by standard histological methods.

While two morphological types of vascular channels were identified in the end plates, it is unlikely that they actually exist in these distinct forms. It is more likely that blood vessels appear in these different configurations as a result of their spatial orientation within the tissue. Those that appear to terminate in the end plate, for instance, would probably continue to course through the disc in a different plane of the section. The variation in the morphology of vascular contacts between medullary bone and cartilage of articular joints in rabbits has been attributed to differences in mechanical forces experienced at different locations (Holmdahl and Ingelmark, 1950). Although this was not tested in the sheep model, it could also be relevant to the intervertebral disc, particularly across the central and lateral zones of the end plate which would be expected to experience a range of mechanical forces

during motion. The uncertainty surrounding the morphological variation of vascular channels could be resolved by systematically comparing sections from multiple levels through these blocks. The possibility also exists that these blind-ending vessels represent evidence of neovascularization of the end plate, particularly in view of the calcification that was seen in association with these vessels. The potential relevance of this observation is discussed later in this thesis.

2.6 FURTHER WORK ON THE VASCULARITY OF THE CARTILAGE END PLATE IN THE SHEEP MODEL

2.6.1 INTRODUCTION

The work in the preceding section established the validity of identifying vascular channels in the end plate using morphological criteria. Since a major part of this thesis involved the estimation of end plate vascularity in sheep following spinal surgery, it was important to understand the normal appearance of the discs in this animal model, to be able to appreciate any changes that result from surgery or ageing. In the section that follows, three separate, but closely related experiments are described. The first of these verifies the reproducibility of the manual histomorphometric technique used extensively in the thesis. The second study examines the distribution of blood vessels across the end plate in normal animals. Finally, the changes associated with ageing are investigated, with a brief reference to differences that occur between levels of the spine.

2.7 REPRODUCIBILITY OF THE MANUAL HISTOMORPHOMETRIC METHOD

2.7.1 AIM

To validate that the manual method of quantifying blood vessels is reliable and reproducible.

2.7.2 METHOD

Ten stained tissue sections were selected at random from an experimental group, and quantified again by the same observer, after an interval of at least twelve months. The counts from each slide were reported as a single value, representing the pooled estimate from the three end plate regions analyzed, and averaged across the cranial and caudal end plate. The difference between the consecutive counts was analyzed to determine the reproducibility of the method, using the measures of bias and random error (Grubbs, 1948).

2.7.3 RESULTS

The results of this analysis are summarized in Table 2.1. The counts varied from 4.4% to 9.8% on the first pass, and from 4.7% to 9.3% on the second pass. The differences between the two counts varied from -1.7% to 1.6%, with a mean difference of 0.05%, indicating the level of bias. The standard deviation of the difference was 1.10%, indicating random error.

37

TABLE 2.1

End plate blood vessel counts in ten lumbar discs from sheep selected at random. The second counts were made by the same observer at least 12 months after the first.

SHEEP/SLIDE	1st COUNT	2nd COUNT	(1st-2nd)
O12/A5	7.7	6.4	1.3
O16/B2	9.8	9.3	0.5
O24/B2	4.4	5.4	-1.0
O32/C5	7.1	8.3	-1.2
O34/C2	5.8	5.3	0.6
O21/A5	8.2	7.9	0.3
O13/B2	8.8	7.2	1.6
O33/B2	5.9	7.6	-1.7
O16/D2	8.5	9.0	-0.5
O30/B1	5.3	4.7	0.6

2.7.4 DISCUSSION

There was no statistically significant difference between the counts made on two separate occasions, indicating that the point-counting method used is highly reproducible. This was an important finding as it validated the histomorphometric technique used extensively in subsequent experiments in this thesis.

It should be noted that only the intra-observer variation has been reported here, since there is general agreement that feature identification represents the most significant source of error in the case of individual operators (Kimmel and Jee, 1983). This method of quantifying features in slides is relatively standard in histology, and it is generally accepted that, when a single observer can reliably identify the feature of interest, the counts would not be expected to vary significantly on separate occasions.

2.8 VASCULARITY OF NORMAL SHEEP DISCS

2.8.1 AIM

To determine the extent of vascularity within the cartilage end plate of the normal adult sheep intervertebral disc.

2.8.2 METHODS

Eight adult Merino wethers, aged approximately 2 years and weighing between 45 and 50 kg were used. The lumbar spines were harvested immediately after death by barbiturate overdose, and prepared in the same manner as described previously (Section 2.3.2). Parasagittal slices were taken from the left and the right sides of each disc, and counts were made of the vascularity at each region (namely, adjoining the anterior and posterior annulus fibrosus, and the nucleus pulposus) along the cranial and caudal end plates. The results from all spinal levels were pooled since the effect of different levels was not being investigated. Students t-test (PC-SAS, SAS Institute) was used to determine if differences existed between the three regions of the end plates. Data were expressed as mean ± standard error of mean. Statistical significance was set at $P<0.05$.

2.8.3 RESULTS

In the lumbar discs of normal adult sheep, there were significantly more blood vessels in the end plate region adjoining the nucleus pulposus than adjoining either the anterior or posterior annulus fibrosus ($P<0.05$) (Figures 2.20; 2.21). The data from the left and the right sides of the discs were almost identical. There was no significant difference between the counts from the anterior and posterior annulus. The cranial and the caudal end plate counts did not differ significantly at any of the end plate regions.

FIGURE 2.20

Cartilage end plate vascularity on the left side of lumbar discs from young normal adult sheep.
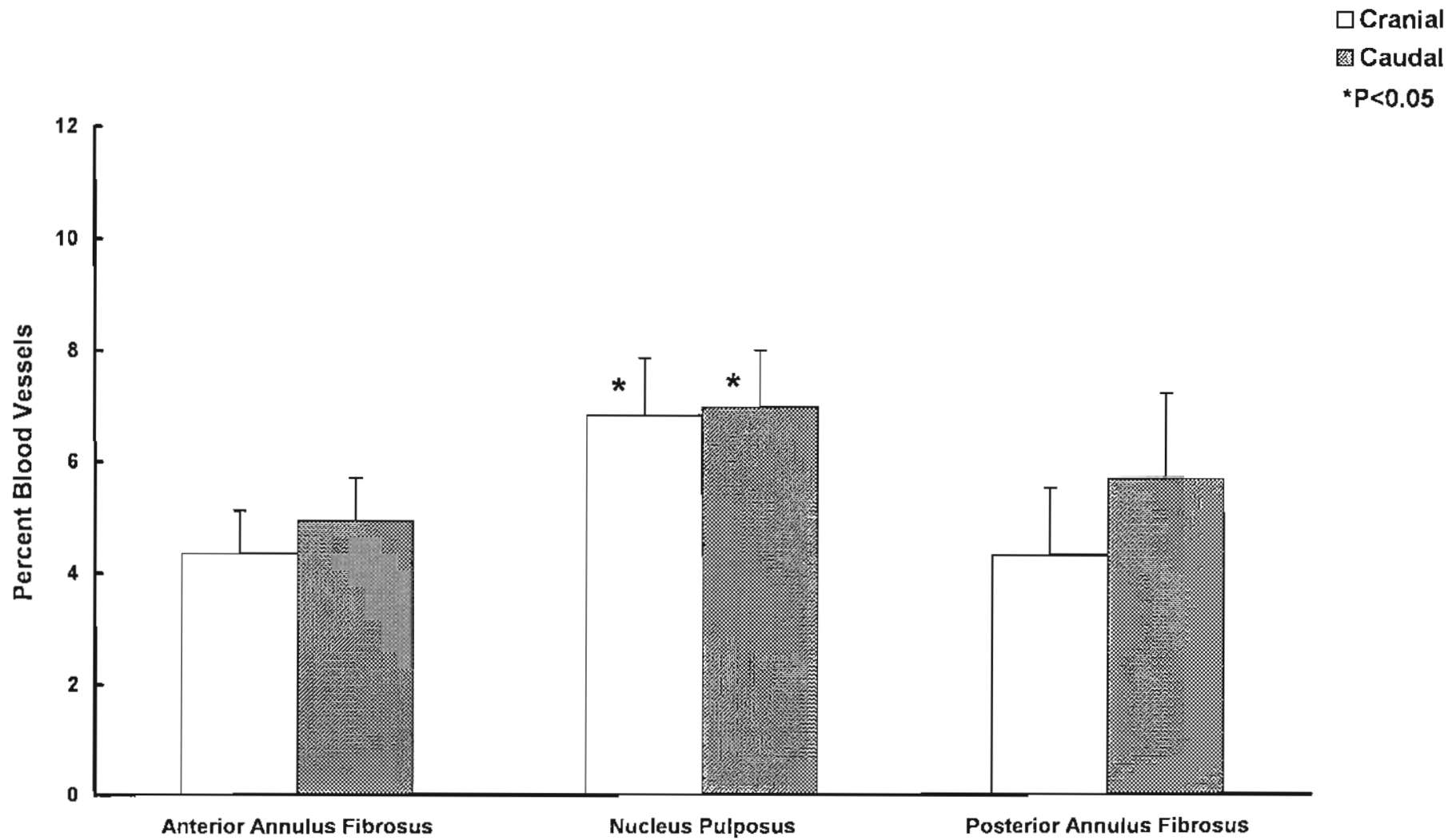
FIGURE 2.21

Cartilage end plate vascularity on the right side of lumbar discs from young normal adult sheep.
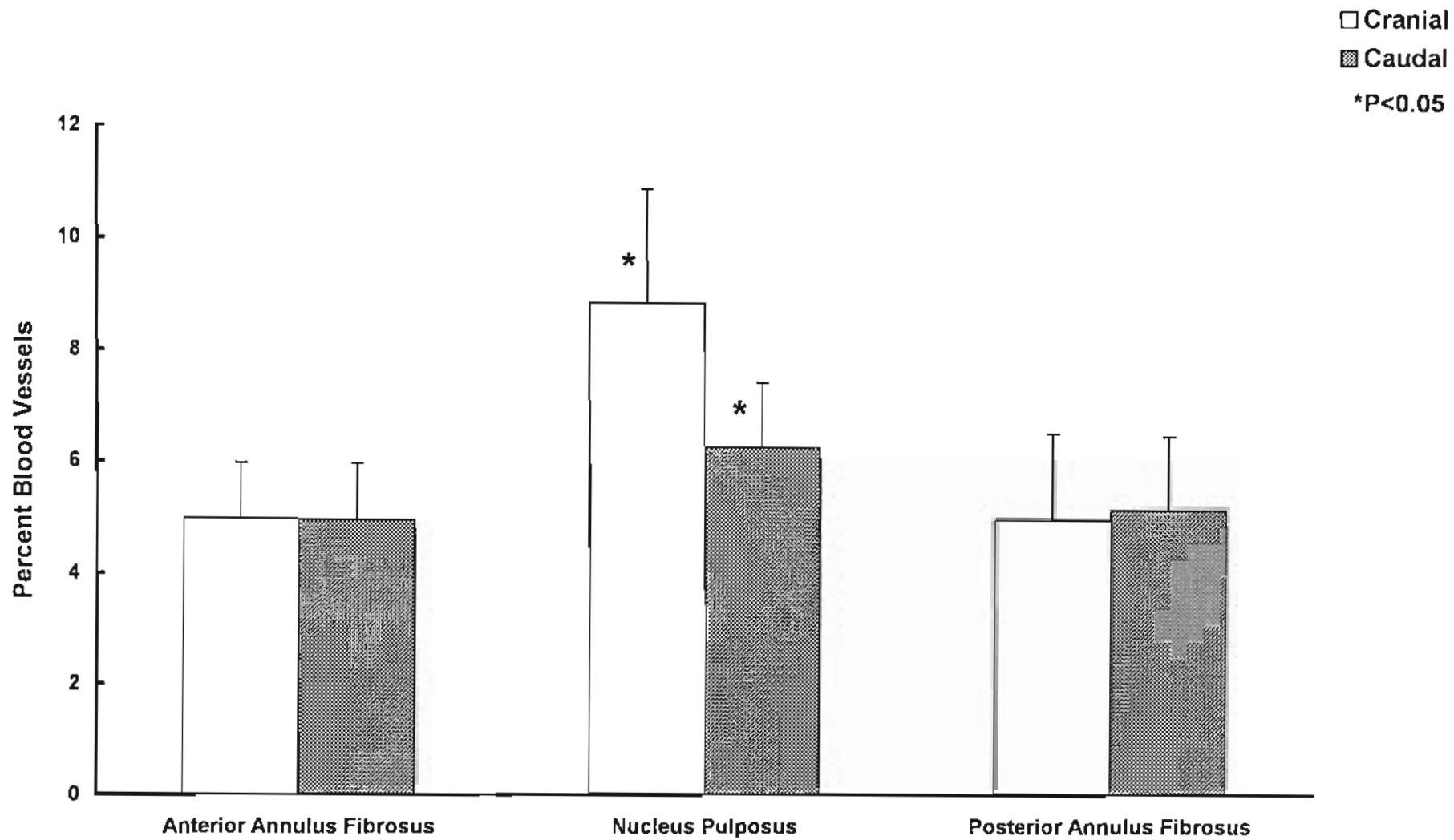
2.8.4 DISCUSSION

Tissue sections from opposite sides of the disc were analyzed to determine if, in the normal sheep, there is any significant difference in the extent of vascularization within the cartilage end plate in any spatial plane. The findings show that the distribution of blood vessels in the end plate is symmetrical, both laterally and in the cranio-caudal direction. They confirm previous observations in human discs that the central region of the end plate, immediately adjoining the nucleus, is significantly more vascularized than the lateral regions adjoining the annulus (Maroudas et al., 1975; Roberts et al., 1989). Further, they suggest that the central region is potentially more important than the lateral zones for the supply of nutrients to, and disposal of waste products from, the disc matrix, which is normally avascular.

It was important to verify that the arrangement of end plate vessels in the disc is symmetrical from one side to the other, since in subsequent experiments the extent of vascularization was evaluated following a unilateral surgical procedure. A different outcome would have confounded the interpretation of subsequent findings.

2.9 VARIATION IN VASCULARITY ALONG THE LUMBAR SPINE OF NORMAL SHEEP

2.9.1 AIM

To determine if there is any variation in the extent of end plate vascularity between the lumbar discs of young and old sheep.

2.9.2 METHODS

Two groups of experimental animals were used - six two-year-old and 5 five-year-old Merino wethers. The lumbar spines were prepared by the histological methods described earlier (Section 2.3.2).

Histomorphometric counts from the cranial and caudal end plates, and from the left and right sides of each disc were pooled, since it was shown in the previous experiment that they were not significantly different in young normal sheep. Student's unpaired t-test (PC-SAS, SAS Institute) was used to determine if there were any significant differences between the counts from the lumbar levels of the two groups. Student-Newman Keuls test (PC-SAS, SAS Institute) was used to test for differences between the lumbar levels of each group Data were expressed as mean ± standard error of mean. Statistical significance was set at $P<0.05$

2.9.3 RESULTS

The data are summarized in two graphs (Figures 2.22; 2.23). There was a significant reduction in the number of end plate vessels associated with increasing age ($P<0.05$). Discs from the older sheep showed a reduction of about 40% in the number of vessels when compared with those from the younger animals. As expected, there were no significant differences in the values from the right or left side from each group. While there was a trend towards a progressive reduction in the number of blood vessels from the upper

44

FIGURE 2.22

Cartilage end plate vascularity on the left and right sides of lumbar discs from normal young (two years) and old (five years) sheep.
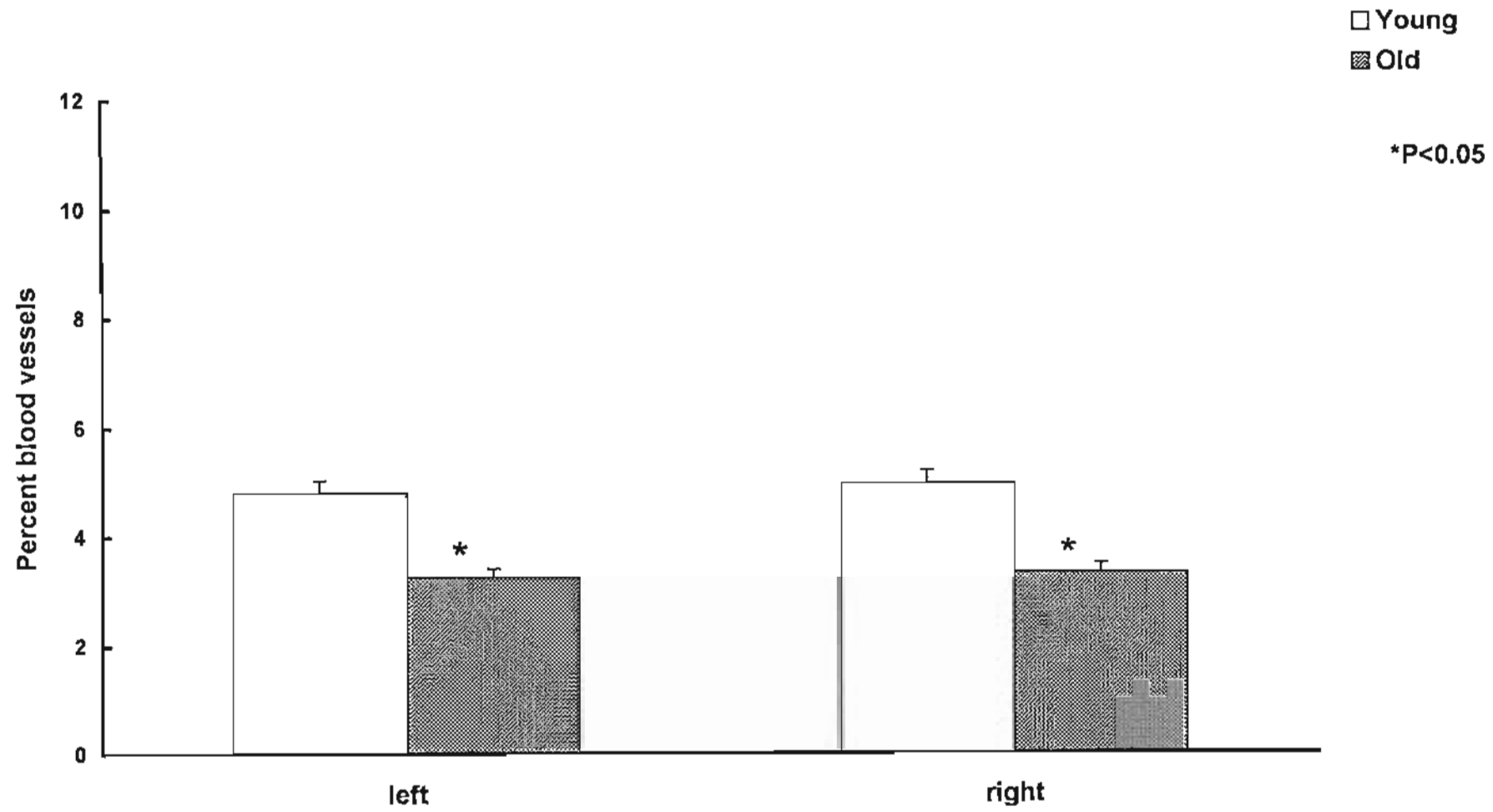
FIGURE 2.23

Cartilage end plate vascularity in the lumbar discs from normal young (two years) and old (five years) sheep. There was a statistically insignificant decline in the extent of vascularity at the distal levels at both ages.
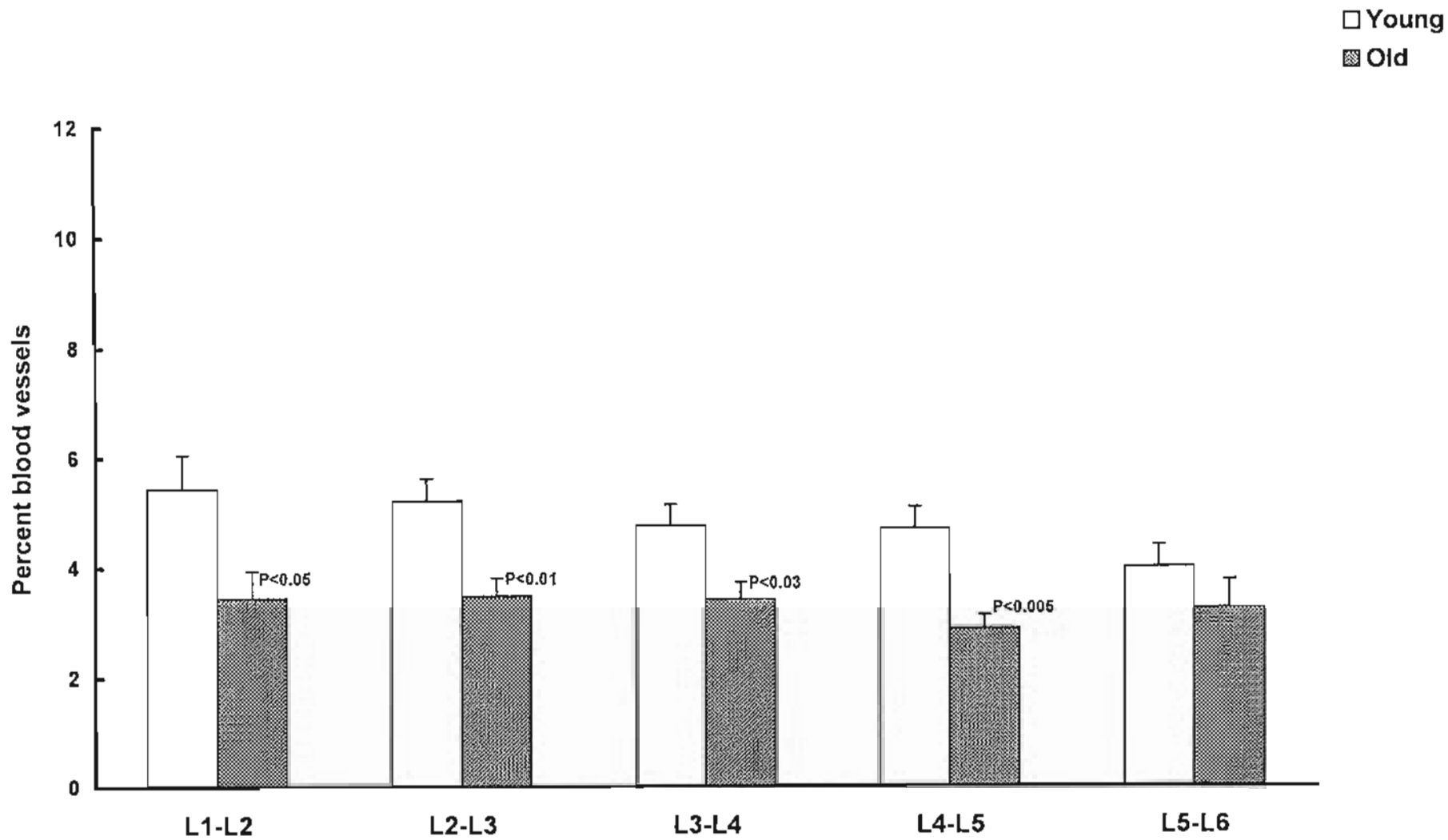
to the lower end of the spine, this was not statistically significant.

The discs from the older group were markedly narrower in sagittal section (that is, disc height was reduced) and showed histological evidence of "age-related degeneration" in the nucleus. In particular there was some evidence of chondrocyte clustering and dehydration which appeared as large cracks through the body of the nuclear matrix. There was also some evidence of chondroid metaplasia in the annulus fibrosus.

2.9.4 DISCUSSION

The sheep used in this study were older than those generally available, since the average sheep bred for commercial purposes in Australia is not allowed to live for more than 24 months. The vascularity of the end plate in the adult sheep disc was noted to diminish with age and, on the basis of this limited study, may reach a critical lower limit which is sufficient for basic disc nutrition in the sheep less than 24 months old. The relatively advanced pathology of older discs may indicate that end plate vessels in the sheep after this age are insufficient to maintain the disc in a healthy state.

This study has validated the random selection of lumbar discs for surgery in the subsequent study, since there was no significant difference (in either age group) in the degree of vascularity of the lower lumbar levels. The surgery performed in the experiment which is reported in Chapter 3, was principally at levels L2-L3 to L4-L5, where the vascular counts were not significantly different between these levels.

2.9.5 CONCLUSIONS

The cartilage end plate of the sheep disc contains a network of blood vessels which communicate closely, if not directly, with the disc matrix. It has been shown that, despite some initial problems in identifying these blood vessels in some histological preparations, they can be quantified in a reproducible manner.

The number of these blood vessels has been examined extensively in groups of normal sheep and it has been shown that they are distributed along the cranial and caudal end plates in equal proportions. It has also been shown that they are distributed equally on the left and right sides of the disc. The extent of end plate vascularization is relatively constant along the length of the lumbar spine of each sheep, but diminishes slightly with age and concurrent degenerative changes.

CHAPTER THREE

VASCULARIZATION OF THE CARTILAGE END PLATE
FOLLOWING AN OUTER ANNULAR TEAR

3.1 AIM

To determine the effect of a superficial cut in the outer annulus fibrosus on the vascularity of the cartilage end plate and the architecture of the subchondral vertebral bone of the sheep.

3.2 METHODS

3.2.1 Induction of annular tears

Thirty one mature (two-year-old) Merino wethers, with an average weight of 50 kg were used in this study. Sodium thiopentone (1 g) was injected into the external jugular vein and, after insertion of an endotracheal tube, general anaesthesia was maintained using nitrous oxide, isoflurane and oxygen. The lumbar spine was exposed using a left-sided retroperitoneal approach and, in three randomly selected lumbar discs, a cut was made in the left antero-lateral annulus. This was parallel and adjacent to the inferior end plate of the cranial vertebra (Figure 3.1). The depth of the cut was restricted to 5 mm by a guard on the scalpel blade, and the width was maintained at 5 mm. The cut left the inner two-thirds of the annulus and the nucleus pulposus intact, as the average thickness of the anterior annulus in the sheep lumbar spine is 7.5 mm.

The annulus cut closely reproduced the rim lesion previously described in human spines (Schmorl and Junghanns, 1971; Vernon-Roberts and Pirie, 1977).

For an average period of three days following surgery, the sheep were kept in individual pens in an indoor animal care facility, during which time they also received daily intramuscular injections of methadone (25 mg). After this time, they were released at a regional field station and were permitted unrestricted activity while being observed on a daily basis.

50

FIGURE 3.1

Low power sagittal view of a sheep lumbar disc with an outer annular cut adjacent the cranial end plate. The cut was restricted to the peripheral annulus and did not penetrate the nucleus pulposus.
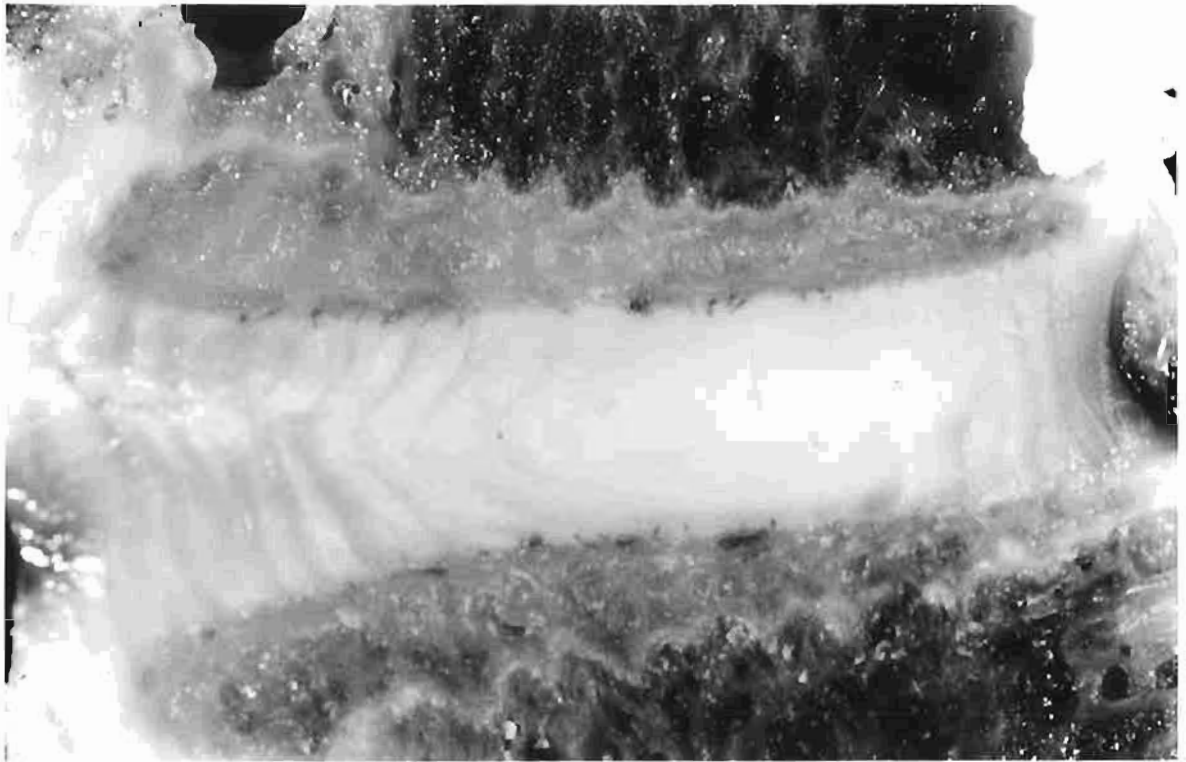
At the time of surgery, the sheep were allocated randomly to different groups in relation to the time interval between operation and sacrifice. Survival times were one to two months ("early"); four to twelve months ("intermediate"); and eighteen to twenty-four months ("late"). A further eight non-operated ("control") animals, in which no annular lesion had been created, were also included in this study. The spines from these animals were examined in the same manner as those from the operated sheep.

3.2.2 Pathological examination of discs

The spines were processed in the manner described previously (Section 2.3.2). The annular cut, which was almost invariably present in the second slice from the left, was examined using a dissecting microscope and photographed to provide a permanent record of its macroscopic appearance. Two of the six slices, the one containing the annulus lesion (slice 2) and a contralateral slice (slice 5), were dehydrated in alcohol and embedded in wax for histological examination and histomorphometric analysis. The innermost and outermost slices were not analyzed in this study. The cranial and caudal end plates were analyzed separately to determine if there was significant variation due to this factor. Histomorphometric estimates of the vascularity of the cartilage end plates were made as described previously (Section 2.3.3). The histomorphometric changes and pathologic findings were assessed by separate observers without knowledge of the sheep groups.

The trabecular bone of the vertebral body immediately adjacent the disc was photographed to ensure that the bone could be clearly distinguished from the fat and cellular marrow elements. The image from the photographic print was captured with a video camera interfaced with a Quantimet 520 Image Analyzing Computer (Cambridge Instruments, UK). The image threshold was adjusted so that only osseous tissue was detected and displayed on the video monitor. The region of interest was bordered by the cartilage end plate on one

52

side and the epiphyseal growth plate on the other. It is this bone which would have some influence on, or would itself be influenced by, pathological changes in the disc. (The growth plate persists in the sheep vertebra for at least five years despite apparent maturation of the long bones). This area was circumscribed manually on the digitizing pad with the light pen, and analyzed by a computer program which had been developed to estimate a range of trabecular bone morphometric parameters, including bone volume, BV/TV (%); bone surface, BS/TV (mm^2/mm^3); trabecular thickness, TbTh (μm); trabecular spacing, TbSp (mm); and trabecular number, TbN (#/mm). Tb.Th and Tb.Sp were calculated according to the rod model of trabecular architecture. These parameters were expressed in the nomenclature as proposed by an international committee on standardization (Parfitt et al., 1987).

Histomorphometric estimates were made from both end plates (cranial and caudal) of the slices from both sides (left and right) of the discs. Multiple lumbar levels were analyzed, representing repeated measurements from each animal.

3.2.3 Statistics

All statistical comparisons of end plate vessels were made using Student's paired (within disc) and unpaired (between disc) t-test. Where the data did not conform to a normal distribution, they were log-transformed prior to statistical analysis. Shapiro-Wilks test was applied to assess normality of the bone morphometric data. Non-parametric statistics were used since two of the parameters, TbTh and TbSp, were not normally distributed. Wilcoxon two-sample test was used to test if the estimates from the three post-operative survival groups differed from the non-operated group. Kruskal-Wallis test was used to test for time-related changes in the operated groups. Repeated measures analysis of variance was used to determine the relative contribution of "spinal level" and "end plate" to any differences that were observed post-operatively.

All analyses were performed using the PC-SAS statistical package (SAS Institute Inc). Morphometric data were expressed as mean ± 1 standard error of mean. Significance was set at P<0.05.

3.3 RESULTS

3.3.1 End plate histology and histomorphometry

There was evidence of a healing response in the outer one-third (1.5 mm) of the defect in the anterior annulus, but the inner two-thirds of the defect showed no repair at any stage. With time, the inner annulus which had not been cut during surgery, showed medial extension of the surgical defect so that, in some instances, there was a degree of internal protrusion of nucleus pulposus towards the site of the surgical incision (Figures 3.2; 3.3).

On the side of the disc ipsilateral to the cut (slice 2), the blood vessel counts along the length of both the cranial and caudal end plates were significantly higher than from the non-operated control animals (P<0.0001, all post-operative groups). However, when compared with the three non-operated discs from the same animals, no significant differences were found. The counts in this ipsilateral slice decreased with time from 9.94 ± 1.53% (cranial) and 9.39 ± 1.61% (caudal) at two months after surgery, to 7.92 ± 0.52% (cranial) and 7.28 ± 0.55% (caudal) after twelve months (P<0.05). Thereafter, the counts remained virtually unchanged for the duration of the experiment (Figure 3.4).

FIGURE 3.2

Low power sagittal view of a sheep lumbar disc one month following surgery to create a 5 mm deep annular incision. There is evidence of a healing response in the peripheral annular layers, but there is no apparent disruption of the inner annulus or nucleus (This figure was reproduced with the kind permission of Mr OL Osti).
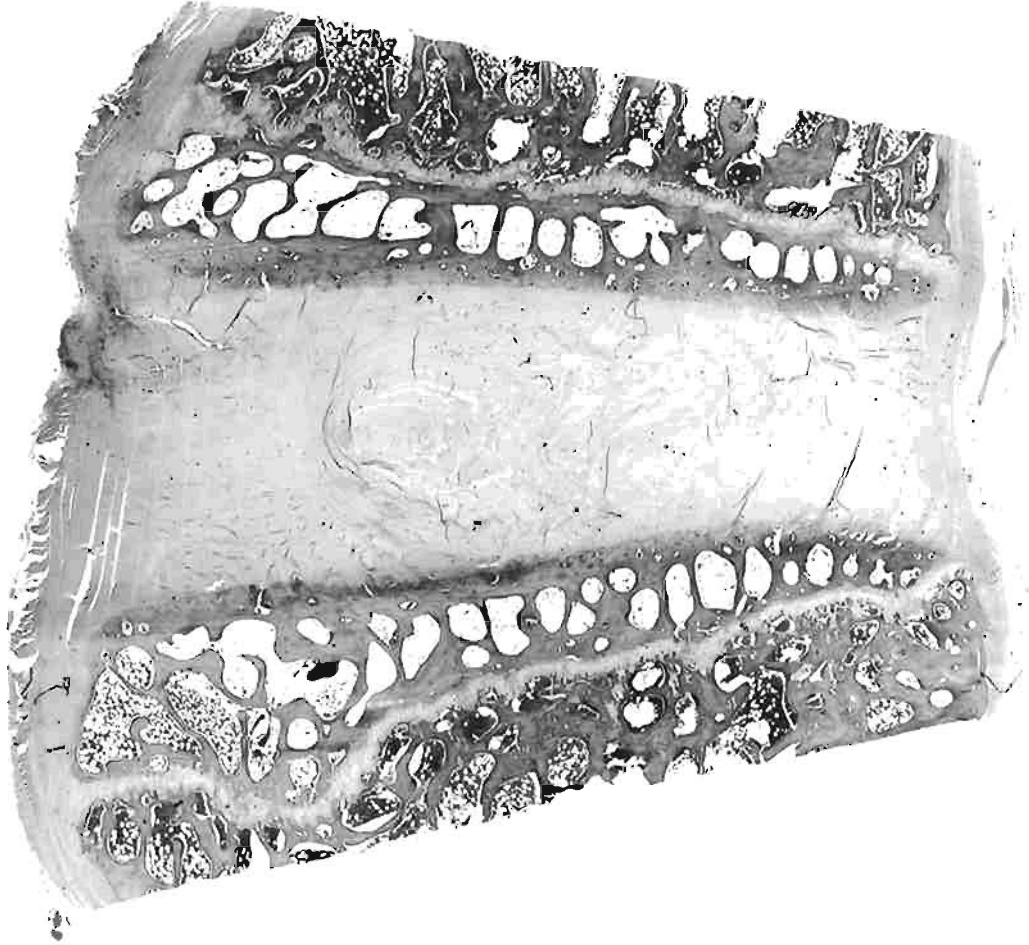
FIGURE 3.3

Low power sagittal view of a sheep lumbar disc twelve months following surgery to create a 5 mm deep outer annular incision. There is medial extension of the cut and the nucleus is displaced towards the site of the original incision. There is evidence of a healing response in the peripheral fibres of the annulus (This figure was reproduced with the kind permission of J.B. Lippincott Co.).

FIGURE 3.4

Cranial and caudal end plate blood vessel counts on the left side of sheep lumbar discs up to two years post-operatively.
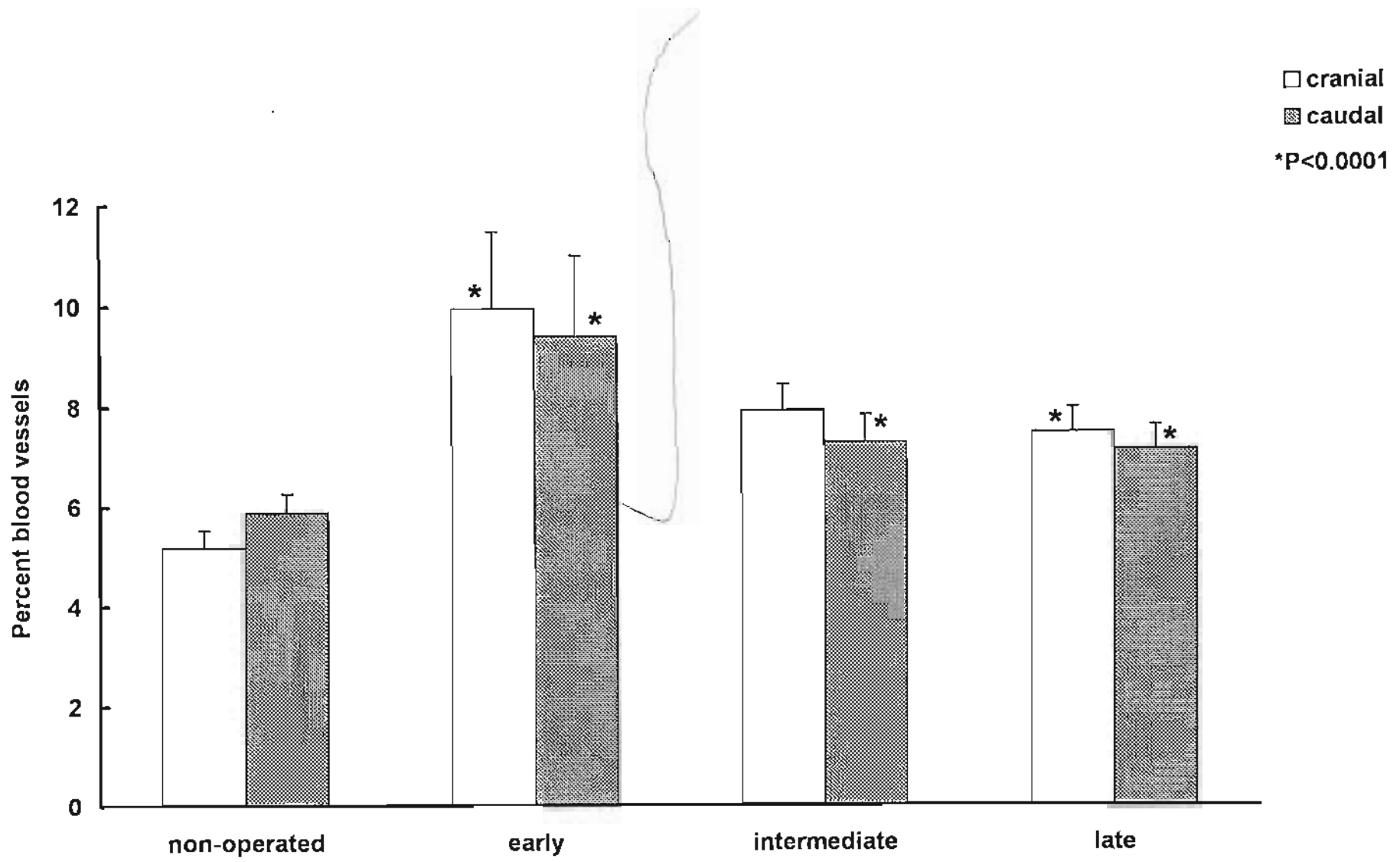
On the side of the disc contralateral to the cut (slice 5), the cranial and caudal vessel counts did not differ significantly from the control values of either the operated or the non-operated control groups, with the exception of the intermediate post-operative group, in which the values were elevated on the caudal end plate alone (P<0.05). The counts from both end plates on this contralateral side of the disc remained relatively constant throughout the course of the study (Figure 3.5).

In the control discs of the operated sheep, there was no significant difference between the cranial and caudal end plates of any group on either the left or the right side of the disc. In the non-operated control group, the cranial end plate had fewer vessels than the caudal end plate on the left side (P<0.05), whereas the cranial end plate had more vessels on the right side (P<0.05).

In those discs with an outer annular cut, the left (ipsilateral) side showed significantly more vessels than the right (contralateral) side, in both the cranial and caudal end plates (p<0.05, Figure 3.6). Of these discs, the inferior end plate in the intermediate post-operative group failed to reach significance (P=0.0776), but maintained the trend of higher blood vessel counts on the left side of the discs.

In the operated discs, the increased vascularity was not confined to the area adjacent the initial cut (antero-superior end plate), but was seen across the entire end plate on the left (operated) side (Figures 3.7; 3.8; 3.9; 3.10). On both sides of the discs, however, the relative distribution of blood vessels was unchanged from the non-operated state, so that all three end plate regions responded to the annulus cut in similar manner. Accordingly, the end plate adjacent the nucleus pulposus maintained a relatively higher vascularity compared with the periphery adjacent to either the anterior or posterior annulus fibrosus. The cranial and caudal end plates were identical in this regard.

FIGURE 3.5

Cranial and caudal end plate blood vessel counts on the right side of sheep lumbar discs up to two years post-operatively.
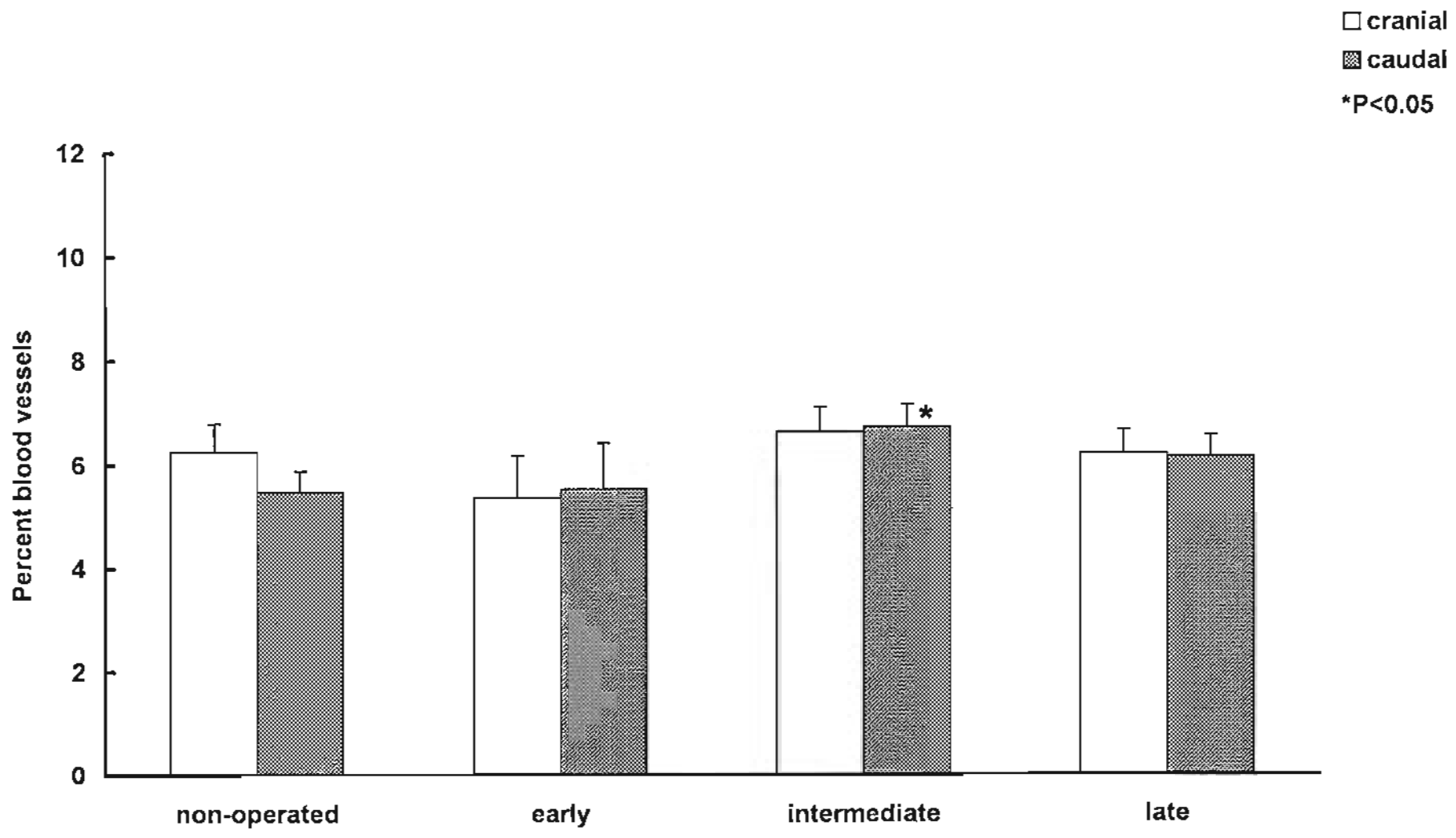
FIGURE 3.6

Combined cranial and caudal end plate blood vessel counts from the left and right sides of lumbar discs from sheep up to two years post-operatively.
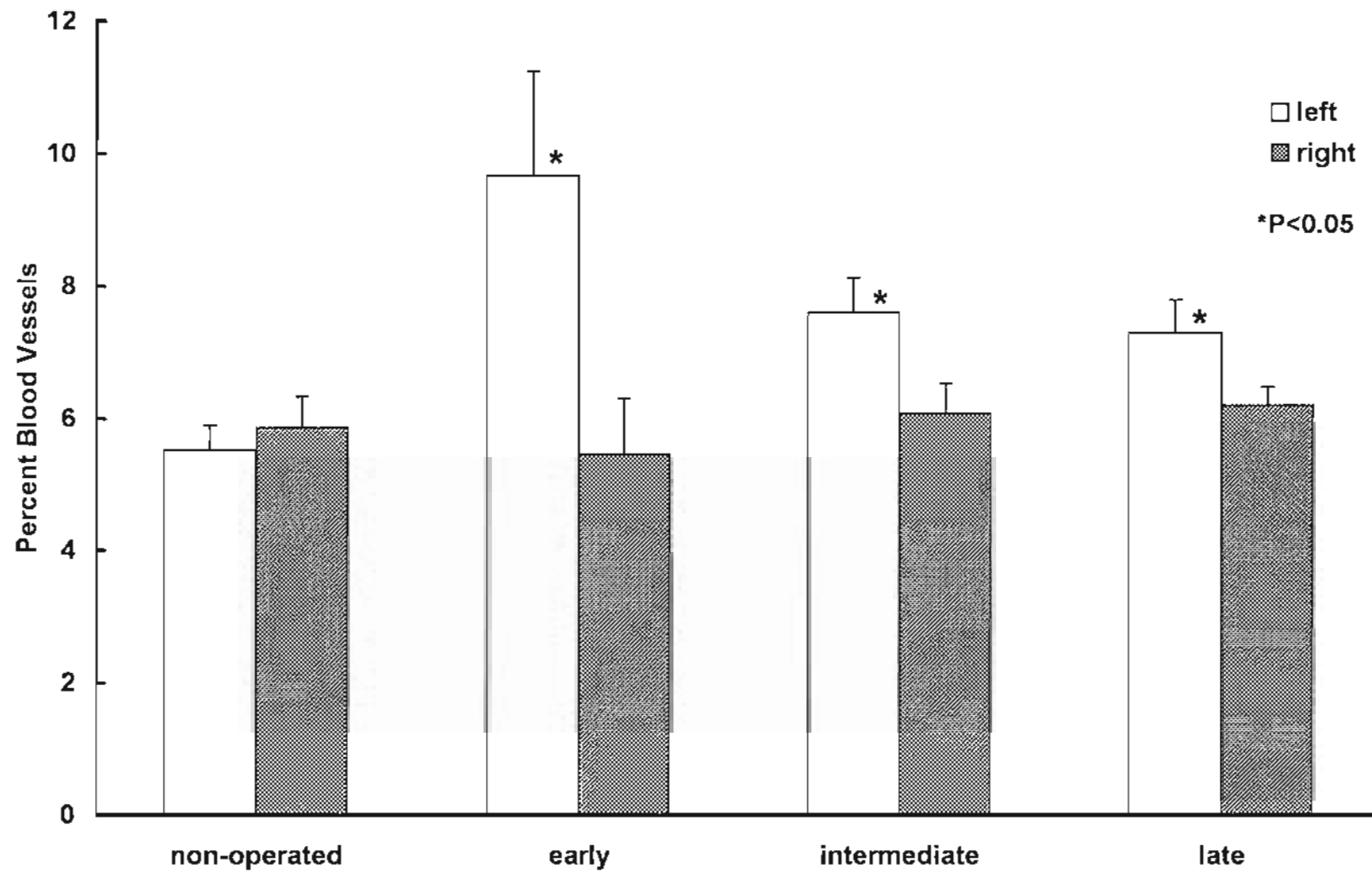
FIGURE 3.7

Blood vessel counts from the superior end plate on the left (operated) side of operated discs.
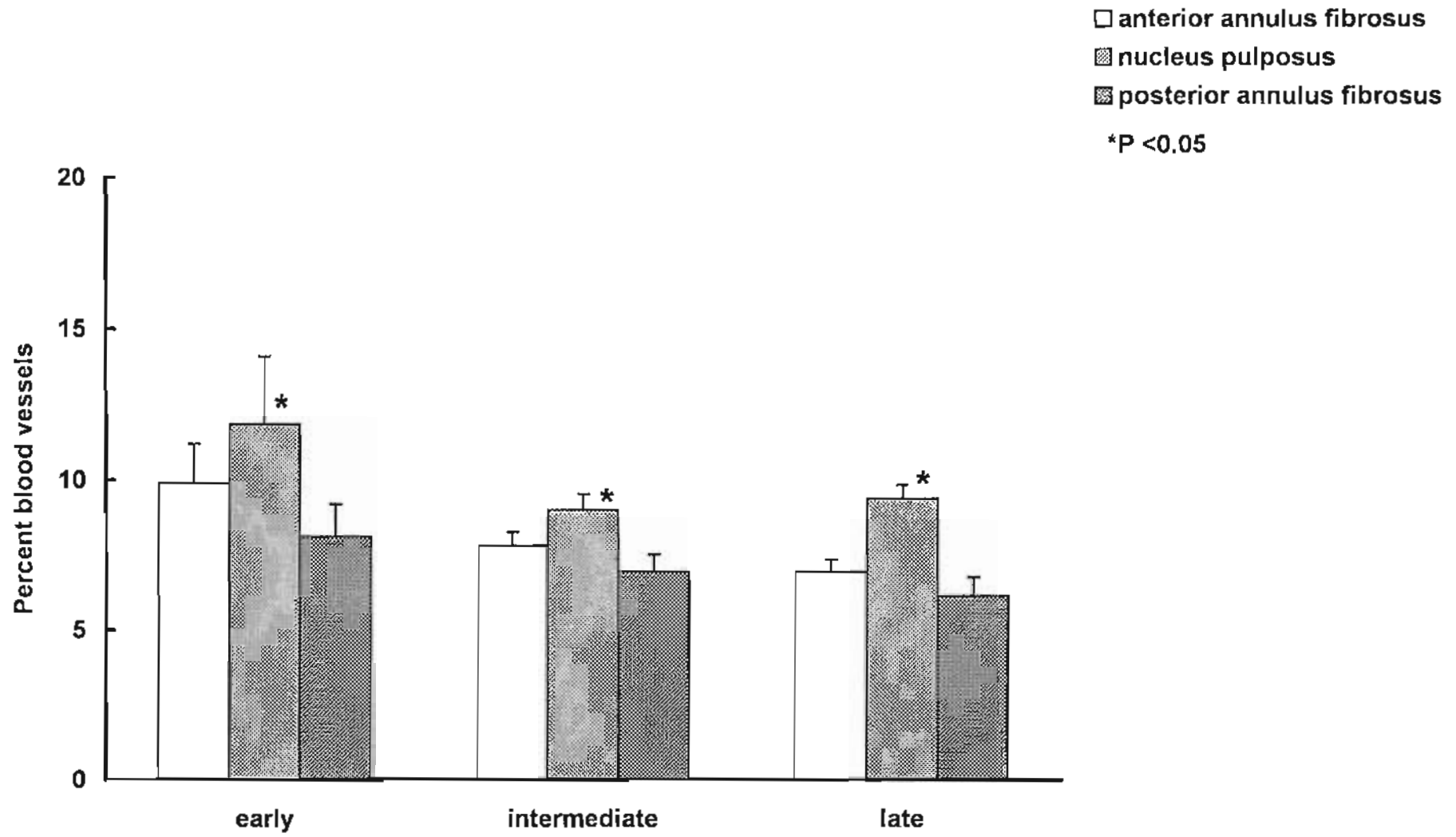
FIGURE 3.8

Blood vessel counts from the inferior end plate on the left (operated) side of operated discs.
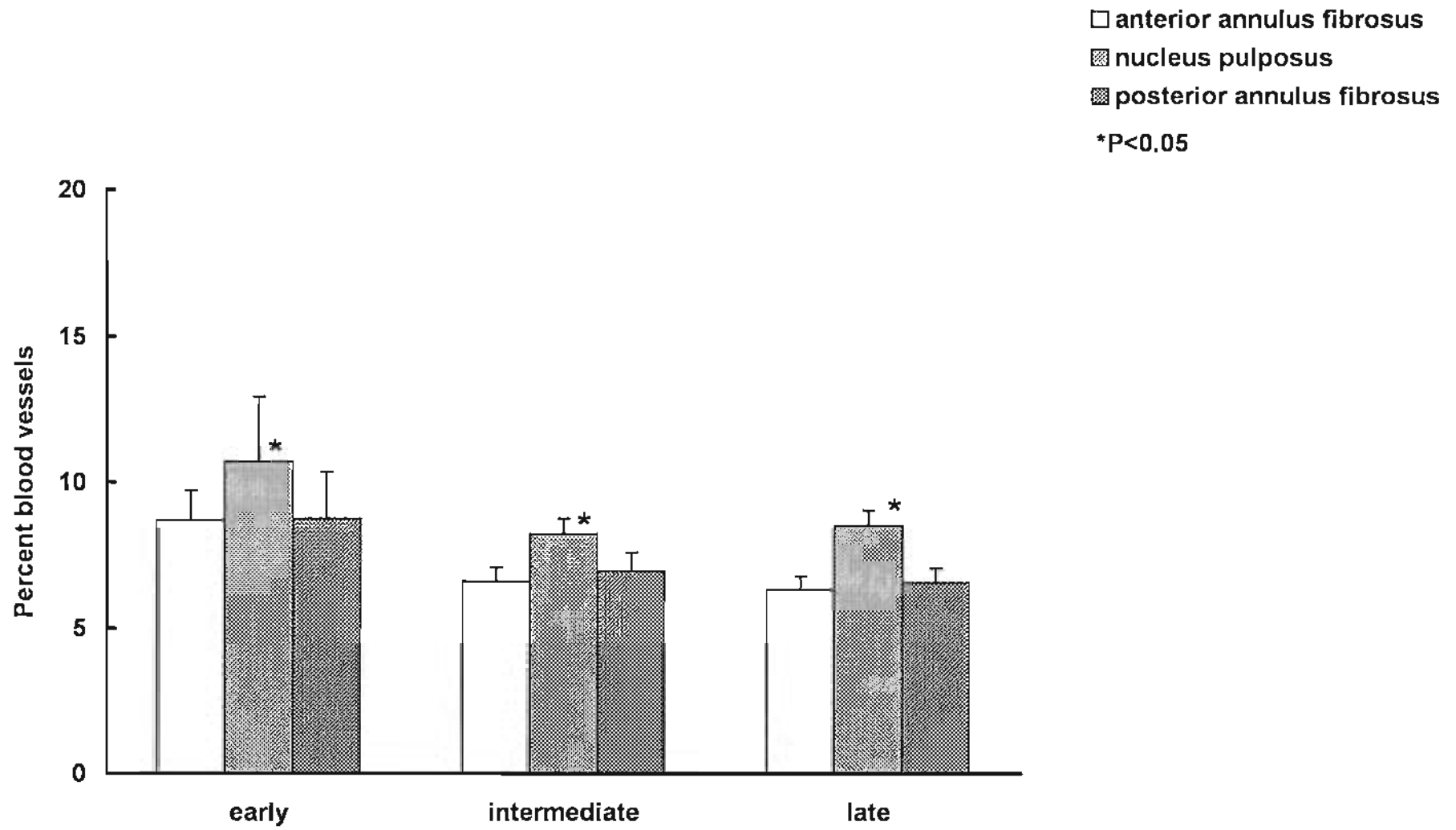
FIGURE 3.9

Blood vessel counts from the superior end plate on the right (non-operated) side of operated discs.
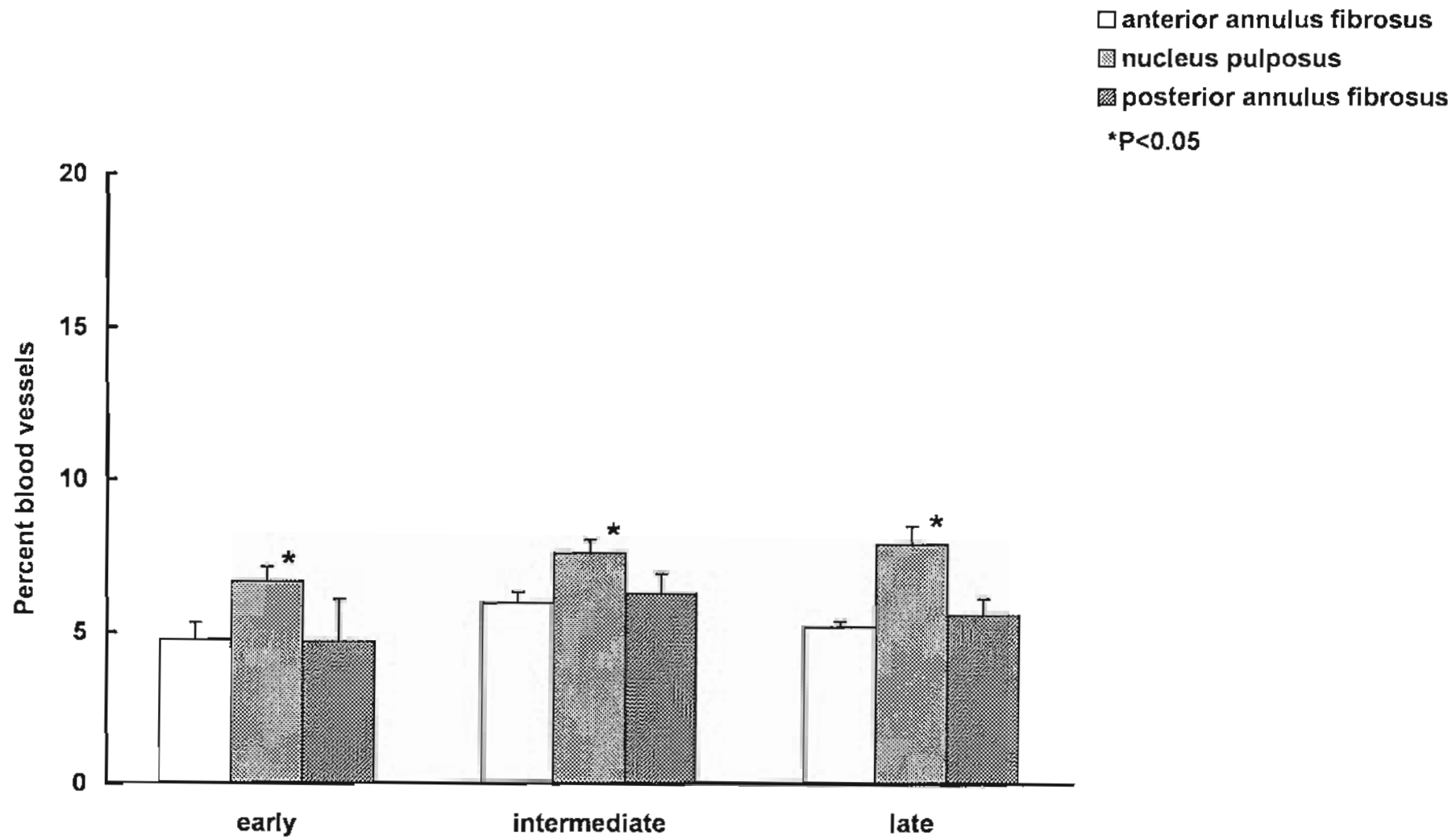
FIGURE 3.10

Blood vessel counts from the inferior end plate on the right (non-operated) side of operated discs.
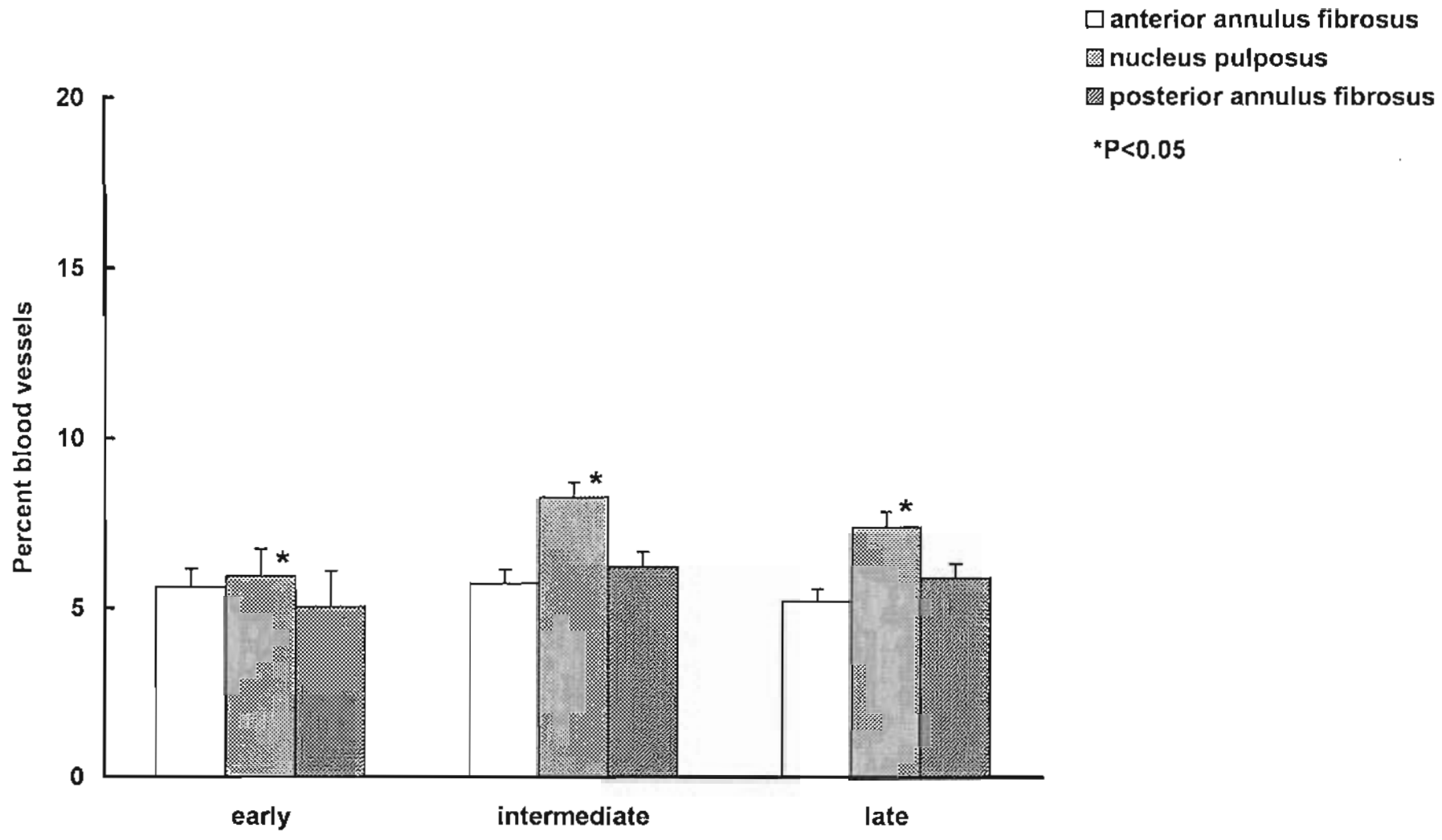
3.3.2 Vertebral histomorphometry

When compared with the measures from the non-operated animals (24.3 ± 1.3% [left] and 18.3 ± 1.4% [right]), trabecular bone volume in the vertebrae adjacent the operated discs was increased to 33.5 ± 2.3% (left/ipsilateral to the cut, P<0.0001) and 33.1 ± 1.2% (right/contralateral, P<0.0001) at two months. There was no significant change in these elevated values over the following two years (Figure 3.11). Trabecular bone surface measures were also significantly increased on both sides of the disc at each time interval. In the non-operated animals, the estimates of BS/TV were 3.2 ± 0.2 mm^2/mm^3 (left) and 2.9 ± 0.2 mm^2/mm^3 (right). In the operated group, this increased to 4.2 ± 0.2 mm^2/mm^3 (left, P<0.0001) and 4.1 ± 0.1 mm^2/mm^3 (right, P<0.0001) two months after the operation. This parameter also remained at this increased level for two years following operation (Figure 3.12).

There was significant thickening of the trabecular elements following the annular cut, but this was confined to the right side of the vertebrae only. The thickness increased from 125 ± 6 µm to 163 ± 4 µm (P<0.0001), and remained consistently higher for two years. Trabeculae from the left side of the non-operated animals were slightly thicker than on the right, but this difference was not statistically significant (Figure 3.13).

The spacing between individual trabecular elements in the vertebrae was reduced on both sides following the operation, decreasing from 0.55 ± 0.06 mm (left) and 0.64 ± 0.06 mm (right) in the non-operated animals to 0.34 ± 0.02 mm (left, P<0.002) and 0.34 ± 0.02 mm (right, P<0.0001) at two months. As with the other parameters, this change was still evident two years after surgery (Figure 3.14).

FIGURE 3.11

Trabecular bone volume (BV/TV) in the vertebrae adjacent lumbar discs of sheep up to two years following operation.
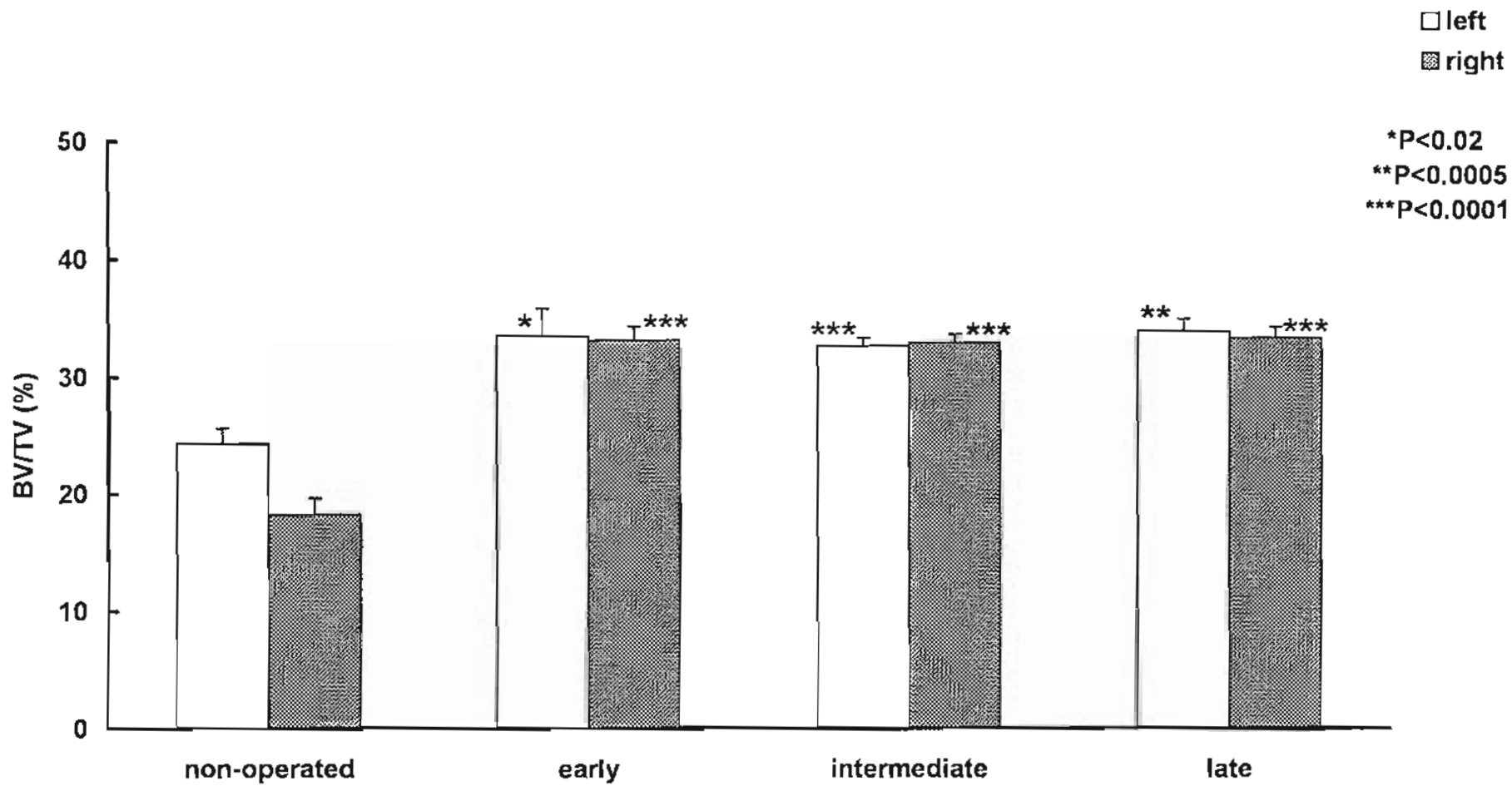
FIGURE 3.12

Trabecular bone surface (BS/TV) in the vertebrae adjacent lumbar discs of sheep up to two years following operation.
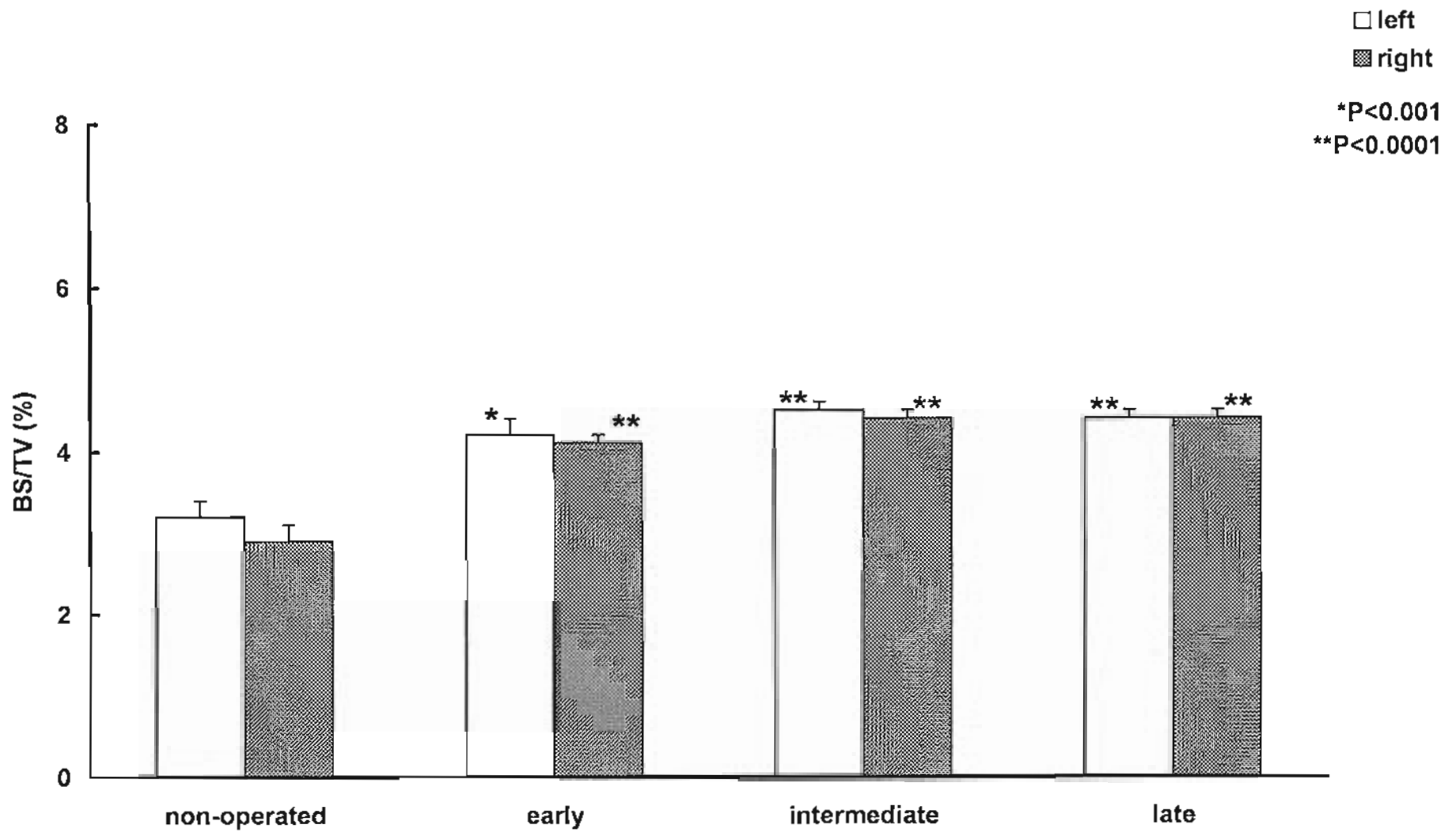
FIGURE 3.13

Thickness of vertebral trabecular bone elements (Tb.Th) adjacent lumbar discs of sheep up to two years following operation.
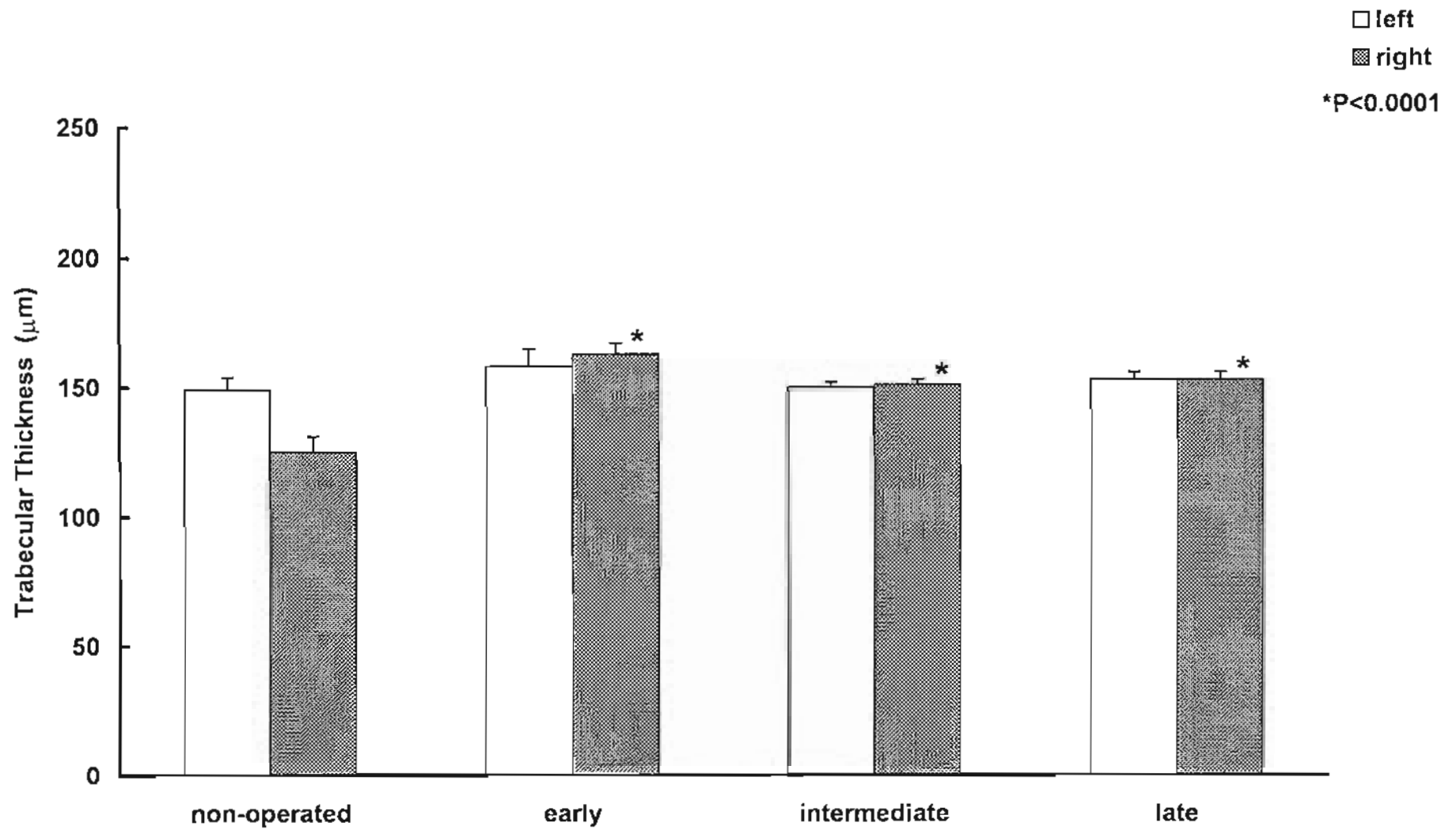
FIGURE 3.14

Spacing of vertebral trabecular bone elements (Tb.Sp) adjacent lumbar discs of sheep up to two years following operation.
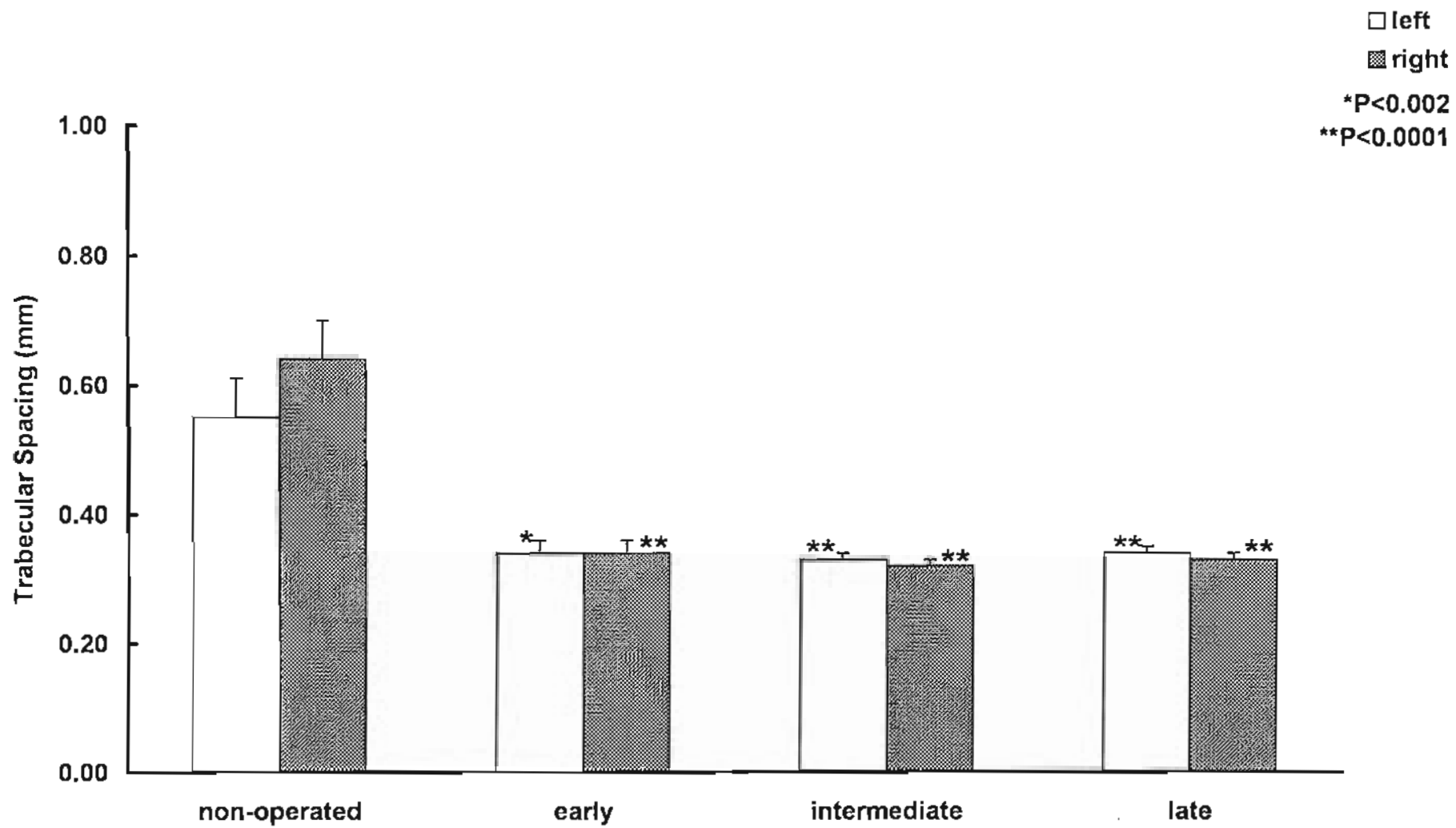
The surgical procedure also caused a rapid increase in the number of trabecular elements within the sampled region of the vertebral body, from 1.62 ± 0.08 /mm (left) and 1.44 ± 0.08 /mm (right) in the non-operated animals to 2.1 ± 0.09 /mm (left, P<0.001) and 2.05 ± 0.07 /mm (right, P<0.0001) after two months. Again, this increase was maintained for two years (Figure 3.15).

The repeated measures analyses revealed no significant effect in the early post-operative group due to end plate or to spinal level. Nor was there any significant interaction between the two factors. In the intermediate and late post-operative groups, there was a significant effect attributable to end plate differences in the estimation of BV/TV. The difference between the cranial and caudal end plates was 0.7% (P<0.05). In the late group, the separate end plate estimates of BV/TV differed by 2.2% (P<0.03). In the late group, too, TbSp estimates at the two end plates differed by 0.02 mm (P<0.04).

3.4 DISCUSSION

Using a variety of techniques, previous studies have shown that the end plates of the intervertebral discs play a vital role in the exchange of metabolites between the vascularized vertebral body and the largely avascular disc matrix (Nachemson et al., 1970; Crock and Yoshizawa, 1977; Holm et al., 1981; Ogata and Whiteside, 1981). The current study has utilized detailed histoquantitation methods which confirmed the findings of others (Maroudas et al., 1975; Roberts et al., 1989) that the central portion of the end plate is relatively better nourished than the peripheral region adjacent the annulus fibrosus, by virtue of being more highly vascularized. The observations in the sheep are consistent with those in humans (Roberts et al., 1989) that the superior and inferior end plates are most likely to be equally important for disc nutrition, since they possess almost identical numbers of vascular channels. These findings further suggest that the quadruped posture does not have a significant effect on the distribution of vessels in the end plate, and hence on

FIGURE 3.15

Number of vertebral trabecular bone elements (Tb.N) adjacent lumbar discs of sheep up to two years following operation.
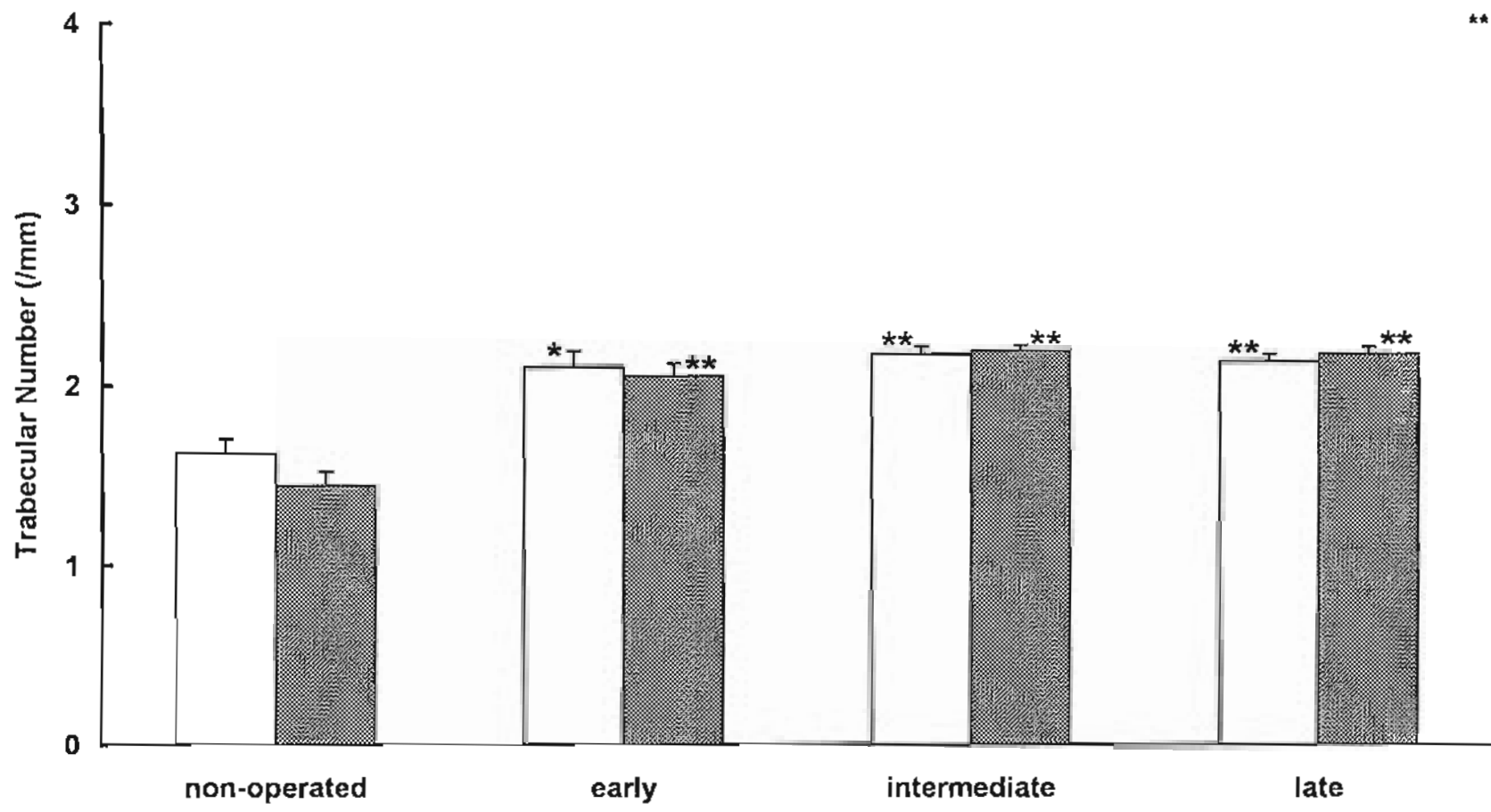
nutrition of the disc.

The degree of end plate vascularization in the sheep disc is similar to that reported for the human disc (Roberts et al., 1989). This observation, together with reported similarities in pathological changes in human and sheep discs (Butler, 1988; Osti et al., 1990), lends support to the choice of the sheep as an experimental model for the investigation of the pathogenesis of human intervertebral disc lesions.

No consistent histoquantitative differences were found in the proportion of blood vessels between the cranial or caudal end plate in any of the discs from the sheep with annulus cuts. This suggests that the pathological response to the annulus tear is generalized rather than simply confined to one or other of the end plates, despite the fact that the cut was created closer to the cranial end plate in all cases.

In the operated discs, there were substantially more end plate vessels on the left (operated) side than on the right (non-operated) side. Given that this asymmetry did not exist in the control discs, it is clear that the annulus incision was responsible for the ipsilateral change. The increase in vascularity was maintained over the two years of the study, although it diminished progressively after four months, to the extent that the difference between the ipsilateral and contralateral sides became less obvious, but statistically significant, nevertheless.

The early increase in the number of vascular channels in the operated discs, with their subsequent disappearance, is consistent with the time course for wound healing in other tissues (Cotran et al., 1989). Moreover, the blood vessel measurements correlate well with the other histological changes in these discs (Osti et al., 1990), in that the peripheral one-third of the annulus is the only portion of the annulus that possesses blood vessels in normal discs (Taylor and Twomey, 1988), and heals relatively quickly. By contrast, the normally non-vascularized inner annulus does not heal, despite a small number of new

blood vessels extending along the outer margin of the unhealed part of the incision. This absence of inner annular healing has been a hallmark of experimental studies investigating disc degeneration following outer annular disruption (Key and Ford, 1948; Smith and Walmsley, 1951; Osti et al., 1990) Furthermore, it is consistent with the observed lack of repair of spontaneously occurring annular tears in human spines (Vernon-Roberts, 1988). It may be postulated, therefore, that lack of healing seen in the inner part of the incision is due to a combination of insufficient vascularization and continued movement between the cut surfaces.

Although there were markedly different histological features in the discs with annular lesions compared with the non-operated control discs in the same sheep, the blood vessel measurements in these two sets of discs were not statistically different. Nevertheless, since end plate vascularity was increased in both operated and non-operated discs of operated animals, but not in non-operated sheep, it is evident that the presence of annular lesions had a significant influence on the blood supply to all discs in the lumbar spine. It is possible that the operation on selected discs induced biomechanical changes that affected all of the discs, whether operated or not, and contributed to the vascular changes in the end plates. An alternative possibility is that the surgical trauma caused the release of vaso-formative mediators which affected both the operated and control discs.

The bone within the vertebrae immediately adjacent the end plates showed significant remodelling in response to the annular cuts. The most obvious change to the local architecture was a generalized increase in the total amount of trabecular bone. This change was not confined to the operated side of the disc, but was also evident on the opposite side, some distance from the original surgical incision. The initial interpretation of this result was that the bone had become more dense in response to some alteration in its micro-environment. Hypertrophic changes such as these are common in osteoarthritis

affecting large joints (Collins, 1949; Hutton et al., 1985). However, in this case, the thickening of trabeculae was confined to the contralateral side of the vertebrae, while the trabeculae in the environment immediately adjacent the original cut were relatively unaffected. This observation is difficult to explain. It is most likely that the increase in bone volume, on both sides of the vertebrae was due to the creation of new trabecular elements during the course of a major remodelling phase in which the motion segments adapted to an altered structural and functional environment.

The appearance of concomitant changes above and below the operated discs is consistent with the symmetrical changes in vascularity observed earlier in the cranial and caudal end plates. Even though the original cut was made immediately adjacent the end plate of the cranial vertebra, it propagated through the inner annulus, often communicating directly with the end plate on the other side of the disc by way of nuclear fissures and cracks. This is similar in many regards to the type of pathology frequently encountered in older human autopsy spines (Osti et al., 1992). The pathological changes that occur at the same time within the disc matrix and the adjacent vertebrae indicate that these tissues are closely related to each other, and that apparently minor pathology in one component will almost invariably affect others.

In direct contrast to the eventual disappearance of end plate blood vessels, the vertebral boney changes did not revert to normal after two years. This may be due, in part, to the fact that the remodelling of bone occurs at a significantly slower rate than other tissues, and that major architectural alterations take longer to appear. Nevertheless, it remains somewhat paradoxical that while the end plate vessels disappear, the disc itself continues to degenerate. Perhaps the surgical manipulation has damaged the disc matrix to such an extent, that complete resolution is not possible. An alternative hypothesis is that the disc matrix is unable to regenerate after maturity, because there are few or no primitive stem cells within the population. This theory,

however, is contested in a recent study which showed that regeneration of disc matrix is possible following chemonucleolysis (Kiester et al., 1994).

CHAPTER FOUR

PLATE FIXATION OF AN OUTER ANNULAR TEAR

4.1 AIM

To determine if the healing response of a disc with an outer annular tear can be influenced by fixation with a rigid plating device.

4.2 INTRODUCTION

The studies described in Chapter 3 suggested that continued movement between the cut surfaces of a disc with an outer annular tear may have prevented the normal healing of the non-vascularized inner layers of the annulus. To test the hypothesis that healing of the inner annulus would take place if this movement could be restricted, a straight four-hole vitallium plate was selected as a fixation device for use in this model. The plate was considered suitable with respect to the size and anatomical contours of the sheep spine, and could be positioned directly over the disc to achieve fixation of two adjacent motion segments. This allowed the evaluation of its influence on healing of the annular lesion, and whether healing of the tear could prevent the disc degeneration that had been observed in non-plated motion segments with annular tears.

4.3 METHODS

4.3.1 Surgery

Fifteen two-year-old Merino wethers were used in this study. Under general anaesthesia, the lumbar spine of each animal was exposed using a left-sided retro-peritoneal approach. In the L2-L3 and L4-L5 discs, a cut measuring 4 mm deep and 10 mm wide was made in the left lateral annulus fibrosus, parallel and adjacent to the inferior end plate of the upper vertebra. The incision was made slightly more laterally than in earlier studies to reduce the amount of dissection required, and to enable the plate to be located directly over the annular incision. The disc (L3-L4) between the two operated discs remained

intact. A four-hole vitallium plate (48 mm long) was positioned directly over the annulus incision at the plated (P) level and secured with 25 mm long 8G screws into the adjacent vertebral bodies (Figures 4.1; 4.2). The screw holes were created with an air-powered drill prior to insertion. The other operated level (NP) was not plated. To avoid operator bias, the allocation of each animal to a follow-up group was not determined until the completion of surgery. Three of the animals were killed immediately following the procedure, to serve as base-line control subjects. The remaining twelve were held under observation in an indoor animal-care facility for one week, during which time they received regular intra-muscular doses of methadone (25 mg). The animals were then returned to a field station for the remaining six months.

4.3.2 Pathology

The animals were killed six months post-operatively and the lumbar spines were removed by the method described previously (Section 2.3.2). Discography was performed for a separate study and the spines were then prepared for histological analysis. In each disc the annulus was examined to assess the extent of healing of the outer annular cut and for propagation of the tear through the disc matrix. Nucleus degeneration was histologically graded as absent, mild, moderate or severe. The zygapophyseal joints were also sectioned and examined for evidence of degenerative changes, including cartilage fibrillation and fissuring, end plate changes and marginal osteophyte formation.

FIGURE 4.1

Macerated sheep spine demonstrating the position of the plating device across a lumbar disc and fixed into the adjacent vertebral bodies.
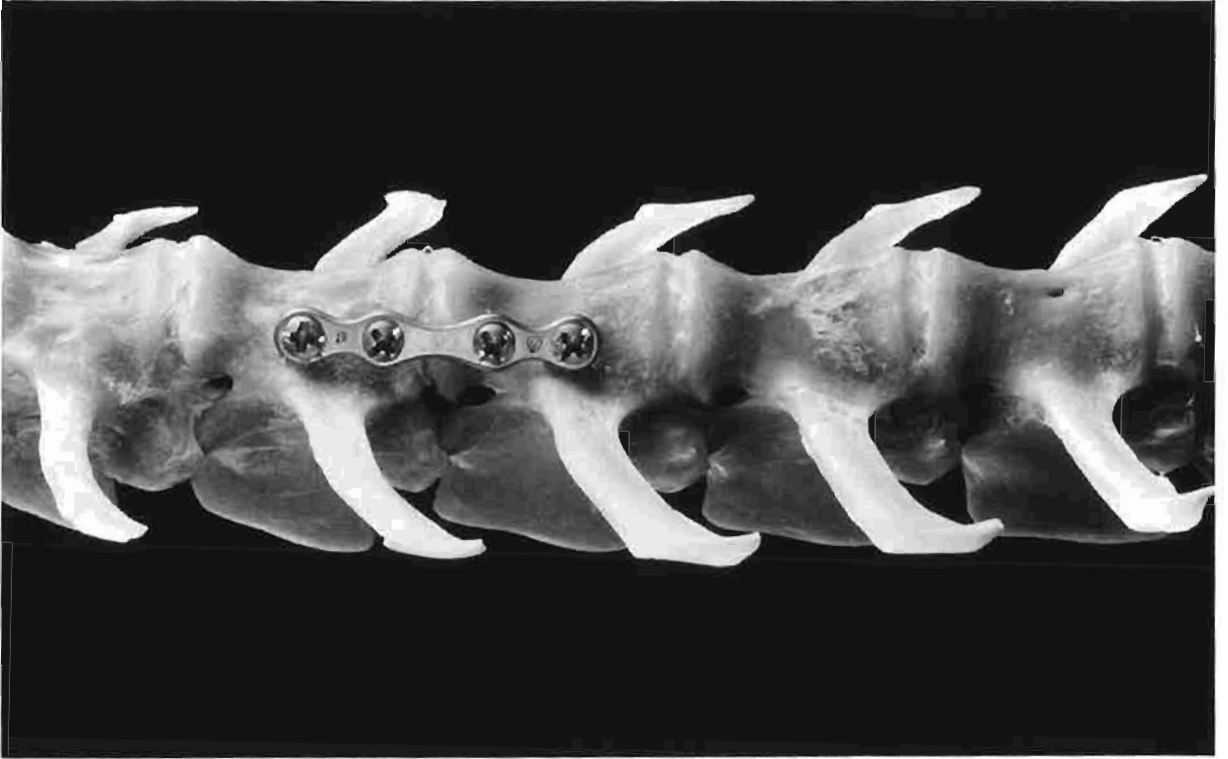
FIGURE 4.2

Plain X-ray film showing the position of the device *in situ*.
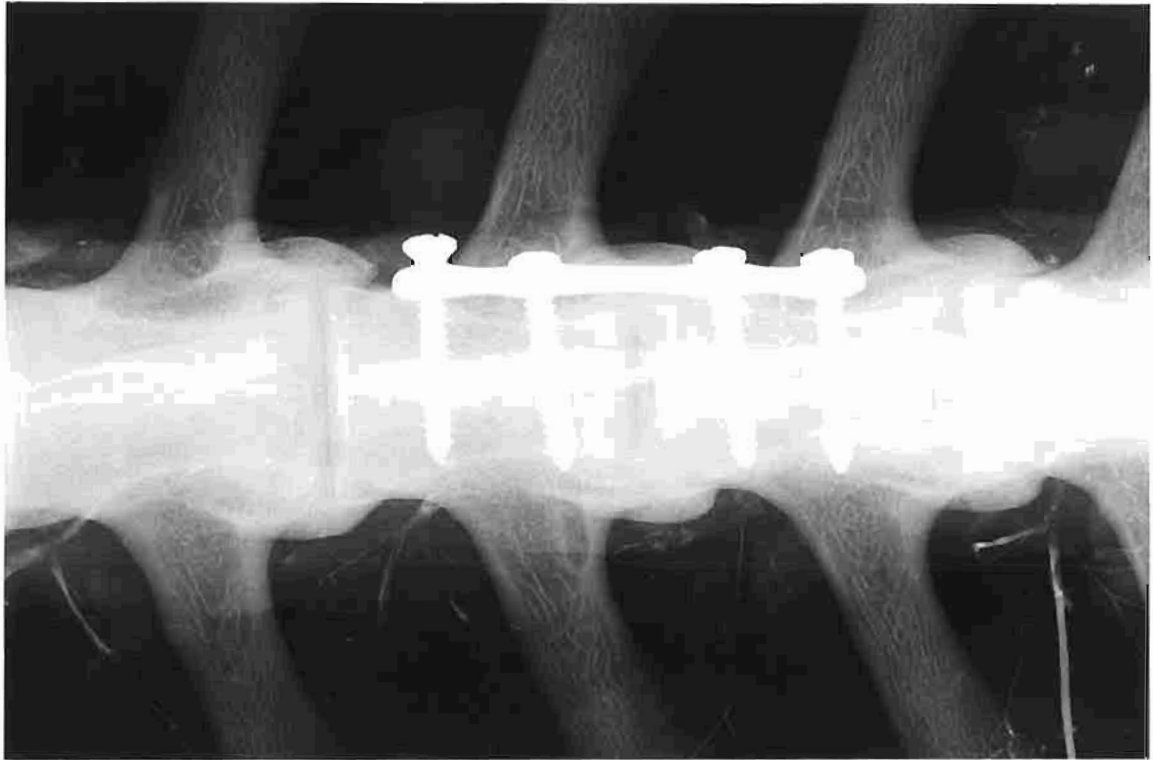
4.3.3 Quantitative histomorphometry

Estimates of blood vessel counts in the cartilage end plates were made as previously described (Section 2.3.3). Data from the cranial and caudal end plates were pooled, as the effect of the annulus cut was previously shown to be similar in both (Section 3.3.1). Data were analyzed with respect to side of the disc (operated/non-operated), fixation (plated/non-plated) and post-operative survival time (0/6 months).

4.3.4 Statistics

Student's t-test (PC-SAS, SAS Institute Inc.) was used to detect differences between the plated and non-plated levels at each time period, and also to determine if plating for six months had a significant effect on end plate vascularity. Statistical significance was set at $P<0.05$.

4.4 RESULTS

Apart from the outer annulus cut, the plated and non-plated discs from the three animals killed immediately after operation showed no significant macroscopic or microscopic abnormality. There were minimal degenerative changes, including the appearance of minor clefting and chondrone formation confined to the nucleus of only one sheep. (Table 4.1).

In the six months following operation, the remaining animals showed no physical impairment which could be attributed directly to the surgical procedure. Histology showed that the original cut had extended through the inner layers of the anterior annulus in eleven of the twelve non-plated discs and in ten of the twelve plated discs (Table 4.1). While the nucleus had migrated both internally and anteriorly, resulting in a slight degree of annular bulging in most operated discs, external herniation of nuclear material was not observed.

In all operated discs, regardless of plating, there was healing confined to the peripheral zone of the annulus tear. This was characterized by invasion of

81

vascular granulation tissue into the defect created by the cut, and scar tissue formation. However, healing of the remaining portion of the cut was not observed in any animal. All operated discs showed evidence of nuclear degeneration, the principal feature of which was the appearance of fissures and clefts. This was most prominent on the operated (left) side of the discs. Chondrones, formed by proliferating clusters of individual chondrocytes were also seen. Histologically, the plated discs were indistinguishable from the non-plated discs at six months.

The zygapophyseal joints of all sheep showed articular abnormality at six months, the most prominent feature being fibrillation and fissuring of the hyaline cartilage which frequently extended down to the osteochondral junction (Table 4.2). Osteophyte formation at the joint margins were also common findings. At six months, the plated levels showed fewer degenerative changes than the non-plated levels, although this difference was not statistically significant.

In the discs from animals killed immediately after operation, there was no significant difference between end plate vascularity on the operated side (6.1 ± 0.9% [NP] and 6.8 ± 0.4% [P]) and the non-operated side (5.5 ± 0.7% [NP] and 5.5 ± 0.5% [P] (Figure 4.3). In addition, there was no significant change in end plate vascularity on the non-operated side of the disc (6.3 ± 0.8% [NP] and 5.7 ± 2.8% [P]) after six months (Figure 4.4). On the operated side, however, the vascularity had increased after six months to 8.9 ± 1.1% [NP] and 9.9 ± 0.9% [P], (P<0.05 and P<0.01, respectively). There was no significant difference in vascularity between plated and non-plated segments either immediately after operation or after six months.

82

TABLE 4.1

Summary of pathological features observed in operated discs following surgery.

The data are presented as the number observed/total number in the group.

*nuclear material was lost from one disc during processing.

		0 Months Post-Operation		6 Months Post-Operation	
		Non-Plated	Plated	Non-Plated	Plated
Inner Annular Failure		0	0	11/12	10/12
Nuclear Degeneration	absent	3/3	2/3	0	0
	mild	0	1/3	6/11*	7/12
	moderate	0	0	4/11*	5/12
	severe	0	0	0	0

TABLE 4.2

Percentage of zygapophyseal joints which exhibited arthritic changes six months after surgery to the disc at that level. In general, changes were more severe at the non-plated levels, but this was not statistically significant.

	Cartilage fibrillation and fissuring	Fissures to osteochondral junction	Marginal osteophytes
PLATED	45%	0	64%
NON-PLATED	54%	21%	79%

FIGURE 4.3

Area of cartilage end plate occupied by vascular channels on the left (operated) side of discs in young adult sheep. There was a significant increase in vascularity on this side of the disc after six months. There was no significant difference in vascularity between plated and non-plated segments either immediately after operation or after six months.
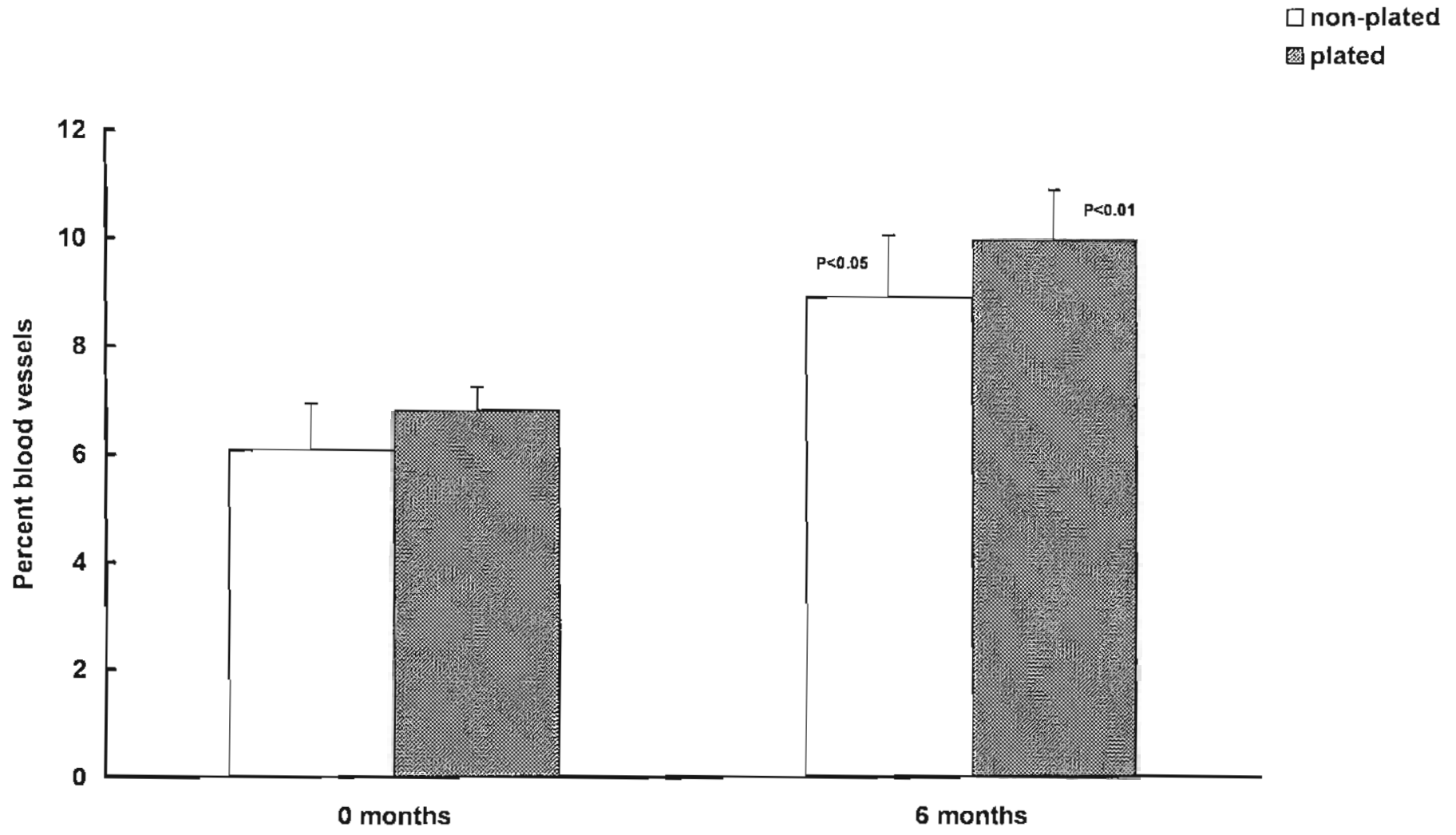
FIGURE 4.4

Area of cartilage end plate occupied by vascular channels on the right (non-operated) side of discs in young adult sheep. There was no significant change in vascularity after six months on this side of the disc. There was no significant difference in vascularity between plated and non-plated segments either immediately after operation or after six months.
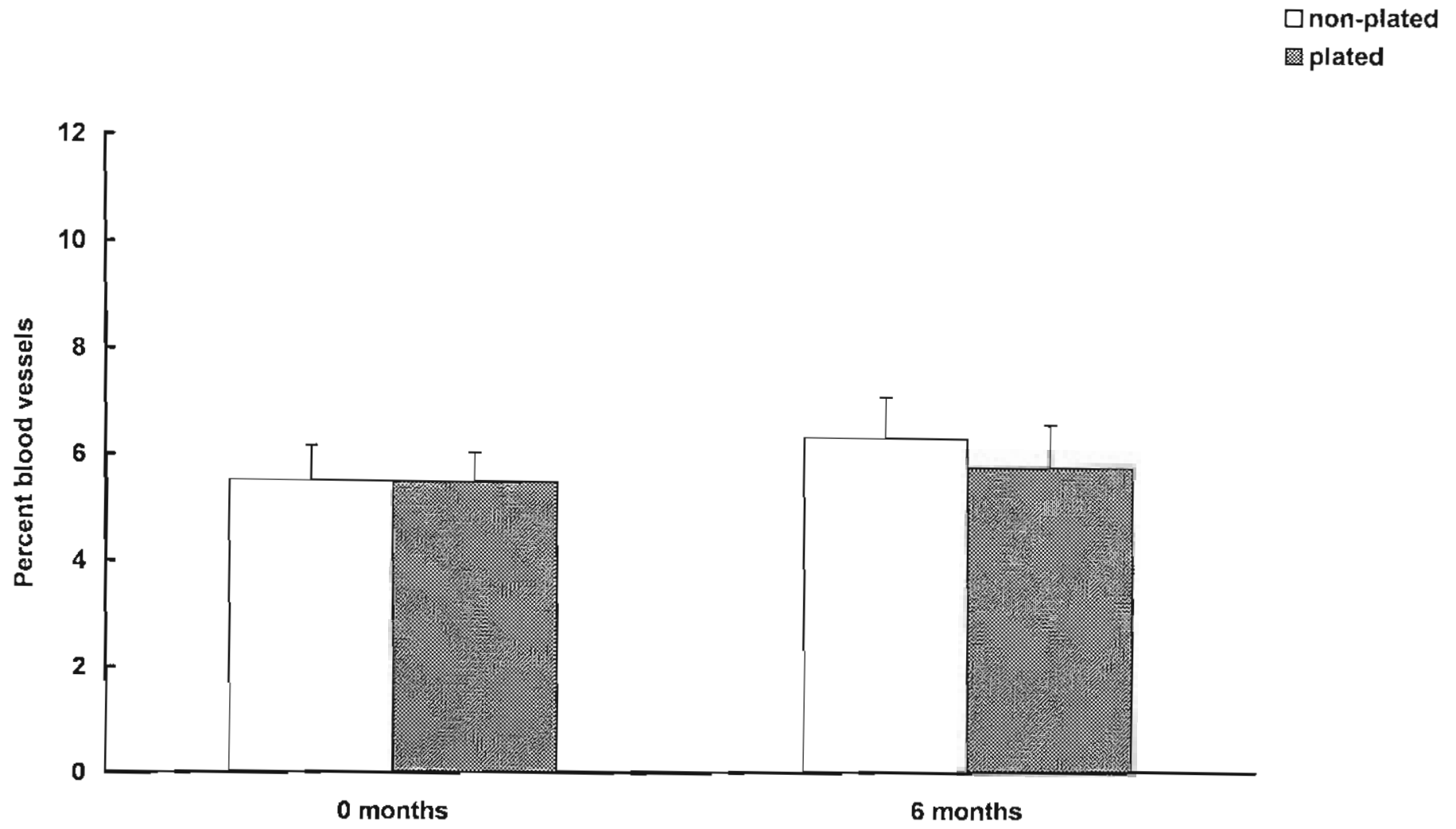
4.5 DISCUSSION

Previous experimental work using the Merino sheep as a model has demonstrated that a shallow cut made in the outer annulus results in rapid and progressive degeneration of the disc (Osti et al., 1990). While the normally vascularized part of the cut contained within the outer one-third of the annulus underwent healing, the inner portion of the cut did not heal. The inner two-thirds of the annulus, which normally is not vascularized, failed after approximately six months, with the result that nuclear material migrated towards the site of the original cut.

As shown in Chapter 3, the progressive degeneration of the disc is accompanied by florid vascular proliferation within the end plate cartilage in the vicinity of the cut. This neovascularization is considered to be an unsuccessful attempt to repair the tissue damage which resulted from the cut, and that movement between the cut surfaces of the annulus prevented healing taking place. For this reason, it was appropriate to ascertain whether restriction of movement between the margins of the cut would allow the inner annulus cut to repair, and prevent the subsequent degeneration of the disc. However, there were no significant morphologic differences between plated and non-plated discs six months after operation. In fact, most of the discs demonstrated inner annular failure, and there was advanced degeneration of the nucleus, similar to that observed in the earlier study in which plating was not undertaken. It was apparent that this plating system had neither allowed healing, nor had it prevented the original superficial cut from progressing to involve the inner annular fibres. While there was some anterior annular bulging in most cases, nuclear herniation was not observed. The outer annular repair, with limited ingrowth of vascularized tissue, was not different between plated and non-plated motion segments, indicating that it developed independently of any stabilizing influence of the plate.

87

It is acknowledged that the vascular channels within the cartilage end plate, particularly in the central region adjacent to the nucleus, are vital for nutrition of the disc matrix (Nachemson et al., 1970; Maroudas et al., 1975; Urban et al., 1978; Holm et al., 1981). While the increased vascularity of this region following outer annular injury may indicate an attempt to repair the damaged disc, previous studies indicated that this response was only temporary, since the initial vascular proliferation returned almost to normal pre-operated levels within 24 months of initial injury (Chapter 3). Meanwhile, the discs became progressively more degenerate. The results of the present experiment are entirely consistent with the original study, despite the attempt to stabilize the incised annulus.

Clearly, the increased vascularity seen in the experimental model could be attributed to fixation of the plate into the vertebrae. However, since there was an increase in the vascularity after six months, regardless of plating, it may be concluded that it resulted solely from the original cut and not from the fixation. Nevertheless, this issue could be tested in future studies by placing screws alone in vertebrae adjoining non-operated discs. Furthermore, there can be no doubt that the plates were firmly fixed within the vertebral bone. There was no plate failure and there was no evidence that any of the components had become loose. In fact, when the plates were removed at the completion of the study, most of the screws were found to be embedded in solid callus and considerable effort was required to remove them.

Biomechanical testing conducted in a parallel study showed that the annular cut alone caused an immediate measurable loss of stiffness in flexion and extension in the operated motion segment (Latham et al., 1994). At six months, this loss of stiffness was not observed in plated discs, indicating a positive response to fixation. In torsion, however, the plated levels were not significantly stiffer than non-plated controls, either immediately following

operation or after six months. It is clear therefore, that the plating system used did not prevent all movement within the operated discs.

One of the fundamental principles of primary union in wound healing is that the cut surfaces must be maintained in close proximity and relatively immobile to enable sprouting blood vessels to bridge the wound gap. Having consistently observed outer annular healing in both plated and non-plated motion segments, it would appear that either motion in the outer annulus is less than in the inner annulus, a concept difficult to sustain, or that other factors are involved in the non-repair of annular tears observed in both the sheep model and the human spine. For example, it has been known for some time that there are factors in hyaline cartilage which prevent its vascularization (Eisenstein et al., 1973; 1975), and it would not be surprising to find similar factors operating within the tissue of the intervertebral disc. However, given the unique structure of the annulus, it may be the case that movement within the inner annulus, not prevented by the plates used in the present study, is an important factor in preventing healing. Support for this conclusion comes from the fact that deep clefts in human discs often show rich vascularity in the cleft margins, but evidence of healing by scar tissue formation is lacking (Vernon-Roberts, 1988).

The outcome of this study suggests that attempts to completely inhibit motion within sheep discs having annulus cuts by using a more effective plating system could resolve the issue of whether lack of healing is intrinsic to the disc or the result of micro-motion.

The changes in the zygapophyseal joints of the discs are of interest, as the joints from the plated levels showed relatively less degeneration than those from the non-plated levels. This suggests that the plates provided at least some protection against zygapophyseal joint changes while not protecting the disc from degeneration.

The starting hypothesis that restricting movement of the motion segment would allow healing of an annulus tear, and thereby prevent disc degeneration,

has not been substantiated by this study. Importantly, the significant increase in end plate vascularization in the region of the tear did not affect the outcome in respect of annulus healing, annulus failure or nuclear degeneration. While it seems worthwhile to now proceed to devise instrumentation which promotes torsional stiffness, as well as maintaining stiffness in flexion and extension, to allow the hypothesis to be tested, it seems likely that healing of tears in the annulus may critically involve other intrinsic factors apart from movement.

CHAPTER FIVE

DEVELOPMENT OF MORPHOLOGICAL CHANGES IN
LUMBAR INTERVERTEBRAL DISCS

5.1 AIM

To describe the development of histological changes within the lumbar discs of the human spine.

5.2 INTRODUCTION

There is a wide array of changes encountered in the pathological examination of the intervertebral disc. Degeneration affects all components of the disc to some extent, and it is very likely that changes to the annulus, for example, will affect the morphological appearance of the nucleus. Despite considerable investigation in the past, it still remains unclear, however, what constitutes the initial lesion or lesions in the degenerative process. To determine the aetiology of these morphological changes and to understand their significance, it is necessary to describe the microscopic appearance of the disc in the normal population.

This study describes the development of lumbar disc pathology in a wide cross section of the populations from metropolitan Adelaide and the Whitechapel area of London. The cases were selected from an autopsy pool, ranging in age from 19 to 78 years. In describing the many changes to the disc, particular attention has been given to those features that may participate in the extrusion of sequestrated fragments from within the disc, since this is regarded as a major cause of low back pain in the general population, with significant economic and social consequences.

5.3 METHODS

Lumbar spines were removed at autopsy from 96 subjects and fixed in 10% buffered formalin for at least one week. Individual motion segments from each spinal level were prepared by transecting the vertebrae at the midline in the axial plane with a bandsaw, taking care to preserve the posterior elements.

The blocks were immersed in a solution comprising 10% (v/v) nitric acid and 1% (w/v) EDTA until complete decalcification was confirmed by radiography. The posterior elements were retained for separate histological analysis. The disc units were then cut into multiple parasagittal slices approximately 5 mm thick and processed into paraffin wax by standard methods. Tissue sections, 5 μm thick, were stained by haematoxylin and eosin for histological examination.

The sections were examined for a range of histopathological features, including rim lesions; radiating tears of the annular fibrosus; migration of the nucleus within the disc; formation of nuclear clefts and fissures; fragmentation of the nucleus; and, vascularization of the peripheral layers of the annulus. These features were noted as either present or absent, without recording the "severity" of individual features. The incidence of features was expressed as a percentage of the total number of discs in each group. Data for males and females were pooled for this study, and were divided into three groups based on age ranges (Group A, under 30 years; Group B, between 30 and 60 years; Group C, over 60 years) on the basis that pathological change in the disc may begin within a wide age range and proceed at varying rates, and do not have an age-related basis for the population considered as a whole.

The presence of rim lesions was confirmed by polarized light microscopy Sections with vertebral rim lesions of the annulus were selected for histoquantitation of end plate vascularization in the same way as for the sheep model (Section 3.2.2). Age and sex-matched controls from the study population, in which no rim lesions were identified, were used for direct comparison.

5.4 RESULTS

There were 39 females and 57 males with average ages of 47 and 49 years respectively. The anatomical distribution of the 383 lumbar discs is shown in Table 5.1.

Rim lesions of the anterior and posterior annular attachments to the vertebrae were found in discs from an early age at all lumbar levels (Figure 5.1; 5.2; Table 5.2). In Group A they were found with equal frequency in the anterior and the posterior disc. From the third decade there was a higher incidence of rim lesions which were slightly more common anteriorly than posteriorly. There was no apparent consistent pattern in the distribution of rim lesions at any level of the lumbar spine in any age group.

As multiple parasagittal slices were available from all discs in the study, it was possible to note the distribution of rim lesions within each disc. In those discs in which they were present, rim lesions were observed in all adjacent slices.

The extent of cartilage end plate vascularization in the discs with rim lesions is summarized in Figure 5.3. Since the total number of cases in each group was relatively small, it was not possible to apply meaningful statistical tests, and the data are presented to illustrate trends. In Group A, 7.8% of the total end plate area in the discs with rim lesions was vascularized, compared with almost 5.7% in the controls. In Group B, there was a modest increase in the extent of end plate vascularization in association with rim lesions (9.3%) and a similar increase in the age-matched control group (7.9%). The end plates of the discs in Group C were substantially more vascularized (13.2% with rim lesions; 10.3% without rim lesions).

94

TABLE 5.1

The number of discs examined from each spinal level of the cadaveric spines.

	GROUP A	GROUP B	GROUP C
L1-L2	20	25	20
L2-L3	22	25	20
L3-L4	24	24	21
L4-L5	23	40	30
L5-S1	24	37	28

FIGURE 5.1

Incidence of anterior annular rim lesions in autopsy subjects. Values are expressed as a percentage of all discs examined.

FIGURE 5.2

Incidence of posterior annular rim lesions in autopsy subjects. Values are expressed as a percentage of all discs examined.
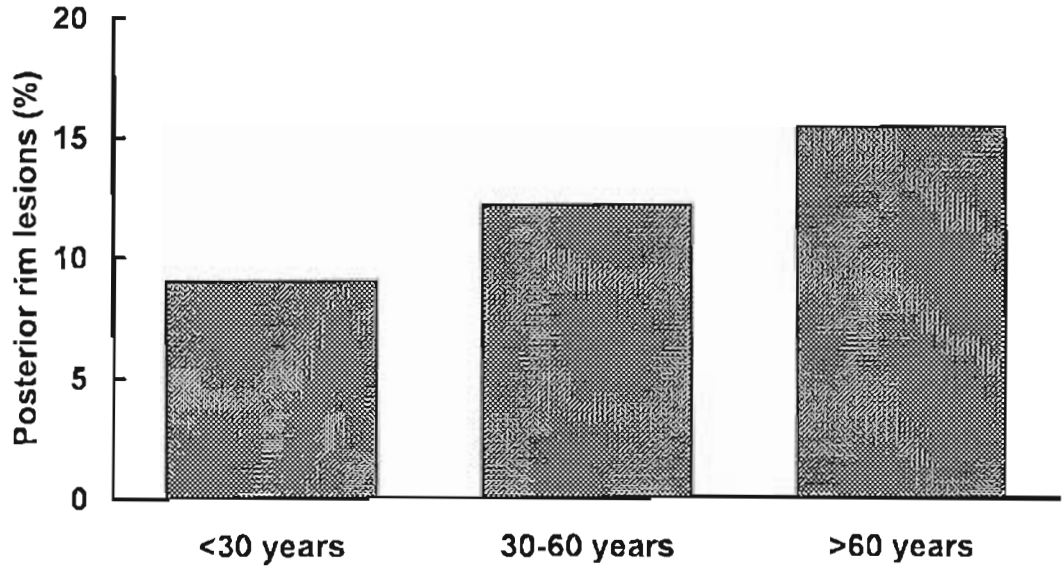
TABLE 5.2

Frequency of pathological features observed in the cadaveric spines examined. The data are grouped according to age and spinal level, and expressed as percentages.

GROUP A (under 30 years)

	Annulus Fibrosus					Anterior Migration			Posterior Migration			Nuclear
	ARL	PRL	ART	PRT	vascularization	subligamentous	transligamentous	sequestrated	subligamentous	transligamentous	sequestrated	clefts
L1-L2	10.0	5.5	—	—	—	10.1	—	5.6	5.6	—	—	50.0
L2-L3	9.5	3.9	3.9	—	—	15.0	—	—	13.3	7.5	—	30.4
L3-L4	9.0	17.7	3.4	3.4	—	—	—	—	12.2	6.5	3.4	15.6
L4-L5	12.0	8.9	5.0	—	—	—	—	—	5.0	5.0	5.0	45.8
L5-S1	3.5	8.9	—	—	—	—	—	—	14.5	9.0	8.9	24.5

GROUP B (between 30 and 60 years)

	Annulus Fibrosus					Anterior Migration			Posterior Migration			Nuclear
	ARL	PRL	ART	PRT	vascularization	subligamentous	transligamentous	sequestrated	subligamentous	transligamentous	sequestrated	clefts
L1-L2	22.0	12.7	4.5	3.6	4.6	7.1	—	—	3.6	8.1	—	32.5
L2-L3	12.0	8.1	10.7	3.6	7.2	—	7.0	—	15.3	15.2	—	26.0
L3-L4	22.0	20.0	4.0	3.9	13.0	4.5	3.9	—	20.0	20.0	—	34.3
L4-L5	22.0	6.3	6.3	15.7	10.4	10.4	8.4	—	16.7	16.7	5.2	35.4
L5-S1	8.0	13.7	7.2	15.0	7.9	5.7	2.2	—	24.6	21.0	5.7	30.3

GROUP C (over 60 years)

	Annulus Fibrosus					Anterior Migration			Posterior Migration			Nuclear
	ARL	PRL	ART	PRT	vascularization	subligamentous	transligamentous	sequestrated	subligamentous	transligamentous	sequestrated	clefts
L1-L2	23.0	26.8	4.5	—	13.7	5.6	—	4.6	4.6	4.6	13.7	45.0
L2-L3	15.7	14.6	21.2	20.7	19.2	15.7	11.1	4.6	20.2	20.2	—	48.5
L3-L4	26.4	13.9	19.5	15.3	9.7	25.0	9.7	—	27.8	27.8	4.4	12.5
L4-L5	29.0	9.8	9.8	24.2	13.6	16.5	5.9	—	27.2	27.2	9.8	36.0
L5-S1	4.6	12.1	7.5	15.0	27.0	—	7.5	3.0	20.9	20.9	26.8	26.8

ARL = anterior rim lesions; PRL = posterior rim lesions; ART = anterior radiating tears; PRT = posterior radiating tears

FIGURE 5.3

Extent of cartilage end plate vascularization in autopsy discs with and without annular rim lesions.
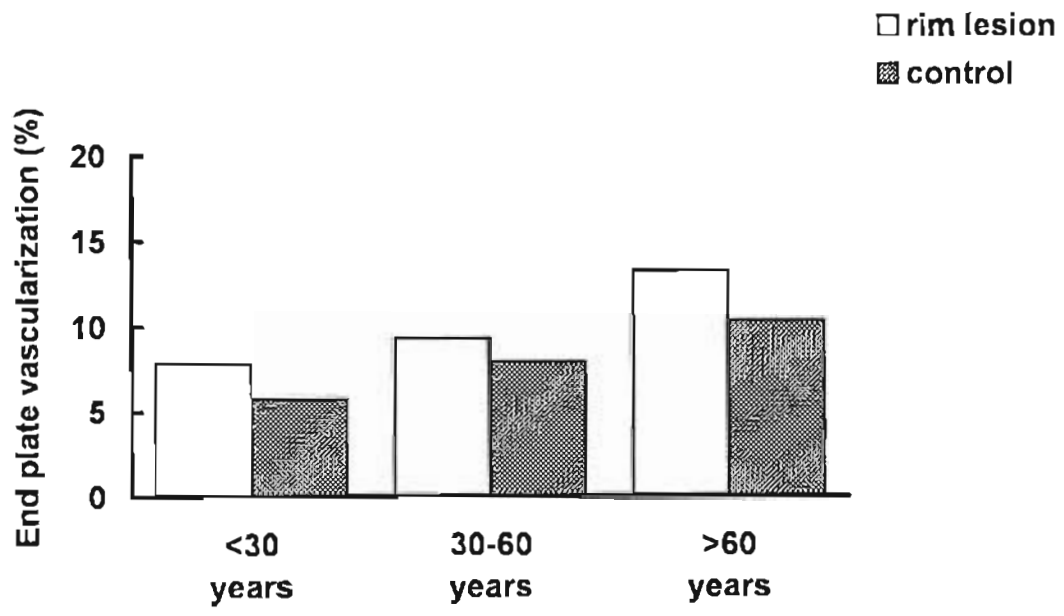
The migration of nuclear matrix either anteriorly or posteriorly within the disc was observed to occur in three distinct stages. The first of these was termed a "subligamentous nuclear bulge" and was characterized by forward or backward displacement of part of the nucleus. Frequently, the inner annular fibres on the opposite side showed reversal of their usual orientation (Figure 5.4). Posterior bulging was evident in the discs from Group A and increased steadily in frequency with age (Table 5.2; Figures 5.5). Posterior disc bulges were uncommon at L1-L2 and were relatively evenly distributed between the lower lumbar levels. Anterior disc bulges were rare in the Group A and showed an increasing incidence with age, although they remained only half as common as posterior bulges (Figure 5.6).

Radiating tears of the annulus were rare in young discs, but were more common in discs at a later age, particularly at L3-L4 and below, and were associated with contained nuclear protrusions or extruded prolapses (Figures 5.7; 5.8). The tears appeared to originate in the nucleus, mid-way between the two end plates, and extended through the annulus, often deviating towards either the superior or inferior rim, with no apparent preference for one or the other (Figure 5.9). In the few discs with radiating tears in which no bulge or protrusion was seen, the nuclei showed evidence of clefting, in some cases, with formation of isolated fragments.

FIGURE 5.4

Reversal of normal inner anterior annulus fibre orientation associated with a posterior subligamentous bulge in this L5-S1 disc from a 58 year old male subject (H&E, x 6).
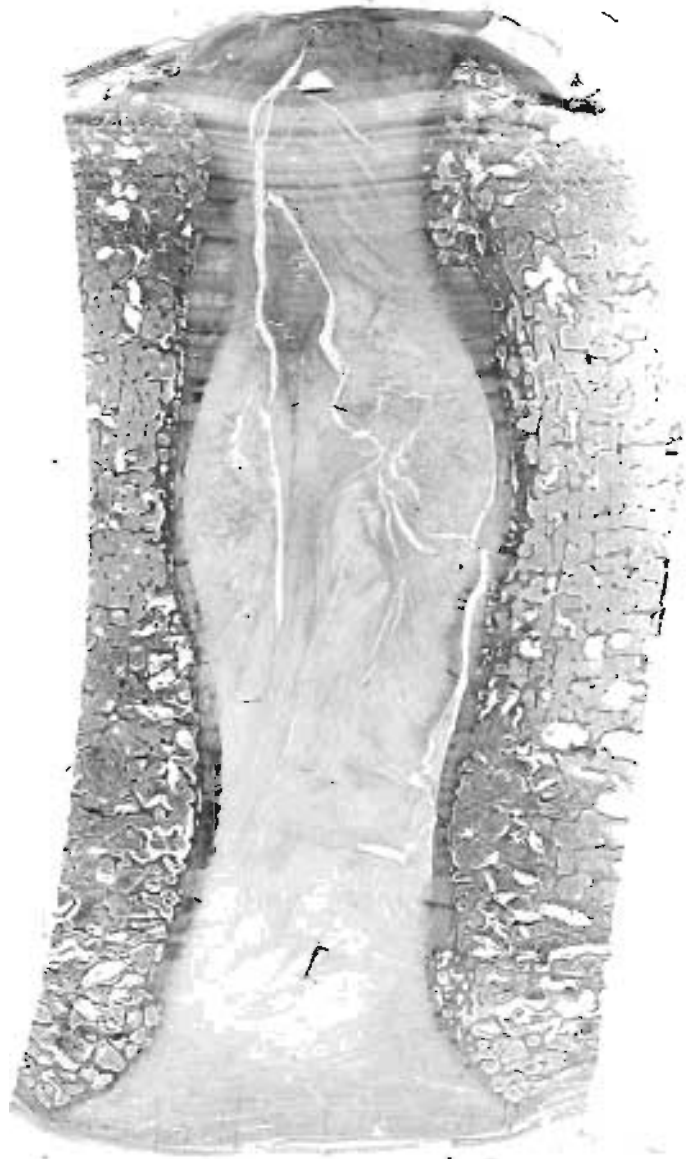
FIGURE 5.5

Incidence of posterior subligamentous annular bulges in autopsy subjects. Values are expressed as a percentage of all discs examined.
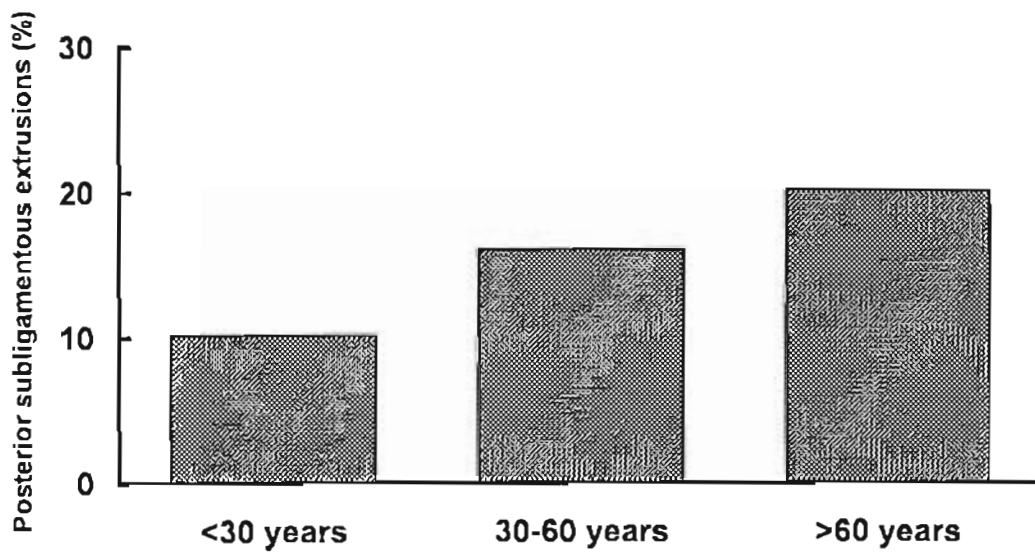
FIGURE 5.6

Incidence of anterior subligamentous annular bulges in autopsy subjects. Values are expressed as a percentage of all discs examined.
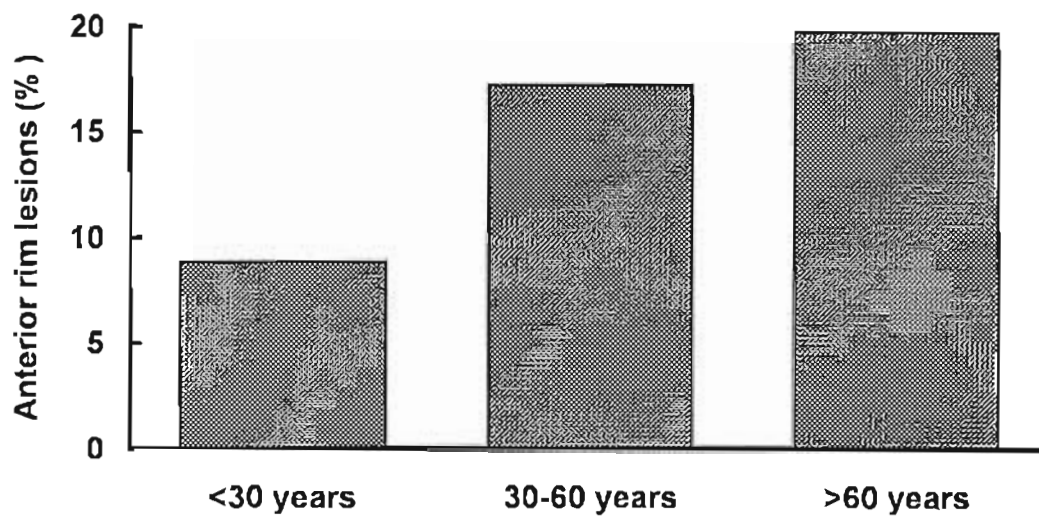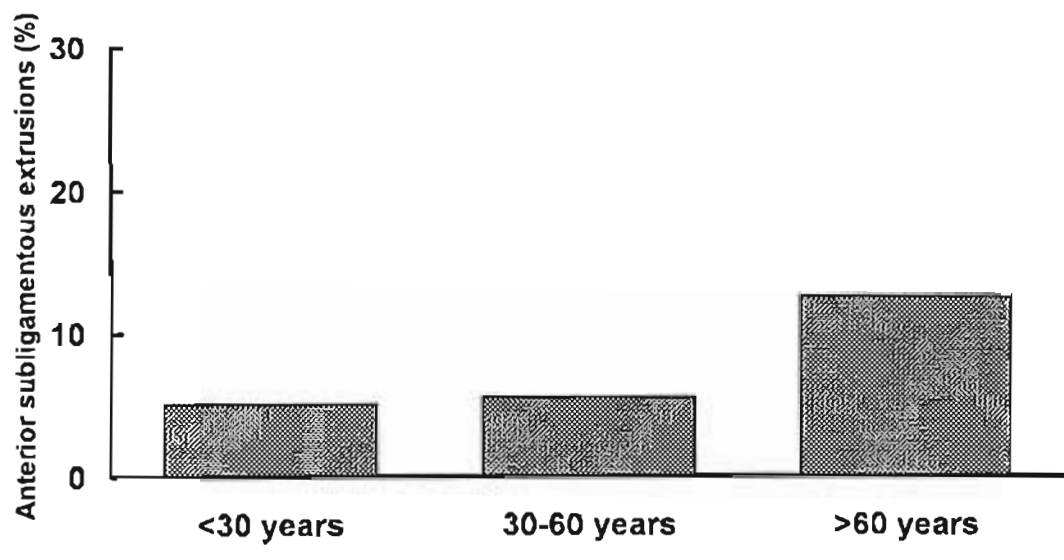
FIGURE 5.7

Incidence of posterior radiating annular tears in autopsy subjects. Values are expressed as a percentage of all discs examined.
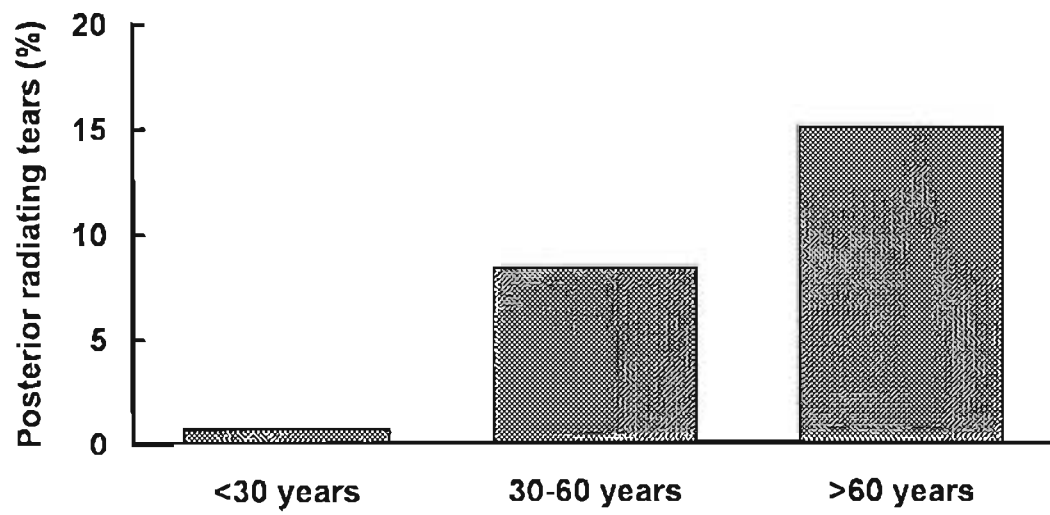
FIGURE 5.8

Incidence of anterior radiating annular tears in autopsy subjects. Values are
expressed as a percentage of all discs examined.
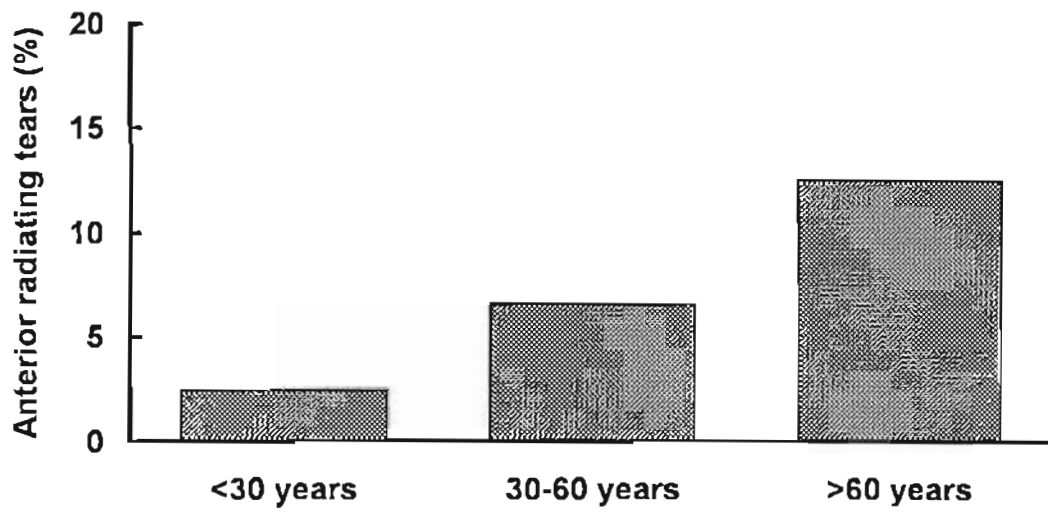
FIGURE 5.9

Low power sagittal view of multiple radiating tears of the posterior annulus fibrosus coursing towards the superior vertebral rim region in this L4-L5 disc from a 27 years old male subject. There is also evidence of anterior annulus infolding associated with posterior migration of the nucleus (H&E, x 6).
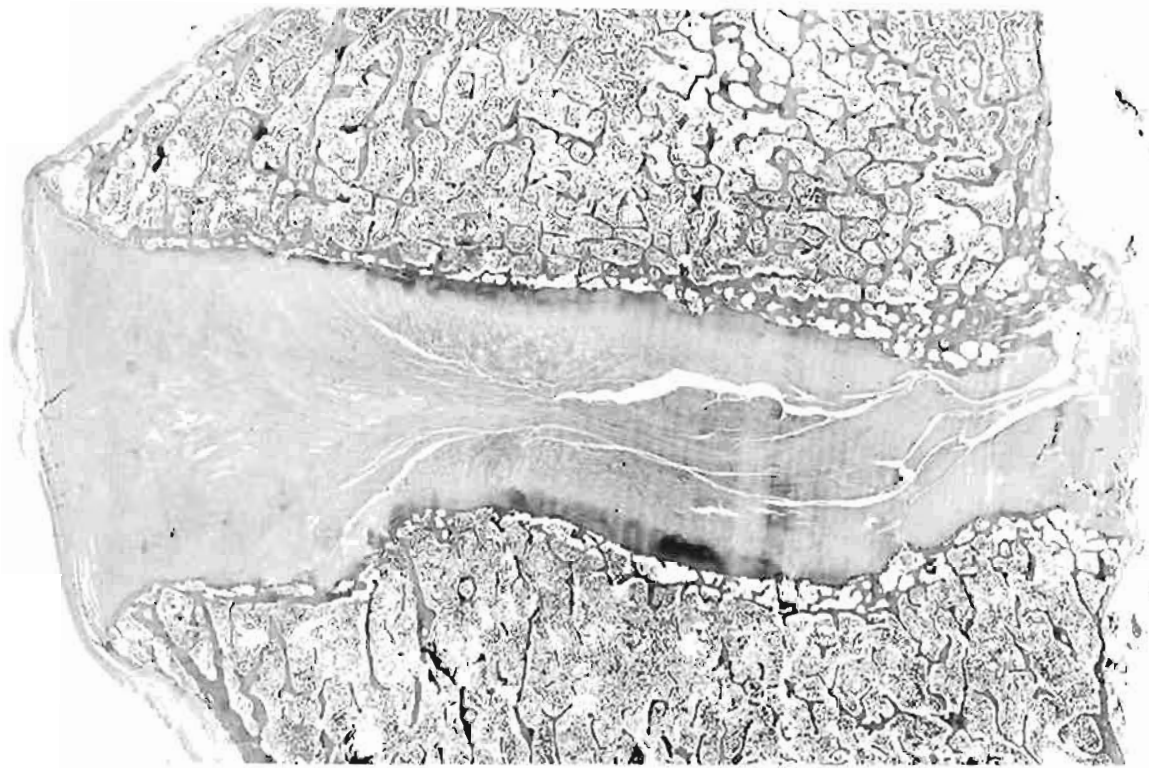
The second stage of prolapse that was categorized was the "transligamentous nuclear protrusion". This was characterized by the protrusion of semi-continuous or discrete fragments of nucleus along a radiating cleft beyond the anterior or posterior longitudinal ligaments (Figures 5.10; 5.11; 5.12). Occasionally, a small portion of end plate, annulus or fibrocartilage was also seen admixed with the nuclear fragments. Posterior transligamentous protrusions were most commonly observed at the three lowest lumbar levels, while anterior transligamentous protrusions were most frequent from L2-L3 and L3-L4. Anterior protrusions did not appear until almost a decade after the first posterior protrusions were seen (Table 5.2).

The final stage of disc prolapse followed disruption of the posterior longitudinal ligament and sequestration of isolated fragments. In older discs, the nucleus pulposus at this stage was very degenerate and contained many discrete fragments, some of which were still seen within radiating tears. Sequestrae were seen infrequently at the anterior aspect of the disc. Posteriorly, sequestrated fragments were rare in the upper lumbar levels until the seventh decade, when they became a predominant feature of the lumbosacral level (Figures 5.13, 5.14, 5.15; Table 5.2).

As a whole, almost 20% of the discs examined contained posterior herniations, of which one-third had a prolapse involving more than one disc. When present, radiating tears of the annulus were often associated with nuclear migration, either through or beyond the longitudinal ligament.

Nuclear clefting, which is a reliable indicator of matrix desiccation, was prominent in all discs. It was seen in one-third of all lumbar discs in the youngest group. There was no significant change in the level of nuclear clefting with age (Table 5.2; Figure 5.16).

FIGURE 5.10

Incidence of anterior transligamentous extrusions in autopsy subjects. Values are expressed as a percentage of all discs examined.
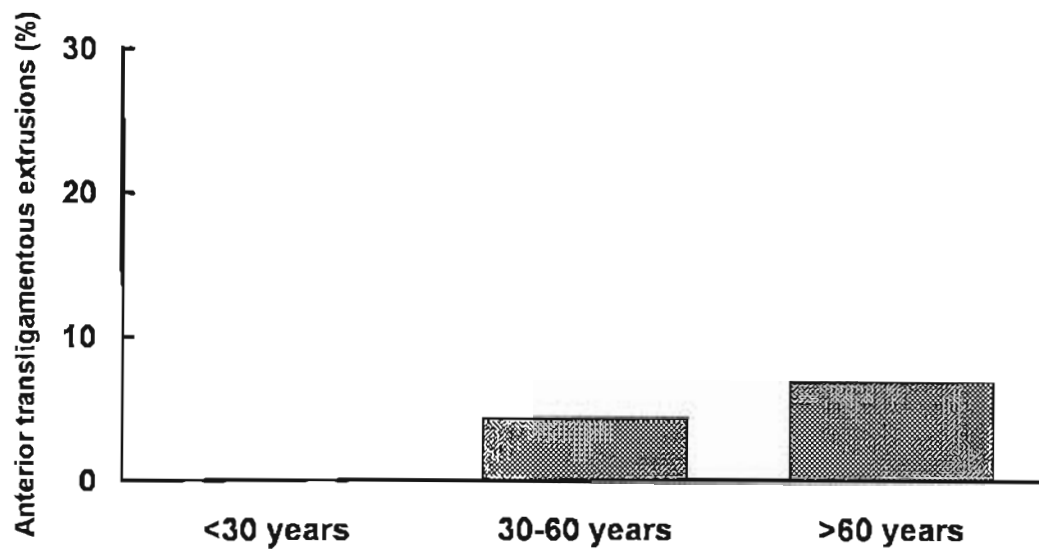
FIGURE 5.11

Incidence of posterior transligamentous extrusions in autopsy subjects. Values are expressed as a percentage of all discs examined.
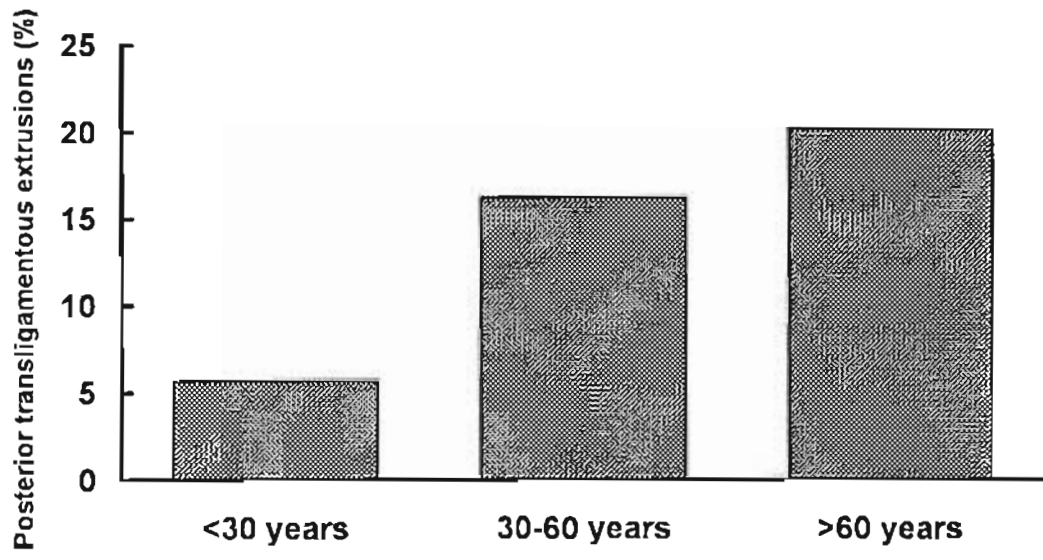
FIGURE 5.12

Transligamentous extrusion of disc material through the posterior annulus fibrosus in this L4-L5 disc from a 79 years old male subject. A large circumferential tear originating at the upper anterior rim communicates with a complex radiating tear between the anterior and the posterior margins of the disc (H&E, x 6).
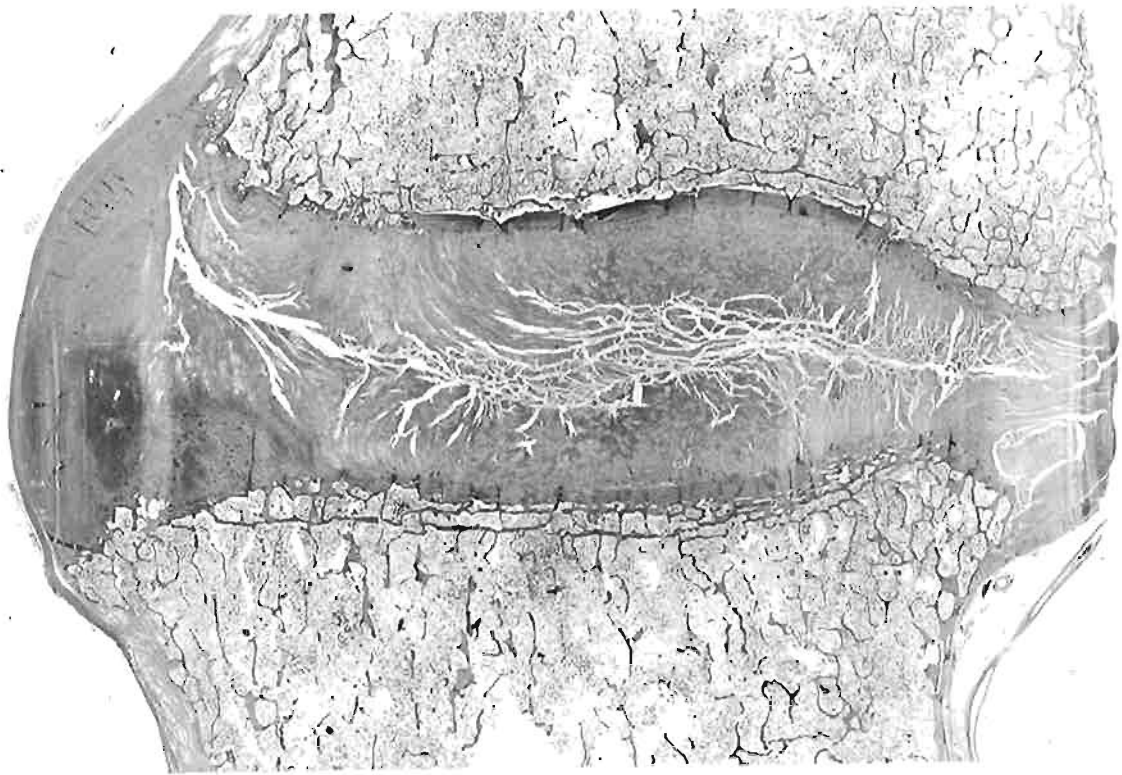
FIGURE 5.13

Incidence of anterior sequestrations in autopsy subjects. Values are expressed as a percentage of all discs examined.
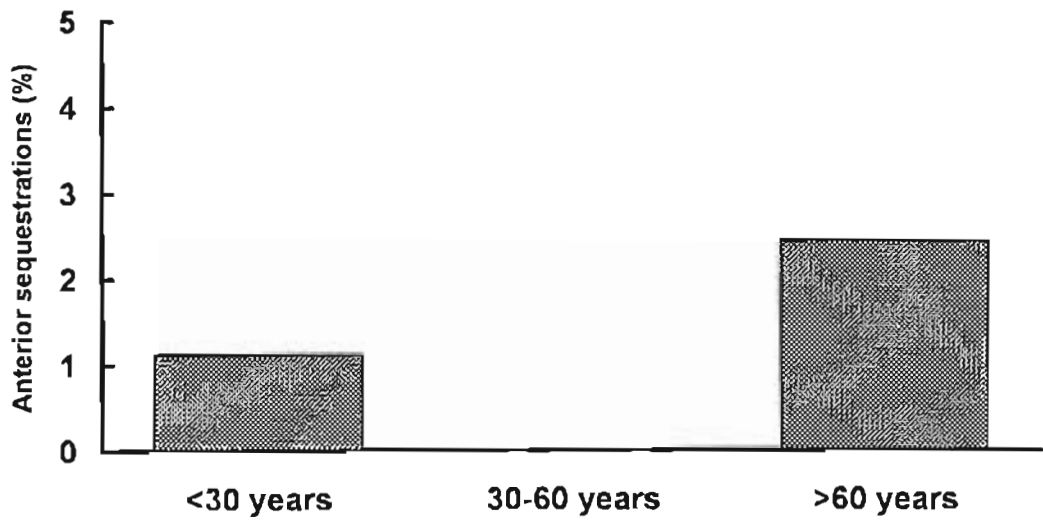
FIGURE 5.14

Incidence of posterior sequestrations in autopsy subjects. Values are expressed as a percentage of all discs examined.
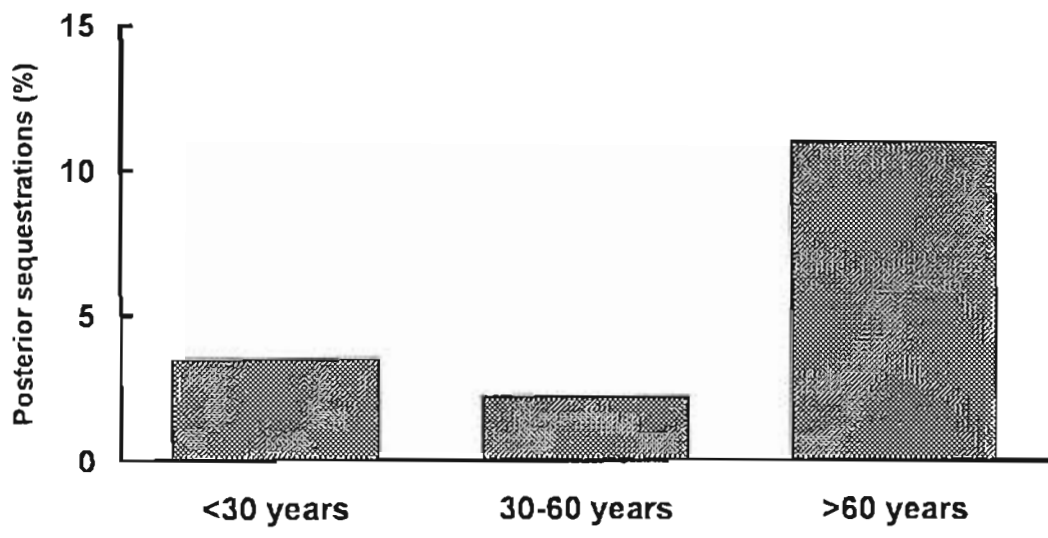
FIGURE 5.15

Early sequestration of nuclear fragments in the posterior and anterior annulus of this L4-L5 disc from a 53 year old female subject. Large cracks in the centre of the disc are contributing to the formation of a separate isolated fragment (H&E, x 6).
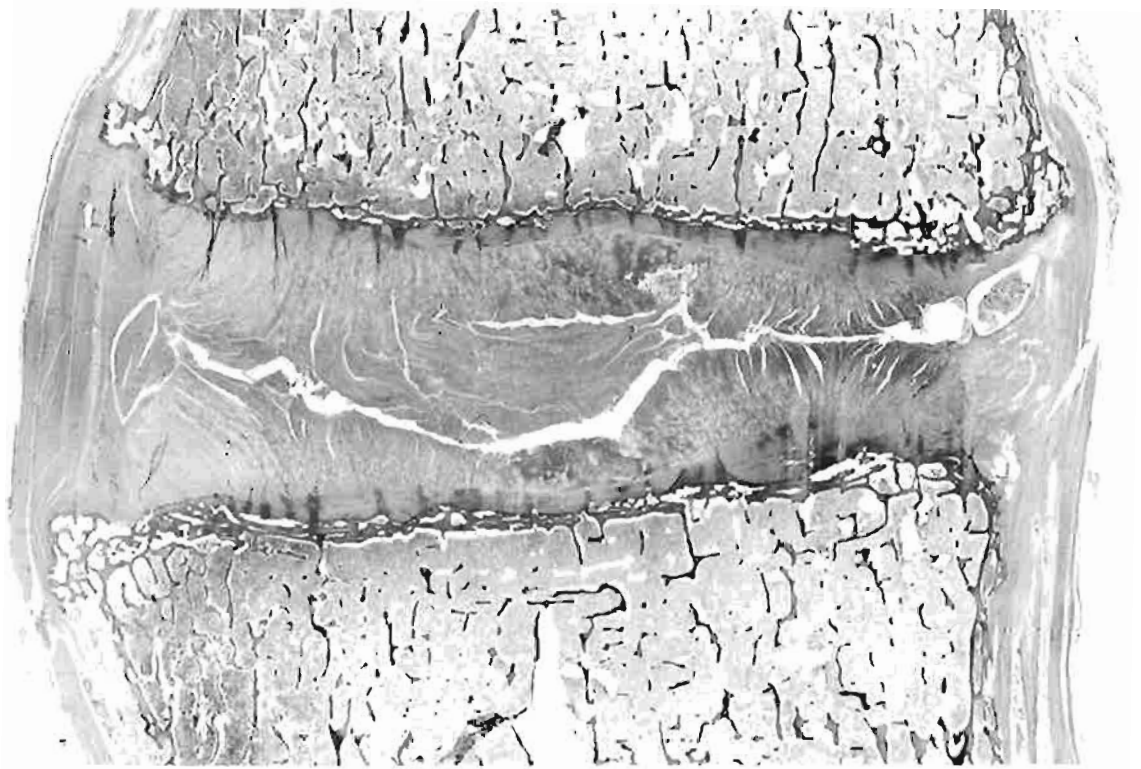
FIGURE 5.16

Incidence of nuclear clefting in autopsy subjects. Values are expressed as a percentage of all discs examined.
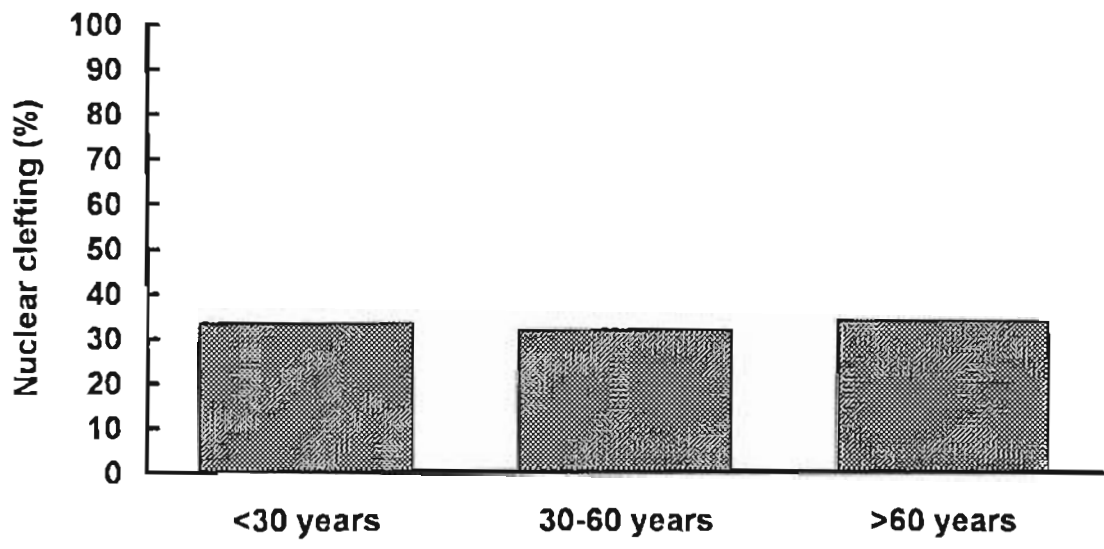
Peripheral vascularization of the normally avascular annulus fibrosus developed after the age of 30 years. Approximately 9% of the lumbar discs from subjects aged between 30 and 60 years showed vascularization of the annular fibres. This increased to over 16% of the discs in the oldest age group. Most of the vascularization was seen in the posterior annulus in association with radiating tears of the fibres (Table 5.2, Figure 5.17).

5.5 DISCUSSION

Several authors have offered hypotheses regarding the pathogenesis of disc herniation in relation to degenerative changes in the disc (Brown, 1971; Coventry et al., 1945a, 1945b, 1945c; Helfet and Gruebel Lee, 1978; Hickey and Hukins, 1980; Shirazi-Adl, 1989; Yasuma et al., 1986; Yu et al., 1988). While most agree that degenerative changes appear in the nucleus towards the end of the second decade, the distinction between the normal ageing process and true pathology is less clear. The classical view, that the turgid nucleus is extruded under pressure following a traumatic rupture of the annulus, may explain the few cases of herniation seen in the young. But, the fact that the mean age of presentation of clinically symptomatic disc protrusions exceeds forty years, at a stage when the nucleus has lost its turgescence, indicates that, in middle age and beyond, herniation is associated with age-related disc degeneration.

Disruption of the annulus and the formation of radiating tears can be due, respectively, to mechanical tearing or degenerative changes. It has been postulated that nuclear degeneration imparts abnormal stress on the annular fibres (Bijlsma and Copius Peereboom, 1972; Kurowski and Kubo, 1986; Pearce et al., 1987). Adams and Hutton (1982) proposed that activities involving low loads on the spine may lead to chronic mechanical fatigue of annular fibres, and a slow progression to prolapse. Vernon-Roberts and Pirie (1977) suggested that the annular radiating clefts may develop as a result of

115

FIGURE 5.17

Incidence of peripheral annular vascularization in autopsy subjects. Values are expressed as a percentage of all discs examined.
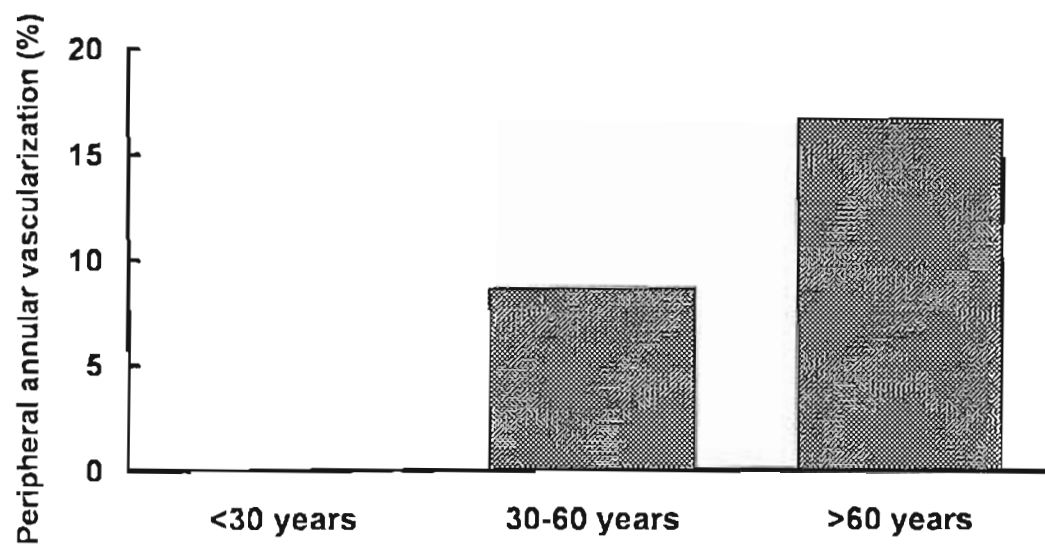
shearing forces, rather than degeneration *per se.* Osti et al. (1990) proposed that rim lesions may initiate the formation of radiating annular tears.

Mechanical tearing refers to those movements that place abnormal stress on the annular fibres, causing sudden rupture. Such movements could include torsion with flexion (Hickey and Hukins, 1980), axial rotation and lateral bending (Shirazi-Adl, 1989), hyper-flexion beyond physiological limits (Adams and Hutton, 1982), or cyclic flexion, compression and rotation (Gordon et al., 1991). The findings of the present study indicate that, at least in adult subjects, nuclear fragments migrate along pre-existing radiating tears of the annulus. In the autopsy discs examined, nuclear degeneration was seen from the second decade. As described previously (Coventry et al., 1945a, 1945b; Schmorl and Junghanns, 1971; Vernon-Roberts and Pirie, 1977) degeneration becomes evident first as large clefts towards the periphery of the nucleus as it becomes less hydrated and shrinks, and is followed by nuclear clefting and fragmentation which are well established by the third decade.

The two transligamentous prolapses that were observed in the younger discs in this study were associated with significantly less nuclear degeneration. This is consistent with the concept that mechanical rupture may play a greater role in herniations in young people. Whether tearing of the ligament is an extension of the degenerative process or an acute traumatic event, is unclear. It is possible that, in adults, the former plays a greater role in the formation of the annular clefts, while the latter is more involved in disruption of the longitudinal ligament. Posterior herniation was most common at L5-S1, affecting almost 30% of discs from subjects over 60 years of age. In total, almost 20% of spines examined had prolapses, over one-third of which had affected discs at more than one level. This is similar to the frequency of prolapse in autopsy spines reported by others.

Vascularization of the various disc components that are not normally endowed with blood vessels appears to be related to attempted repair of

117

pathological processes within the disc. For example, the annulus exhibits an apparent reparative process in the peripheral fibres that are involved with radiating tears and rim lesions. This study demonstrated an absence of annular vascularization in the young discs in which relatively few pathological changes were observed in the annulus. However, with advancing age, the markedly increased incidence of rim lesions and radiating tears was accompanied by a similar increase in the extent of peripheral annular vascularization as well as end plate vascularization. It appears likely, therefore, that the vascular ingrowth represents attempts at repair occurring in the disc regardless of whether structural changes result from natural ageing or from a pathological process. Despite attempts at repair, however, the discs undergo prospective and irreversible changes, which are collectively designated as "degeneration", both in the human spine and in the sheep model (described in Chapter 3).

This autopsy study of end plate vascularization draws certain parallels with the sheep model. In both studies there is evidence of advancing disc degeneration in the presence of annular lesions. In fact, there is evidence from the present study and others (Osti et al., 1990) that annular tears may be the initiating factor in disc degeneration. In the animal model, end plate vascularization increased significantly within one to two months of a vertebral rim defect being created, and began to return to pre-operative levels after 18 months. In the human study, however, there was no indication that the elevated levels of vascularization would resolve with time, as vascularization continued to increase with age. It could be argued, therefore, that this contradicts the animal model. However, it must be remembered that, in the human study, it was not possible to determine how long the annular lesions had been present prior to examination. In addition, the annulus lesion was not initiated in "elderly" sheep. It is a significant observation that the estimates for end plate vascularization in the younger human discs are of the same magnitude as those

found in the sheep study. This further validates the sheep as a suitable model for the study of human disc pathology.

CHAPTER SIX

VASCULARIZATION OF TISSUE FRAGMENTS EXTRUDED DURING LUMBAR DISC HERNIATION

6.1 AIM

The aim was to establish if there is any significant relationship between the histological appearance of herniated lumbar disc fragments and a range of clinical symptoms in patients who have had surgery for low back pain.

6.2 INTRODUCTION

With the exception of the most peripheral annular fibres, young healthy adult disc tissue normally shows no sign of vascularization. However, neovascularization has been reported in isolated fragments that have been extruded from the body of the disc (Weidner and Rice, 1988; Yasuma et al., 1993). Furthermore, it has been claimed that this feature can be considered as a reliable histological indicator that the excised material originated from within the disc (Weidner and Rice, 1988).

Prolapsed disc fragments are frequently removed at surgery following extended periods of sciatic pain. It remains uncertain, however, whether or not this tissue is directly responsible for such pain, since not all patients experience relief of pain after this procedure. To date, the existence of any relationship between neovascularization of extruded fragments and associated clinical features has not been thoroughly explored.

This retrospective study of human intervertebral disc material aimed to examine the relationship between neovascularization of herniated fragments and symptoms and signs of sciatica in a series of patients who had undergone surgical removal of a disc protrusion. An attempt was made to correlate pain symptoms with the histological appearance of blood vessels in the material collected from surgery.

6.3 METHODS

A consecutive series of one hundred patients was reviewed following spinal surgery to remove extruded or sequestrated intervertebral disc herniations at one or more lumbar levels. A total of one hundred and twenty tissue samples of excised material were analyzed. The presence of disc herniation had previously been confirmed in all cases by radiologic modalities including magnetic resonance imaging, computed tomography (CT) or CT/myelography.

The herniated fragments were classified by the surgeon at the time of operation as 'subligamentous protrusions' if they remained attached to the body of the disc but did not extend beyond the posterior longitudinal ligament; 'transligamentous protrusions' if they had passed through the posterior longitudinal ligament, but retained a point of attachment to the disc; or 'sequestrations' if they were found to be lying free within the spinal canal. Following removal, the samples were fixed in 10% neutral buffered formalin for at least 24 hours and processed into paraffin wax using standard automated techniques. Tissue sections 5 μm thick were stained with haematoxylin and eosin prior to histological examination by light microscopy. Additional sections were incubated overnight with a monoclonal antibody to the phosphorylated form of neurofilament protein (NFP, SMI-31), diluted 1 in 6000 and stained with diaminobenzidine (Sigma) with avidin-biotin peroxidase (Vector stain, ABC kit), and finally counterstained with haematoxylin.

The excised disc components were classified according to fundamental histological criteria. The distinctive collagen fibre arrangement of the discs components was confirmed by polarized light microscopy. Cartilage end plate was identified by its characteristic hyaline cartilage features. Annulus fibrosus was identified by the light and dark lamellae of collagen fibres oriented at alternating angles under polarized light. Nucleus consisted of a homogeneous matrix containing chondrocytes either as single cells or as clusters within a

random arrangement of collagen fibres. The relative amount of each component was rated using an arbitrary three point scale.

Specimens were examined for evidence of peripheral neovascularization characterized by endothelial-lined capillaries, and the maximum depth of vascular ingrowth (μm) was measured perpendicular to the outer margin using an ocular-mounted graticule at an overall magnification of 400x.

Clinical data comprising the patients' age, sex, spinal level involved, extent of straight leg raising restriction by leg pain (SLR), signs of nerve root impairment (power, sensation or reflexes) and details of clinical outcome were obtained by examination of case notes. Clinical follow-up was conducted after at least six months in all cases. Patients were excluded if there was any other spinal pathology apparent, or if there was a history of previous spinal surgery. The duration of pre-operative sciatica was recorded. Clinical outcome was assessed as 'poor' if the patient reported persistence of incapacitating pain after surgery; 'fair' if the overall outcome was considered good, but patients still had persisting radicular symptoms and had not returned to pre-injury status, or; 'good' if there were no significant pain symptoms at the time of the follow-up interview.

Patient age, the extent of neovascularization, the interval between onset of sciatica and surgery, and SLR were compared using a correlation matrix. One-way ANOVA (Excel version 4, Microsoft Corp., Redmond, WA) was used to determine if there was any significant difference between the mean measurements of neovascularization when grouped on the basis of the clinical parameters. Statistical comparisons were considered significant when $P < 0.05$.

6.4 RESULTS

There were 63 males and 37 females included in 100 patients entering this study. The average age of patients was 46 years (range 18-85 years). Fifty percent of the prolapsed disc specimens were from L5-S1, 47% were from L4-L5, and the remainder were from L3-L4. The average period of time between onset of leg pain and surgery was 38.4 weeks, (range 3-574 weeks). Mean SLR was 40.5° (range 10-90°).

Sequestrated fragments were the most abundant (71%) followed by extruded subligamentous (18%) and extruded transligamentous (11%). The origin of the fragments from within the disc is summarized in Table 6.1. Thirty-four percent of the 120 specimens consisted of nucleus pulposus tissue alone. None consisted solely of annulus. Fourteen percent contained both nuclear and annular material, and of these, the great majority were predominantly nuclear. Twenty-nine percent contained both nucleus and end plate, and the majority of these contained a greater proportion of nucleus. Nineteen percent contained all three types of disc tissue, and the vast majority of these had predominantly nuclear material. One specimen contained only scar tissue of unknown origin and another consisted of end plate with scar tissue.

Overall 98% of the specimens contained some nucleus pulposus, either alone or in conjunction with other material. Annular material was seen in one-third of specimens, and 49% contained some end plate. Nucleus was the principal tissue in 89% of all specimens, whereas none consisted predominantly of annulus and only 3% consisted of mainly end plate. Twenty-two percent of specimens, excluding one which consisted solely of scar tissue, contained small amounts of adipose, scar or bone tissue. No evidence of acute or chronic inflammation was observed in any of the specimens.

124

TABLE 6.1

Summary of the origin of 120 extruded disc fragments from 100 consecutive surgical patients. AF=annulus fibrosus; NP=nucleus pulposus; EP=cartilage end plate

ORIGIN OF MATERIAL	NUMBER OBSERVED	
Nucleus Only	43	
Annulus Only	0	
End Plate Only	1	
Nucleus + Annulus	17	
NP > AF		16
NP = AF		1
NP < AF		0
Nucleus + End Plate	35	
NP > EP		29
NP = EP		5
NP < EP		1
Nucleus + Annulus + End Plate	23	
NP predominates		19
AF predominates		0
EP predominates		1
NP = EP > AF		2
NP = EP = AF		1
Connective Tissue Only	1	
Total		**120**

Areas of peripheral neovascularization were observed in 89% of the disc fragments examined. Vascularization was located focally in some fragments but was relatively widespread in others. A characteristic feature of all such regions was small capillaries lined with endothelial cells that were set within disc tissue derived principally from nucleus pulposus. Occasionally annulus and cartilage end plate were also identified. Clusters of chondrocytes (chondrones) were noted in about half of the fragments. Vascularization extended to a depth of up to 250 μm but there was not a significant difference between the three surgical classifications of prolapse (Table 6.2).

There was no significant difference noted in the mean age of patients with the different types of protrusions (Table 6.2). Patients with sequestrated fragments experienced pain, on average, for longer (mean 43.9 weeks) than the patients with extruded subligamentous (mean 25.5 weeks) and extruded transligamentous fragments (mean 22.8 weeks), but this was not significantly different because of wide variability in the data. There was no significant difference in SLR between these groups (Table 6.2).

The correlation matrix comparing the depth of neovascularization, age, duration of pain and SLR showed no significant relationship between any of the variables (Table 6.3). There was a weak negative relationship between depth of vascularization and duration of pain, but this was shown, using a t-test with unequal variances, not to be significant.

The depth of vascular tissue penetration was compared between the groups of patients as designated by the surgical classification of the fragments. There was no significant difference with respect to neovascularization or age between the three groups (Figure 6.1). Furthermore, no statistically significant differences were found in the mean depth of vascularization or age when the patients were grouped according to clinical outcome assessment (Figure 6.2), or by signs of nerve root impairment (Figure 6.3).

TABLE 6.2

Basic clinicopathological data from the surgical cases of disc prolapse. Mean (standard error of mean).

	Age (years)	Vascularization (μm)	Pain (weeks)	SLR (°)
Classification:				
Extruded subligamentous	43.5 (3.1)	40.2 (8.9)	25.5 (6.6)	41.4 (5.6)
Extruded transligamentous	46.6 (6.2)	50.6 (33.6)	22.8 (6.1)	46.7 (9.1)
Sequestrated	46.9 (1.8)	40.4 (5.0)	43.9 (15.7)	39.0 (3.2)
Outcome:				
Poor	42.9 (4.8)	43.8 (13.5)	68.8 (50.0)	39.3 (10.4)
Fair	46.6 (2.8)	41.7 (8.1)	49.9 (27.2)	50.0 (5.2)
Good	43.9 (2.7)	42.7 (13.1)	15.2 (3.0)	34.2 (3.8)
Neural Deficit:				
Power	44.6 (2.4)	61.9 (11.3)	12.0 (1.8)	34.8 (4.4)
Sensation	45.2 (1.9)	47.5 (7.9)	24.4 (7.8)	37.4 (3.1)
Reflex	47.2 (2.9)	47.7 (12.9)	44.6 (27.3)	43.6 (5.7)

TABLE 6.3

Correlation matrix between clinical data and vascularization.

	Age	Vascularization	Duration of pain	SLR
Age	1.000			
Vascularization	-0.129	1.000		
Duration of pain	-0.032	-0.198	1.000	
SLR	0.454	0.059	0.268	1.000

FIGURE 6.1

Relationship between age of patients and extent of neovascularization in fragments separated according to surgical classification.
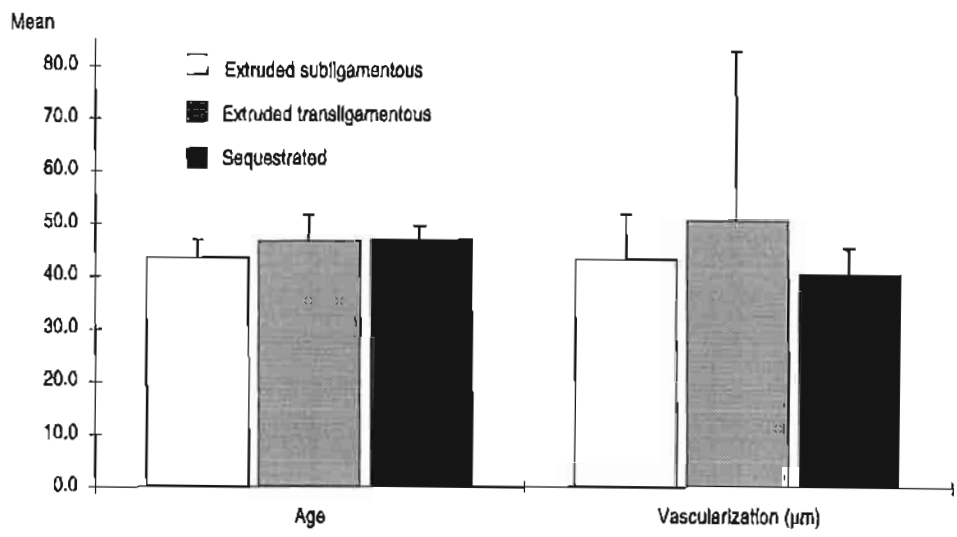
FIGURE 6.2

Relationship between age of patients and extent of neovascularization in fragments separated according to clinical outcome.

Mean
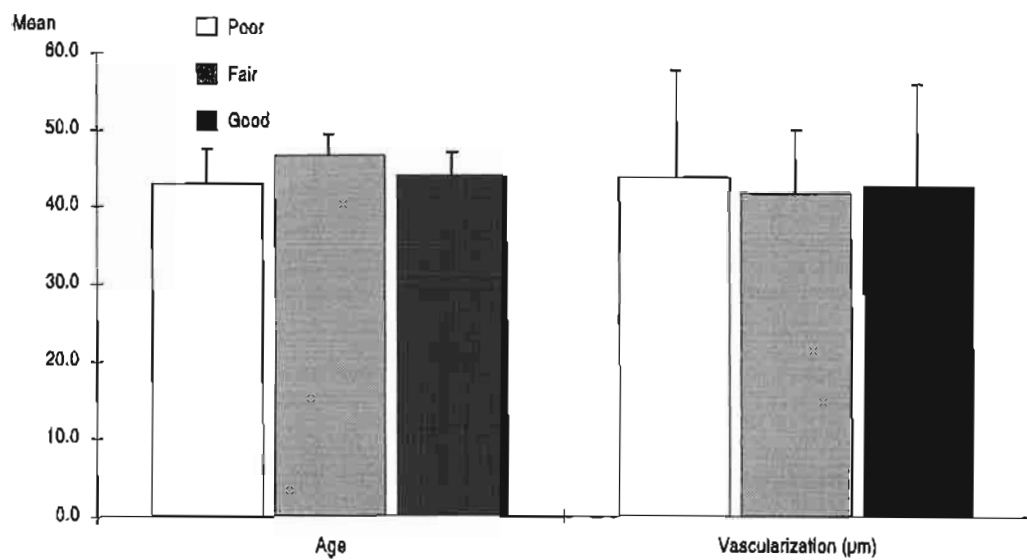
FIGURE 6.3

Relationship between age of patients and extent of neovascularization in fragments separated according to clinical neural deficit.
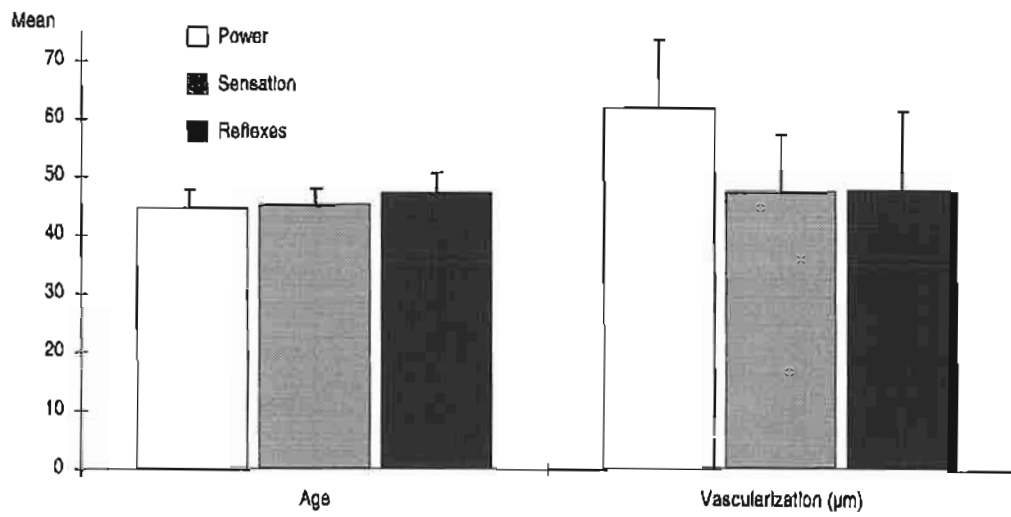
Tissue sections demonstrated neural tissue adjacent small blood vessels (Figure 6.4), which was confirmed by a positive staining reaction for neurofilament protein (NFP) (Figure 6.5).

FIGURE 6.4

Low power photomicrograph of a portion of extruded disc tissue showing an elongated strip of neural tissue (arrow) adjacent a cluster of three small blood vessels (H&E, x 100).

FIGURE 6.5

High power photomicrograph of the neural tissue stained positive for NFP (SMI-31, x 200).

6.5 DISCUSSION

This study was undertaken to determine if there is any significant relationship between a range of clinical symptoms and the histological features of herniated disc fragments removed during lumbar spine surgery. In this particular group of patients there appears to be no demonstrable correlation between neovascularization at the periphery of herniated fragments and several important clinical indicators of disc prolapse.

In a recently published study which established the clinical importance of characterizing the nature of prolapsed disc material (Weidner and Rice, 1988), neovascularization was the only one in a range of descriptive histological criteria claimed to be a reliable indicator of disc prolapse. Fifty percent of the curetted specimens showed some evidence of peripheral neovascularization, and, although the statistical sensitivity was low, the authors concluded that it was the only unique feature which indicated that herniation of disc material had occurred. This view, however, was challenged by the authors of a later study, who found evidence of vascularization in only slightly more sequestrated fragments than in material which was described as non-sequestered "routine material" (Brock et al., 1992). Exceeding any results previously reported, the present study found neovascularization in the great majority (89%) of samples, suggesting that it is a common feature of herniated disc material, regardless of the individual characteristics of that material.

The differences between the present study and those previously published are important. One explanation could be in the histological interpretation of the material examined - annulus derived from the transitional zone of the disc could easily resemble nucleus. It is for this reason that polarized light was used in the present study to verify the birefringent nature of the collagen fibres of the annulus. An alternative explanation is that surgical excision of disc bulges may have also included small portions of annular material that was attached to the offending nuclear fragments.

Invasion of herniated fragments by blood vessels would most likely occur when tissue from the central region of the disc, which is normally avascular, comes into direct contact with outer annulus or epidural tissue, both of which normally have a low degree of vascularization (Brock et al., 1992). In a situation analogous to a foreign body reaction, the expected natural history of this phenomenon would be eventual removal of the prolapsed fragment by macrophages able to migrate into the tissue as vascular invasion occurs (Lindblom and Hultqvist, 1950; Nohara and Tohmura, 1993), or the formation of a fibrous scar which potentially could persist for an indefinite time. An alternative view derived from studies on cadaveric discs, indicates that at least a portion of the vascularization in these fragments is derived from the original disc tissue as the material is extruded (Yasuma et al., 1993). While there is no doubt that the peripheral layers of the annulus do contain a small amount of blood vessels, it is unlikely that they are directly involved in herniation, as the bulk of the material examined in the present study is nuclear in origin. As discussed earlier, it is possible that, in these other studies, bulging outer annulus may have been collected at the same time as the prolapsed tissue was biopsied.

While pain may be expected to arise from direct contact of the herniated fragment on the innervated structures in the region of the nerve root due to a direct pressure effect, the role of vascularization in the propagation or exacerbation of pain symptoms is speculative. Recent radiologic evidence indicates that vascularization leads to resorption of extruded disc fragments (Komori et al., 1993; Nohara and Tohmura, 1993). Therefore, in the event that a fragment undergoes complete resorption, whether or not it is responsible for mechanical deformation of the spinal cord or nerve root, there should eventually be cessation of the pain. However, while the fragment persists, and with it, extensive vascularization, the pain itself may persist. The evidence from the present study indicates that resorption is not a major factor in the resolution

138

of these fragments since, in some of the patients the pain was present for a considerable time, even though the fragments showed only peripheral vascularization. If resorption does occur, it is likely that there would be release of degradative enzymes to reduce the bulk of the fragment, which in turn would liberate intra-cellular proteins and other chemical species as potential irritants (Weinstein et al., 1988; Franson et al., 1992).

Clearly, the significance of vascularization in this clinicopathologic study relates to its potential role in pain production. Radicular pain in cases of disc protrusion is usually attributed to a direct pressure effect resulting from nerve root compression. Moreover, surgery usually fails to reveal convincing evidence of inflamed or irritated nerve roots. It is well established that neural tissue ingrowth frequently accompanies blood vessels as they penetrate newly vascularized tissue, and this was confirmed in the present study, using a monoclonal antibody to neurofilament protein. It is still uncertain, however, if the nerve fibres that have been identified are genuinely nociceptive. If this were possible, this would confirm the association between these two features in the production of pain by prolapsed disc fragments.

The average depth of vascularization that was measured in the disc fragments did not differ significantly between the three surgical classifications of prolapse. It is probable that sequestration of fragments represents a more advanced stage of a degenerative process in the disc than the non-sequestrated fragments, and while the data are not statistically significant due to wide variability, they suggest that patients with sequestrated fragments experienced sciatic pain for longer periods than the other groups.

It has been suggested that sequestrated fragments show fewer histological features of degeneration than non-prolapsed material, because the peripheral vascularization would provide better nutrition of a tissue previously accustomed to an avascular environment (Brock et al., 1992). The finding of chondrones in many of the fragments indicates pre-existing degeneration in the

139

nuclear material, but, regardless of their surgical classification, the disc fragments showed little evidence of the vacuolation or necrosis noted in the study of Brock et al. (1992). By inference, it is most likely that they were well nourished. It should be noted that the extent of vascularization was measured in the two dimensions of the tissue section only, and further levels were taken only if the initial section failed to show evidence of blood vessels. This point demonstrates the fact that it may be necessary to undertake extensive sampling through the entire block of tissue in order to be confident that an accurate assessment of vascularization has been made.

Brock et al. (1992) further observed that patients with sequestrated disc fragments were significantly older than patients with prolapsed and protruded fragments. The data from the present study do not support that finding. The relationship between patient age and the physical characteristics of the extruded fragments can be interpreted in a number of ways. On the one hand, sequestrated fragments may be more common in younger individuals, who are, in general, physically more active. The relatively higher fluid properties of younger discs is the result of greater tissue hydration which may render them more vulnerable to extrusion following acute annular disruption. On the other hand, it could be argued that younger individuals would have more resilient annuli which should resist extrusion of nuclear material. Since, in this study, there was no age difference between patients with sequestrated discs and those with other forms of protrusion, there may be evidence that sequestrae form within the disc early, as the matrix becomes dehydrated, and are extruded subsequently.

As in the present study, Eckert and Decker (1947) found no correlation between any of their microscopic observations (including vascularization of the extruded material) and clinical outcome in their study, and were unable to provide an explanation for a large group of patients who experienced continued post-operative pain with sciatic radiation. A group of patients in the present

140

study also continued to experience pain following surgery. From a substantial post-operative analysis, Ross and Jelsma (1952) attributed residual pain to a loose piece of cartilage compressing the nerve root at a level other than that of the prolapsed disc. More recently, others have compared the outcome of surgical and non-surgical management of clinically detectable herniated discs (Weber, 1983; Saal et al., 1990), but little further attempt has been made to correlate pathology with clinical outcome.

If neovascularization of disc fragments contributed to pain production in these patients, greater ingrowth in patients with a poorer clinical outcome, or greater depth of invasion in those patients with a longer duration between onset of pain and surgery would be predicted. In this study, however, there was no statistical difference between vascularization in patients with a poor, fair or good clinical outcome, suggesting that vascularization has no predictive value for clinical outcome. However, on the basis of this study, it is not possible to determine whether vascularization is either directly or indirectly related to pain production.

It is concluded that pathologic features of herniated disc material are, at best, imprecise markers for clinical characteristics. Vascularization of extruded disc fragments is not related to the length of time between onset of pain and surgery, the type of herniation that has occurred, or the clinical outcome. On the basis of this study is not possible to confirm or exclude vascularization from having a role in the production of sciatic pain.

CHAPTER SEVEN

CONCLUDING REMARKS

This thesis has demonstrated that the normally avascular components of the mammalian intervertebral disc are capable of supporting a blood supply, as a result of pathological change. The benefits of this new blood supply to components of the disc, however, remain doubtful. In the sheep, for example, the observation of increased cartilage end plate vascularization following a small experimental outer annular tear gave the impression that it represented a reparative process, and that given sufficient time, the disc had the potential to recover from such an injury. However, the integrity of the disc matrix was not restored at any stage during the subsequent twenty-four months of that study. In fact, despite extensive neovascularization following injury, the operated discs became progressively more degenerate, with medial extension of the outer annulus cut and breakdown of the nucleus pulposus. In humans, too, proliferation of blood vessels in the cartilage end plate and the peripheral annulus were notable features of discs which showed evidence of increased degeneration with advancing age.

In most circumstances, the presence of neovascularization would be regarded as a favourable indicator of tissue repair with the potential for resolution of injury in time. The role of blood vessels in providing valuable nutrients and mobilizing particular cell types at the injury site to re-establish normal tissue function is well understood. However, the ironic situation revealed by this thesis is that the disc undergoes progressive and irreversible degeneration despite the proliferation of blood vessels. In the operated sheep discs, as well as in the human autopsy discs examined in this study, it was assumed that the neovascularization was detrimental to the structure by further weakening the connective tissue components of the annulus and promoting the degenerative process.

There is now abundant evidence indicating that the breakdown of the disc matrix, whether it is the result of normal ageing or artificially induced in animal experiments, is related to a family of molecules known as angiogenic

143

factors (Folkman and Klagsbrun, 1987). One of the better known of these factors, the low molecular mass endothelial cell stimulating angiogenesis factor (ESAF), has been shown to be mitogenic to endothelial cells of new blood vessels within tissue matrices, particularly in relation to tumour growth (Weiss et al., 1979). ESAF is highly specific in its action on small capillaries, and is inactive towards the endothelial cells of much larger blood vessels, such as the aorta (Schor et al., 1980). It is known to activate latent metalloproteinases (MMPs), including collagenase, gelatinase and stromelysin, and other degradative enzymes which are essential to prepare the tissue for invasion by small blood capillaries (Sedowafia et al., 1982; Weiss et al., 1983; Melrose et al., 1987). There is recent evidence that T-cell cytokines are also intimately involved with the control of MMP release (Liu et al., 1991; Lacraz et al., 1992). In addition to angiogenic factors, there is a further controlling mechanism in the form of one or more tissue inhibitors of metalloproteinases (TIMP), which, under normal circumstances, suppress the activity of the MMPs by forming inactive complexes (Welgus et al., 1985).

The evidence from this thesis is that while the injured disc makes substantial attempts at repair by promoting vascular invasion of the matrix through the end plate and other routes, this process has actually become uncontrolled, with the result of further damage and consequent deterioration of the disc. It is reasonable to assume that the outer annular tear in the sheep progresses medially as a result of altered biomechanics of the motion segment. It remains uncertain, however, why the strong vascular response at the periphery and at the end plates fails to proceed to complete tissue repair. One explanation could be that the affected discs develop an imbalance of the levels of MMPs, TIMP and other relevant factors, resulting in a cascade of events that leads to the destructive, pathological end stage described in the preceding chapters. The role of these factors should be investigated further to understand the molecular processes involved with degenerative disc pathology.

The potential for TIMP to prevent matrix breakdown presents interesting possibilities for the treatment, or even the prevention of pain symptoms resulting from disc degeneration. As TIMP occurs naturally in the disc, there is, presumably, a regulatory mechanism controlling its normal activity. It may be possible, therefore, to promote the synthesis or expression of the TIMP protein, using gene therapy to increase the number of copies of the appropriate gene. With advances in the understanding of the genetic basis for disease, it may become evident that individuals who are susceptible to disc degeneration do not produce TIMP, or, at best, produce it in low quantities. Alternative treatments such as the administration of purified TIMP or a synthetic analogue, may become possible, in an effort to combat the degenerative process. It may also be possible to target MMP production by "anti-sense oligonucleotide therapy", in which synthetically-derived oligonucleotides complementary to specific sequences of messenger RNA are hybridized to the RNA responsible for MMP synthesis, resulting in its degradation by RNase, and subsequent lack of protein production.

The significance of the various MMPs, ESAF and TIMP in the study of neovascularization of disc components is clear when considered in the context of the histological observations reported in Chapter 2. It would be convenient, therefore, in attempting to account for the appearance of blood vessels in an environment that is normally avascular, to be able to demonstrate these molecular entities in tissue sections of disc material. Immunohistochemistry could be applied to demonstrate gene products, and *in situ* hybridization to demonstrate messenger RNA in thin tissue sections. Since conventional decalcified histology, as described in Chapter 2 (Section 2.3.2), may inactivate the tissue components of interest, resin embedding of non-decalcified tissue may be necessary.

The investigations outlined here assume that the molecules of interest are produced by cells that reside in the disc. There is recent evidence, however,

145

which indicates that, under pathological conditions, these molecules may be transported into the disc from an external site, possibly from cells within the vertebral bone marrow (Fujita et al., 1993). Furthermore, it is possible that pathological processes may alter the phenotype of the existing cells, with simultaneous modulation of their behaviour. That is, the cells begin to produce and secrete factors which are different to those normally produced. When the origin of these molecules has been determined, it may be possible to regulate their synthesis or modify their behaviour *in vivo*. It is clear that this work would also have relevance to the study of osteoarthritis, in which vascularization, degradation of cartilage and new bone formation are all prominent features.

The precise role of neovascularization in the production and transmission of pain signals requires further investigation. It is known that vascular repair in tissue is frequently accompanied by the establishment of neural elements which may be both proprioceptive and nociceptive. Specific neuropeptides and nerve tissue have been demonstrated previously in some components of the mammalian disc (Malinsky, 1959; Yoshizawa et al., 1980; Bogduk et al., 1981; Groen et al., 1990; McCarthy et al., 1991, 1992; Ashton et al., 1994), and in Chapter 6 of this thesis, neural tissue was identified adjacent blood vessels in sequestrated disc fragments. It is not known, however, if these neural elements were directly responsible for the symptoms associated with low back pain or sciatica in that group of patients, or if they were only incidental pathological findings. Structures resembling nerve fibres and staining positive for the neuropeptide Substance P (Beaman et al., 1993), and vasoactive intestinal peptide (VIP) (Ashton et al., 1992), both strongly believed to have a role in nociception (Liesi et al., 1983) have been demonstrated in human lumbar facet joints. Within the peripheral (annular) portions of the disc itself, nerve fibres have been demonstrated staining positive for Substance P, VIP and calcitonin gene-related peptide. In the present study, however, in which NFP

146

was demonstrated, the principal tissue examined was sequestrated nucleus pulposus, suggesting that this tissue is capable of becoming innervated as well as vascularized under pathological conditions. These findings may indicate the need for immediate surgical removal of tissue where possible, or by wholesale ablation of nerve tissue *in situ* to alleviate pain symptoms arising from extruded disc fragments.

While the outer annular lesion in the sheep model appears to have initiated a course of irreversible disc degeneration, there is evidence from the autopsy study (Chapter 5) of other pathological changes, such as nuclear desiccation, which may also have a role in what may be a complex sequence of events. It has been proposed in this thesis that these changes are related to each other and occur in a relatively predictable sequence with advancing age. Appearing almost invariably in this sequence of events is an element of neovascularization, either along the cartilage end plate, or in one of the disc components which ultimately may be extruded from the body of the disc. It is clear, however, that although this vascularization may begin as an innocent and potentially beneficial repair process in response to an injury, it eventually proceeds to the point where it causes the demise of the disc. The delicate balance that must be maintained to ensure that, in the normal situation, angiogenesis is controlled, appears to be disturbed during the process of ageing and disc degeneration, although the trigger that alters the stable state, is not clear. If this process can be controlled, however, it may be possible to offer patients some relief from the pain that accompanies disc degeneration.

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154

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ADDENDUM

The surgical procedures reported in this thesis were conducted by surgeons and Research Fellows from the Spinal Unit of the Department of Orthopaedic Surgery (Royal Adelaide Hospital) under the direction of Professor Robert Fraser. In particular, I acknowledge the expertise of Dr Mario Penta (Chapter 2); Mr Orso Osti (Chapter 3) and Dr Jeremy Latham (Chapter 4). The candidate assisted in all of these operations.

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