AN INVESTIGATION INTO THE EFFECTIVENESS OF COLLAGENASE FOR THE PERCUTANEOUS DISCOLYSIS OF THORACOLUMBAR INTERVERTEBRAL DISCS IN THE DOG

A thesis presented in partial fulfilment

of the requirements for the degree of

Master Of Veterinary Science

at Massey University

JONATHAN PETER BRAY

1993

Abstract

This investigation compared the effectiveness of chemonucleolysis with 500 units of collagenase, to lateral fenestration of the thoracolumbar intervertebral discs of the non-chondrodystrophoid dog. Effectiveness was based on the amount of nuclear material removed from the disc and the associated modifications to intervertebral disc structure, as determined by histological examination. The object was to determine whether the percutaneous injection of collagenase enzyme could be an alternative to fenestration as a prophylaxis against herniation of a degenerate intervertebral disc.

Eight one year old, non-chondrodystrophoid mongrel dogs were used in the experiment. Apart from two dogs which remained untreated, the remaining six dogs were from two litters which had been sired by the same animal. Two of these dogs had six intervertebral discs (T10/11 to L2/3) injected with 500 units of collagnease VIIs delivered percutaneously. Another two dogs had their equivalent discs surgically fenestrated by the lateral approach as described by Flo and Brinker. The remaining two dogs were subjected to a placebo injection of physiological saline.

The animals were examined clinically, neurologically and radiologically before treatment and at regular intervals following treatment. Six months following treatment, the dogs were euthanased.

The results showed that collagenase caused almost complete removal of normal nuclear material from within the disc. The centre of the disc was replaced by a variable combination of fibrocartilage and hyaline cartilage, which appeared to develop from the collapsed inner lamellae of the annulus fibrosus. Complications were recorded in only one dog, who suffered a transient hind limb paralysis in the two days immediately following injection. A massive dorsal extrusion of nuclear material was observed in one disc at post-mortem in this dog and was believed to be the cause of the paralysis. The dog recovered without treatment and remained normal on clinical, neurological and radiological examination six months after injection. The annulus fibrosus, dorsal and ventral longitudinal ligaments remained intact in all other dogs.

Fenestration was found to cause a variable disruption to the normal architecture of the nucleus pulposus. In most discs, cellular aggregations from the normal nucleus pulposus were undergoing a transformation to fibrocartilage. These cell groups were separated by an increased amount of amorphous matrix material which stained moderately with alcian blue. In the remaining discs (3/12), an increased fibrosus of the nucleus pulposus was seen, but no other

disruption to the normal architecture was recorded. No complications occurred in these dogs. The injection of the discs with physiological saline caused remarkably similar histological effects to the disc as did fenestration.

The investigators concluded that collagenase appeared to be an attractive alternative to fenestration for the prophylaxis of intervertebral disc herniation, on the basis of its completeness of removal of nuclear tissue, and its simplicity, cheapness, non-invasiveness and the lack of medium and short term complications. Since intervertebral disc protrusions occur more commonly in chondrodystrophoid breeds of dog, the effect of collagenase should be studied in degenerate disc of these breeds before it can be recommended for clinical use.

Acknowledgements

I would like to thank my supervisors for their work in the preparation of this thesis: Hilary Burbidge for her constant support, guidance and perpetual good humour; Brian Goulden for his desire for excellence, and his clear guidance and thinking; Elwyn Firth and Keith Thompson for their positive comments and criticims. I would also like to thank Andy Bray for his assistance with the layout design of this thesis.

The collagenase used in this experiment was supplied by Sigma laboratories, and their support is acknowledged.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

SECTION 1.0

SECTION 2.0

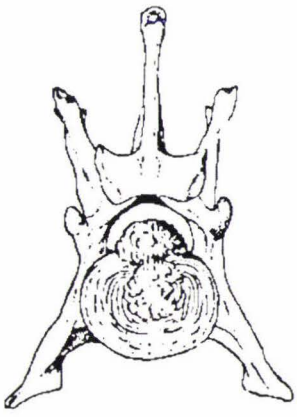
SECTION 3.0

SECTION 4.0

SECTION 8.0

SECTION 9.0

1.0



INTRODUCTION

1.1 AN INTRODUCTION

Intervertebral disc disease is a common, frequently debilitating and painful disease of dogs.[39,54,102,126,145,146,149,186,221,232,256,298,299] Although current figures on its incidence are not available, there is some evidence to indicate that the disease is becoming more prevalent. Forty years ago, disc related disease accounted for 0 - 1% of clinic patients[145] and this incidence had apparently increased to 2.3% by the 1970's[102]. In one study, the frequency of disc related problems requiring veterinary attention in the dog climbed annually during a 10 year period[39], although this increase could be attributed to an improved recognition of the disease by veterinarians, changes in breed popularity, and a more aged population due to improvements in animal care.

(i) Breed Incidence

Intervertebral disc disease has a widespread distribution throughout most breeds of dogs, although some are apparently more susceptible to the disorder than others. The Dachshund has an obvious predisposition to disc related disease[39,102,118,145,233]. (Figure 1.1) It has been calculated that the risk of intervertebral disc disease in this breed is 10 - 12.6 times greater than that for all other breeds combined[118] and accounts for 45 - 70% of all canine cases of intervertebral disc disease. Moreover, it has been estimated that 19% of Dachshunds suffer from

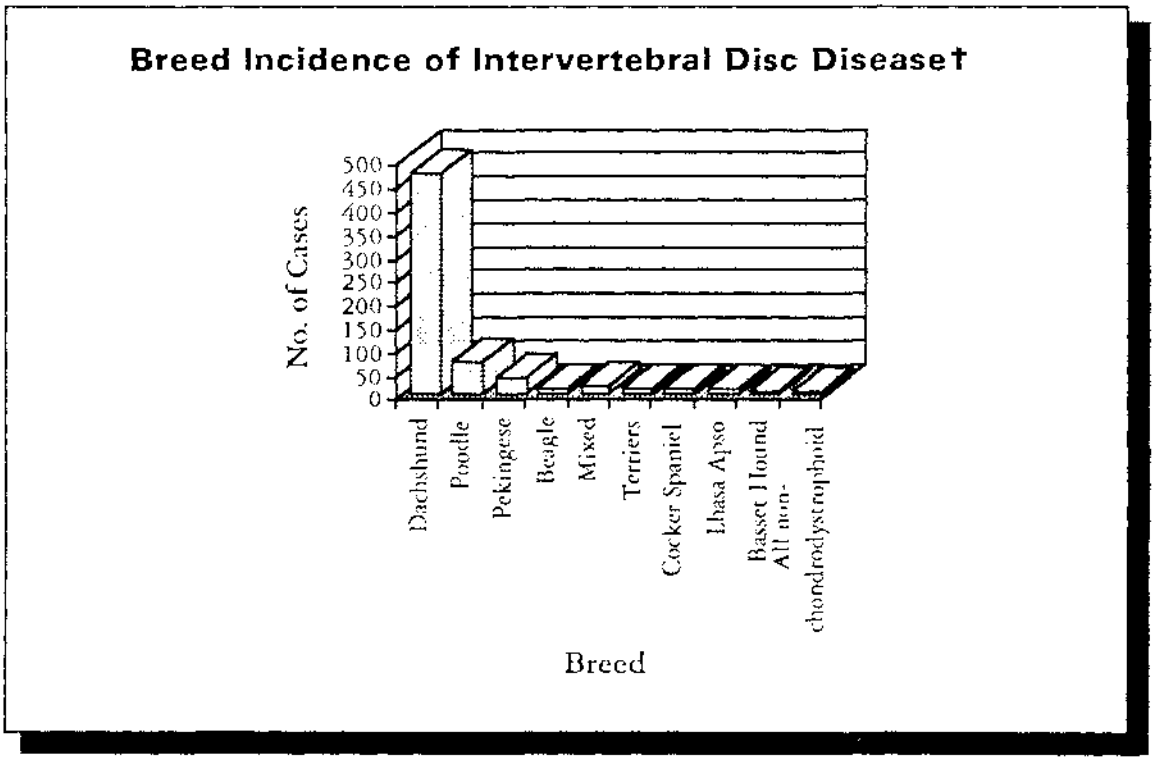


Figure 1.1: *The relative breed incidence of intervertebral disc disease in the dog. († with data from Gage ED (1975)[102])*

this problem[10]. While it is considered that there is an hereditable basis for this predisposition, the actual mechanism of inheritance is not known[10]. Other breeds considered to be susceptible to intervertebral disc disease are the Pekingese, Beagle, Shih Tzu, Lhasa Apso and Cocker Spaniel[102, 145, 233].

(ii) Sex Incidence

The relationship between the gender of a dog and its propensity to suffer intervertebral disc disease has not been clearly established. Most authors believe that males and females are equally affected[102, 118, 145], although in two reports[19, 233] a small, but statistically significant increase in the disease was found in males ($p \leq 0.01$). The investigators in these studies concluded that the recorded differences may be due to variations in body weight between the sexes, and the possible protective influence of oestrogen on the ground substance of the intervertebral disc[226, 233].

(iii) Site of involvement with intervertebral disc disease

The intervertebral disc between the twelfth (T_{12}) and thirteenth (T_{13}) thoracic vertebrae has the highest incidence of clinically obvious disease[102, 126, 145, 179]. (Figure 1.2) Indeed, this and the immediately caudal discs (T_{13}/L_1 and L_{1-2}) are implicated up to 70% of all clinical cases

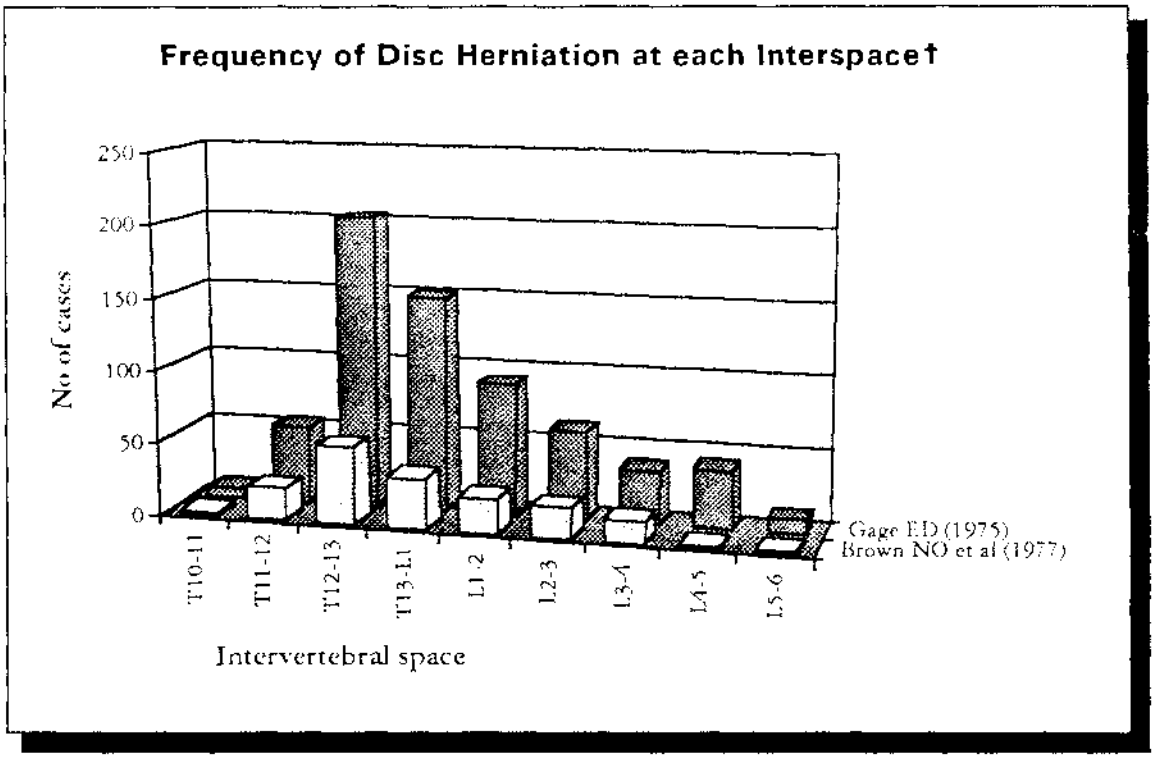


Figure 1.2. *Number of herniations recorded at each thoracolumbar intervertebral space in the dog. († from Brown NO et al (1999)[28] & Gage ED (1975)[102])*

of thoracolumbar disc protrusions[102, 126, 145, 186]. Incidentally, these discs occupy the region of most motion in the canine vertebral column[261], an observation which will become of increasing significance in the later parts of this dissertation.

Calcification of intervertebral discs, which indicates previous disc disease or degeneration, occurs throughout the length of the vertebral column[126, 145, 205]. It has its greatest incidence between T_{10-11} and T_{11-12} and frequently, several calcified discs may be identified in most adult dogs[126, 145, 265]. Using radiographic evidence, Stigen (1991)[265] reported an average of 2.3 (8.8%) calcified discs were present in a random population of Dachshunds, although a much higher incidence (30 - 60% of discs, equivalent to an average of 11.7 discs) was observed at post-mortem by Horlein (1953)[145]. This difference probably reflects the relative imprecision of radiography in detecting calcification.

(iv) Onset of clinical signs

The onset of clinical signs of intervertebral disc disease has been reported in dogs as young as one year and as old as 15 years of age, but according to Gage (1975)[102] 81.3% of cases occur between three and seven years of age. This finding is in agreement with other authors[145, 186, 208]. The average time of onset of intervertebral disc disease in all dogs is five years of age[39, 138, 145, 305]. There is some evidence to indicate that the peak clinical incidence varies between the chondrodystrophoid and non-chondrodystrophic dogs, the former being affected at an earlier age[235].

1.2 CLINICAL SIGNS OF INTERVERTEBRAL DISC DISEASE

Intervertebral disc disease is a consequence of degeneration of the nucleus pulposus of the intervertebral disc, making it less resilient and therefore, less able to function normally. Clinical signs are related to malfunction of the intervertebral disc and in some cases, rupture of the disc and the herniation of nuclear material into the neural canal[126, 145, 256, 298, 299].

Two different disease processes are recognised in the development of intervertebral disc degeneration[126, 127]. One affects the short, bandy-legged dogs that make up the chondrodystrophoid breeds only, and is believed to be related to the inappropriate maturation of cartilage which typifies these breeds[126]. Almost all intervertebral discs will be affected in these dogs and violent herniations are typical.

The other disease process is typical of all other dogs and is attributable to premature aging of a disc[126, 127]. With this latter form of disc disease, it is uncommon for more than one disc to be

degenerate in the non-geriatric individual. The intervertebral disc disease of man[59,82,86,188] and other species[175,176,192] is similar to this disease process.

1.2.1 Clinical signs: chondrodystrophic breeds

The degenerative process which usually affects many intervertebral discs along the length of the vertebral column in the chondrodystrophic breeds can render the discs quite rigid, with most becoming cartilaginous by three years of age[126,145]. Eventually, some discs may even become calcified. Disc extrusions, when they occur, are explosive and the spinal cord is traumatised by the sudden impact of this hardened nuclear material. (Figure 1.3) Frequently the injury will develop after an apparently minor accident or activity, such as jumping off the sofa, playing with other dogs or breeding. The amount of resultant hind-limb dysfunction reflects the spinal level of the disc herniation, the degree of mechanical trauma and severity of vascular interference to the spinal cord. In many cases the animal is acutely paralysed, unable to support its weight with its hind legs[145].

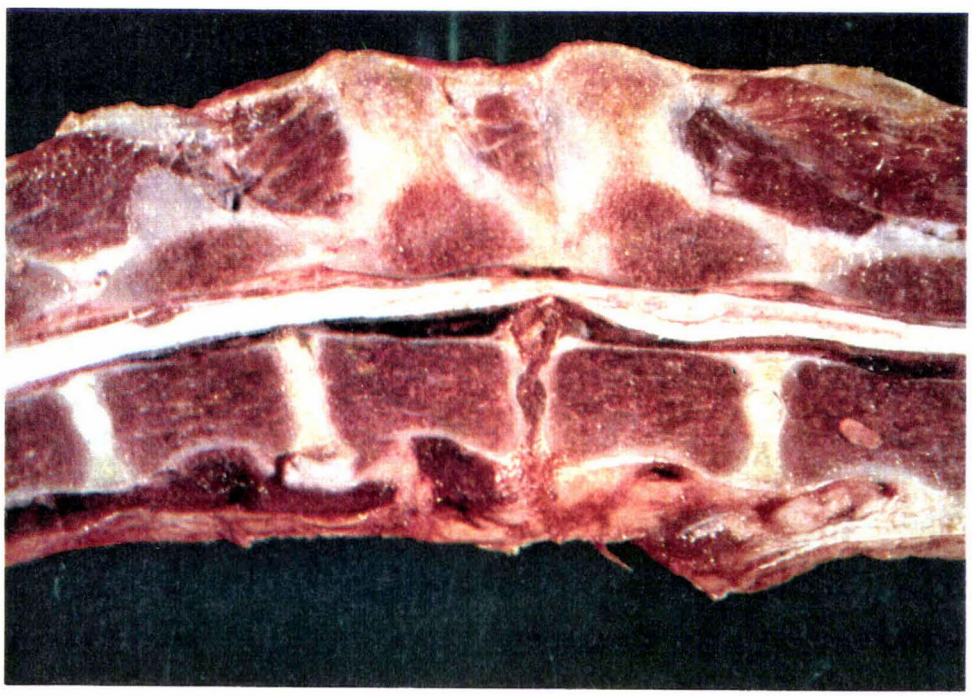


Figure 1.3: *Photograph of a massive Type I disc herniation in a 5 year old Beagle. Note the complete failure of the dorsal annulus with the extrusion of the hardened nuclear material causing considerable compression of the spinal cord within the neural canal. Note also the calcification of nucleus pulposus in the adjacent intervertebral discs, and the developing spondylosis deformans arising from the ventral aspect of the vertebral bodies.*

Pain is a common component in all but the very grave cases of intervertebral disc herniations and is commonly described in the days immediately prior to an acute paralytic episode. Pain may be due to minor compression of the spinal cord by a partially extruded disc or by stretching of

the annular fibres. When in pain the animal will be reluctant to move and will cry out when picked up. The abdominal muscles are often held taut and the animal resents palpation of this region, leading to the frequent misdiagnosis of abdominal pain by the veterinarian. The back

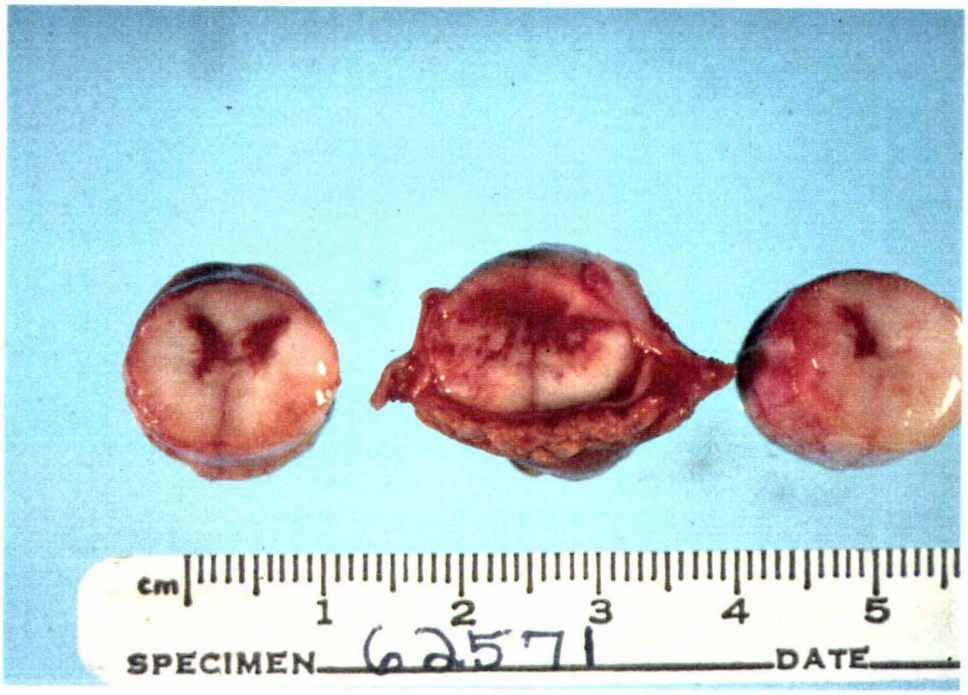


Figure 1.4. *Severe haematomyelia in the spinal cord of a 5 year old corgi following an acute disc herniation.*

may also be arched (kyphosis), probably in an effort to decrease the dorsal projection of the disc. Occasionally, a lateral spinal curve (scoliosis) can be seen due to spasm of the epaxial muscles, particularly when the disc has herniated to one side.

Lameness in one leg may be exhibited if the disc material impinges on the dorsal root ganglia carrying pain fibres to the affected limb. This radicular, or root pain, is also known as the root signature and occurs particularly if the disc herniates in a predominantly lateral direction.

Frequently, the animal may recover spontaneously from these acute episodes of pain and/or paresis only to suffer one or more relapses. Alternately, sudden deterioration of a previously low grade complaint may occur, reflecting the progressive extrusion of further degenerate nuclear material[126, 145, 222].

In most instances, the animal will remain systemically normal in spite of the severity of the spinal injury. Rarely, a progressive, ascending vascular disturbance develops subsequent to very severe disc herniations. In these animals, the acute episode is followed by a progressive infarction

of the remaining portions of the spinal cord (Figure 1.4) which ultimately causes respiratory failure, due to paralysis of the diaphragm and intercostal muscles[146, 256, 309].

1.2.2 Clinical signs: non-chondrodystrophic breeds

Disc degeneration in the non-chondrodystrophic breeds is associated with senile maturation of the intervertebral disc[126]. Trauma to the disc can hasten this process and this premature aging can result in the formation of a poorly functioning disc in an otherwise athletic animal. Perhaps because of this traumatic aetiology, it is uncommon for more than one disc to be degenerate in dogs less than eight years of age[126]. This aetiology appears to be similar to the disc degeneration which affects most other species, including man[84, 86].

Acute, explosive herniations of the disc are uncommon in these animals. Indeed, intervertebral disc disease is typified by the slowly progressive development of clinical signs. Pain is the most common manifestation and is associated with bulging of the dorsal annulus due to altered biomechanical function and/or partial protrusion of the disc. (Figure 1.5) The pain in these animals is considered to arise from three sources: pain receptors within the disc itself, the nerve roots, and the meningeal coat of the spinal cord. Continued spinal cord compression from this slowly developing lesion may ultimately result in more apparent neurological deficits[222] (for example, paresis, either from motor or sensory dysfunction, may develop).

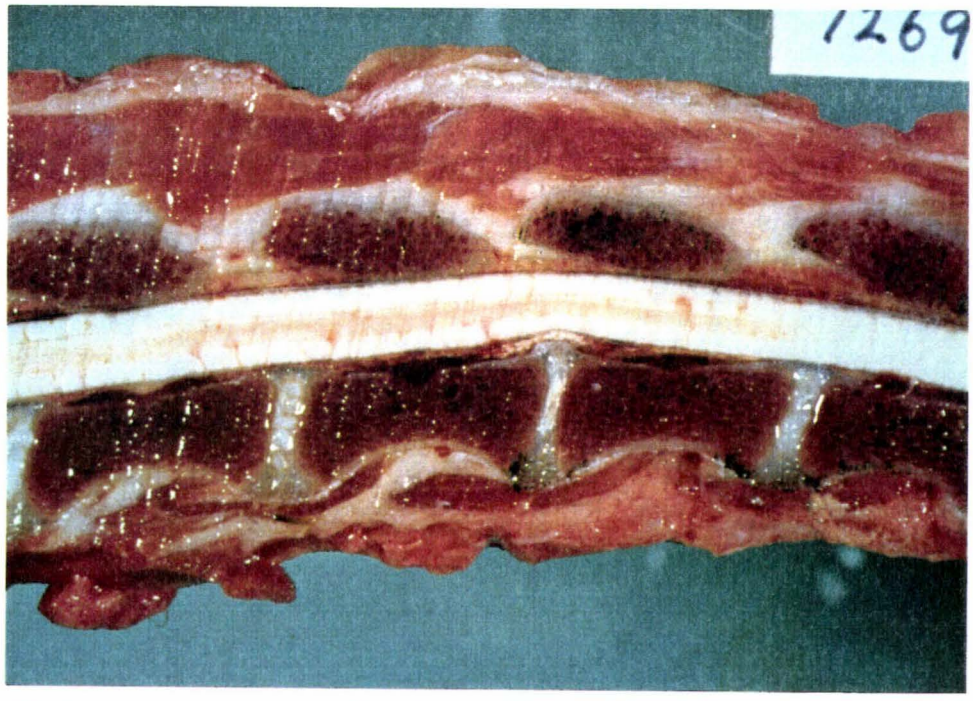


Figure 1.5: *This photograph shows a severe Type II lesion in a 9 year old Border Collie. In comparison with Figure 1.3, note the continued integrity of the dorsal annulus in the protruding disc, the* ***fibrous*** *nucleus pulposus, and the relatively normal, gelatinous intervertebral discs of the adjacent spaces.*

Altered function of the intervertebral disc can also result in changes to adjacent tissues, in particular the vertebral body and the articular facet joints. Ventral spondylosis results from calcification of the surrounding soft tissue, particularly the ventral longitudinal ligament[189,237] (Section 2.3.2). This calcification results in bony outgrowths from the cranioventral and caudoventral edges of the vertebral body[205]. In some cases, complete bony bridging of the ventral intervertebral space can occur.[205,237] Degenerative joint disease of the synovial articular facet joint can develop due to inappropriate load-bearing with continued dysfunction of the intervertebral disc and vertebral column[50,169]. In man, these two conditions are considered an important cause of non-discal pain in the syndrome of back disease[160,166,181,187,189,234]. By comparison, in the dog the influence of ventral spondylosis and articular facet arthritis on the development of clinical signs have been poorly investigated[237]. Whilst similar lesions occur to a relatively high frequency in the older non-chondrodystrophoid dog[126,237], their contribution to the 'age-related' factors of reluctance to exercise and slowness in rising is difficult to identify in a species which does not verbalise its general aches and pains.

1.3 TREATMENT OF INTERVERTEBRAL DISC DISEASE

The treatment of intervertebral disc disease can be considered to comprise two general categories, based on the clinical effects of the disease. The differences in the therapeutic protocol reflect the chronic (more typical of non-chondrodystrophic dogs and some chondrodystrophic dogs) and acute (more typical of chondrodystrophic dogs) nature of disc disease.

Treatment of clinical signs related to simple intervertebral disc degeneration is aimed at relieving the clinical signs attributable to the disc as it bulges into the neural canal. The principle objective of this treatment is to minimise the protrusion of disc material into the neural canal, and thereby reduce the degree of spinal cord compression[222,232,243].

Treatment of dogs with sudden onset of neurological signs attributable to an explosive disc herniation is similar, but is complicated by the massive spinal injury which has occurred. In these cases, the objective of therapy is to attempt to control and limit the self-perpetuating cycle of events which develop in the spinal cord as a result of injury through the use of decompressive surgical techniques and pharmacologic manipulation[162,182,232]. Concomitant retrieval of disc material at the time of surgery is thought to permit a more rapid and complete resolution of clinical signs in these dogs.

Despite this slight difference in therapeutic philosophy for acute and chronic disc herniations, treatment of intervertebral disc disease is achieved, in variable degrees, by a combination of conservative and surgical regimes. The presenting condition of the animal, the experience of the clinician, the facilities available and the financial status of the client dictate the treatment ultimately given to the patient.

1.3.1 Conservative management

Conservative management of intervertebral disc disease consists of supportive nursing care and medical therapy. Nursing care is aimed at relieving further herniation of the degenerate disc by enforcing strict cage rest. The mechanical and chemical irritation of the spinal cord, nerve root and meninges by extruded disc material is also alleviated by this means. Resolution of mild clinical signs can frequently be achieved with this therapy although the herniated disc material remains extruded and may continue to contribute to recurrent clinical signs[22, 221, 232].

The medical management of intervertebral disc disease targets the inflammatory process incited in the spinal cord and surrounding tissues as a result of the distorted mechanical function of the disc[32, 55, 69]. Corticosteroids are most commonly used due to their potent anti-inflammatory action. The beneficial effect of corticosteroids is well accepted in the veterinary profession[162, 243, 258]. Most experimental work strongly supports the ability of corticosteroids to significantly improve the speed and completeness of the functional recovery following spinal cord injury in the dog[15, 32, 55, 69, 162]. Improvement can be noted even when the corticosteroid is given up to 24 hours after the injury[69, 162].

Corticosteroids appear to limit spinal cord damage by suppressing the development of oedema, enhancing the spinal cord blood flow, inhibiting the inflammatory response and stabilising cell membranes, thereby limiting the development of free oxygen radicals and preventing the release of lysosomal enzymes[69, 162]. Non-steroidal antiinflammatory products, for example aspirin and phenylbutazone, can provide palliative analgesic activity for mild intervertebral disc lesions[243].

1.3.2 Surgical management

The basis of surgical intervention following acute or chronic intervertebral disc herniation is to relieve excessive spinal cord compression and/or oedema. Retrieval of herniated disc material from the neural canal should be attempted, provided this does not induce significant iatrogenic spinal cord injury. The surgical management of intervertebral disc disease includes

decompressive procedures (laminectomy)[21, 56, 100, 153] and the removal of degenerated disc material from within the confines of the intervertebral disc (fenestration)[21, 24, 71, 92, 103, 221, 255, 308]. The indications for decompressive surgery include evidence of spinal cord compression on myelogram, spinal cord oedema sufficient to cause constriction by the neural canal, a lack of response to conservative management after 48 hours, or a sudden worsening in condition despite appropriate medical treatment[148, 256]. Techniques to achieve variable removal of the dorsal and dorsolateral bony walls of the neural canal have been described[99, 153].

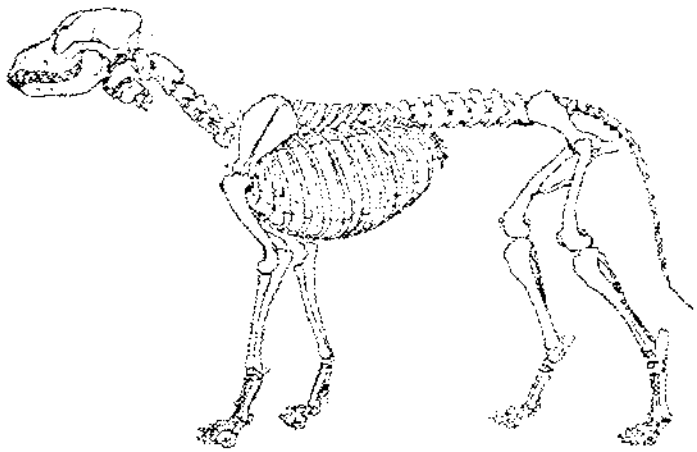
Many facets of intervertebral disc disease make an accurate assessment of the effect of treatment unachievable. Its variable presentation and course, its propensity for spontaneous resolution, the individual animal response to drugs and surgery, the varying abilities of surgeons and many other factors preclude accuracy in this assessment[259, 278]. However, it is reported that with judicial use of pharmacologic and surgical therapy, the chances of a paralysed dog regaining ambulatory function is greater than 50%[39]. Up to 90% success has been reported for intervertebral disc lesions when appropriate management is instituted within 48 hours of injury[39, 100, 132, 179]. Even greater success is reported for treatment of slowly developing disc lesions due to the propensity for the spinal cord to adjust without serious compromise[222].

Medical treatment of intervertebral disc disease is palliative only and recurrence of clinical signs is common[39]. Surgical relief of spinal compression, whilst frequently curative, is invasive and associated with considerable post-operative pain and management, potential complications and financial commitment. For these reasons, removal of the degenerate material from within the confines of the intervertebral disc is advocated for both the treatment and prevention of further episodes of this debilitating disease[92]. Surgical fenestration is the currently accepted method but considerable controversy surrounds its use[90].

The purpose of the present investigation is to compare surgical fenestration with a less invasive and less expensive technique, where the disc is enzymatically dissolved with a proteolytic enzyme administered percutaneously. This procedure, called chemonucleolysis, has been poorly accepted by the veterinary profession despite the favourable theoretical basis for its use in intervertebral disc disease[18, 19, 240]. Controversy surrounds its use in humans, despite reports of clinical success[65, 70].

To fully comprehend the problems associated with the treatment and prevention of intervertebral disc disease in the dog one must have an understanding of the structures of the vertebral column and their contribution to the biomechanical function of the spine. This information will be presented in the following chapters.

2.0



ANATOMY

2.1 INTRODUCTION

The mammalian skeleton can be divided into the axial part, which consists of the skull, the vertebral column, ribs and sternum, and the appendicular part, which comprises the bones of the limbs. The focus of this dissertation is on the vertebral column.

The vertebral column surrounds and protects the spinal cord. It is assembled from a number of individual building blocks, or vertebrae, of which there are at least thirty in the dog (not including the tail). A variety of ligaments and muscles join the individual units together and permit slight flexibility of the column, whilst assuring stability against excessive movement. Mobility between adjacent vertebrae is governed by a hydraulic joint, called the intervertebral disc and two diarthrodial joints, the articular facets.

For descriptive convenience the vertebral column can be divided into five sections: cervical, thoracic, lumbar, sacral and coccygeal (Figure 2.1). This discussion is mostly limited to the thoracic and lumbar regions and has, in the most part, been taken from three comprehensive anatomical reviews[70, 80, 278].

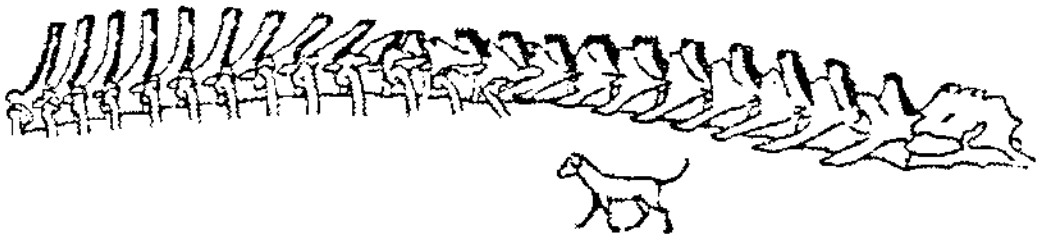


Figure 2.1: *The thoracolumbar spine of the dog.*

2.2 STRUCTURE OF THE VERTEBRA

2.2.1 Introduction

The dog has 7 cervical, 13 thoracic, 7 lumbar, 3 sacral and some 20 coccygeal vertebrae. Only numbers in the latter section are prone to significant variations in number. The total length of the vertebral column, and thus individual vertebrae, will of course vary greatly between breeds.

All vertebrae are composed of the same basic structure of a body, neural arch, articulations and processes. (Figure 2.2) As a reflection of the differing mechanical requirements, the vertebrae in a given region have certain anatomical characteristics which distinguish them from vertebrae of another region. Moreover, some vertebrae have distinct individual characteristics which allow them to be readily identified.

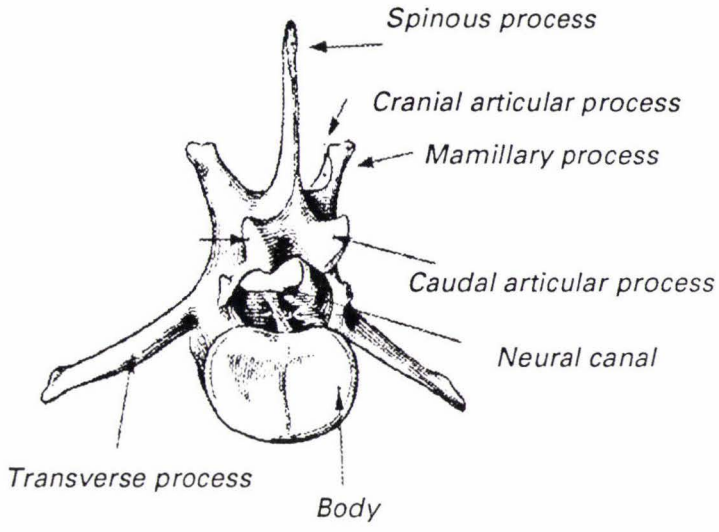


Figure 2.2: *Structure of the fifth lumbar vertebrae, caudal lateral aspect (from Evans and Christensen, Miller's Anatomy of the Dog[20])*

2.2.2 The vertebral body

The body of the vertebra (Figure 2.2a) is a cylindrical mass upon which other parts of the vertebra are constructed. The inner core of cancellous bone is surrounded by a shell of cortical bone. In cross section, the trabecular pattern of this inner core is roughly parallel to the long axis of the vertebral body.

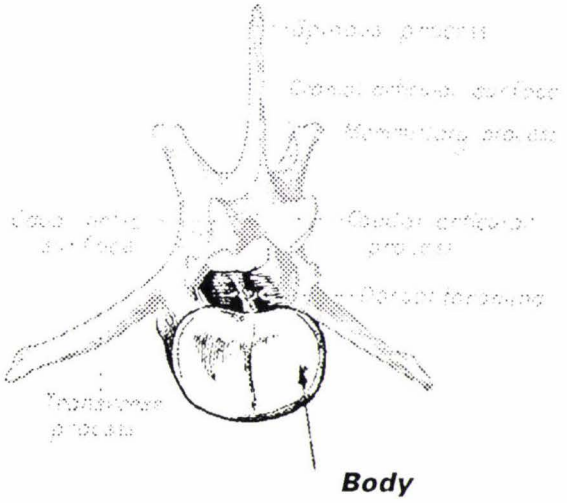


Figure 2.2a: *The Vertebral Body (adapted from Evans and Christensen, Miller's Anatomy of the Dog[20])*

The dorsal surface of the body tends to be a little flattened. This represents the ventral boundary of the neural canal, through which the spinal cord passes. The ventral and lateral aspects of the body are in close association with various muscles or mesothelial lining. The body is bounded cranially and caudally by the fibrocartilaginous intervertebral disc.

2.2.3 The neural arch

The neural arch (Figure 2.2b) is attached to the dorsal surface of the body. In contrast to man, in dogs it is bigger than the vertebral body, resulting in a comparatively spacious neural

canal in this species. Additionally, the size of the canal is proportionately wider in the smaller breeds of dog.

Each lateral half of the neural arch consists of a ventral pedicle and a dorsal lamina. Notches cut into the cranial and caudal borders of each ventral pedicle contribute to the formation of the vertebral foramina when paired with the ventral notch of the adjacent neural arch. Spinal nerves, lymph and blood vessels pass through each vertebral foramen.

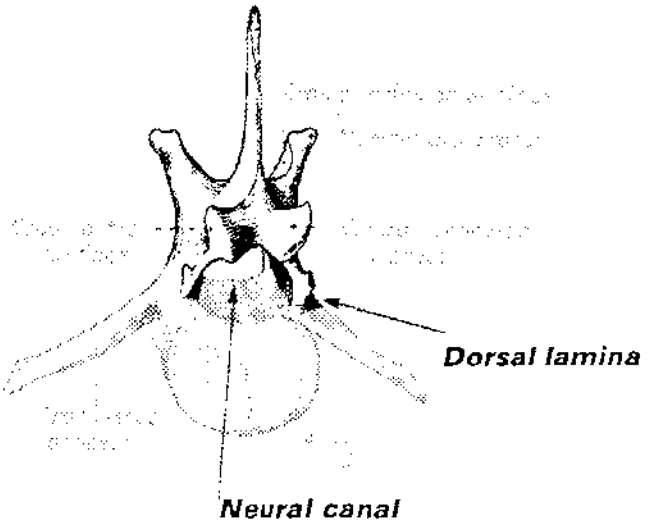


Figure 2.2b: *The Neural Arch (adapted from Evans and Christensen, Miller's Anatomy of the Dog[20])*

An important mechanical strut, the dorsal spinous process, projects dorsally from the centre of the neural arch. This structure will be discussed in more detail later. (Section 2.2.6)

2.2.4 Articular facet joints

The articular processes, or facets, project from either side of the borders of the arch (Figure 2.2c). Two processes are present cranially and two caudally. The cartilage-lined surfaces of the articular processes from adjacent vertebrae articulate with one another and are surrounded by synovial tissue. This is the only true diarthrodial joint in the vertebral column and its role in providing stability and limited flexibility to the spine has become the focus of increasing investigation.

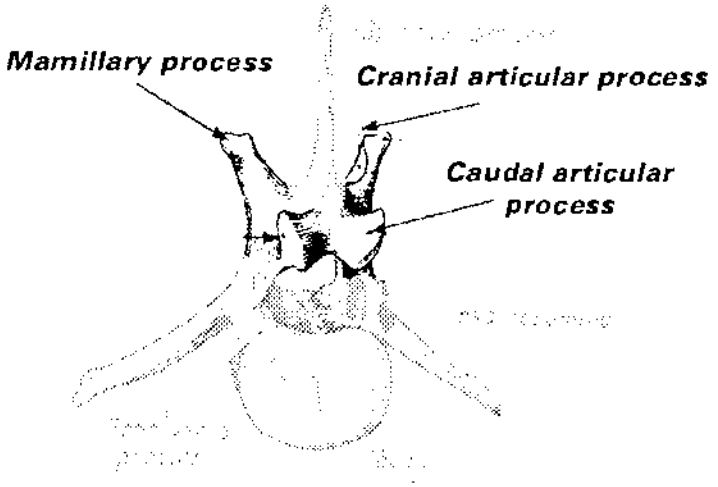


Figure 2.2c: *The articular facets (adapted from Evans and Christensen, Miller's Anatomy of the Dog[20])*

The orientation of the articular surfaces of the facets change at the tenth thoracic vertebra, the so-called diaphragmatic vertebra. At this site the horizontal orientation of the articular surface

of the thoracic vertebrae changes to a vertical direction. This transition, which occurs over the single vertebra defines the point on the vertebral column caudal to which rotation about the longitudinal axis is no longer possible[139, 261, 301]. The development of interlocking cranial and caudal accessory processes in the lumbar region, and the lack of the *rotatores mm.* (Section 2.5) combine to form a very efficient wedge system[261]. This renders significant torsional movements almost impossible in the lumbar region of the vertebral column[86].

2.2.5 Transverse process

Two transverse processes project laterally from the sides of the arch, or from the junction of the arch and the body (Figure 2.2d). In the cervical region only, each process is pierced by the transverse foramen which allows passage of the vertebral artery, vein and a plexus of sympathetic nerves. In the thoracic region each transverse process has a facet for articulation with the tubercle of a rib whilst in the lumbar region, the transverse processes are large and plate-like and are directed cranioventrally. Their size increases maximally to the sixth lumbar vertebrae.

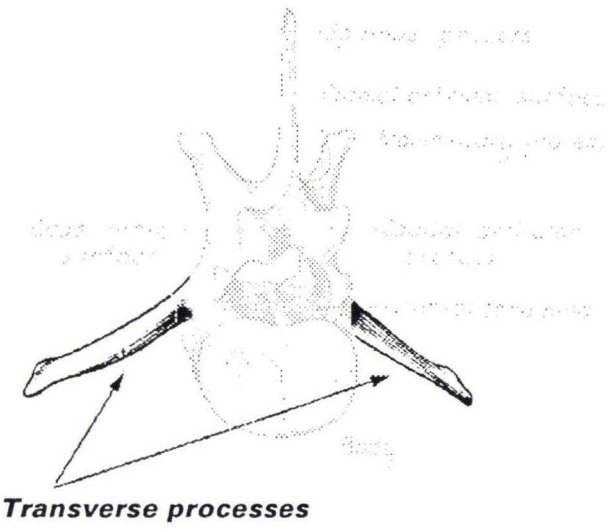


Figure 2.2d: *The transverse processes (adapted from Evans and Christensen, Miller's Anatomy of the Dog[80])*

The transverse processes provide important attachment sites for the surrounding epaxial muscles of the spine, including the *multifidus, longissimus* and *iliocostalis* muscles.

2.2.6 Dorsal spinous process

The dorsal spinous process projects from the middle of the neural arch. It varies greatly in form, size and direction in different vertebrae, due in part to its primary role as a mechanical strut for the insertion of ligaments and muscles.

The eleventh thoracic vertebrae is the anticlinal vertebrae (Figure 2.3) and marks the transition from backward sloping to forward sloping dorsal spinous processes. Animals with pronounced angulation of the dorsal spinous processes at the anticlinal vertebrae typically have flexible

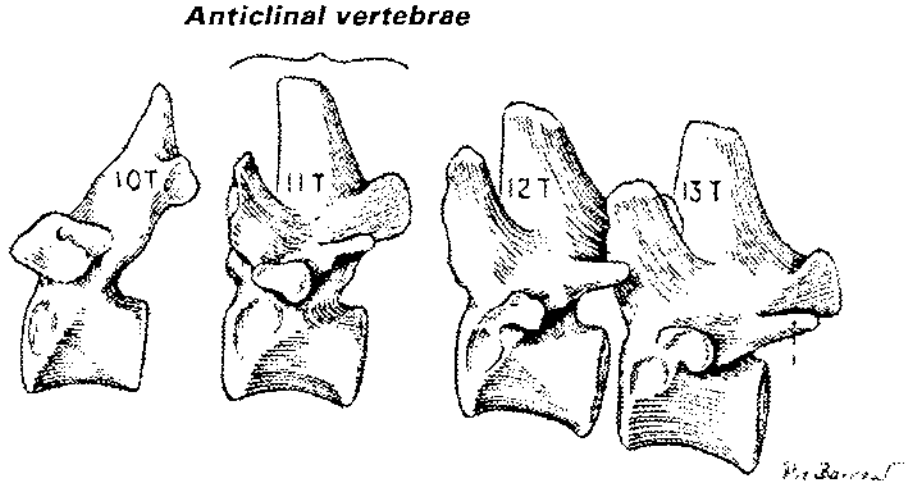


Figure 2.3: The anticlinal vertebra (from Evans and Christensen (1979)[80])

vertebral columns[161]. The greatest motion of the vertebral column occurs about this area[139, 261]. (see Section 3.3.2)

2.3 LIGAMENTS OF THE VERTEBRAL COLUMN

2.3.1 Introduction

The principle function of the ligaments in the vertebral column is to permit motion between individual vertebrae. They must also protect the spinal cord from injury by restricting this motion to well-defined limits[81, 226].

Ligaments are best suited to withstand tensile forces and, like rubber bands, they buckle when compressed[81, 302]. By surrounding the vertebral column with sets of ligaments, resistance to tensile forces can be assured even when it is subjected to loading from many different directions[81, 226, 301].

There are seven ligaments in the vertebral column; three long ligaments traversing the length of the spine and four short sets, which act across each intervertebral joint (Figure 2.4). The long and short ligaments will be described separately.

2.3.2 Long ligaments of the Vertebral Column

i) Ventral Longitudinal ligament: The ventral longitudinal ligament lies along the ventral surface of the vertebral bodies and passes from the axis to the sacrum. It is most distinct

over the thoracic region. Although firmly attached to the edges of the vertebral bodies, it is only loosely connected to the annular fibres of the intervertebral disc.

ii) Dorsal longitudinal ligament: The dorsal longitudinal ligament is larger than the ventral ligament and lies on the floor of the neural canal. It attaches to the rough ridges on the dorsum of the vertebral bodies and to the outer fibres of the intervertebral disc. This ligament is of variable width, being narrowest over the middle of the vertebral bodies and wider over the intervertebral disc spaces. It extends from the dens of the axis to the end of the vertebral canal in the coccygeal region.

iii) Nuchal ligament and the Supraspinous ligament: The nuchal ligament is a characteristically yellow, elastic ligament which attaches to the heavy spinous process of the axis and extends caudally, eventually intermingling with the supraspinous ligament to the level of the tenth thoracic vertebra.

The supraspinous ligament extends from the spinous process of the first thoracic vertebrae to the third coccygeal vertebrae. It is a heavy band especially in the thoracic region, and attaches to the apices of the spines as it passes from one to another. In the thoracolumbar region, the fibres of the supraspinous ligament blends with the dense lumbodorsal fascia.

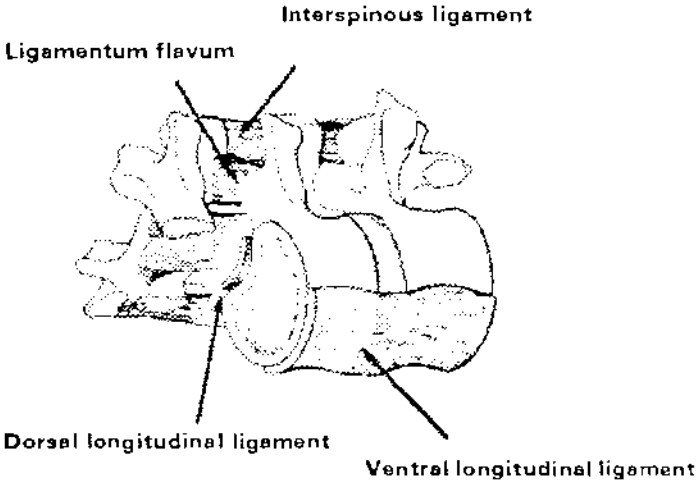


Figure 2.4: The ligaments of the vertebral column (from White and Panjabi (1978), Clinical Biomechanics of the Spine[23])

2.3.3 Short Ligaments of the Vertebral Column

i) Interspinous ligaments: The interspinous ligaments connect adjacent vertebral spines and consist of laterally compressed bands of fibrous tissue interspersed with muscle bundles of the *interspinalis mm.* These ligaments run from the base of the spines and decussate as they insert on the opposed caudal and cranial borders of adjacent spines near their tips.

ii) Intertransverse ligaments: These ligaments consist of bundles of fibres which unite the craniolaterally directed transverse processes of the lumbar vertebrae. In the dog, they are not distinct in other regions of the vertebral column.

iii) Ligamentum flavum (Interarcuate ligaments): This ligament is a thin elastic sheet which lies between the neighbouring arches of adjacent vertebrae, and is positioned dorsally in the neural canal. In the thoracic region it is narrow, but are considerably wider in the cervical section of the spine. Ventral to this ligament is the epidural space, which separates the ligaments and the arches of the vertebrae from the dura covering the spinal cord.

2.4 MUSCULATURE OF THE VERTEBRAL COLUMN

The function of the muscles that surround the vertebral column is to flex and extend the spine[105, 139, 261]. Lateral movements are possible when the muscles act on one side only. Extension of the spine is achieved by the action of the dorsal group of muscles, called the epaxial system. The ventral hypaxial system permits flexion of the spine. Though similar muscle groupings are present within all animal species, modification of the origin and insertion points of the muscles, as well as various fusions between individual muscle bellies, have occurred in different species in response to the locomotory demands of the animal[261]. These variations are covered in more detail elsewhere in this thesis (section 3.3.2).

2.4.1 The Epaxial Spinal Musculature

The dorsal epaxial muscle group comprises three longitudinal muscle masses which extend the length of the vertebral column. The most lateral muscle mass is the *m. iliocostalis* group and the *m. longissimus* system lies medial to this (Figure 2.5). The most medial group, the *m. transversospinalis* system rests between the articular facets and the dorsal spinous processes (Figure 2.6).

(i) Iliocostalis system: The iliocostalis muscle group (Figure 2.5) extends from the ilial wing to the seventh cervical vertebrae and can be divided into two regional divisions: the *m. iliocostalis lumborum* (lumbar region) and the *m. iliocostalis thoracis* (thoracic region). The *m. iliocostalis lumborum* originates from the pelvic surface of the ilial wing, the iliac crest and partly from an intermuscular septum, which itself has strong attachments to the lumbodorsal fascia. The cranial portion of the muscle is quite distinct from the *m. longissimus* whereas the most caudal portion of the *m. iliocostalis lumborum* is fused to the *m. longissimus* to form the *m. erector spinae*. The *m. iliocostalis lumborum* inserts, through fleshy serrations, on the thirteenth, twelfth, eleventh and tenth ribs. Occasionally a long, delicate tendon provides insertion onto the ninth rib. The *m. iliocostalis thoracis* has its origins deep to this lumbar portion. Individual segments arise from the cranial border of the vertebral end of the ribs, eventually fusing into one long,

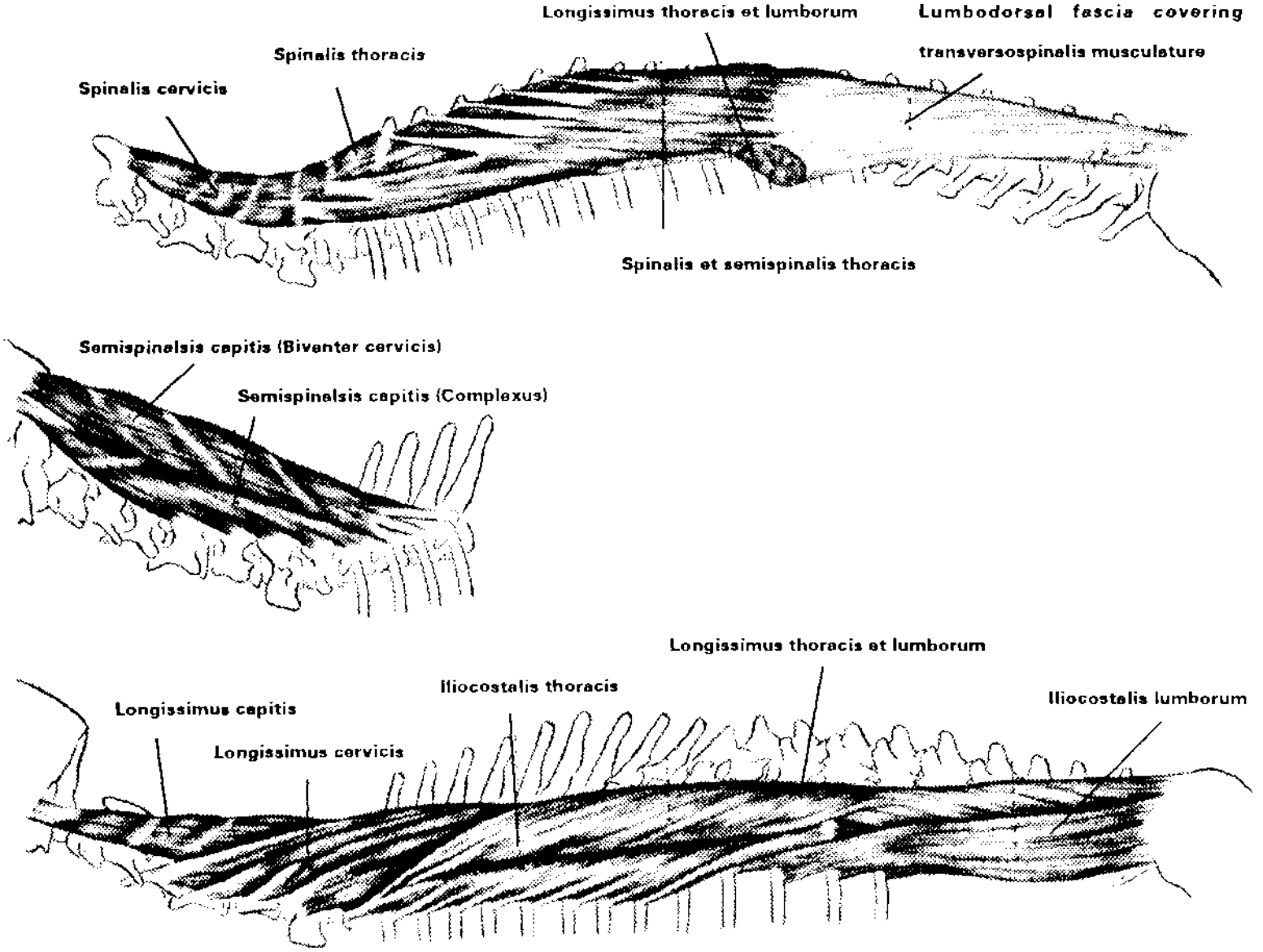


Figure 2.5: The superficial epaxial muscules of the dog. (from Evans and Christensen (1979))

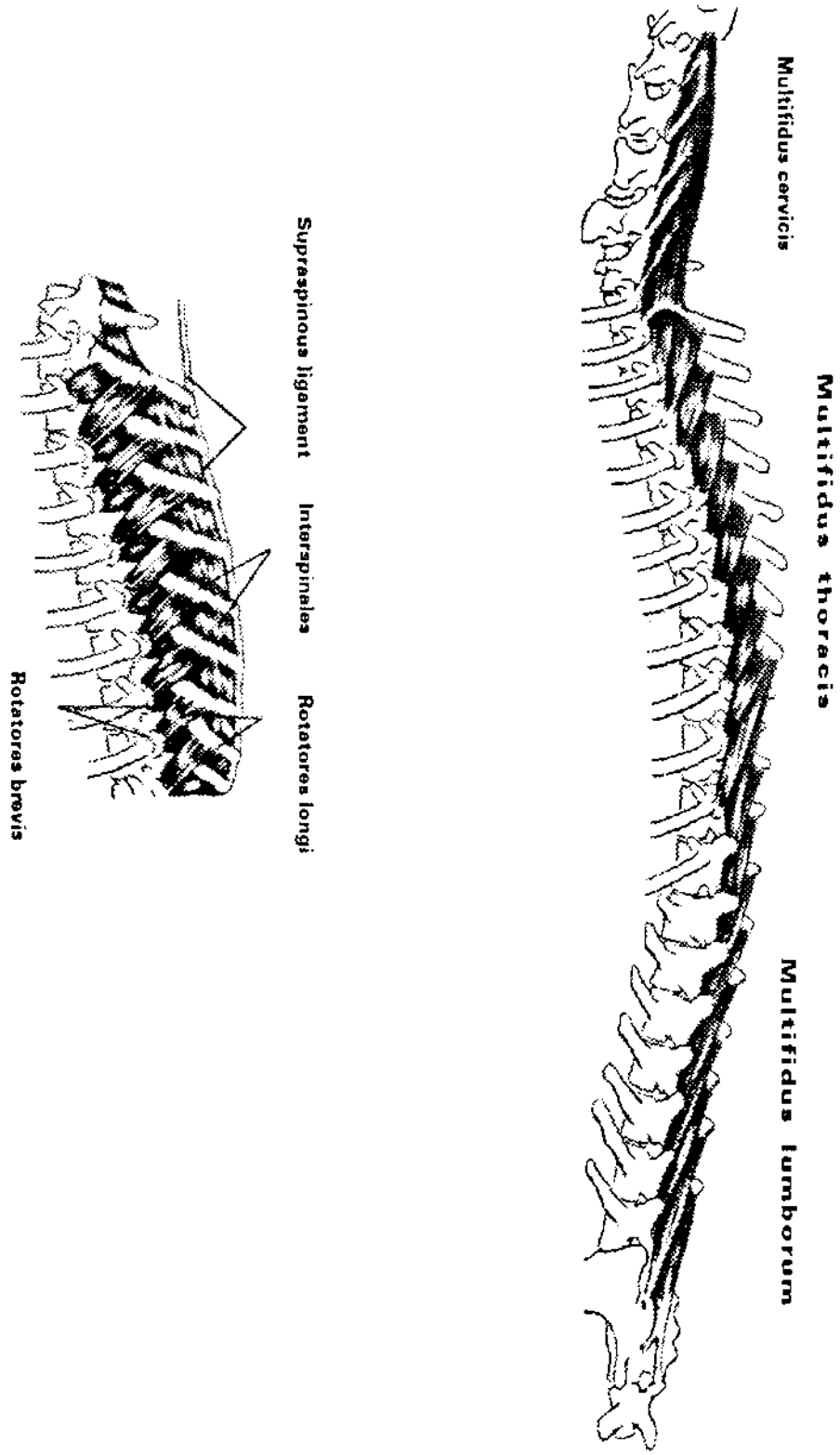


Figure 2.6: *The deep epaxial muscles of the dog. (from Evans and Christensen (1979)[20])*

narrow muscle belly. Terminal serrations arise from this belly to insert, via long tendons, onto the costal angle of the ribs and the transverse process of the seventh cervical vertebrae.

(ii) Longissimus system: This intermediate muscle group can be divided into three regional divisions: thoracolumbar, cervical and capital (Figure 2.5). The *m. longissimus thoracis et lumborum* is the strongest of the epaxial muscles and extends from the ilium to the caudal cervical vertebrae. Fibres also arise medially from the dorsal spinous processes and supraspinous ligament throughout its length. In the lumbar region the surface of the muscle is particularly aponeurotic and is divided into several distinct tendinous bands. Between the seventh and eleventh thoracic ribs this aponeurosis serves as an origin for the *m. spinalis* which courses cranially. Throughout its length, the *m. longissimus thoracis et lumborum* sends off serrated insertion fascicles to accessory processes, articular facets and vertebral margins of the ribs. The terminal insertion of the *m. longissimus thoracis et lumborum* is to the transverse process of the sixth and fifth cervical vertebrae and the longissimus system is continued in the cervical region by the *m. longissimus cervicus* and *m. longissimus capitus*. The former is composed of four serrations which insert on the transverse processes of the third to sixth cervical vertebrae. The *m. longissimus capitis* is a strong muscle which lies medial to the *m. longissimus cervicis*. It originates by separate bundles from the transverse processes of the first three thoracic vertebrae and the caudal articular process of the seventh to fourth cervical vertebrae. The muscle narrows cranially and inserts, by means of a strong tendon, on to the mastoid process of the temporal bone.

(iii) Transversospinalis system: This most medial and deepest epaxial muscle mass consists of a number of different fascicles which join one vertebra (Figure 2.6). Though the nomenclature varies between authors, the most consistently described members of this group include the *spinalis, semispinalis, multifidus, rotatores (longi et breves), interspinalis* and *intertransversarii* muscles. Due to the intimate relationship between the *spinalis* and *semispinalis* muscles, they are frequently described under the compound name: *m. spinalis et semispinalis*.

The *m. spinalis et semispinalis* arises from the aponeurosis of the *m. longissimus thoracis* and the spinous processes of the first few lumbar and first six thoracic vertebrae. Some fibres also arise from the mammary processes of the first two lumbar and last thoracic vertebrae. This muscle passes unsegmented in the thoracic region but divides into two muscles in the neck: the *m. biventer cervicis* which courses medially to insert ultimately in the external occipital protuberance and the *m. complexus* whose fibres run craniad to a tendinous attachment on the dorsal nuchal line. The *mm. multifidus* consists of lumbar, thoracic and cervical portions which lie on the sides of the vertebral spines. (Figure 2.6) This muscle consists of numerous bundles which originate from the transverse, articular and mamillary processes of the vertebrae. These fibres course

craniodorsally and attach to the caudal edge of the dorsal spinous process of at least the second vertebra craniad to the point of origin.

The *mm. rotatores* consists of long *(mm. rotatores longi)* and short *(mm. rotatores brevis)* bundles which lie deep to the multifidi in the cranial three-quarters of the thoracic region. (Figure 2.6) They are arranged like the multifidi but are more vertical in orientation: the long bundles pass between alternate vertebrae, whilst the short bundles run between adjacent vertebrae only. Due to the orientation of the articular facets in the cranial thoracic region, the action of these muscles results in rotary movements of the vertebral column.

The *mm. interspinales* are present in all regions of the vertebral column. (Figure 2.6) Their horizontal fibres pass between contiguous edges of the dorsal spinous processes and aid in fixing the vertebral column.

The *m. intertransversarii* are deep segments split off from the longissimus system and are present in all regions of the vertebral column. Their bundles span one or two vertebrae, passing between accessory, mamillary and transverse processes. This muscle group is more prominent in the cervical region.

2.4.2 The Hypaxial musculature

The muscles of the caudal ventral vertebral column include the *m. quadratus lumborum* and the *m. iliopsoas* (Figure 2.7). The *m. quadratus lumborum* originates from the last three thoracic vertebrae. This thoracic portion of the muscle is composed of incompletely isolated tendinous bundles but these become more fleshy as the muscle passes caudolaterally, inserting on the transverse processes of the lumbar vertebrae and the medial surface of the ilium. Throughout its length it is covered ventrally by the *m. iliopsoas*, but the lateral portion may overhang the transverse processes of the lumbar vertebrae.

The *m. iliopsoas* consists of the *m. psoas major* and the *m. iliacus*. (Figure 2.7) At its origin the *m. psoas major* is narrow and attaches to the last six lumbar vertebrae. As is continues caudally it becomes thicker and wider and is reinforced by muscle fibres from the ventral surface of the ilium which constitute the *m. iliacus*, and together these muscles insert onto the trochanter minor of the femur.

The *m. psoas minor* is the most ventral of the sublumbar muscles. (Figure 2.7) It is widest at its origin from the last thoracic vertebrae, which is more craniad than that of the *m. psoas major*. As is passes caudally it becomes narrower and more tendinous, to insert on the shaft of the ilium.

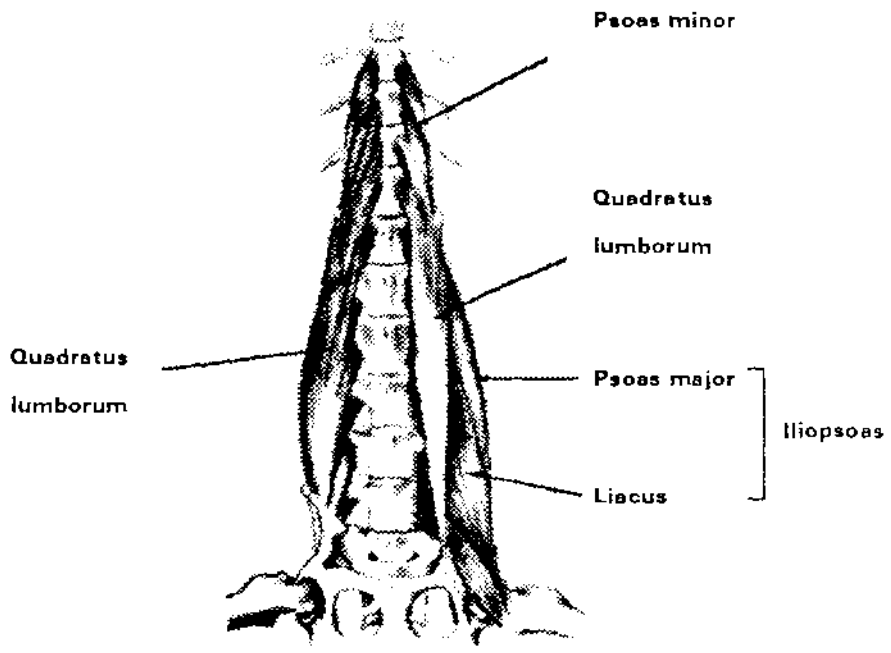


Figure 2.7: The hypaxial muscles of the dog (from Evans and Christensen (1979)[6])

2.5 THE SPINAL CORD

2.5.1 Meninges

The spinal cord is surrounded and protected by three covering layers: the *dura mater*, the arachnoid membrane and the *pia mater* (Figure 2.8).

The ***dura mater*** is the most superficial of the meningeal coats. It forms a tough, fibrous sheath that encloses the spinal cord and nerve roots. It accompanies the spinal nerve roots and is continuous with the periosteum at the intervertebral foramen. This attachment serves to anchor the spinal cord laterally. Caudally, in the sacral and coccygeal areas, the *dura mater* tapers in the shape of a cone and attaches to the periosteum of the caudal vertebrae. The epidural space lies between the *dura mater* and the inner lumen of the vertebral canal. This space is filled with epidural fat, but also contains the internal vertebral venous plexus.

The arachnoid membrane is a thin, almost transparent sheath that lies in contact with the inner surface of the *dura mater*. The arachnoid is connected to the deeper *pia mater* by web-like

connective tissue projections which pass across the subarachnoid space. This space is filled with cerebrospinal fluid which functions to protect the spinal cord during traumatic shock.

The *pia mater* is a highly vascularised layer which is firmly attached to the spinal cord and nerve roots, following the contours of these structures closely. The blood vessels passing within this layer give off branches which penetrate deep into the spinal cord. They are accompanied by the surrounding *pia mater* which ultimately forms the perivascular connective tissue. The *pia mater* is thickened along the lateral margin of the spinal cord, forming the dentate ligaments. Each of these has attachments to the *dura mater*. This arrangement effectively suspends the spinal cord in a liquid medium of cerebrospinal fluid.

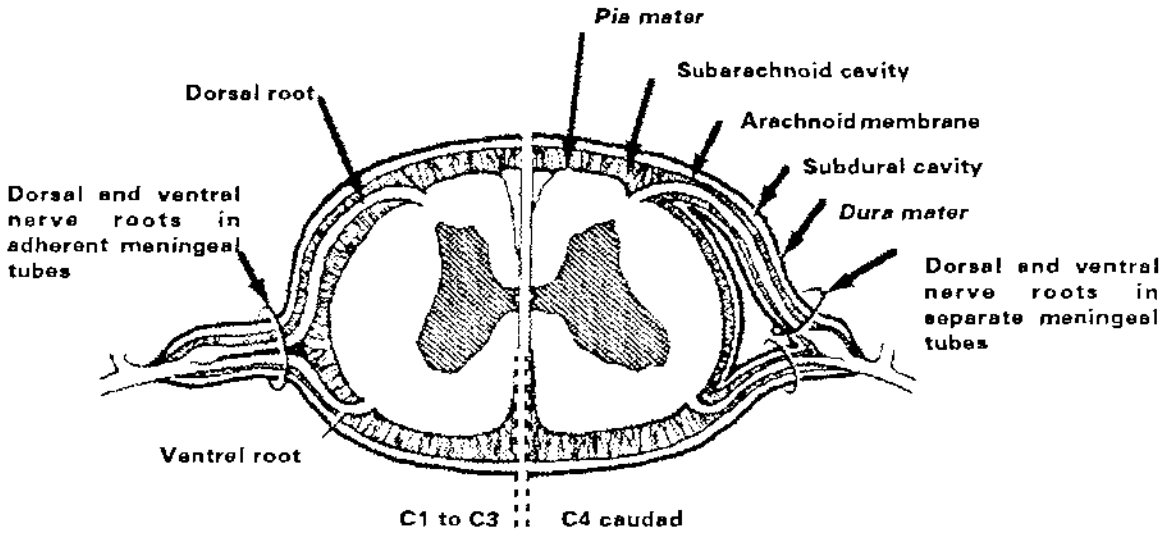


Figure 2.8: *A cross-section of the spinal cord showing the individual meningeal coats and related structures (from Evans and Christensen (1979), Miller's Anatomy of the Dog[40])*

2.5.2 Spinal cord

The spinal cord is a part of the central nervous system which is continuous with the *medulla oblongata* of the brain and exits from the cranium at the *foramen magnum*. Throughout its length, it is entirely enclosed within the vertebral canal. The spinal cord performs three general functions[53]:

1. It monitors receptors in the skin, muscles, joints and viscera and discharges impulses that control muscles and glands.
2. It functions as a reflex centre, continuously integrating afferent information and initiating appropriate responses in muscles and glands.

3. It conducts information to and from the brain through an elaborate system of fibre tracts, by which the central nervous system can regulate posture, movement, secretion and afferent activity.

As with other components of the vertebral column the spinal cord can be broken into regional sections: the cervical, thoracic, lumbar, sacral and caudal (or coccygeal) parts. Further to this, the spinal cord can also be broken into segments named according to where a pair of spinal cord nerve roots enter and leave the cord. Thus, there are eight cervical (C1 - C8), thirteen thoracic (T1 - T13), seven lumbar (L1 - L7) and three sacral (S1 - S3) segments. The segments between C_6 to T_2 and L_4 to S_1 contribute nerve roots to the fore and hind limbs respectively. The cord is swollen at these sites due to the high numbers of nerve fibres and cells.

The spinal cord is divided into symmetrical halves by the dorsal and ventral median fissures. The ventral fissure is better developed in most species and distinct separation of the spinal cord into two ventral funiculi is detectable. In the dorsal region, only a thin membrane of *pia mater* extends ventrally from the dorsal median fissure into the grey matter encircling the central canal, thus segregating the two dorsal funiculi. The lateral margins of the dorsal and ventral funiculi are delineated by the emergence of the nerve rootlets. An intermediate groove in the cranial thoracic and cervical regions of the cord, divides the dorsal funiculus into gracile (medial) and cuneate (lateral) funiculi.

In transverse section, the central grey matter of the spinal cord, so named because of its gross colouration, displays a butterfly shape - a central zone joining two wings. The central intermediate substance has at its core the central canal, which contains cerebrospinal fluid and a proteinaceous material, Reissner's fibres. Immediately surrounding the central canal is a concentrated accumulation of glial cells, and this region is termed the *substantia gelatinosa centralis*. Each wing of the grey matter is divided into ventral and dorsal horns. Occasionally, the overlap between the ventral and dorsal horns is referred to as the lateral intermediate substance. This is continuous with the central region.

The cell bodies of the peripheral nerves are located within the grey matter of the spinal cord. Descending axonal fibres from the cerebral cortex interface with these cells and transmit motor control of the peripheral nerves. Sensory fibres from the body also terminate in the grey matter. Extensive ramifications between cells allow coordination of movement by a feed-back mechanism. The grey matter can therefore be viewed as an active electrical 'switchboard' for signals coming into a specific spinal cord segment. Reflex movements are triggered through a locally acting arc within the grey matter, following input from receptors in the body via the sensory dorsal nerve

roots. The segmental pattern of innervation to the body is an important concept in the identification of spinal cord injury, and can be used to specifically localise the site of the lesion. The high cellular content of the grey matter is nourished by an abundant capillary bed.

The white matter of the spinal cord completely surrounds the grey matter. It is composed of many densely packed axons, whose lipid-rich myelin sheaths furnish the colour for which this tissue is named. Within each segment, the white matter is composed of nerve fibre tracts which comprise the dorsal and ventral nerve roots, and the longitudinal fibre tracts which carry information to and from the brain. These long tracts can be more or less divided into bundles (or *fasciculi*) which are closely related functionally and are named according to the site of origin and termination of the fibres. In general, the ascending (sensory) tracts tend to be located more peripherally about the spinal cord than the descending (motor) tracts. Only the major tracts considered to have the greatest clinical significance will be considered in this review.

(i) Sensory (ascending) tracts

Three main sensory tracts will be discussed; the spinocerebellar, dorsal and spinothalamic tracts (Figure 2.9) . Apart from the more complex spinothalamic tract, information is generally carried on the same (ipsilateral) side of the spinal cord, ultimately crossing in the mid-brain region.

The spinocerebellar tract consists of about four discrete fibre bundles ascending in the lateral funiculus of the white matter. These fibre bundles carry unconscious proprioceptive information to the cerebellum, and facilitate the coordination and regulation of motor function. Injury to these tracts can result in ataxia and incoordination of the gait. Due to the superficial location of these fibre tracts in the spinal cord, these clinical signs are among the first to develop following progressive compression of the spinal cord. Furthermore, tracts from the hind limbs are carried more superficially in the cord making them more susceptible to injury.

The dorsal sensory tracts are located in the dorsal funiculus of the spinal cord and carry conscious proprioception information to the cerebral cortex, enabling the animal to correct the limbs when placed in abnormal positions relative to the body. Some large pain fibres, producing sharp, localised pain may also ascend in this system. Division into fore and hind limb tracts is again apparent, with the *fasciculus gracilis* (from hind limb and tail) passing medial to the *fasciculus cuneatus* (from fore limb and neck), in some places separated by the dorsal intermediate septum. Lesions in the dorsal funiculus produce ipsilateral proprioceptive deficits in the affected limbs.

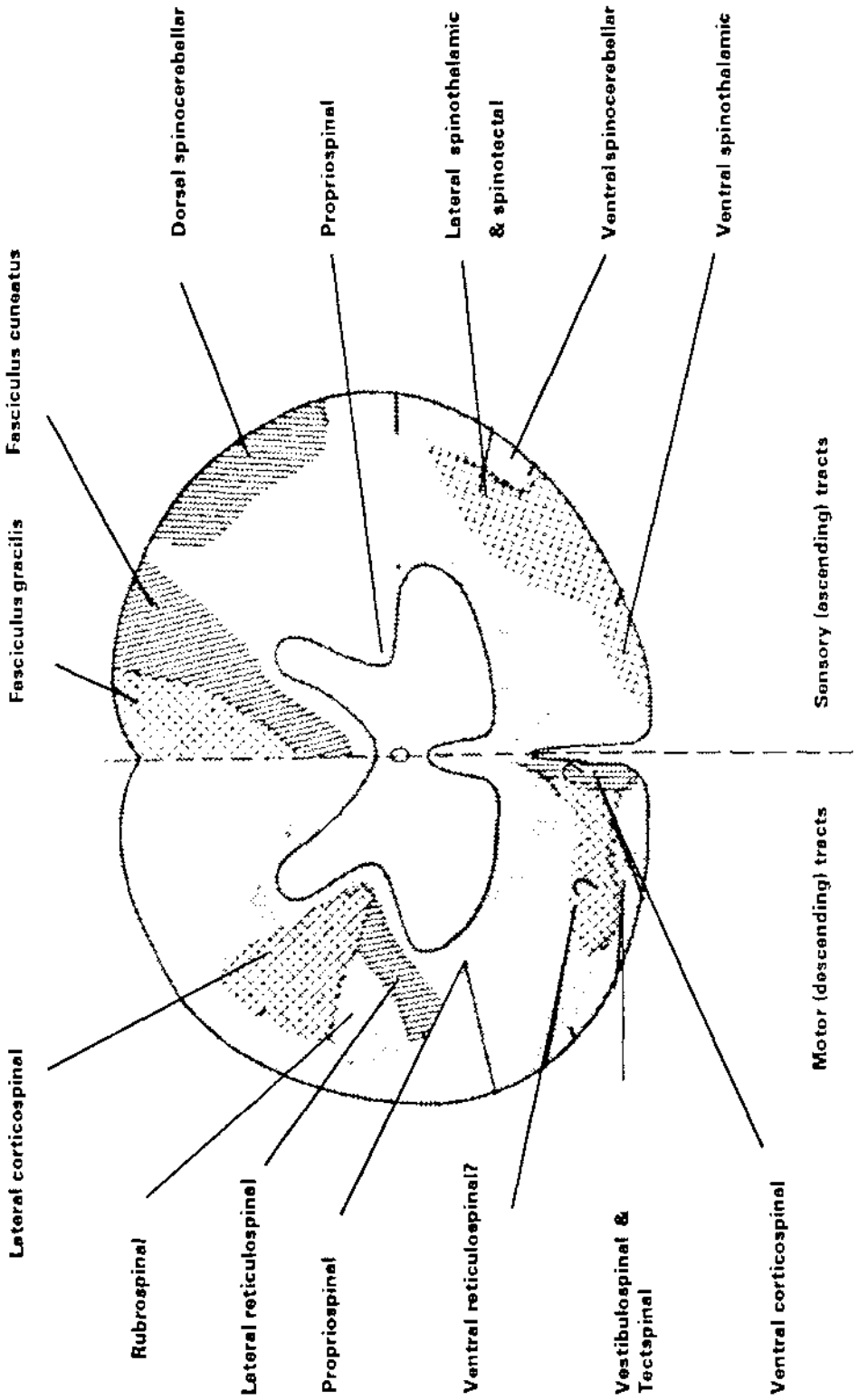


Figure 2.9: *Cross-section of the spinal cord showing the relative positions of the sensory (ascending) and motor (descending) tracts (from Chrisman CL (1982), Problems in Small Animal Neurology[63])*

The third sensory system is the spinothalamic tract, which carries fibres responsible for the dull, non-localised ache characteristic of deep pain. This system is better developed in animals than man, and comprises a complex, multisynaptic pathway, with frequent crossing-over of the related fibre tracts. Extensive ramifications of the spinothalamic tracts within the reticular system of the mid-brain trigger motivationally affected processes, through connections with the autonomic reflex centres, limbic system (emotional control) and the cerebral cortex. In contrast to other sensory tracts, the spinothalamic tract is located deep within the ventral funiculus of the spinal cord. Consequently, interference to deep pain sensation occurs only after severe bilateral, deep spinal cord disease.

(ii) Motor (descending tracts)

The motor tracts can be divided into two functional groups: those that supply muscles of voluntary activity (flexors), and those supplying the muscles of postural support and gravitational-resistance (extensors). Two tracts in each group are considered of clinical significance: the rubrospinal and corticospinal tracts (voluntary activity) and the vestibulospinal and reticulospinal tracts (postural/involuntary activity) (Figure 2.9).

The rubrospinal tract descends from the red nucleus of the mid-brain. Crossing immediately, it travels down the spinal cord on the same side to which it innervates, passing medial to the spinocerebellar tracts. The rubrospinal tract is the most important motor tract for voluntary movement. Loss of function following spinal injury results in ipsilateral paresis and paralysis. In progressive external compression of the spinal cord, these signs usually follow ataxia.

The corticospinal tract, also known as the pyramidal tract, originates from the motor area of the frontal lobe of the cerebral cortex. While this tract is very important in man, its significance in other animals is less apparent. The corticospinal tract crosses over at the level of the mid-brain, and descends in the lateral funiculus of the spinal cord. Because it is situated quite near the rubrospinal tract, these two tracts are frequently affected together in spinal cord compression.

The vestibulospinal and reticulospinal tracts maintain postural tone within the body, allowing it to actively resist the effects of gravity. Involuntary alterations in muscle tone ensure preservation of balance following the initiation of a voluntary movement. This control is achieved through extensive connections with the vestibular system of the ear, cerebellum, and ascending spinoreticular systems.

The vestibulospinal tract arises from the vestibular nuclei of the mid-brain following multisynaptic connections with the oculomotor nerve providing sensory input from the balancing system of

the inner ear. This tract descends uncrossed in the ventral funiculus of the spinal cord. Loss of function in these tracts, which can happen early in the course of spinal compression, results in the loss of ability by the animal to support its own weight (ie weakness).

The reticulospinal tracts also arise in the midbrain and descend uncrossed in the lateral funiculus of the spinal cord. Though primarily associated with maintenance of postural tone, some parts of the tract synapse with, and influence the effect of, the voluntary motor tracts.

2.5.3 Blood supply to the Spinal cord

(i) Arterial supply: In almost all species, three arteries traverse the entire length of the spinal cord: one is positioned ventrally and the other two dorsolaterally. In the dog, these arteries are continuous throughout their length, but appear to narrow significantly in the thoracic regions.

The **ventral spinal artery** lies at the base of the ventral median fissure. It is supplied by radicular branches (*rami spinales)* which accompany the spinal nerve through the intervertebral foramen of each vertebra. In the cervical region the radicular branches are derived from the vertebral artery as it passes through the transverse foramen of the cervical vertebrae. These radicular branches, in the thoracic region, are supplied by the dorsal intercostal and, for the first three thoracic vertebrae only, the thoracic vertebral artery. In the lumbar region, the lumbar artery branches off the abdominal aorta. It divides, and one division, the ventral spinal branch, passes through the intervertebral foramen to anastomose with the ventral spinal artery.

The number of radicular branches supplying the ventral spinal artery varies widely between sections of the spinal cord. The cervical section has the greatest contribution of radicular branches, with on average 14.2 of a possible 16 spinal arteries (88.8%) penetrating the intervertebral foramen from the vertebral artery. The thoracic region in contrast is poorly supplied, with less than a third of the 26 segments receiving radicular supply. The lumbar region received only 45% of the possible maximum 14 spinal arteries, but frequently a large artery can enter the L_5 intervertebral foramen (usually unilaterally).

The two **dorsolateral spinal arteries** receive radicular contributions from the ventral spinal artery. The relative number of anastomotic branches in each section of the spinal cord shows a similar pattern of distribution to that previously described, with 88.1%, 49.6% and 60% of the maximum occurring in the cervical, thoracic and lumbar regions of the spinal cord respectively.

The parenchyma of the spinal cord is supplied by central arteries which arise from the dorsal and ventral spinal arteries. The central arteries are thin, tortuous vessels which may supply only the left, only the right, or both halves of the spinal cord in an apparently random fashion. This irregularity is most commonly observed in the thoracic region, where up to three unilateral branches may be observed sequentially. An arterial density of 4.5 arteries/cm^2 is described in this region. In the cervical and lumbar regions, a more alternate pattern of arterial distribution is usual, with an arterial density of about 6.0 arteries/cm^2.

Normal spinal blood flow to the grey matter of the cord is approximately five times that of white matter reflecting the high metabolic requirement of this cellular region. This level of blood flow is maintained within fairly narrow limits, despite fluctuations in mean arterial blood pressure. This autoregulation of blood flow in the spinal cord is less efficient than the cerebral arrangement, and begins to fluctuate markedly at meao arterial pressures below 50 mm Hg, stopping altogether at 30 mm Hg. Blood flow also varies directly with the Pa_{CO2}. Autoregulation will also be affected by spinal cord trauma[55, 162].

The significant variability in the location, size and arrangement of the arterial supply to the spinal cord of the dog (and indeed, all animals) may be an important factor in the ability of the spinal cord to respond appropriately to a pathological stimulus. This fact will ultimately influence the degree and severity of the resultant ischaemia in the spinal cord, and therefore the degree of secondary disruption to spinal cord function[32, 55, 69, 162].

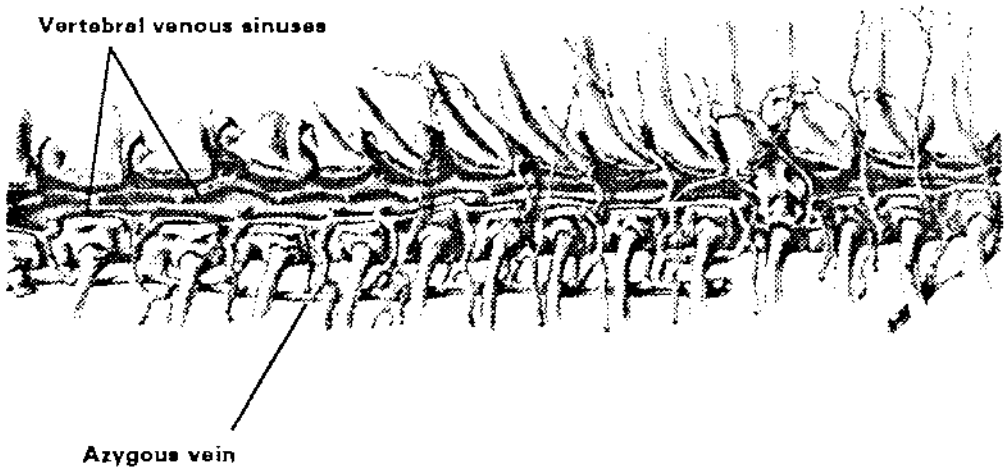


Figure 2.10: Thoracic and Lumbar vertebral veins, right lateral aspect (from Evans and Christensen (1979), Miller's Anatomy of the Dog[85])

(ii) Venous drainage: Venous drainage of the spinal cord is accomplished by the paired longitudinal vertebral venous sinuses, which extend from the skull to the caudal vertebrae (Figure 2.10). These thin-walled, flattened, valve-less vessels lie on the floor of the vertebral canal, nestled in the epidural fat. As they pass over the vertebral bodies, the left and right vertebral sinuses widen and approach each other, but diverge over the intervertebral spaces. Frequent anastomoses occur between the left and right channels. Significant contributions to the venous sinuses are also provided by the interarcuate branches in the cervical and thoracic regions. These branches drain blood from the epaxial musculature and enter the spinal canal dorsally, through the interarcuate space. No interarcuate veins have been recorded between the ninth thoracic and seventh lumbar vertebrae, nor in the caudal section.

Communication between the internal vertebral sinuses and the extravertebral veins is provided by the intervertebral veins, which pass through every intervertebral foramen on both sides of the spinal cord. Frequently these branches are double, providing a vascular cushion for the emerging spinal nerve which lies between them. The intervertebral veins will subsequently anastomose with the main central veins adjacent to the emergent region, for example, the vertebral, thoracic vertebral, azygous, caudal vena cava, internal iliac and gluteal veins. Because of this broad anastomotic organisation, the vertebral venous sinus system can act as an alternate route for the return of blood to the heart, effectively bypassing the caval system. Being valveless, blood within the spinal (and cerebral) venous sinuses can flow cranially or caudally, depending on pressure relations.

2.7 THE ANATOMY OF THE INTERVERTEBRAL DISC

2.7.1 Introduction

An intervertebral disc exists between each pair of vertebrae along the entire length of the spinal column (except between C_1 and C_2). The disc functions as a deformable section in the spine, permitting slight mobility to occur between individual vertebrae. The thickness of the disc is greatest in the cervical and lumbar regions, with the thickest being between the most caudal of the cervical vertebrae[126].

The external appearance of the intervertebral disc offers little insight to the inner structure and function of this small, but significant tissue. Dorsally and ventrally, the outer portions of the intervertebral disc are bound by, and in places continuous with, the dorsal and ventral longitudinal ligaments respectively[80,126]. Laterally, the smooth fibrocartilaginous surface of the disc is visible.

Sectioning in the median plane reveals three distinct anatomical regions within the disc: the annulus fibrosus, the nucleus pulposus and two cartilaginous end plates[126] (Figure 2.11). Each distinctive portion imparts a unique functional characteristic to the disc. Collectively, this results in a structure which is capable of resisting and providing stability against deforming loads in six directions, yet still permitting flexibility of the spine when these loads are within physiological limits[142].

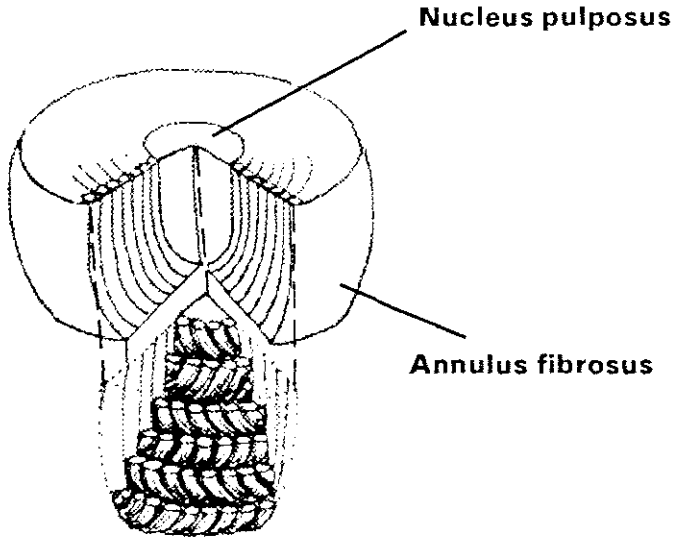


Figure 2.11: *The Intervertebral Disc. Note the alternating arrangement of the annular lamellae (stylised) as they pass between the end plates.*

2.7.2 GROSS ANATOMY OF THE INTERVERTEBRAL DISC

(i) Annulus Fibrosus

The annulus fibrosus (Figure 2.12) is a fibrous basket that envelops the nucleus pulposus. In transverse section, it appears as concentric rings of fibrous tissue, encircling the nucleus pulposus. When transected in the median plane, these rings impart a banded appearance to the annulus[104, 126, 197]. Each parallel layer is firmly anchored into the neighbouring cartilaginous end plates. The obliquity of each layer increases towards the centre of the intervertebral disc and, in the depression revealed when the nucleus pulposus is removed, a mesh-like pattern can be discerned.[126] At the periphery of the annulus, the layers become parallel to, and continuous with, the fibres of the ventral and dorsal longitudinal ligaments[52, 57, 126, 197].

(ii) Nucleus pulposus

The nucleus pulposus (Figure 2.12) is an embryological remnant of the notochord[57, 60], which is an early phylogenetic development of the back-bone[274]. In a young animal, the nucleus pulposus is a gelatinous globule, slightly translucent in colour, persistently sweating moisture from its cut surface[57, 126]. In most dogs, there is a distinct demarcation from the annulus fibrosus, and the nucleus pulposus can be scooped out of a shallow depression in this surrounding fibrous basket. The nucleus pulposus is bounded dorsally and ventrally by the annulus fibrosus, but lies in close contact with the cartilaginous end plates at the cranial and caudal boundaries[57, 90]. In the cervical and lumbar regions especially, the nucleus pulposus is located slightly eccentrically in the intervertebral disc su that the ventral portion of the annulus fibrosus is two to three times as wide as the dorsal portion[57, 169].

(iii) Cartilaginous End Plates

The cartilaginous end plates (Figure 2.12) represent the cranial and caudal boundaries of the intervertebral disc and are in contact with the associated vertebral body. In the young animal, the surface of the cartilaginous end plates is lined with a soft, translucent material which resembles hyaline (or articular) cartilage[57]. Being about 1 - 2 mm thick at the periphery, this cartilaginous surface thins towards the middle of the disc and may become barely discernible. A slight concavity in this central portion of each cartilaginous end plate coincides with the zone where the nucleus pulposus lies in intimate contact with it[57].

2.7.3 MICROSCOPIC ANATOMY OF THE INTERVERTEBRAL DISC

(i) Annulus Fibrosus

The banded structures visible in the gross specimen are revealed under the microscope to be a series of intricate fibrocartilaginous lamellae, each composed of numerous parallel fibrous bundles. Each lamella arises from the cartilaginous end plate and surrounding vertebral body, and runs a roughly parallel course between adjacent vertebrae[60, 104, 128]. Each lamella is quite separate and distinct from another, and no interconnection is visible at the optical microscope level[159, 197].The directional arrangement of the fibrous bundles alternates in sequential lamellae. (Figure 2.12)

The number and thickness of the lamellar layers in the annulus fibrosus of dogs is not known, but in human lumbar discs between 15 and 38 distinct lamella layers have been reported[52, 60, 128, 159, 197]. These layers are thinnest in the lateral parts of the disc and thickest in the dorsal and ventral areas. The lamellae become thicker in aged specimens[52, 128, 159, 197, 288].

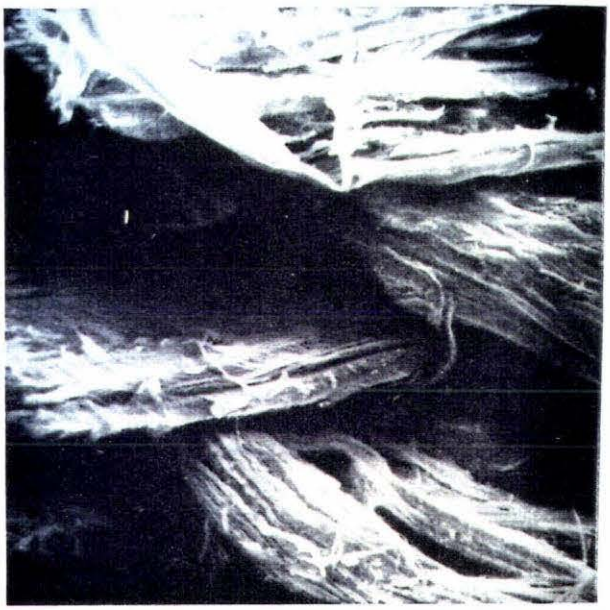


Figure 2.12: *Scanning electron micrograph of the annulus fibrosus. The individual lamellae can be distinctly seen. Note the alternating arrangement of the collagen fibre direction. (from Inoue (1973) Arch Histol Jpn 36: 39 - 56)*

On average, about 53% of the lamellar bands in the human lumbar intervertebral disc do not form complete rings around the nucleus pulposus and this percentage increases significantly in the older disc[197]. These bands are interrupted most frequently at the dorsolateral aspects of the annulus fibrosus and it is suggested that the high level of lamellar discontinuity may induce an inherent weakness at this point[197].

The lamellae are composed of numerous fibrillar bundles which are uniformly arranged within each layer. The thickness of individual fibril bundles in the human intervertebral disc have been variably described to range from 0.03 mm - 140 mm[60, 128, 138, 159, 288], and so it is clearly apparent that gross inadequacies exist in this aspect of knowledge on the intervertebral disc.

Elastic fibres passing between the lamellar layers of the annulus fibrosus have been described in the intervertebral disc of man[42, 110, 128, 138]. These structures are only visible at the electron microscopic level and occur in all regions of the intervertebral disc. Johnson et al (1982)[164] described a three-dimensional elastic fibre meshwork, with the fibres arranged circularly, longitudinally and obliquely within the lamellae of the annulus fibrosus. Individual elastic fibres pass between the lamellar layers and it is considered that they impart some dynamic flexibility to the tissue. The elastic mesh is better developed in regions of the disc which connect to the adjacent vertebrae, and thus are less commonly observed in the inner regions of the disc. Similar investigations have not been conducted in the dog.

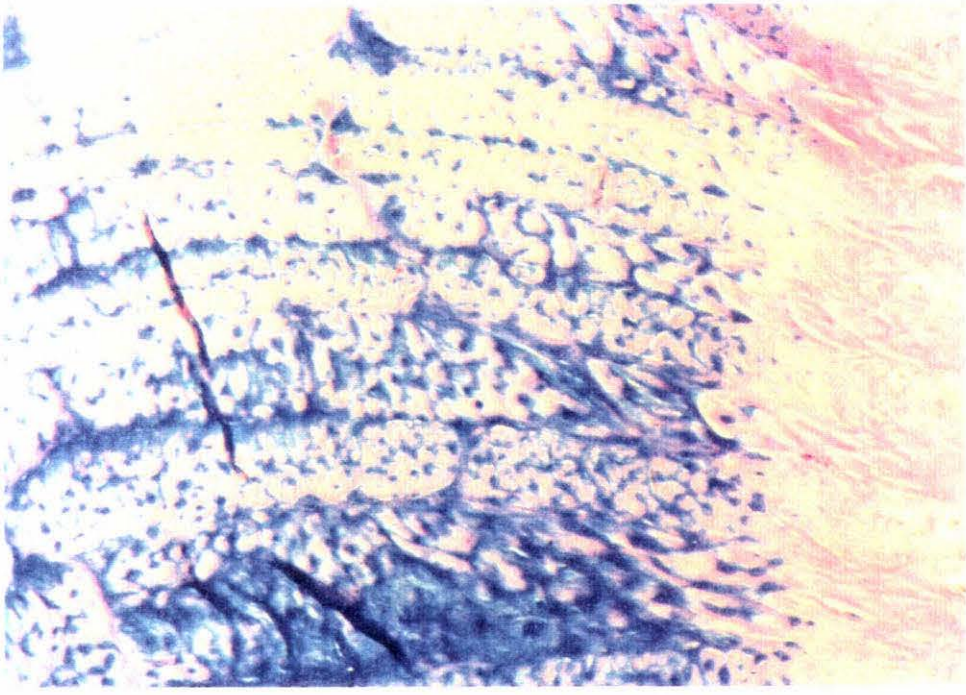


Figure 2.13: *Photomicrograph (40x) of the normal annulus fibrosus, showing the junction between the lamellae and the cartilaginous end plate (CEP). Note the intense staining with alcian blue between the individual lamellae.*

Fibres from the annulus fibrosus insert directly into surrounding anatomical structures to form stabilising attachments[60] (Figure 2.13) . Some of the fibres pass from the outer two-thirds of the annulus fibrosus to become interwoven with the lamellae of the bony trabeculae of the vertebral bodies, thereby forming a strong bond between the two structures[159]. This intimate weaving of fibrous elements of connective tissue and bone is typical of most soft:hard tissue attachments in the body, and are known as Sharpey's fibres[60]. Other fibres from the more peripheral regions of the annulus fibrosus intermingle with the fibre bundles of the dorsal and ventral longitudinal ligaments, and with the periosteal fibres of the vertebral bodies[60, 104]. Still others, from the inner third of the annulus fibrosus, attach to the cartilaginous end plate of the vertebral body by becoming intermingled with the collagen fibres of this structure[159].

The fibrous tissue of the annulus fibrosus is produced and maintained by the cellular elements which are located between fibrous bundles[57, 60]. The cells are bi-convex in shape and are typical of fibroblasts found in other tissues about the body[57, 60, 126]. Under electron microscopy, fibrils can be observed to stream off from the ends of the cells into the general matrix. Towards the inner margins of the annulus, cellular elements become comparatively more numerous and the nucleus of the cell becomes bigger, and slightly rounded in appearance. Enmeshed in the fibrils are dense granules which also occur to a lesser extent in the intercellular matrix[159]. These granules have been identified as proteoglycan[16]. Whilst this substance is present in all regions of the annulus fibrosus, it is predominantly localised pericellularly[12, 16].

The peripheral third of the annulus fibrosus in the dog and man is innervated by a number of fine nerve endings[23, 60, 94, 160]. Dissection of the human lumbar intervertebral disc has revealed that the posterior (dorsal) region of the annulus fibrosus is innervated by branches from the sinuvertebral nerve which also supplies other structures in the vertebral canal. Innervation of the lateral and anterior (ventral) regions of the disc come from multiple branches of the ventral primary rami which arises from the caudal lumbar plexus. Other branches may also arise from the sympathetic trunk. In one study, these nerve endings were observed to penetrate to a depth equivalent to one third the total thickness of the annulus fibrosus. No nerve endings have been described in the inner regions of the annulus fibrosus or nucleus pulposus.

(ii) Nucleus Pulposus

Microscopically, the demarcation between the annulus fibrosus and the nucleus pulposus is less distinct than that observed grossly[126]. The lamellae of the annulus fibrosus progressively become more disorganised at the transitional zone between these two regions and eventually disintegrates into an irregular, three-dimensional lattice of collagen fibres[126]. Ground substance is present in greater quantity than seen in the annulus fibrosus, resulting in wide spaces between individual fibrils[57, 60]. Granules of ground substance can frequently be observed adherent to the fibrils[159].

The fibrils of the nucleus pulposus insert directly into the cartilaginous end plates, through interconnections similar to that described for the inner regions of the annulus fibrosus[159]. A more orderly arrangement of fibres is present in the area immediately adjacent to the cartilaginous end plate[52, 159].

Similar deficiencies as those described for the annulus fibrosus exist in the recording of diameter of the fibrils of the nucleus pulposus. Nevertheless, it is generally agreed that the fibrils of the nucleus are finer in diameter than those of the annulus fibrosus[57, 60, 159]. Fibril diameter is

reported to increase with age in the nucleus pulposus[57,58,59]. These findings are consistent with the trends previously reported for the annulus fibrosus.

Water is the principal constituent of the nucleus pulposus and in early life, a water content of 80 - 88% is frequently reported[57,60,135]. Water is attracted to, and bound within, the disc by the proteoglycan constituents of the ground substance[49,82,135,214]. In the human, diurnal variations in the height of the intervertebral disc, as measured by radiograph, have been reported. In the morning, the intervertebral disc is thicker than the same disc measured at the end of the day[1]. It has been shown that a constant flux of water occurs from the disc with constant load-bearing, contributing to the reduction in disc width[219]. Removal of the load allows water, and thus disc width, to be regained[1,135].

A variety of cell types have been described in the nucleus pulposus[165,287]. These include chondrocytes, fibroblasts and notochordal cells. Intermediate cell types are also recognised[165]. The predominant cell type of the young nucleus pulposus is the chondrocyte[57,60,126,173,287]. Unlike the cells in the annulus fibrosus, these cells are typically rounded, and the intracytoplasmic granules are scattered throughout the periphery of the cytoplasm. (Figure 2.14) Sometimes, a number of cells may form into groups[126]. The granular matrix between these cells is detectable under light microscopy and contains extremely fine fibres[273].

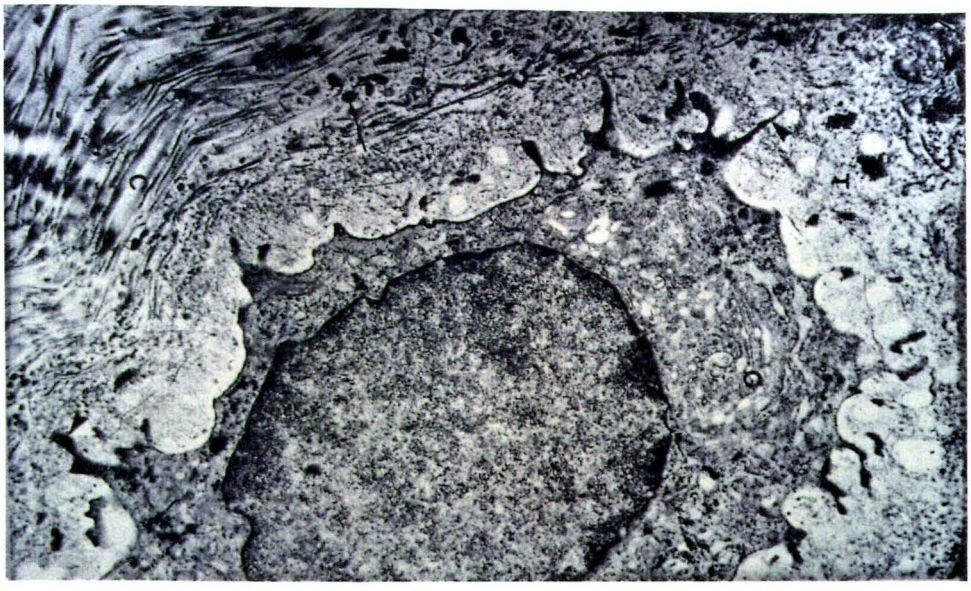


Figure 2.14: *Electron micrograph of a cell from the nucleus pulposus of a normal, human intervertebral disc. Note the collagen fibrils forming outside the cell (C), and the occasional globule of matrix products (arrow).*

In the very immature disc, notochordal cells may persist, sole remnants of this phylogenetic structure in the vertebrate skeleton[60, 165, 288]. Though uncommon, these cells may be found in clusters within the nucleus pulposus of the foetus, but such clusters decrease in frequency with increasing age[165, 288]. The distinctive feature of the notochord cell is the abundance of glycogen found densely packed within its cytoplasm[165]. The membranes of neighbouring cells commonly interdigitate, and a variety of cell-to-cell junctions are described[165].

Fibroblast cells are also reported in the nucleus pulposus, but more frequently in the adult disc[126, 165, 287]. It is suggested that their appearance represents a senile phenomenon. Further credence to this suggestion is the observation of many intermediate forms of mesenchymal cells in the aging intervertebral disc[165]. These intermediate cells have features common to both chondrocyte and fibroblastic cells.

Johnson et al (1982)[165] has also reported an additional cell type, whose location appears to be confined to the matrix abutting the junction of the nucleus pulposus and the adjacent cartilaginous end plate. These spindle-shaped cells resemble fibroblasts, but are characterised by unusually long cytoplasmic processes, terminating at bulbous swellings. Pericellular vesicles in the matrix about these end-terminals appear to be similar to intracellular structures. It is suggested that these long cytoplasmic processes allow the cell to maintain the matrix of the central regions of the avascular nucleus pulposus, whilst keeping the cell body close to nutrients which diffuse through the cartilaginous end plate.

With advancing age, the number of cells showing signs of degeneration, characterised by pyknotic and disintegrating nuclei, begins to increase[57, 59]. However, despite this rise in the proportion of non-viable cells, the *absolute* number of cells in the nucleus pulposus appears to increase with age[59, 214]. This finding is supported by analysis of the DNA content of the human nucleus pulposus, which increases progressively with age from 0.30 mg DNA/gm in the seven year old disc to 0.61 mg DNA/gm by 42 years of age[214].

(iii) Cartilaginous End Plate

The histologic appearance of the cartilaginous end plate is similar to articular cartilage. Under electron microscopy the very tight collagen framework can be seen to be arranged parallel to the cranial and caudal surface of the vertebral body[60, 159]. Aside from the perpendicular insertion of peripheral annular fibres into the vertebral body via Sharpey's fibres, no fibrillar connection can be observed between the cartilaginous end plate and subchondral trabeculae of the vertebral body[159]. It is suggested that this arrangement would impart a proficient shock absorptive function to the cartilaginous end plate during axial loading[159]. However, it would

also render it susceptible to horizontal shearing forces, and segmental separation of the cartilaginous end plate from the vertebral body has been described in the human[202].

The cartilaginous end plate has frequently been implicated in the nutrition of the intervertebral disc through diffusion of nutrients through its surface[57, 60, 199, 210]. *In vitro* and *in vivo* investigations have revealed that only the central portion of the cartilaginous end plate is effectively permeable[199, 210, 291]. Frequently, vascular channels can be observed in this portion of the cartilaginous end plate and these appear to permit direct communication with the marrow spaces of the vertebral body[82]. Large venous sinuses are occasionally present within the vertebral body, adjacent to the osteochondral junction at the central portion of the cartilaginous end plate[159].

2.7.4 MOLECULAR FRAMEWORK OF THE DISC

Full understanding of the functional design of the intervertebral disc requires separate consideration of the two integral components of its' structural framework: collagen and proteoglycan. The following discussion is but a brief summary of the current knowledge on these two biologically important molecules. Except where specifically stated, the information has been obtained from two comprehensive reviews on this area[110, 128].

(i) Collagen

Collagen is one of the most abundant molecules in the body. It is an important constituent of fibrous tissue, which is the single most prevalent tissue in the body. In a variety of forms such as bone, tendon, cartilage and fascia, it gives form and structural rigidity to the body. Collagen also plays a significant role in reparative processes which occur by the production of fibrous tissue.

Collagen is made from numerous repeating units of a basic building block, the tropocollagen molecule. The molecular weight of tropocollagen is approximately 270,000 daltons, which puts it in the class of large proteins. Each molecule is 1.4 nm in diameter and about 300 nm in length. The tropocollagen molecule has several fundamental qualities that identifies it from other protein molecules, thereby imparting the collagen fibres with several significant features. These characteristics are:

1. Each molecule consists of three linear peptide chains containing about 1000 amino acid residues per chain and, due to the location of certain consistent amino acid

residues, each strand is twisted in a right hand helix. This three chain assembly is itself twisted into a left-hand 'super-helix'.

2. The amino acid 'glycine' is located at every third position along the peptide chain, resulting in the repeating triplet *Gly-X-Y,* where X and Y may be any other amino acid.

3. The amino acids hydroxyproline and hydroxylysine are unique to the collagen molecule. They occur only in the Y position of the tri-peptide *Gly-X-Y* and it is the interaction of these amino acids on other molecules which impart the helical structure to the molecule. The structural importance of these molecules is reflected in their comparatively high content within the collagen molecule

Production of the collagen molecule begins within the fibroblast (or its related mesenchymal counterpart) through protein synthesis on the endoplasmic reticulum. Each newly synthesised peptide chain is longer than ultimately required for the collagen molecule, and this extra length acts as a registration area, enabling three individual peptide chains to bind together. Once bound, the proline and lysine residues become hydroxylated and the helical structure is adopted through the influence of electrical forces. Ascorbic acid (vitamin C) acts as a catalyst for the hydroxylation of lysine and proline. A deficiency of this vitamin can severely weaken the collagen molecule, due to the lack of strong bonds between the individual peptide chains.

The helical 'procollagen' molecule is now secreted from the fibroblast and the registration ends are cleaved by the action of two enzymes, resulting in the formation of the tropocollagen molecule. Tropocollagen is the fundamental unit of collagen, but is short-lived as a singular entity with the formation of collagen fibrils occurring spontaneously. Many tropocollagen molecules arrange themselves in a staggered array, with the 'tail' of an adjacent molecule overlapping the 'head' of another by about 10%. In cross-sectional analysis, the tropocollagen molecules are arranged in a pentagonal fashion, which facilitates this quarter stagger array to continue in all directions, resulting in the gradual increase in the thickness of the collagen fibre. This polymer aggregate is stabilised by strong intermolecular covalent bonds between, in particular, the lysine and hydroxylysine residues of adjacent tropocollagen molecules. These covalent bonds will form only if the participating groups are within 14 - 15 Å of each other. It is suggested that with continued deposition of collagen molecules, the inner core of the fibril becomes more tightly packed, facilitating the formation of these strong bonds. Until these covalent bonds form, the polymer is held by comparatively weaker electrostatic bonds.

Ultimate fibril diameter is dictated by the number of tropocollagen molecules which bond together. This process appears to be coordinated in some way as each tissue and each species may have one or more characteristic fibril diameters. The influence of the ground substance on the control of fibril growth is becoming increasingly recognised, and is believed to occur through electrical interaction with the binding sites of the tropocollagen molecule. The proteoglycan constituents of the ground substance are considered particularly significant in this role due to the interaction of their carbohydrate side-chains with certain amino acid residues.

Proteoglycans are also implicated in determining the orientation of fibre direction during fibrillogenesis. It is suggested that the surface of the fibroblast has a layer of proteoglycan whose charge and orientation directs the aggregation and ultimate orientation of the collagen polymer. Cellular shape appears to be significant in this respect. Further discussion of this is covered in later sections (Section 4.5.2)

Due to their influence on fibril growth, proteoglycans play an important role in dictating the ultimate qualities of the collagen fibril. Many different types of collagen molecules have been isolated from tissues throughout the body. These types differ predominantly in the order and proportions of amino acid residues in their peptide chains. These differences appear to impart certain individual characteristics to each fibre type, resulting in variation in the solubility, carbohydrate content, fibre size and organisation of tropocollagen molecules. Different collagen molecules are designated numerically using roman numerals. Only Types I and II are of importance in the intervertebral disc, although small amounts of Type III have been isolated from the periphery of the annulus fibrosus. Type I collagen is the predominant structural protein and is found in the skin, tendons, muscle and is the principal reparative collagen type in the body. Type II collagen is found predominantly in cartilage throughout the body.

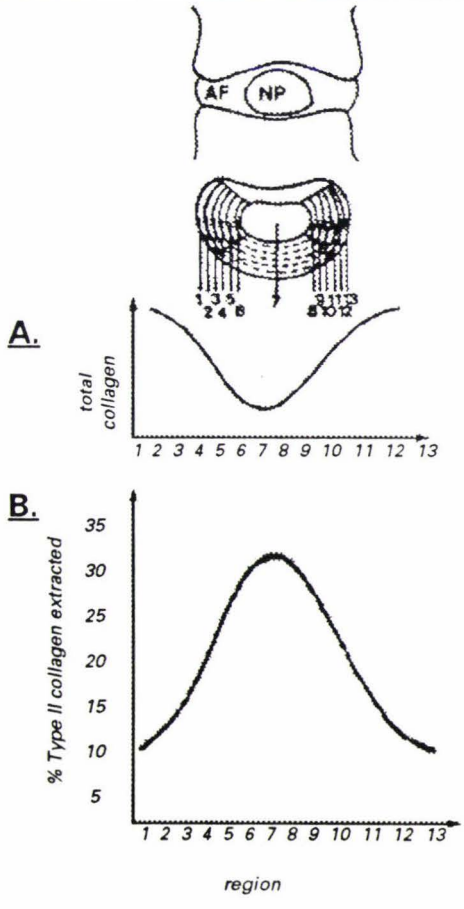


Figure 2.15: *[A] Stylised graph portraying the relative amount of collagen in the annulus fibrosus and nucleus pulposus.* ***[B]*** *The nucleus pulposus contains considerably more Type II collagen than the annulus fibrosus, where Type I collagen predominates.*

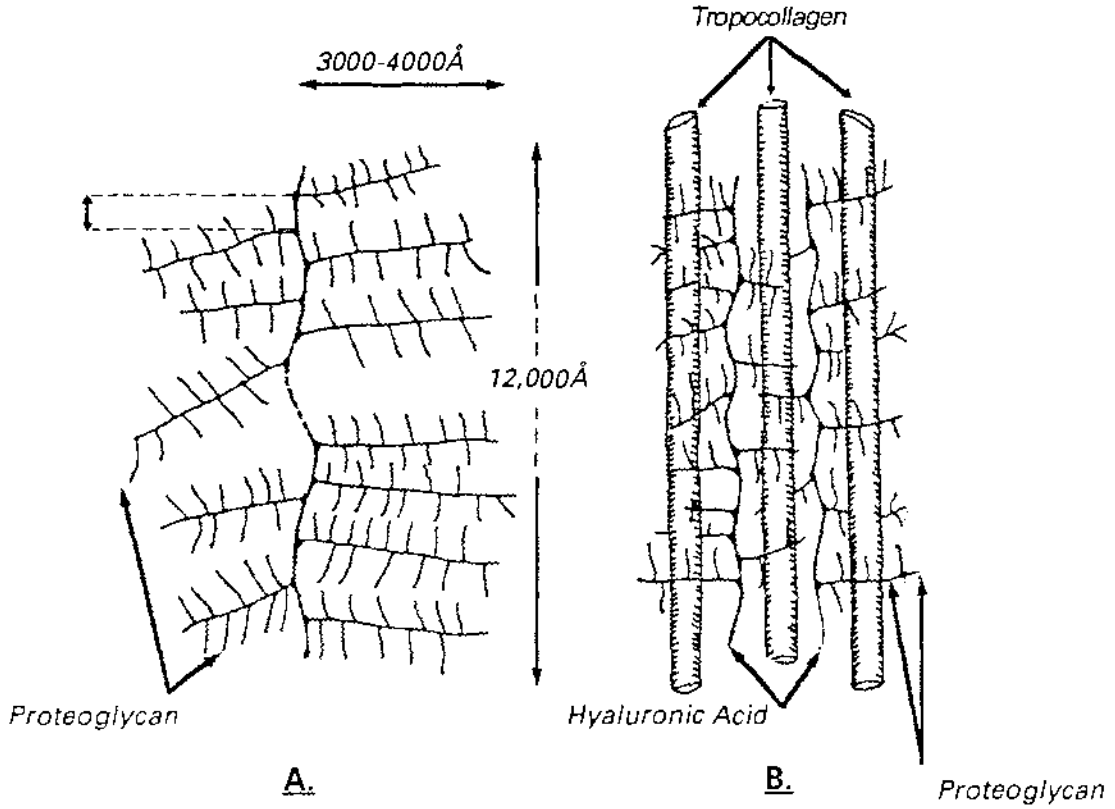


Figure 2.16: *[A] Drawing of the proteoglycan molecule (with the side-chain glycosaminoglycans giving a bottle brush appearance) linked to a single hyaluronic acid molecule (aggregation). [B] Schematic representation of the proteoglycan aggregate and its close relationship with the collagen molecule. Such an arrangement, which is stabilised through covalent bonds between the carbohydrate moieties of both molecules, has important consequences on the ultimate fibril orientation, diameter and qualities of the collagen fibre. Type II collagen, in contrast to Type I collagen, contains a high proportion of proteoglycan, which may influence its ability to better withstand compressive loads.*

Collagen is the principal component of the annulus fibrosus and may comprise 70% of its dry weight. The collagen content of the nucleus pulposus is, by comparison, significantly less. (Figure 2.15a) The type of collagen present in the intervertebral disc varies between the peripheral areas and the centre. At the periphery of the disc, Type I collagen exists, but a gradual transition to Type II collagen occurs as the more central regions are approached so that in the nucleus pulposus, Type II collagen predominates. (Figure 2.15b) As will become clearer in subsequent sections of this thesis, this organisation utilises the special properties of each collagen type to protect the disc against the biomechanical forces acting upon it.

Two of the three peptide chains in Type I collagen are identical, and are referred to as a1(I). Type II collagen contains three identical peptide chains and these share some basic structural similarities with the a1 peptide of Type I collagen. These chains are referred to as a1(II). Type II collagen contains about nine times as much hydroxylysine, which is known to readily interact with carbohydrate molecules. Because Type II collagen has a high degree of association with proteoglycan in comparison to Type I collagen, it is speculated that extensive glycosylation of

the hydroxylysine residues would promote collagen-proteoglycan interactions. It is anticipated that this relatively high concentration of proteoglycan, intimately bound to the Type II collagen fibre, would have a significant influence on the ultimate qualities of the tissue. (Figure 2.16) To understand why this should be so, one must have knowledge of the structure and qualities of the proteoglycan molecule.

(ii) Proteoglycans and the Glycosaminoglycans

The proteoglycan molecule, or monomer, consists of a protein backbone from which numerous polysaccharide side-chains, or glycosaminoglycan molecules, arise. (Figure 2.16) Each glycosaminoglycan molecule is composed of an alternating sequence of two monosaccharide units. One of these monosaccharide units is always a derivative of an aminohexose, usually D-glucosamine or D-galactosamine. Differences in the remaining monosaccharide will alter the properties of the proteoglycan molecule. Seven different glycosaminoglycan molecules are described in the body: chondroitin, chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate, heparin sulphate, keratan sulphate and hyaluronic acid. Each displays some specificity to certain locations in the body. The glycosaminoglycans of importance in the intervertebral disc include chondroitin-6-sulphate and keratan sulphate and, to a lesser degree, hyaluronic acid[16, 45, 49, 77].

The repeating disaccharide unit of chondroitin-6-sulphate includes galactosamine and glucuronic acid. The galactosamine sugar is sulphated at the sixth carbon residue, which imparts a double negative charge to each repeating unit. Chondroitin-6-sulphate has a molecular weight of 2×10^4. Keratan sulphate is a smaller molecule, with a molecular weight of $5 - 20 \times 10^3$ and contains the monosaccharides galactose and glucosamine. The glucosamine molecule is also sulphated at the sixth carbon residue[16, 45, 49, 77].

In all cartilaginous tissues, including the intervertebral disc, the basic structure of the proteoglycan molecule is the same[16]. This structure comprises many chondroitin-6-sulphate and keratan sulphate glycosaminoglycan molecules, which are bound to a central protein core. The chondroitin-6-sulphate molecules are not distributed evenly along the protein core, but occur in groups or clusters of 2 - 8 closely spaced chains. Towards one end (the N-terminal) of the protein core molecule, no chondroitin-6-sulphate are present, and a keratan sulphate-rich region exists. Keratan sulphate molecules are also distributed throughout the rest of the chain. The resultant appearance of the proteoglycan molecule resembles a bottle-brush, because the negatively charged monosaccharide units repel each other[16, 45, 49, 77].

The N-terminal end of the protein core has a binding site which allows the proteoglycan monomer to aggregate with hyaluronic acid[129,130,132]. Up to 100 proteoglycan monomers may bind to the hyaluronic acid chain, separated by a distance of 20 nm. The binding site on the hyaluronic acid molecule is very specific, and this bond is stabilised by a glycoprotein link fraction[129]. Aggregation occurs extracellularly, following the secretion of proteoglycan monomers into the pericellular environment by chondrocytes. There is evidence that the rate of incorporation of newly synthesised proteoglycan into stable aggregates diminishes in the skeletally mature animal[130]. The purpose of aggregation is not clearly understood, but it is believed that the production of such a large molecule would help bind the proteoglycan subunits in the matrix. Loss of aggregation ability in the mature animal would therefore permit greater dispersion of the proteoglycan monomers away from the matrix. (Figure 2.17)

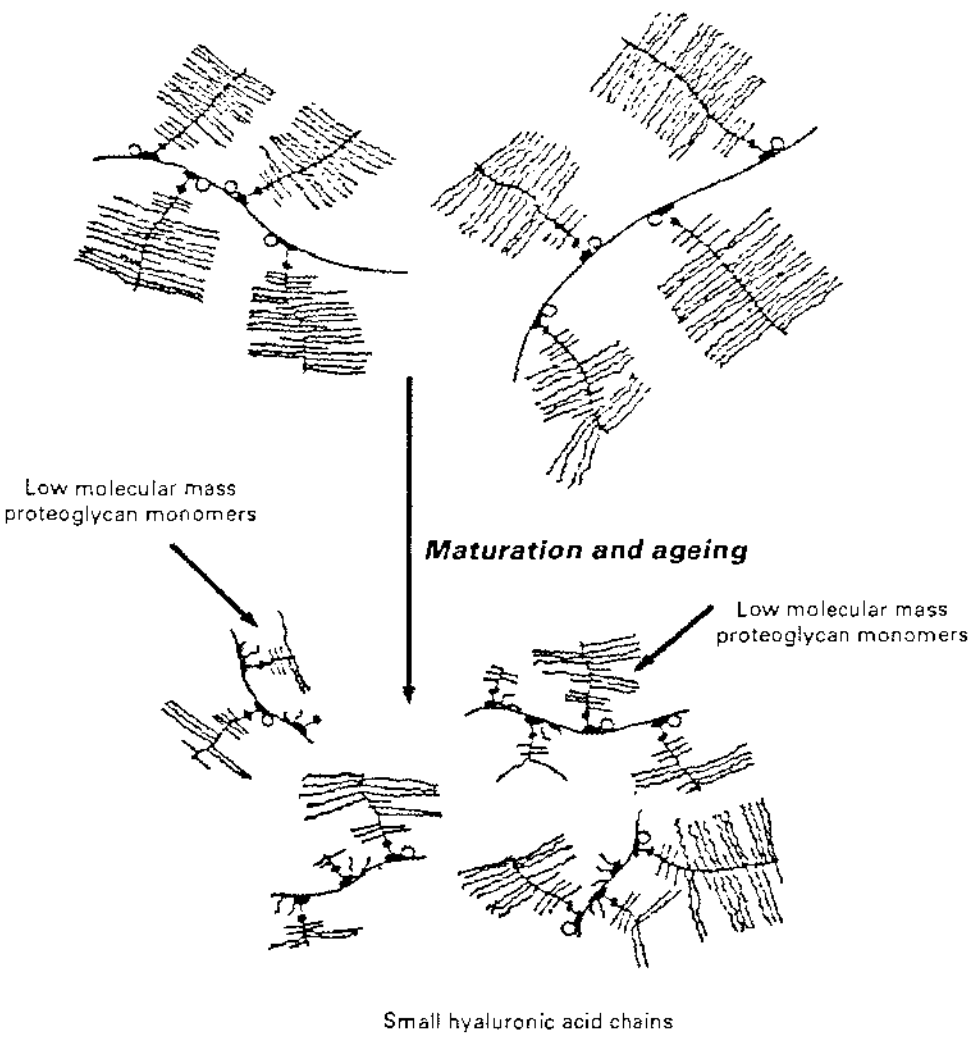


Figure 2.17: *Scheme showing the major age-related changes in aggregating proteoglycan structure. Note the shorter chain length of the side-chain proteoglycans, the fragmentation of the hyaluronic acid 'back-bone' resulting in smaller aggregates. (from Scott JE (1990)[45])*

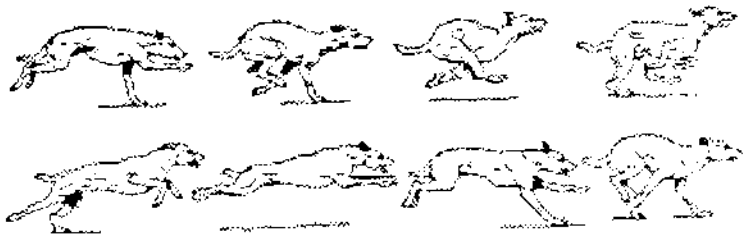
Buckwalter et al (1985)[45] found that the number of aggregates in the nucleus pulposus of the human intervertebral disc decreases 46% from birth to the age of eight months. Similar work in the adult disc has corroborated these findings[11,49], indicating a continuing loss of aggregating ability with age. This loss appears to be associated with a loss of the binding region on the core protein of the proteoglycan, as disc concentration levels of the link glycoprotein and hyaluronic acid remain adequate[111,74].

By comparison, the proteoglycans of the annulus fibrosus have a higher affinity for aggregation, and Buckwalter et al (1985)[45] reported only a 20% decrease to eight months of age. The aggregates that form in the annulus fibrosus were longer, with wider spaces separating the proteoglycan monomers on the hyaluronic filament. About one-third of the aggregates in the nucleus pulposus are similar to these long aggregates, whilst the remaining two-thirds are short, with densely packed proteoglycan monomers.

The most significant quality of the proteoglycan aggregate is the very high negative charge that they impart to the matrix under physiologic conditions[135]. This fixed charge density determines the movement of charged solutes between the matrix and plasma[140], and directly influences the characteristically high water content of the cartilaginous matrix. The variation in the quality of proteoglycan aggregation in the immature and adult intervertebral disc would probably have important consequences on the movement of fluid within the intervertebral disc.

In the following section it will become apparent how the quality of the collagen and proteoglycan molecules can influence the biomechanical function of the intervertebral disc.

3.0



BIOMECHANICS

OF THE VERTEBRAL COLUMN

3.1 INTRODUCTION

The vertebral column must be rigid to support the animal against the influence of gravity[139, 261]. However, in order to facilitate locomotory movements, it must also be flexible[105, 139, 261]. These seemingly opposing mechanical functions are achieved by its complex design.

Form and function are inextricably linked in all mechanical systems. Consequently, interference to form will result in modification to the function of a particular structure. By understanding the reason for a particular design, one can begin to appreciate the anatomical requirements, both structural and ultrastructural, demanded to fulfil a function. Furthermore, one can appreciate the effects that damage or deterioration in structure may have on the ultimate function of the 'machine'.

The form and function of the vertebral column has long been the subject of speculation and analysis[67, 105, 139, 261, 302]. A rigid horizontal beam provides the best support against gravitational forces and in this context, the vertebral column has been likened to a bridge[67, 139, 261]. Unfortunately, this theory did not provide for the flexibility required to enable locomotion[261]. The currently accepted theory of vertebral column function equates it to a bow and string[139, 261]. This theory incorporates the influence of the surrounding musculature on the vertebral column and adequately reflects the dynamic nature of the system. Nevertheless, the concepts of the previous theories are worth reviewing, as they provide some added comprehension of the requirements of intervertebral disc function.

3.2 THE VERTEBRAL COLUMN AS A HORIZONTAL BEAM

3.2.1 The Bridge Theory

In the early half of this century several pictorial analogies likened the vertebral column to a variety of bridge designs[139, 261]. The theory which gained the most favour with the veterinary and medical professions of the time depicted the spine as a cantilever-bridge; the neural spines represented the vertical braces rising from the road bed (the vertebral bodies), held by the suspension apparatus of the nuchal and supraspinous ligaments. (Figure 3.1) Whilst these analogies allowed graphical representation of the profile of the vertebral column, the theories failed to accurately illustrate the true design of the individual components of the vertebral column. Moreover, movement of the vertebral column, one of its fundamental qualities, was completely ignored![261]

Clearly the bridge theory, presenting a passive, static state was not commensurate with the requirements for an active, dynamic living system. Nevertheless, certain theoretical aspects of the bridge theory provide some insight into the form and function of the vertebral column.

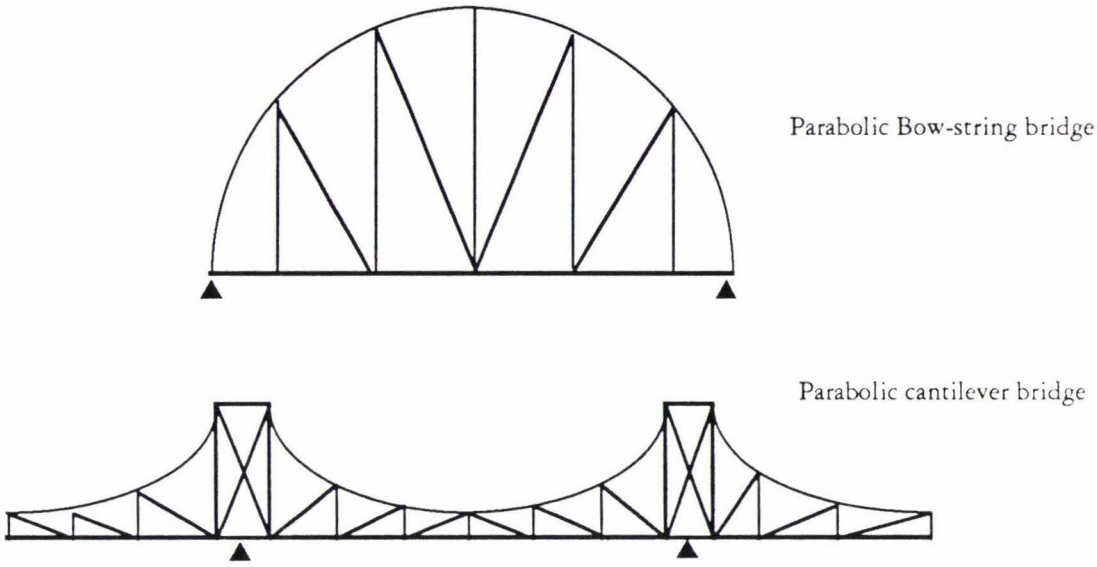


Figure 3.1: *The 'bridge theory' attempted to graphically mimic the structure of the vertebral column of animals to enable an explanation of function. The inability of the theory to accomodate motion led to its eventual rejection.*

3.2.2 Theoretical Considerations

If the vertebral column is regarded as a rigid girder, the 'fore' and 'hind' limbs can be represented as supporting struts located at two points down its length. (Figure 3.2a) The fore-leg support is located some distance from one end of the girder, resulting in a cantilevered configuration at this point. Such an arrangement is similar in structure to a carpenters 'saw-horse' and is characterised by considerable inherent stability[67, 261].

Two opposing forces can be considered to act on this structure: gravity, acting downwards upon the whole structure, and an equal reactive upward force from the ground which is transmitted solely through the two limb struts[67, 139, 261]. Between these two struts, the unsupported regions of the girder have a tendency to dip; this becomes greater as the distance from the support increases. The tendency for any particular point along the girder to bend can be calculated. This 'bending moment' is a product of the force applied to the girder multiplied by the shortest distance from the point to of force application. A graphical display of the bending moment versus distance along the girder represents the stress diagram for that structure. (Figure 3.2b)

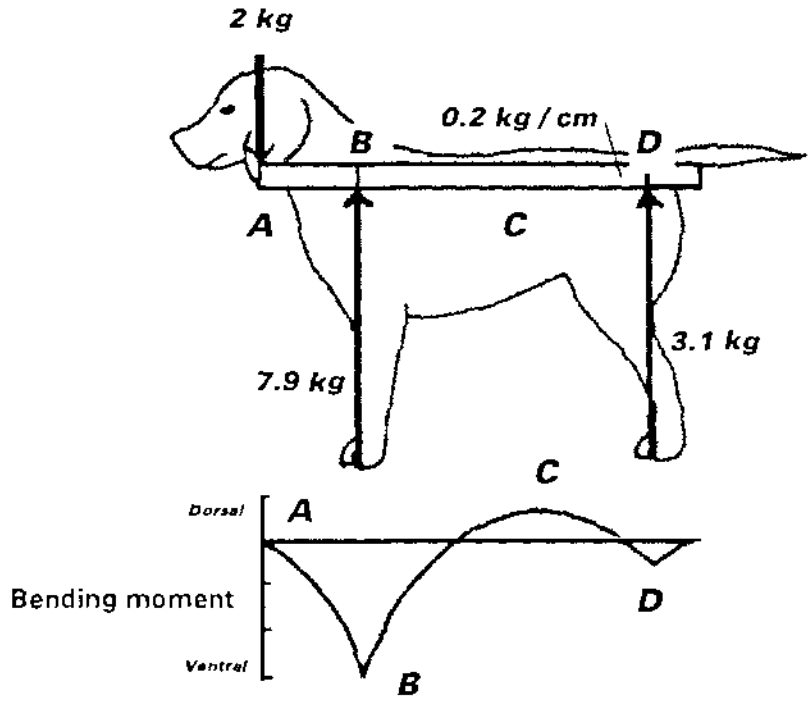


Figure 3.2: *The trunk of the dog is represented by a horizontal beam A - D (45 cm long) with a uniformly distributed load of 0.2 kg/cm, together with a concentrated load cranially of 2 kg (the head). The total weight is therefore 12.1 kg. With the limbs placed as shown, the opposing reaction forces are 7.9 kg in the forelimb, and 3.1 kg in the hindlimb.* ***b)*** *Bending moments along the vertebral column are shown graphically in the lower figure. (from Davies AS (1982)[61])*

If bending moments are calculated for a hypothetical vertebral column, a stress diagram (Figure 3.2b) can be produced[65, 159, 261]. Over the fore and hind limb supports, the bending moment is maximum in a positive sense due to the upwardly-acting reactive force at these sites, resulting in a ventrally-concave bend in the girder. As the distance from the supports increases, so does the tendency for the beam to sag under the influence of the gravitational force. This dorsally concave bend in the girder reaches a maximum about two-thirds the distance between the two supports (this theoretical model approximates the unequal load-sharing by the fore and hind legs in real life).

Effective counteraction of these inherent bending moments can be achieved by the application of basic engineering principles to the girder. Bending in the ventrally concave sense is opposed by first altering the shape of the beam to produce a dorsally concave beam at the forelimb support. This curve is supported by a series of struts (the spinous processes of the cervical and thoracic vertebrae) and stays (the nuchal and supraspinous ligaments and the epaxial muscles of the neck). Bending in a dorsally concave sense is opposed again by altering the shape of the beam, thus providing a ventrally concave curve in the thoracolumbar region. In a similar manner, this curve is supported by a series of struts (the rib cage and sacrum), and stays (the ventral thoracic and abdominal muscles and the sacrotuberous ligament). Thus, when an animal

stands at rest, the vertebral column is in a state of equilibrium, and only slight muscular activity is required to maintain this posture[105,139,261]. A lateral projection of these curves in the vertebral column of the resting animal is termed the *eigenform*, and this profile can show significant variation between the species[261]. The importance of these differences on ambulation will be discussed later. (Section 3.3.2)

3.3 THE VERTEBRAL COLUMN AS A FLEXIBLE BEAM

If the rigid, horizontal girder presented in the previous analogy is broken into numerous individual segments, stability is lost and an almost snake-like flexibility is produced in the beam. Such a structure would of course offer no resistance to gravitational forces, and it would freely collapse between the two supporting struts. However, if this segmented unit is surrounded by elastic stays, stability can be imparted to the structure without sacrificing its inherent flexibility[261].

This concept can be extrapolated to illustrate the design of the vertebral column; the individual segments are equivalent to the vertebral bodies and the elastic stays are the surrounding epaxial and hypaxial musculature, ligamentous supports and abdominal musculature. The combined action of this elastic apparatus at rest stabilises the vertebral column by adopting the balanced *eigenform* particular to that animal. Movement of the animal is possible by coordinated contraction and relaxation of the surrounding musculature. Such a design has been compared to a bow-and-string, and this theory is currently accepted to provide suitable explanation of vertebral column function[105,139,261].

3.3.1 The Bow-and-String theory

The bow-and-string theory considers the vertebral column and the pelvis, with their muscles, to represent a 'bow', bent in a dorsal direction (ventral concave) by a 'string', consisting of the sternum, the abdominal muscles (especially the *rectus abdominus*) and the connective tissue of the *linea alba*. The weight of the abdominal organs and the elasticity of the bow tries to extend the bow, but this extension is prevented by the stress or tension maintained in the abdominal musculature[139,206,261]. Hyperflexion of the vertebral column is resisted by the interspinous, dorsal spinous and longitudinal ligaments[81,226,301], as well as the epaxial musculature[7,80,263].

The significance of the abdominal muscles in maintaining the ventral concavity in the thoracolumbar vertebral column is clearly shown by the observation that, in a dead body, the vertebral column extends only if the abdominal muscles are cut[261]. Similarly, there is a definite

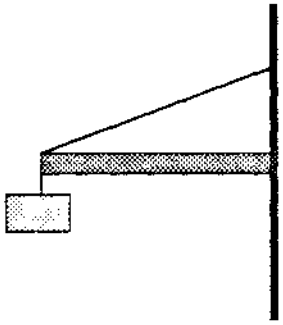


Figure 3.3: *A beam, supported at one end only by being built into a wall, is given additional support by a stretched cord. This stucture is analogous to the role of the nuchal ligament in supporting the head.*

sag in the vertebral column of young animals until the abdominal musculature is properly developed: as tension is acquired in the 'string' with increasing age, this sag is transformed into a dorsal curvature[261].

A second bow can be considered in the cervical region. Here the bow has a dorsal concavity, and the string consists of the nuchal ligament and epaxial muscles of the neck. Again, cutting this ligament in the dead animal has the effect of allowing the cervical vertebrae to stretch out, thus demonstrating the tension maintained in the string to fulfil its role[261]. This bow is designed to hold the weight of the head. A comparable construction design is used frequently in sign boards. (Figure 3.3)

3.3.1 (i) The Bow-and-String theory: experimental models

Using human subjects, Morris (1961)[206] was able to demonstrate the rôle of the ventral string during increased loading on the vertebral column. In his experiments, measurement of intra-thoracic and intra-abdominal cavity pressures, and muscular activity in the intercostal, *m. rectus abdominus*, *m. abdominus oblique*, and epaxial muscles of the back were simultaneously recorded whilst a range of static and dynamic loads were placed on the vertebral column. Increased intra-thoracic and intra-abdominal pressure during loading occurred, resulting in the formation of two solid, sturdy cylinders: one containing air (the thorax), the other containing fluid (the abdomen). Both these cylinders are capable of transmitting part of the forces generated during loading, thereby relieving the vertebral column of a significant portion of the weight-bearing burden.

Morris[206] calculated that the actual force on the spine is much less than that which exists when the effect of the intra-cavitary pressures, is omitted. He mathematically calculated this force to be 30 - 50% less than would be present without such support. This finding has been corroborated by the extensive *in vivo* and *in vitro* work of Nachemson[207, 208, 209], as well as other investigators[1, 2, 170, 231].

The function of the abdominal musculature could be considered as 'taking the strain' during loading of the back[206, 261]. The mechanism of increased intra cavitary pressures through contraction of the thoracic cage and related muscle is a reflex activity[206]. Since the intervertebral disc is capable of withstanding much higher loads in vitro, one may conclude that one of the most

significant effects of the increased tension in the extrinsic support is to enhance the rigidity of the vertebral column[206, 208, 211, 225].

3.4 MOTION OF ANIMALS (ROLE OF THE VERTEBRAL COLUMN)

Emergence from the aquatic environment by the ancient ancestors of all current terrestrial animals bought about radical changes in skeletal and other morphological structures. Because of the need to acquire food and escape from predators, locomotory adaptation became a dominant feature of evolutionary development. Despite the wide diversity of habitats available to the terrestrial inhabitant, the evolutionary development of animal locomotion can be fairly accurately traced to one or two distinct design concepts, which in more recent times (evolutionally speaking) have undergone further modification to more appropriately adapt the animal to its chosen environment[105, 139, 200].

The typical gait of the early terrestrials consisted of undulating lateral flexures of the body, reminiscent of the swimming motion of fish and other aquatic species. This mode of locomotion remains the normal gait of reptiles and other amphibians whose limbs are arranged perpendicular to the vertebral column[105, 139, 200].

The adoption of a parasagittal position of the limbs, attributed to the need to dig for food, permitted significant advances in locomotion to be made[105]. The horizontal (lateral) undulations of the spine changed to vertical flexures which permitted the progression of movement by a series of jumps, with the hind limbs being propulsive and the fore-limbs shock absorbers. Further development of this movement saw the inhibition of the forward drift of the hind limbs whilst in the air, with the forelimbs adding a second propulsive input to the gait. This motion is typical of the gallop[105, 139, 261].

Animal species with a high degree of locomotory specialisation are termed cursorial[105, 139]. The evolution of the cursors from simple walkers was the result of several selective advantages[105]. Cursors are able to forage for food over a wide area, seeking new sources of food or water when familiar sites fail or when seasonal variations make a particular habitat unsuitable. It is possible to segregate the evolutionary progress of the herbivores (prey) and the carnivorous (predator) species. The requirements of their chosen habitats placed great demands on the development of their musculoskeletal system.

Herbivores may spend up to 16 hours a day foraging for food and their habitat may be vast. Standing is the customary posture for these animals and indeed sitting, although possible, is an

unusual activity[261]. To provide skeletal support with minimum expenditure of energy, the vertebral column is comparatively rigid[105, 139, 200, 261]. This rigidity is promoted by well-developed ligamentous supports and increased skeletal articulations. In the thoracic region, mobility is chiefly limited by the large dorsal spinous processes which are required to provide mechanical struts for the insertion of the nuchal ligament and epaxial muscles of the head to offset the effect of the large heavy head in these animals. Flexibility of the spine in the caudal thoracic and lumbar regions of the spine is further limited due to broadening of the dorsal spinous process, so that the interspace is minimal. The interspinous and supraspinous ligaments are also thickened in this location. Lateral flexures of the spine are restricted by the broad transverse processes. (Figure 3.4) The only significant movement in the equine vertebral column occurs at the interspace of $T_{1/2}$, which permits grazing activity, and at the lumbosacral area[261, 284].

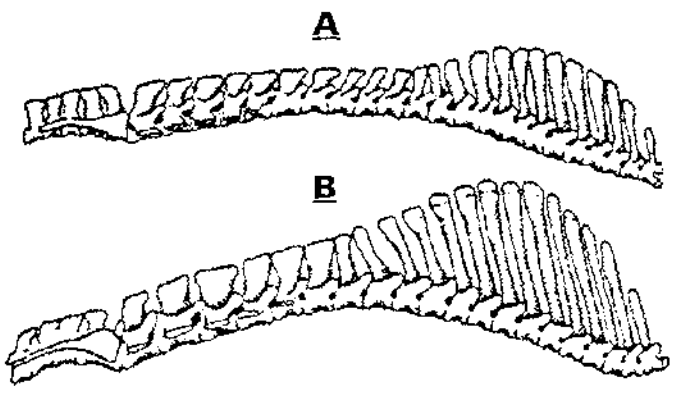


Figure 3.4: *The thoracolumbar spine of the Ungulates (A: horse; B: Bison). Note how the dorsal spinous processes are very tall in the thoracic region, and are laterally flattened in the lumbar area. Also note how the anti-clinal zone is less distinctive in these species compared to the dog (compare to Figure 1.1)*

The unfortunate position of the herbivores in the food chain necessitates the need for rapid retreat from predators. Because of the comparatively inflexible vertebral column, development of speed in these animals has been achieved by lengthening the stride through modifications to the appendicular skeleton[57, 105, 139, 200]. (Figure 3.5) The effective length of the limb has been increased by the animal walking on the distal end of the third phalanx[139]. In the specialist runner, in particular, the skeletal complexity of the distal limb is reduced and all weight is borne by a

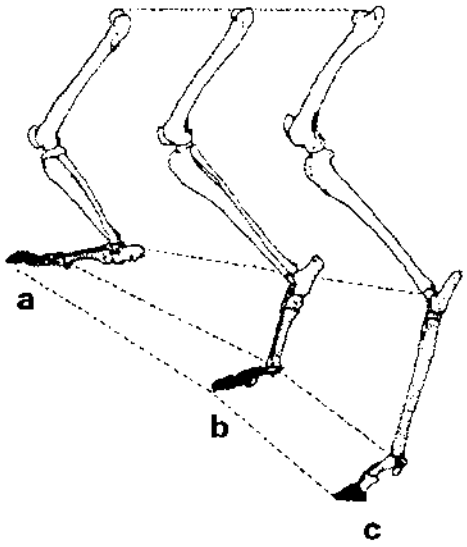


Figure 3.5: *Phylogenetic elongation of the distal end of the mammalian limb. Comparison of the hind-limb of the bear (a), dog (b), and deer (c) shows both a progressive elongation of the distal segments of the limb and a progressive incorporation of the distal segments into the limb pendulum from the* plantigrade *(a) to the* digitigrade *(b) and* unguligrade *(c) stance, as the limb becomes cursorially adapted. (From Davies AS (1982)[81])*

single digit; the remaining four digits become redundant. In other runners whose habitat is especially mountainous, muddy or uneven, this development of the hoof region shows some variation to ensure a firm foot-fall in the rough terrain[105, 139, 200]. Endurance is enhanced by the pendulum action of a comparatively lengthened antebrachial region whose motion is effected by a large muscle mass about the shoulder and hips acting over a shortened length[67, 105, 139]. Comparatively small contractions of the limb musculature create an increased stride length with a resultant increase in speed.

The vertebral column of these animals can therefore be likened to the horizontal beam described earlier. However, complete concrescence of the spine into a single unit has been prevented because this would greatly diminish its' shock absorbing role which is vital with high-speed gaits which have a stage where no limbs are weight-bearing (ie free-flight, typical of all forms of gallop. The intervertebral disc is a central component in this shock absorbent role[135].

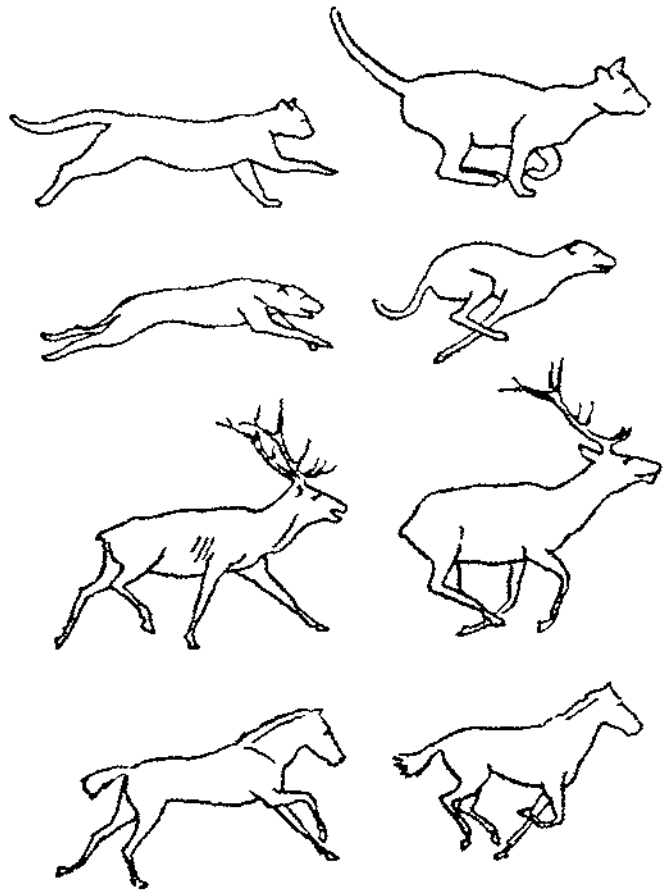


Figure 3.6: *Two different phases of the leaping-gallop (cat, dog) and the horse-gallop (deer, horse). Note the comparative straightness of the back throughout each phase of the gallop in the ungulate species. (from Slijper EJ (1946)[181])*

In contrast with the herbivorous species, the development of the musculoskeletal system in the predator species was dictated by the need to obtain *live* food[105]. The long chase, hot pursuit of the victim at close quarters, prey-seeking in burrows, the catching of live fish, specialisation for hunting small and large animals, group or solitary hunting and feeding on carrion all created special conditions dictating the motion and limb morphology of the carnivore. The need for manipulative functions by the distal limb resulted in the retention of all digits and development of a rotatory action in the fore-limbs[105, 200]. The morphology of this area is greatest in the cat because the claws are used to seize and hold prey[105, 139]. The dog, on the other hand, is more likely to seize prey in its teeth.

A characteristic feature of the carnivore is the flexibility of the spine[105, 139, 261]. This flexibility is most obvious during stalking, in the final lunge and struggle with the victim at the kill, and in the pursuit of prey through winding burrows[105]. The flexibility of the spine is also advantageous during high speed running, when the considerable vertical mobility of the vertebral column promotes an increase in the length of the stride, thereby accelerating the speed of running[67, 105, 139, 261]. (Figure 3.7)

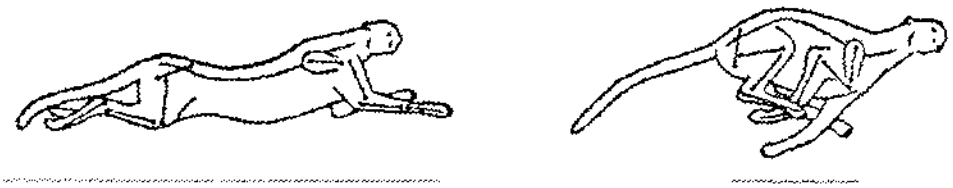


Figure 3.7: *The gallop of the cheetah. Impressive speed is gained by the dramatic increase in stride length provided by the tremendous flexibility of the vertebral column at the diaphragmatic region. (from Gambaryan PP (1974)[105])*

The active vertical mobility of the spine is reflected in the structural design of the vertebral column. The vertebral column of the dog is very mobile in the dorsal direction and fairly mobile in the ventral direction[261]. This mobility is especially large in the diaphragmatic region (thoracolumbar junction). Pivoting of the vertebral column at the diaphragmatic region provides a propulsive thrust to the hind limbs, which are drawn under the body during the gallop[105, 139]. The mobility of the vertebral column is associated with a narrowing of the dorsal spinous processes to increase the interspinous space and a poorly developed supraspinous ligament. In their place, a complicated system of interspinous ligaments and muscles develops[105, 139, 200, 261]. The epaxial musculature is also quite fleshy and well-developed[261]. The intervertebral disc plays an important role in the attenuation of the impacts passing through the vertebral column during running, as well as conferring the spine with its' distinguishing flexibility in these species.

The intervertebral disc in all animals must therefore be able to resist and attenuate a variety of forces. Interestingly, intervertebral disc disease is recorded most frequently at the most mobile region of the vertebral column in all animals. For example, degeneration of the intervertebral disc at T_{1-2} and at the lumbosacral region is recorded in the horse[285], and the latter region is also typically affected in the human[96,126]. (Figure 3.8) However, clinical disease associated with disc degeneration is infrequently recorded in the horse; this is not surprising due to the relative immobility of the vertebral column. Indeed, the more typical complaint is associated with hyperextension of the spine, due to riding and excessive jumping, resulting in impingement of the dorsal spinous processes and arthritic changes in the articular facets[285]. In the dog, degeneration of discs about the diaphragmatic region is more typical[102,126,145,179,186].

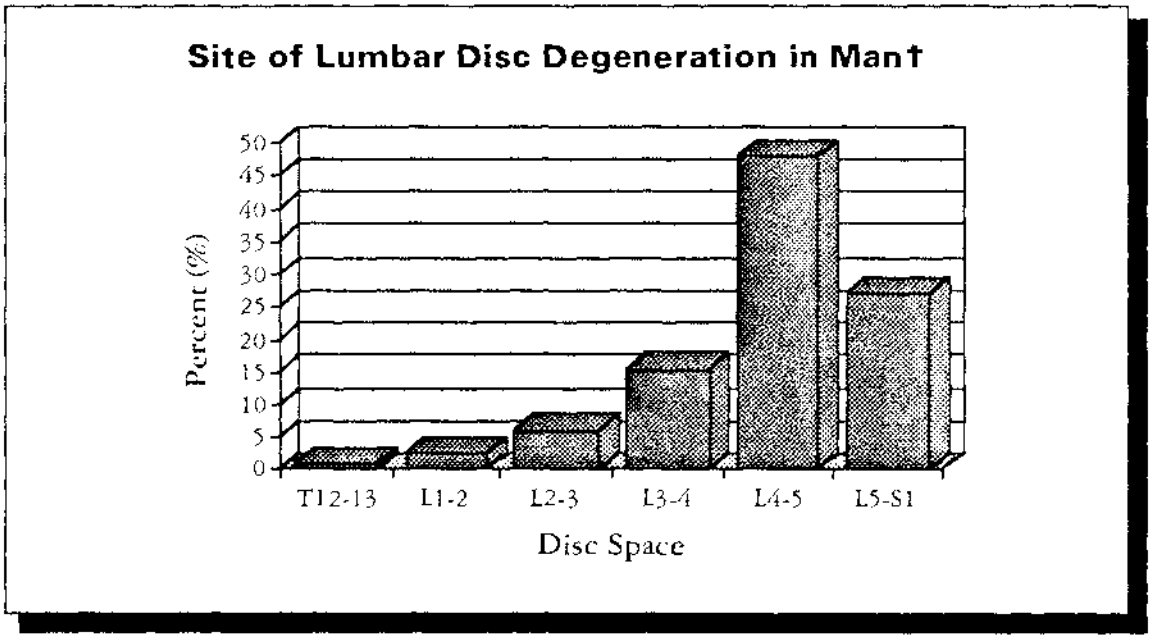


Figure 3.8: *Site of disc herniation in the human. Compare this with the distribution in the dog (Figure 1.2) (†with data from Hansen HJ (1959)[74])*

How the intervertebral disc attenuates and resists a variety of biomechanical forces, and the reasons why it degenerates will be presented in the following sections.

3.5 BIOMECHANICS OF THE INTERVERTEBRAL DISC

To enable mobility of the vertebral column, the intervertebral disc acts as a deformable tissue between the individual vertebral bodies[135]. This role requires it to be flexible enough to permit the extremes of movement associated with locomotion, yet rigid enough to withstand the normal physiological forces acting along the vertebral column. How the intervertebral disc tolerates these apparently conflicting qualities has been the focus of considerable investigation[2,5,40,82,86,93,104,109,140-142,158,166,170,171,181,183,187,198,208,209,211,219,244,254,296,302].

When any structure is bent, the elements on the concave side are compressed whilst those on the convex side are under a state of tension. A gradual state of transition between these two states exists and, at one place within the beam, the tensile and compressive forces are completely balanced. This neutral zone is also referred to as the axis of movement[169]. Investigations in the horse and human have shown that, in the cervical and lumbar regions of the vertebral column, the axis of movement passes directly through the centre of the eccentrically-located nucleus pulposus[169]. In the thoracic region, the axis of movement (and accordingly, the nucleus pulposus) is located more centrally within the intervertebral disc, probably reflecting the influence of the rib cage on vertebral column stability[206].

Under normal conditions, the intervertebral disc can be subjected to five possible loading conditions: compression, tension, bending, shear and torsion[302]. In most cases, a physiological biomechanical force would probably be a combination of several, if not all of these. The results of investigations into the reaction of the intervertebral disc to these loads are summarised below.

The majority of experiments on intervertebral disc function have been performed on isolated but intact disc units, bounded by thin sections of the adjacent vertebral bodies. In most cases, the neural arch and associated soft tissue elements had been removed enabling the investigators to focus solely on the activity of the intervertebral disc. In these experiments, the intervertebral disc unit was subjected to a variety of controlled, compressive loading forces. The applied load was classified as static (a single continuous load, often of increasing intensity, with each loading event followed by a period of recovery) or dynamic (repetitive loading of many cycles per second). The intervertebral disc has been found to react quite differently to these loads, a quality which is quite typical of other elastic or semi-elastic (viscoelastic) structures.

Loading of the intervertebral disc section along its axis results in the generation of a purely compressive force acting through the centre of the nucleus pulposus. The reaction of the disc

to loading is examined by a) measuring the amount of positional displacement which occurs and b) changes in the disc morphology.

Under a gradually increasing static load, the intervertebral disc unit is initially quite flexible and deforms easily. As the load increases, the disc becomes progressively stiffer, with a stable state occurring after several minutes. The micromovement of the disc prior this equilibrium state being reached has been termed creep and is believed to be due to initial rearrangement of the lamellar layers of the annulus, followed by the gradual efflux of tissue fluid from the interstices of the disc. This flux of fluid is directly dependant on the size of the load[170, 219].

A number of studies have indicated that the nucleus pulposus is normally kept under a constant degree of compression[60, 181, 170, 211, 219, 226, 227]. This normal intradiscal pressure, or preload, has been measured in humans and dogs. As a compressive load is applied across the intervertebral disc unit, the force is absorbed mostly by the gelatinous nucleus pulposus, resulting in the generation of a hydraulic pressure within the disc which is radiated in all directions[135, 302]. (Figure 3.9) As the nucleus pulposus is squeezed, the annular fibres react by sliding over each other to form a

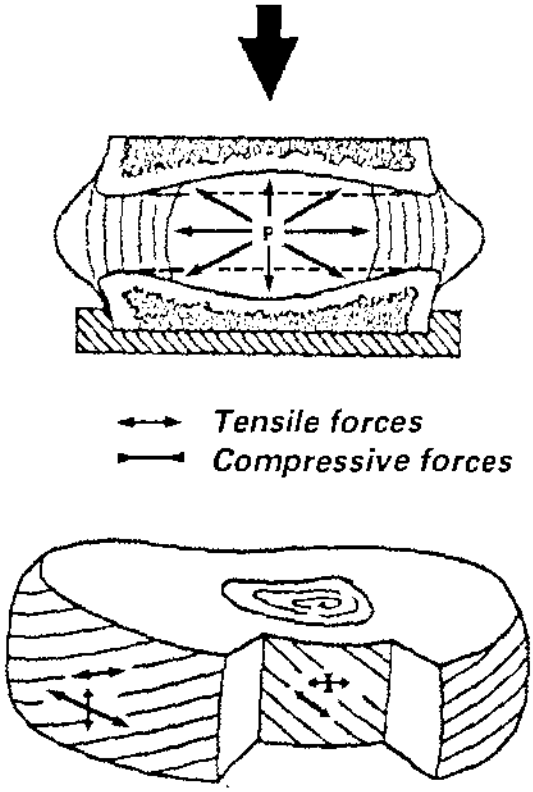


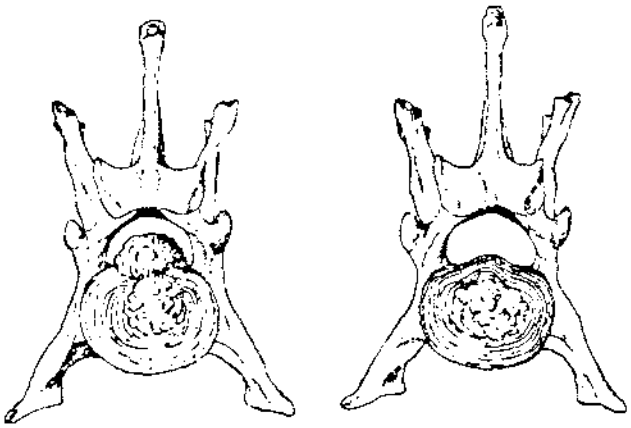
Figure 3.9: *When a compressive load is passed through the normal intervertebral disc, a hydraulic pressure is generated within the gelatinous nucleus pulposus. This is radiated in all directions, and is absorbed by the annular fibres. The compressive force is, in this way, converted to a predominantly tensile force in the annular fibres. This tensile force is largest in the peripheral layers of the annulus. (from White and Panjabi (1978)[302])*

more tightly packed arrangement, thereby restraining the disc circumference in spite of the reduction in disc width[154]. This action results in the generation of large tensile stresses within the lamellar fibres (estimated to be about four or five times the applied compressive load)[208] which makes the annulus fibrosus very stiff, thereby preventing it from collapsing under the weight of the compressive load[135, 154, 302]. Since it has been shown that the annular lamellae are three times stronger when loaded along the direction of their fibres[104], it is presumed that the alternating arrangement of the lamellae in the annulus fibrosus, together with the obliquity of the lamellar fibres, enables them to align themselves in the most efficient manner to counteract the forces generated in the disc during loading[135, 154, 302].

Bending, torsional and shear loads are considered to more closely represent the normal physiological activity of the disc than purely compressive loads, and indeed, experimental studies suggest that it is these loading conditions which are more likely to result in traumatic disruption to the intervertebral disc[86]. Resistance to these loads is aided by the activity of the other stabilising elements of the vertebral column, including the long and short ligaments of the spine and, in particular the articular facets. Moreover, in the live animal, physiological function of the disc is aided by variations in thoracic and abdominal pressures, which have been shown to dramatically influence the biomechanical function of the vertebral column and, in particular, the intervertebral disc[170, 206, 209, 211] (Section 3.3.1). Thus the direct extrapolation of the results of *in vitro* investigations to the live patient must be made with care.

A degenerating nucleus pulposus can have serious consequences on the functioning of the intervertebral disc and vertebral column of the animal. The changes that occur in the intervertebral disc, and in particular the nucleus pulposus, is the focus of the following section.

4.0



DEGENERATION

OF THE INTERVERTEBRAL DISC

4.1 INTRODUCTION

Degeneration of the intervertebral disc in dog[48,111,112,114,117,126,127,145,148,184,205,254,256,268,295] and man[3,4,12,13,17,35,50,56,59,95,135,144,156,173,188,213,214,218,228,275,280,289,307] has been observed and described by many authors. Age-associated changes in the disc and its surrounding structures have been the focus of considerable research. This is especially so in the human because back disease, largely attributable to premature malfunction of the intervertebral disc, results in substantial debilitation and is a considerable financial burden to health sectors throughout the world[202].

Disc degeneration is associated with loss of water from the nucleus pulposus[56,57,69,126,155,214], due in part to a lower concentration of proteoglycan[4,12,13,56,59,77,82,111,112,114,126,135,213,218,228]. This reduces the ability of the disc to function as a hydraulic cushion and consequently interferes with the normal action of the vertebral column[131,135,180]. When this occurs, other structures associated with the function of the spine, such as the articular facets, vertebral bodies, ligaments and musculature can become involved in the disease process[50,160,166,181,187,189,234].

The term 'intervertebral disc degeneration' a commonly used but often inappropriately applied. The expression 'degeneration' has now come to convey a wide variety of clinical, radiographic and pathologic changes that occur in the intervertebral disc. Indeed, Pritzker (1977)[239] stated that "the word is really only a symbol of our ignorance about disc disease", maintaining that it fails to distinguish between age related structural changes that would be expected to occur, and true disc disease.

It is possible that the difference between aging and degeneration is related to the time of onset. *Aging* of the disc would thus represent a gradual maturation and development of the inherent structures, enabling them to respond to changes in the functional requirements of the animal. These changes are directed and created by a viable cell population, which actively secrete sufficient collagen and matrix products to allow the disc to function appropriately under the altering conditions[35,243]. Similar ultrastrutural and biochemical modifications are recorded in other anatomical structures which are subjected to similar loading circumstances as the interevertebral disc[168,243,245,246,247,248,249,293] ***Degeneration***, on the other hand, would represent a decaying of the disc structure and consequently, a higher population of non-viable cells would be expected.

Whilst the apparent morphological changes in aging and degenerating discs are similar, some inciting factor results in an acceleration of the process in the degenerate disc. In man and most

dogs, this factor appears to be trauma and the initial incitement is currently believed to be tearing of the annular rings[84,86]. If of appreciable size, these radial tears do not heal adequately[125] and, ultimately, degenerative changes eventuate in the nucleus. Thus, degeneration appears to begin peripherally, eventually causing central disintegration[86,126]. With progressive degeneration of the nuclear region, the disc may begin to bulge out from the confines of the vertebral space, causing spinal cord or nerve root compression and the onset of clinical signs related to disc disease[126,131,135,302].

A different form of disc degeneration from that described above is known to be associated with chondrodystrophic dogs. In these animals, degeneration of the disc is thought to be a manifestation of the chondrodysplasia which typifies the skeletal defects of these dogs[126]. In this form of degeneration, the initial changes appear centrally within the disc, eventually extending to the more peripheral annular layers in a centripetal fashion. The disruption to disc function that results tends to lead predominantly to complete rupture of the annular fibres and a massive herniation of degenerate nuclear material. Hansen (1952)[126] classified this form of disc herniation as Type I. The previously described form he classified as Type II.

In this discussion, the term 'degenerative changes' is used to describe both senile remodelling and the pathological changes that occur in the intervertebral disc. Where differences are known to occur, these will be detailed.

4.2 DEGENERATIVE CHANGES IN THE NON-CHONDRODYSTROPHOID BREEDS

The non-chondrodystrophoid dogs are those that do not have chondrodysplasia as a breed characteristic. Clinical disease associated with the intervertebral disc in these breeds is less common, and occurs in considerably older individuals[126,145,233]. It is likely therefore that senile remodelling is a factor in this form of disc degeneration[126].

4.2.1 Gross changes

In most non-chondrodystrophoid dogs, the mucoid nucleus described earlier (section 2.7.1) is preserved for much of the animal's life[126]. (Figure 4.1) Only in some older dogs (over seven years of age) does the occasional disc become fibroid in character. These discs lose their transparency, becoming turbid and milky-white in colour. When this occurs, the nucleus eventually becomes indistinguishable from the inner layers of the annulus fibrosus. Dehydration of the disc is associated with these changes and results in a loss of disc width. Only occasionally does calcification of the disc occur[126,145,147].

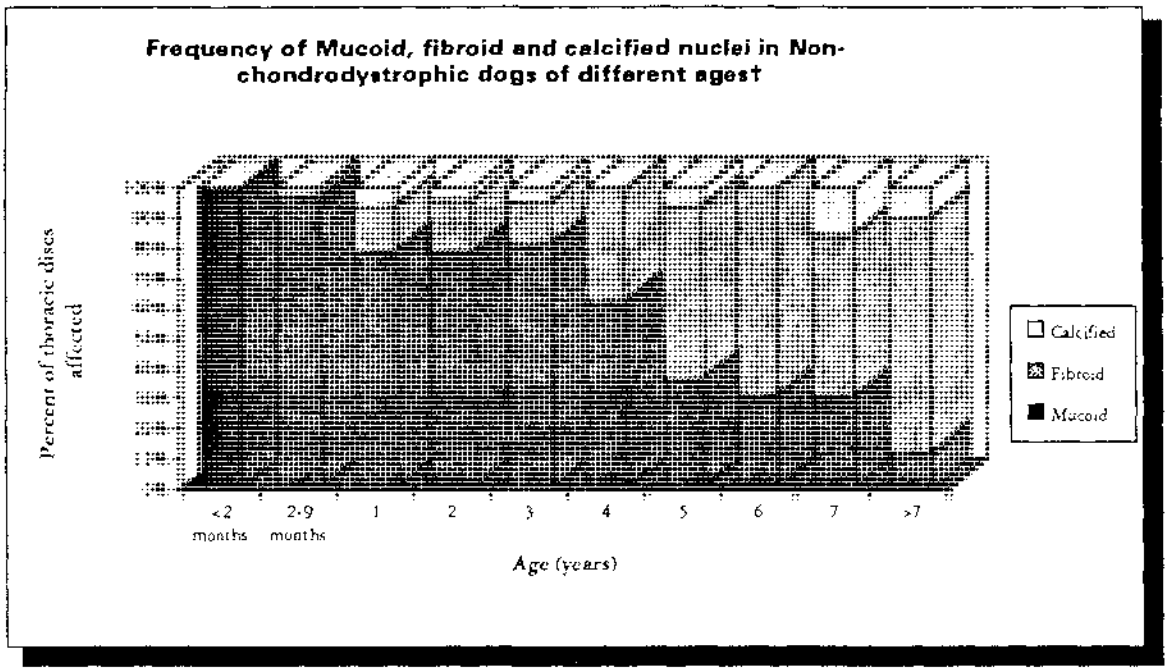


Figure 4.1: *Graph showing the relative proportions of non-chondrodystrophoid discs which may show some signs of degeneration (as indicated by increased fibrosis or chondrification) with increasing age. (†with data from Hansen HJ (1952)[126]) Compare this with the chondrodystrophoid disc [Figure 4.3]*

4.2.2 Microscopic changes in the nucleus pulposus

The first feature of degeneration to be evident microscopically is a focal increase in cellular mitotic activity, especially about the perinuclear region of the intervertebral disc[126]. The newly divided cells gather themselves into variable sized groups, their nuclei arranged in a wheel-spoke pattern. These cells appear to be metabolically active, with collagen being the main cellular product. At times, a loose aggregation of fibrils can be seen surrounding the cells. The concentration of proteoglycan in the matrix, as measured by staining intensity, decreases during this period[126]. The collagenisation of the perinuclear region eventually extends deeper into the nucleus, dividing it into distinct lobules, separated by many collagenous septae[126]. These septae ultimately thicken, and soon the cells of the nucleus pulposus are located in a net of fibrillar bundles. In some of these isolated lobules, degeneration of the cellular features may be apparent.

The probability for these degenerative changes to occur increases in dogs over seven years of age[126], although no studies are available that report the incidence of non-clinical disc degeneration in the non-chondrodystrophoid dog.

The above process of fibroid metamorphosis of the nucleus pulposus could be considered to be a progressive maturation of the intervertebral disc. Viable cellular elements, actively secreting matrix products are visible throughout the disc. Nevertheless, truly degenerative changes are

matrix products are visible throughout the disc. Nevertheless, truly degenerative changes are observed to occur, and these are characterised by cellular and intercellular matrix changes. Cellular death, as evidenced by progressive disintegration of the nucleus, predominates in these degenerative areas. Though certainly more common in the older animal, focal areas of degeneration may be observed in the intervertebral disc at any age[126].

4.2.3. Ultrastructural changes

(i) Collagen: The collagen content of the nucleus pulposus of the non-chondrodystrophoid canine intervertebral disc is age related. In animals less than seven to eight years of age no significant changes in the collagen content have been recorded[113,114]. Around seven to eight years however, the collagen content of the nucleus pulposus of these discs increases dramatically, rising to an average of more than 25%[114]. This change is particularly pronounced in the nucleus pulposus of discs from the lumbosacral region. No such differences in collagen content have been noted in the annulus fibrosus[114].

A similar increase in the collagen content of degenerate and aging intervertebral discs has been recorded in the human[4,13,35,57,82,110,144,228273,293,307]. It has been determined that the increase in collagen content is caused by a relative rise in the Type I collagen content, with a decrease in the amount of Type II collagen throughout the disc[4,35,57,114,228]. More significantly, Brickley-Parsons and Glimcher (1984)[35] identified regional variations in the relative proportions of both Type I and Type II collagen with age. They found that these 'modifications' could be related to biomechanical forces passing through the intervertebral disc, with more Type I collagen developing where tensile forces prevailed, and Type II collagen predominating when compressive forces were more common. These variations appeared to be mediated by an active cellular response to the prevailing internal stresses within the aging (and/or degenerating) intervertebral disc.

*(ii) **Proteoglycan:*** The proteoglycan content of the non-chondrodystrophoid nucleus pulposus is typically very high[110,112,113,114,293]. However, significant variations occur throughout the dog's life. From the immature level of up to 40%, the concentration can fall to as low as 20%, by 10 months of age. However, by eight years of age it has increased again to about 33%[112,114].

Specific analysis of the variation in the glycosaminoglycan content of the degenerating intervertebral disc in both the dog[112] and the human[4,12] indicate changes that have been associated with the normal aging process of other connective tissues in the body[11,125,167,195,196]. By five years of age, the predominant glycosaminoglycan molecule in the matrix has become keratan sulphate, with concentrations localised about the cells[112]. (Figure 4.2)

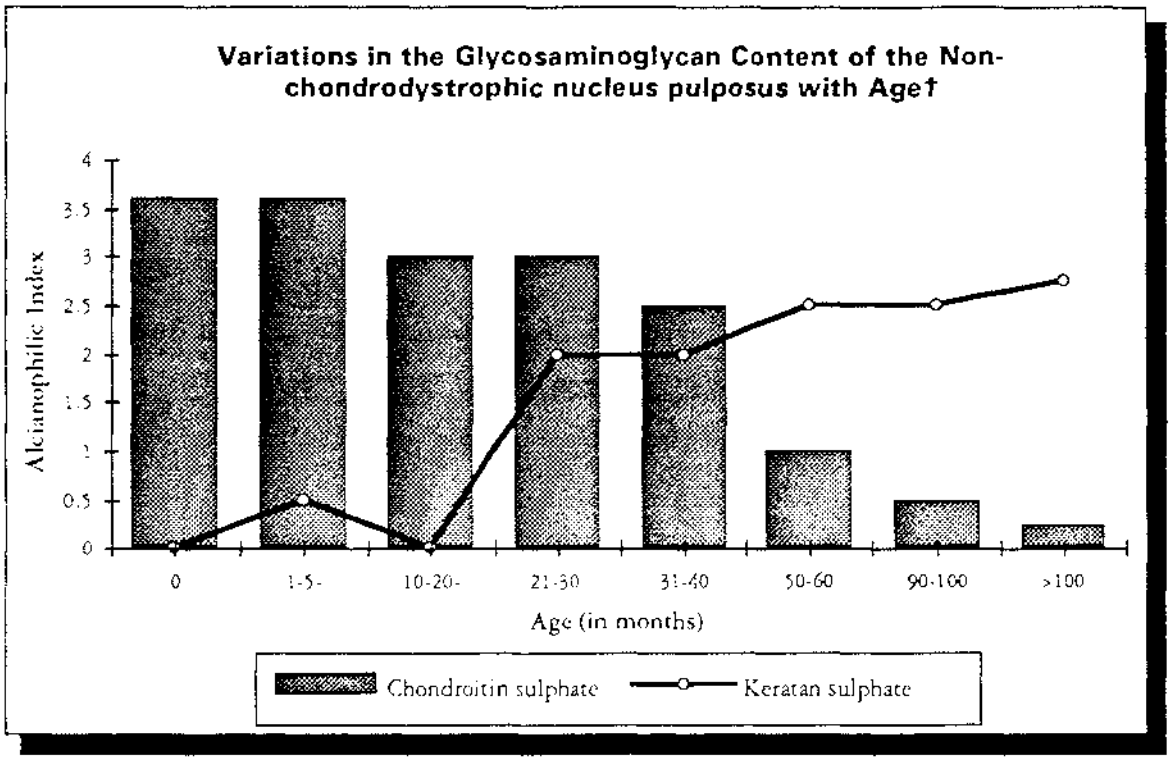


Figure 4.2: *Graph showing the relative concentrations of the major glycosaminoglycans within the non-chondrodystrophoid nucleus pulosus with increasing age. By 60 months (5 years) keratan sulphate is the predominant glycosaminoglycan. Compare this with the chondrodystrophoid disc (Figure 4.6) (†with data from Ghosh P, Taylor TKF, Braund KG and Larsen LH (1978)[111])*

In the aging intervertebral disc, greater concentrations of proteoglycan molecules can be extracted from the matrix[49, 77, 161, 229, 266, 294], implying reduced aggregation of the molecule to hyaluronic acid[129, 161, 229]. As the concentration of the glycoprotein-link molecule remains relatively unchanged over this period[74], this decreased aggregation appears to be due to a loss of the binding region on the glycosaminoglycan molecule.

*(iii) **Variation in the ratio of collagen:proteoglycan:*** In the nucleus pulposus of the canine intervertebral disc, the ratio of collagen:proteoglycan is fairly constant for much of the dog's life[114]. By about 10 years of age it begins to increase, signifying a relative rise in the collagen content[114]. However, in the annular and perinuclear regions of the disc, the levels of collagen:proteoglycan can show considerable variation. Beginning at about two years of age, the ratio can increase considerably, peaking at about five years of age. A gradual decline is then detected. Interestingly, a similar collagen:proteoglycan ratio is observed in all regions of the disc by 10 years of age[114].

4.3 DISC DEGENERATION IN THE CHONDRODYSTROPHOID BREEDS

Chondrodysplasia is an affliction which results in a disturbance of endochondral ossification[30, 126, 157, 236, 260, 277]. It represents a failure of the chondroblasts of the epiphyseal plate to grow and mature. Histological examination of the ossification zone in the Beagle, an example of a chondrodysplastic animal, shows that the cells have failed to undergo dissolution, the trabecula pattern is immature, and calcification is minimal[30]. The effect of this disturbance in growth is a disproportionate dwarfism. The Dachshund is the classic example of this breed, with short, deformed legs and a comparatively long body length. In some breeds, the bones of the head are also affected (Pekingese, Bulldog).

Chondrodysplasia is a heritable disease of man, cattle, poultry and rabbits and it is assumed that the development of the chondrodystrophoid breeds of dogs has been by the direct selection (by human interference) of a pathological trait.

4.3.1 Gross changes

In the newborn individual, the cut surface of the intervertebral disc is glistening and shows little dissimilarity to a disc from a non-chondrodystrophoid dogs at this age[126]. However, changes in the disc occur rapidly and by one year, this 'mucoid' nucleus has been replaced by a cartilaginous tissue which is greyish-white or greyish-yellow in colour, with a consistency of soft caramel[126]. (Figure 4.3) Changes occur in the intervertebral discs of all regions almost

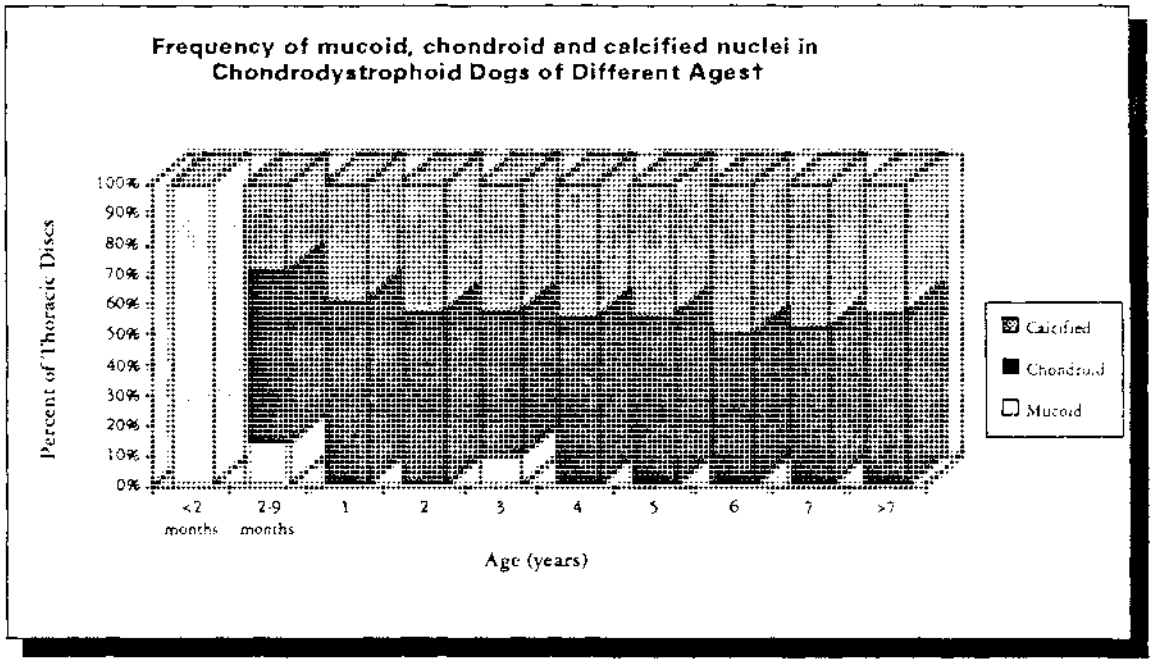


Figure 4.3: *Graph showing the relative proportions of chondrodystrophoid discs which may show some signs of degeneration (as indicated by increased chondrification or calcification) with increasing age. (†with data from Hansen HJ (1952)[126]) Compare this with the non-chondrodystrophoid disc (Figure 4.1)*

simultaneously. The gross distinction between the nucleus and annulus fibrosus gradually becomes less apparent, and physical separation of the structures is nearly impossible.

Partial or complete calcification of the centre of the disc is the next degenerative feature to become apparent. This occurs most frequently in the thoracic region[126, 147]. (Figure 4.4) Calcified discs have been observed in puppies as young as five months of age but their incidence increases with age.[126] The incidence of calcified discs have been examined both by radiographic studies[147, 268] and post-mortem[126, 145]. The radiographic studies indicated, on average, two to three calcified discs per animal (10%), but the more accurate technique of post-mortem examination found between 60-70% of intervertebral discs are calcified in dogs over two years of age.

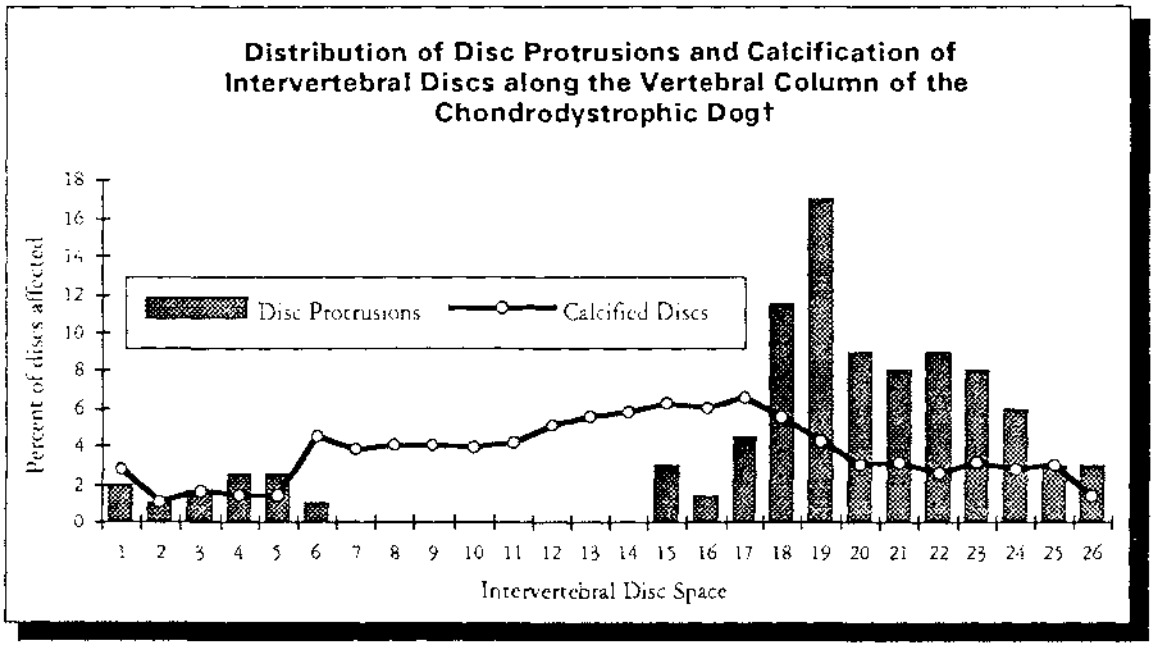


Figure 4.4: *The comparative distribution of disc calcification (line) and disc protrusions (histogram) along the length of the vertebral column of the dog, as revealed at autopsy. Note the peak incidence of disc protrusion and calcification at the diaphragmatic zone (Disc 19 = T13/L1), and the lack of protrusions (but the prevalence of calcification) within the thoracic region. († from Hansen HJ (1952)[126])*

In some discs, the nucleus is composed of a greyish-brown or dirty-brown substance. Hansen (1952)[126] noted that this kind of colouration was observed only when the disc centre has been in communication with surrounding tissues. In these discs, prolapse of the degenerate nuclear material has occurred through complete rupture of the annulus fibrosus. The brown discolouration is attributed to breakdown products of haemorrhage which results from this prolapse[126]. Prolapse has been found to occur in about 20% of discs that show calcification[145]. The most

common direction for prolapse of nuclear material is dorsolateral[126]. The tears in the annulus fibrosus tend to be radial, beginning frequently in the inner layers and eventually radiate towards the dorsal side of the disc[126].

The most common site for disc herniations that cause clinical symptoms in the dog is at $T_{12/13}$[39, 102, 118, 126, 145, 147, 179, 221, 233, 294]. Neighbouring disc spaces also show a greater likelihood of rupture compared to other regions of the vertebral column. One will recall that this is the zone about which greatest movement of the vertebral column occurs. Interestingly, clinical disc disease in man is more common in the low lumbar region of the vertebral column ($L_{5/6}$), which is the zone of greatest movement in this species (see Figure 3.8, page 57).

Whilst clinical disease is more commonly caused by prolapse of intervertebral discs about this 'high-motion' zone, autopsy investigations have shown that disc prolapses in chondrodystrophoid dogs is uniformly frequent throughout the lumbar spine[145]. (Figure 4.5) It is suggested that the severity of the clinical disease which results following failure of a disc is not determined by the size or location of the lesion, but rather by the duration and intensity of the herniation[222]. It is possible that, with a slowly developing lesion, compensatory changes occur in the spinal cord and acute paralysis is less likely.

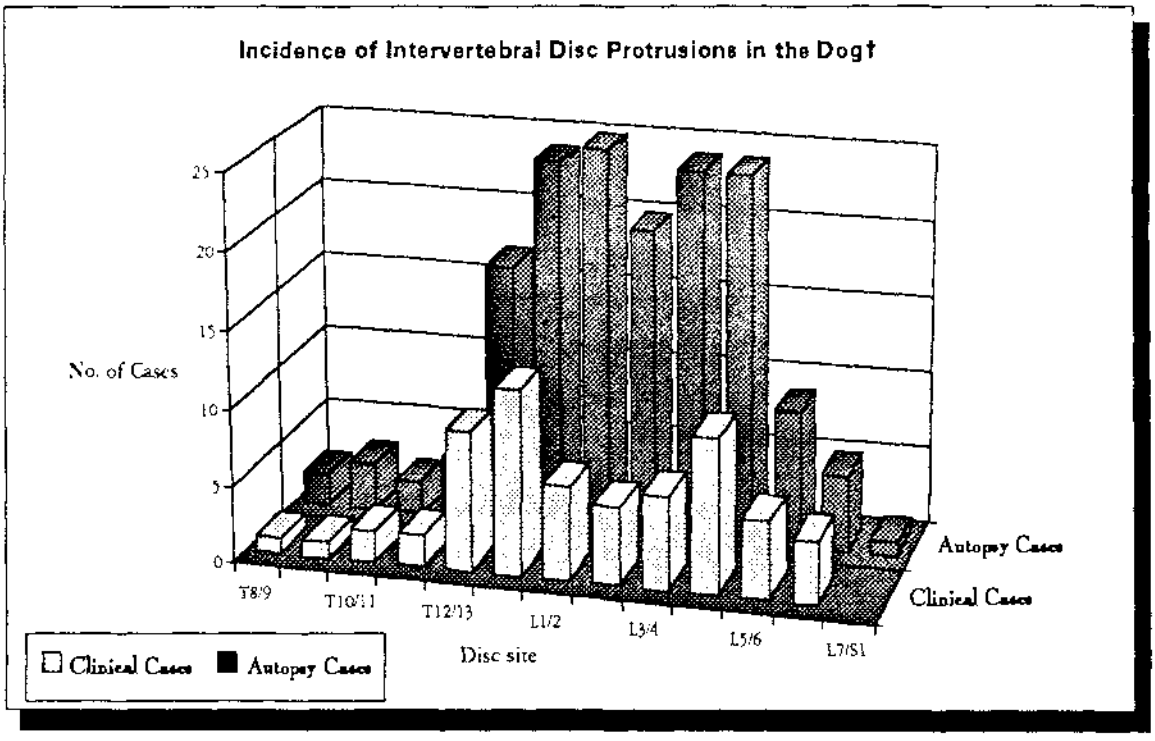


Figure 4.5: Despite the high clinical incidence of disc herniations about the 'high-motion' diaphragmatic region, Hoerlein found a relatively uniform incidence of disc protrusions along the length of the vertebral column at autopsy. (†with data from Hoerlein BF (1953)[145])

4.3.2 Microscopic changes in the Nucleus Pulposus

As with the non-chondrodystrophoid dog, degenerative changes are observed initially within the perinuclear region[126]. An increased mitotic activity in this area results in the formation of active cellular 'nests' and a subsequent increase in collagenisation. These events, which histologically are similar to those described for the non-chondrodystrophoid dog, occur at a much earlier age and may commence as early as two months of age in some dogs[126]. By one to two years of age, the collagen content of the tissue has increased dramatically, the isolated cells adopt a half-moon configuration and the tissue ultimately takes on the histological appearance of cartilage[126].

Progression of the perinuclear activity results in an increase in fibrous tissue which ultimately divides the nucleus pulposus into lobules. In some of these lobules, cellular activity occurs similarly to that described for the perinuclear region, and cartilage-like tissue may develop. In other lobules, degenerative changes are observed, with karyolysis and pyknosis of the nucleus, accompanied by disintegration of the intercellular matrix[126]. In some animals, no interlobular degenerative changes occur and the whole of the nucleus is transformed into a homogenous cartilaginous-like tissue[126].

The development of cartilaginous regions in the intervertebral disc occurs rapidly and the above changes are generally complete by one year of age. This process appears to be mediated by cellular activity and viable cells are still apparent within the tissue. With continued aging of the disc, extensive further changes occur, and signs of cellular degeneration become more widespread[126].

Focal calcification of areas of the perinuclear region follows cellular degeneration and disintegration of the intercellular substance. As these small foci enlarge and coalesce, the central chondroid portion of the nucleus ultimately becomes surrounded by a moat of calcified tissue[126]. Cellular features may completely disappear, or they may remain as pyknotic shells, engulfed in a sea of calcified matrix.

4.3.3 Biochemical changes

(i) Collagen: The collagen content of the nucleus pulposus in the chondrodystrophoid dog is considerably greater than that found in a similarly-aged non-chondrodystrophoid dog[113]. Though already high at birth, the collagen content rises rapidly and by 11 months of age the nucleus pulposus at all spinal levels contains an average collagen content of 25%[114]. However,

following this initial rise from birth to maturity, the concentration of collagen in the disc does not change substantially in the following few years[114].

(ii) Proteoglycan: In the chondrodystrophoid disc, the concentration of proteoglycan is more variable with age although, except in the annulus fibrosus, no overall trend is detectable. In the annular region there is a general decline in proteoglycan concentration from birth up to 5 years of age. At all ages, the quantity of proteoglycan in the chondrodystrophoid intervertebral disc is below that reported for the non-chondrodystrophoid disc[114].

The development of keratan sulphate as the dominant glycosaminoglycan is almost complete in the chondrodystrophoid disc by two and a half years of age[111]. (Figure 4.6)

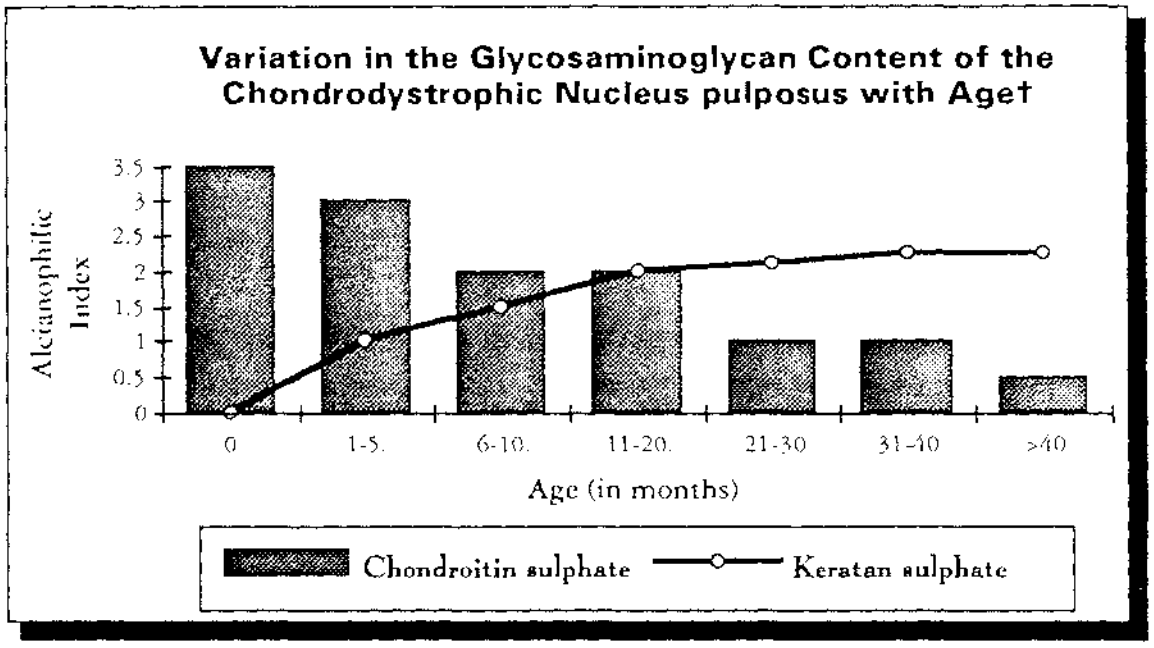


Figure 4.6: *Graph showing the relative concentrations of the major glycosaminoglycans within the chondrodystrophoid nucleus pulposus with increasing age. By 30 months (2.5 years) keratan sulphate is the predominant glycosaminoglycan. Compare this with the non-chondrodystrophoid disc [Figure 4.2] (with data from Ghosh P, Taylor TKF, Braund KG and Larsen LH (1976)[111])*

(iii) Ratio of Collagen:Proteoglycan: The variation of collagen and proteoglycan in the chondrodystrophoid disc shows significant quantitative differences to the non-chondrodystrophoid disc. Even from birth, the nucleus pulposus of the chondrodystrophic disc can contain up to twelve times more collagen than proteoglycan. Similar proportions are recorded in the annulus fibrosus and the collagen content continues to increase further with age. Collagen and proteoglycan content have not been measured beyond 6 years of age in the chondrodystrophoid dog.

'STAGE 1'

The normal intervertebral disc. Apart from a slight increase in stiffness in the disc with age, associated with a rise in the collagen content of the nucleus pulposus at 8 years of age, significant degenerative changes do not occur in the majority of non-chondrodystrophoid intervertebral discs.

'STAGE 2'

Traumatic injury to the disc is believed to be the initial incitement for degeneration of the non-chondrodystrophoid intervertebral disc. The most injurious force is torsion, especially when the disc is already under a compressive load. Torsion causes the radial separation of the outer lamellae and this loss of cohesion between the individual layers affects the ability of the annulus fibrosus to absorb the tensile and shear forces transmitted to it by the nucleus pulposus.

'STAGE 3'

The disruption to the normal biomechanical properties of the annulus fibrosus causes adaptive changes in the nucleus pulposus. These modifications are mediated by a viable cell population in response to the altered biomechanical forces passing through the disc. The ultimate effects of this cellular remodelling is the division of the nucleus into distinct lobules due to the invasion (or proliferation) of fibrocartilaginous septae passing between cellular groups.

'STAGE 4'

In time, more severe degeneration of isolated nuclear lobules may occur resulting in progressive disintegration of the nucleus. These degenerative changes are characterised by cellular death, necrosis and calcification. In other places, progressive fibrosis is occuring through proliferation of fibrocartilage. The continued disruption to the biomechnical function of the disc beacuse of the stiffer nucleus causes bulging of the disc, and the outer annular fibres constantly stretched beyond their elastic limit. Failure of the fibres ultimately occurs.

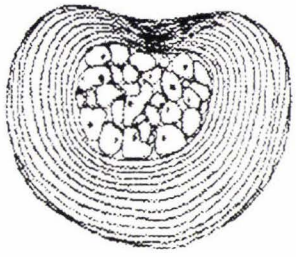
'STAGE 5'

Continued biomechanical loading of the degenerating intervertebral disc may finally cause complete failure of the annular fibres, with extrusion of nuclear material in a dorsal, or dorsolateral direction. Typically, this type of herniation is slow in onset so the resultant clinical signs may be mild due to compensatory nature of the spinal cord.

Figure 4.7: *A schematic representation of the development of disc degeneration in the non-chondrodystrophoid disc.*

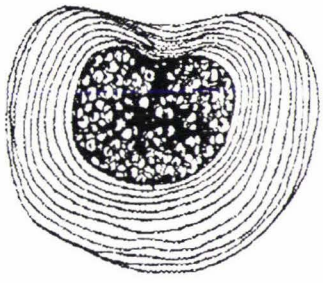
'STAGE 1'

The normal chondrodystrophoid intervertebral disc. Although the outward appearance of the immature disc is similar to the non-chondrodystrophoid disc, biochemical analysis reveals that the immature chondrodystrophoid nucleus pulposus contains up to five times more collagen than a similarly aged non-chondrodystrophoid disc. The proteoglycan content is also considerably below the level found in the non-chondystrophoid disc. Not surprisingly therefore, it would be expected that the chondystrophoid disc would lack normal hydraulic properties ***from birth****. The rapid development of degenerative changes in these discs may therefore be a consequence of the cellular response to the normal biomechanical loading of the discs, to which they are ill-suited to withstand.*



'STAGE 2'

The initial histological changes in the nucleus pulposus are similar to those described for the non-chondrodystrophoid disc, but occur at a much earlier age (and may commence as early as two months of age). These changes are characterised by lobulisation of the nucleus by a thickening of fibrocartilaginous septae which pass between cellular 'nests'. Individual degenerative changes also develop within the separate lobules due to active cellular remodelling, and as a consequence of premature cellular death.

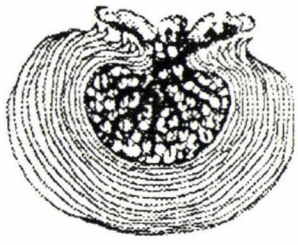


'STAGE 3'

By one year of age, chondrocyte proliferation within the nucleus causes the tissue to take on the histological appearance of cartilage, with the cells residing in individual lacunae which are arranged in a pallisading manner.

'STAGE 4'

With continued aging of the disc, focal areas of the nucleus begin to show more extensive signs of degeneration, characterised by cellular necrosis, disintegration of the intercellular ground substance, and calcification. These foci ultimately enlarge and coalesce, and the central chondroid portion of the nucleus becomes surrounded by a moat of calcified tissue. The poor biomechanical properties of the nucleus pulposus is also causing disruption to the annulus fibrosus, and individual lamellae may become separated or may rupture completely. Small protrusions of degenerate nuclear material may start to push through the inner margins of the annulus fibrosus also.



'STAGE 5'

Progressive disintegration of the lamellar fibres ultimately creates a path through the dorsal annulus fibrosus. The stiff nucleus pulposus may explode through this weakened zone and the hardened, calcified tissue impacts suddenly on the spinal cord and lacerates the venous sinuses. The resultant spinal cord injury, vascular disturbance and continuing spinal compression can cause severe neurological problems in the animal.

Figure 4.8: *A schematic representation of the development of disc degeneration in the chondrodystrophoid disc.*

4.4 CHANGES IN THE ANNULUS FIBROSUS WITH DISC DEGENERATION

Although initially alluded to by Coventry *et al* in 1945[59], only recently have medical researchers agreed that, in man at least, degenerative changes in the intervertebral disc may frequently begin in the annulus fibrosus[84, 158, 190, 223]. Unfortunately, the histological description of changes within the nucleus pulposus are more completely described than those in the annulus fibrosus largely because nuclear degeneration is more grossly apparent and herniations, when they occur, are more noticeably composed of degenerate nuclear material. In many reports, changes within the annulus tend to be lumped in with the general description of nuclear degeneration. Similarly, in those papers that do describe changes in the annulus fibrosus, concomitant changes in the nucleus pulposus are often inadequately described.

According to Hansen (1952)[126], there are differences in the changes that occur in the annulus fibrosus between the chondrodystrophoid and the non-chondrodystrophoid dog. He found that in the chondrodystrophoid dog, changes in the outer portions of the annulus fibrosus are never present without comprehensive changes occurring in the central region. This is in contrast to the non-chondrodystrophoid dog where foci of degeneration are frequently observed within the annulus fibrosus before there are any significant changes within the nucleus pulposus. This has also been reported in the human[84, 86, 158], and confirmed experimentally in rabbits[190, 191, 262] and sheep[223].

4.4.1 The non-chondrodystrophoid dog

One of the earliest changes to occur in the annulus fibrosus of man[86] and the non-chondrodystrophoid dog[126] is slight separation of the lamellae at small foci around the disc; the lamellae remain organised into layers but within each lamella there are gaps and fissures between the collagen bundles. This radial separation is preceded by a disintegration of the ground substance into granular material. Investigations in man have shown that this material contains an increased glycoprotein and decreased proteoglycan content[74, 218].

Concomitant changes in the collagen fibres also occur. These include hyaline degeneration, characterised by swelling of the fibres, homogenisation and subsequent fragmentation of the lamella structure[126]. However, complete cross-rupture of individual lamellae is generally infrequent. Human discs, especially from individuals over the age of 65 years, show similar changes[218]. By the age of 73, only a few individual lamellae are intact and discernible. Increases in glycoprotein and a decreased proteoglycan content are recorded in the associated ground substance. This alteration in the collagen structure is similar to what has been described for other aging human tissues, notably the periodontal ligament and the gingivae[218].

Cellular changes in the annulus eventually parallel those which are seen in the perinuclear region[126]. In the human disc, 32% of the cells show some signs of degeneration by the age of 30 years[218]. The remaining cells, over the following two decades, undergo similar chondroid changes as have previously been described. Calcification of the tissue can occur occasionally[89, 126].

Initially, only isolated, focal areas of annular degeneration occur in the disc. With age, however, individual foci may form a linear passage between the centre of the disc to the periphery. 'Intradiscal protrusions' of nuclear material are frequently seen projecting along this ramified communication between lamellae in all directions[126]. Complete ventral protrusions tend to be prevented due to the greater resistance of the outer annulus fibrosus in this region. Nevertheless, the presence of nuclear material about the ventral longitudinal ligament following intradiscal herniations is implicated in the formation of osteophytes at this site[126, 237] (Section 4.5.1).

Dorsal protrusions of nuclear material can reach further towards the periphery. Frequently the outer lamellae, or the dorsal longitudinal ligament, bulge due to the presence of the underlying prolapse. Reversal of the orientation of the inner lamellae is also reported in conjunction with these protrusions[126, 188, 218].

The dorsal bulging of the disc due to the presence of intradiscal prolapses of nuclear material was classified as a Type II herniation by Hansen[126]. (Figure 4.9) Type II protrusions are characterised especially by their occurrence in more aged individuals.

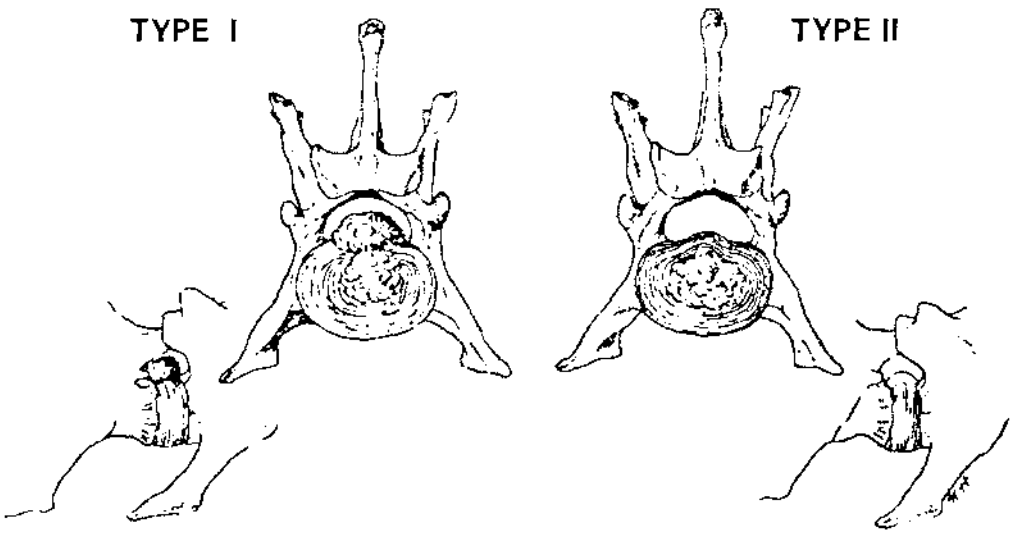


Figure 4.9: Representation of typical type I and type II intervertebral disc herniations. In type I, note the herniation of the nucleus pulposus through the annulus fibrosus and into the neural canal. In type II, note the bulging of the nucleus and annulus, without protrusion of the nucleus. (From Hoerlien BF (1978) [150])

4.4.2 The chondrodystrophoid dog

The degenerative changes that are observed in the annulus of the chondrodystrophoid animal are similar, but more extensive, and occur at a much younger age, than those in the non-chondrodystrophoid animal[126]. These changes involve the lamellae, the ground substance and the cells and have only been described in detail in chondrodystrophoid dogs by Hansen (1952)[126].

Changes in the collagen fibres occur at all regions of the annulus fibrosus. In the outer regions, however, hyalinisation of the fibres predominate initially but, eventually, complete disintegration and fragmentation of the lamella layers is observed. Rupture of disintegrated inner fibres occurs early and, in contrast to the non-chondrodystrophoid dog, cross-ruptures of the fibres are common. At these sites, the ends of the fibres become club-shaped. Because of these ruptures, significant disruption to the continuity of the annular lamellae occurs at an early age.

Degenerate nuclear material may occasionally protrude through the annulus fibrosus in all degrees, ranging from small cross ruptures of the inner fibres with a penetrating narrow point of nuclear material, through to complete disintegration of the annulus fibrosus, particularly in the dorsal direction. Some protrusions may simply follow a dissecting path, finding communications between focal areas of separation. Commonly, however, complete separation of the inner lamellae occurs and the ruptured ends of the fibres protrude together with the herniated nuclear material. These ruptures, which appear more explosive in character, with total cleavage of the peripheral layers of the annulus, occur more typically in the dorsal direction. (Figure 4.9)

4.5 THE PATHOPHYSIOLOGY OF DISC DEGENERATION

It is clear that disc degeneration and subsequent herniation of the intervertebral disc in the chondrodystrophoid dog follows a significantly different course to that seen in the non-chondrodystrophoid dog. The most important difference is the order in which degenerative events occur. (Figures 4.7 and 4.8) In the chondrodystrophoid dog, a poor structural differentiation of the intervertebral disc is present from birth. In comparison to the non-chondrodystrophoid dog, the collagen content of the chondrodystrophoid disc is significantly elevated (Figure 4.10) and the proteoglycan content of the disc is reduced. Degenerative changes, which appear initially to be mediated by active cellular remodelling, predominate about the perinuclear region and the tissue takes on an increasing cartilage-like quality, becoming calcified in many places. From our knowledge of the biomechanics of the disc, it would be possible to say that the chondrodystrophoid disc would probably respond poorly to normal biomechanical loads from

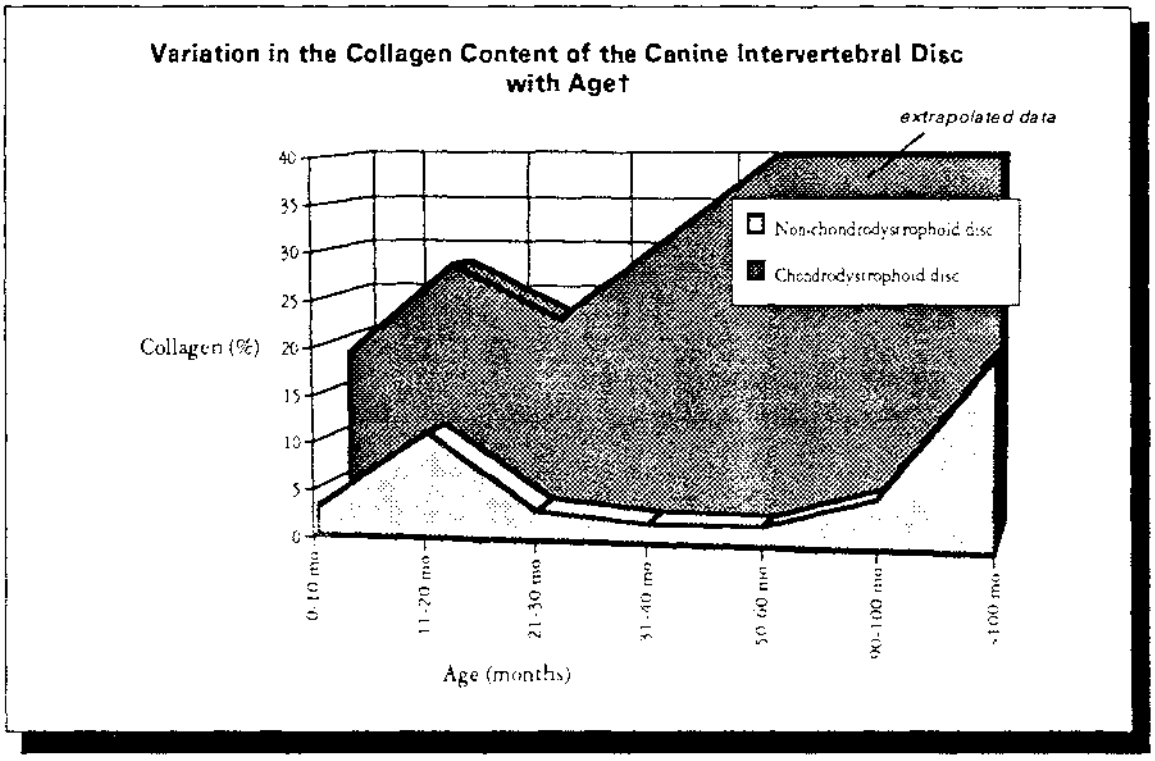


Figure 4.10: *Representation of the variation in the collagen content of the nucleus pulposus of both chondrodystrophic and non-chondrodystrophic discs. Note the considerably high collagen content of the chondrodystrophoid disc from birth, and throughout life, and the very low collagen content of the non-chondrodystrophic disc up until 100 months of age (8.5 yrs) (with data from Ghosh P, Taylor TKF, Braund KG and Larsen LH (1976)[111])*

an early age. It would be less flexible and exhibit very little of the viscoelastic properties that characterise a normal, hydrated disc. Unfortunately, biomechanical testing of the chondrodystrophoid disc has not been performed.

Herniation of degenerate nuclear material occurs following complete failure of the annular rings, providing communication with the neural canal. In the chondrodystrophoid breeds, failure of the annular fibres may be due to two causes, although it is possible that both processes occur concurrently. These processes are:

1. Associated 'chondrodystrophic' abnormalities resulting in aberrant differentiation of the annular fibres.

2. A poorly functioning nucleus which, as a consequence of its comparative inflexibility, would result in a large proportion of the biomechanical load passing through the annular ring.

In the non-chondrodystrophoid dog however, degeneration is a more gradual affair. In most animals, the changes that occur in the disc are associated with senile remodelling, and are the result of cellular remodelling of the substrate. Changes in the annulus and nucleus appear to occur simultaneously in some dogs, whilst in others, significant annular degeneration is present when the nucleus is comparatively normal.

Specific investigation into the pathophysiology of degeneration in the chondrodystrophoid disc has not been made. In comparison, considerable investigations have been made into the causes of degeneration in the human disc[86, 125, 158, 188, 189, 190, 191, 223, 262], whose disc structure and reported degenerative changes closely resemble those described in the non-chondrodystrophoid dog[117, 126]. This work, which has utilised post-mortem and animal model material, has identified a number of individual factors, including trauma to the annulus, excessive cellular remodelling and nutritional effects which together probably contribute to the ultimate degenerative process.

It is probable that these influences also play a role in the degeneration of the chondrodystrophoid disc but, due to the inherent differences in structure, occur more rapidly and to a greater degree than they do in the non-chondrodystrophoid disc.

4.5.1 Annular Tears

The recognition of concentric fissures or tears in the annular lamellae before the onset of substantial changes in the nucleus pulposus has been made by several authors[56, 95, 144, 223]. The suggestion that the link between annular ruptures and disc degeneration represented a separate entity from the normal physiologic aging process of the disc was originally presented as early as 1948[95]. Investigations over the following 40 years have determined that, in the human, two distinct annular tears exist[84]. One occurs in the inner layers of the annulus and is the result of failure of the fibres following degenerative changes. The second occurs towards the periphery of the annulus and appears to be a traumatic rather than a degenerative process. Biomechanical studies have identified that the tensile strain in the lamellar fibres during compression and rotatory stress is highest in the outer regions of the annulus[104, 208, 302]. Experimental work by Farfan created surprisingly similar peripheral tears in intervertebral disc sections which were subjected to failure with torsional loads[86]. These tears, which represented a loss of cohesion between the layers of the lamellae, were limited to the outer region of the annulus; the inner regions remained undisturbed.

Further experimental work in rabbits[190, 191, 262] and sheep[223] has shown that the resultant histological appearance of the disc following an artificially created tear in the annulus closely mimics the changes that are observed during clinical degeneration. These models of disc

degeneration thus allowed closer analysis of the biochemical changes which occur in the disc immediately after trauma.

In these models, the proportions of the collagen, proteoglycan and water content which ultimately developed in the 'degenerate' disc reflected changes previously described for *in vivo* degeneration. However, analysis of the *peracute* changes in the disc provided considerable insight into the response of the disc to injury[190,191]. Immediately following injury, there is an acute loss of water from the disc. Within four days, however, the water content returns to near normal levels. These changes are paralleled by changes in the proteoglycan molecules[191]. A loss in the aggregating ability of the proteoglycan molecules occurs immediately after injury, but this ability is fully restored at about two weeks.

The restoration of proteoglycan and water content to pre-injury level is short lived, and a progressive decline of the aggregating proteoglycans occurs seven weeks after injury[191]. A corresponding gradual loss of water also begins at this time[190,191]. These observations have been recently confirmed using magnetic resonance imaging (MRI)[223].

It has been suggested that the biochemical changes that occur in the intervertebral disc after injury could be attributed to a reparative process[191]. This process is mediated by the cellular elements of the disc which, as have been discussed, are sensitive to changes in the mechanical forces acting through the disc[241,243]. A tear in the peripheral region of the annulus fibrosus would probably affect the confined fluid mechanics of the intervertebral disc, and this is revealed by the immediate loss of water and proteoglycan aggregates from the disc. However, the increased synthesis of proteoglycan by the cells immediately after injury results in the rehydration of the tissue and, if the lesion is small enough, this swelling could effectively close and heal the defect, resulting in a reinstitution of closed fluid dynamics of the disc[191]. If the lesion cannot be sealed by this means, continued derangement of disc function results in the acceleration of degenerative changes, characterised by collagen deposition, alterations in the proteoglycan matrix and continued loss of water[191].

From the findings of these experimental models, it would appear that dehydration of the nucleus occurs late in the development of disc degeneration, and is the consequence of progressive biochemical changes in the disc. These biochemical changes are in turn mediated by altered cellular turnover of the proteoglycan components of the matrix due to a shift in the forces acting through the disc.

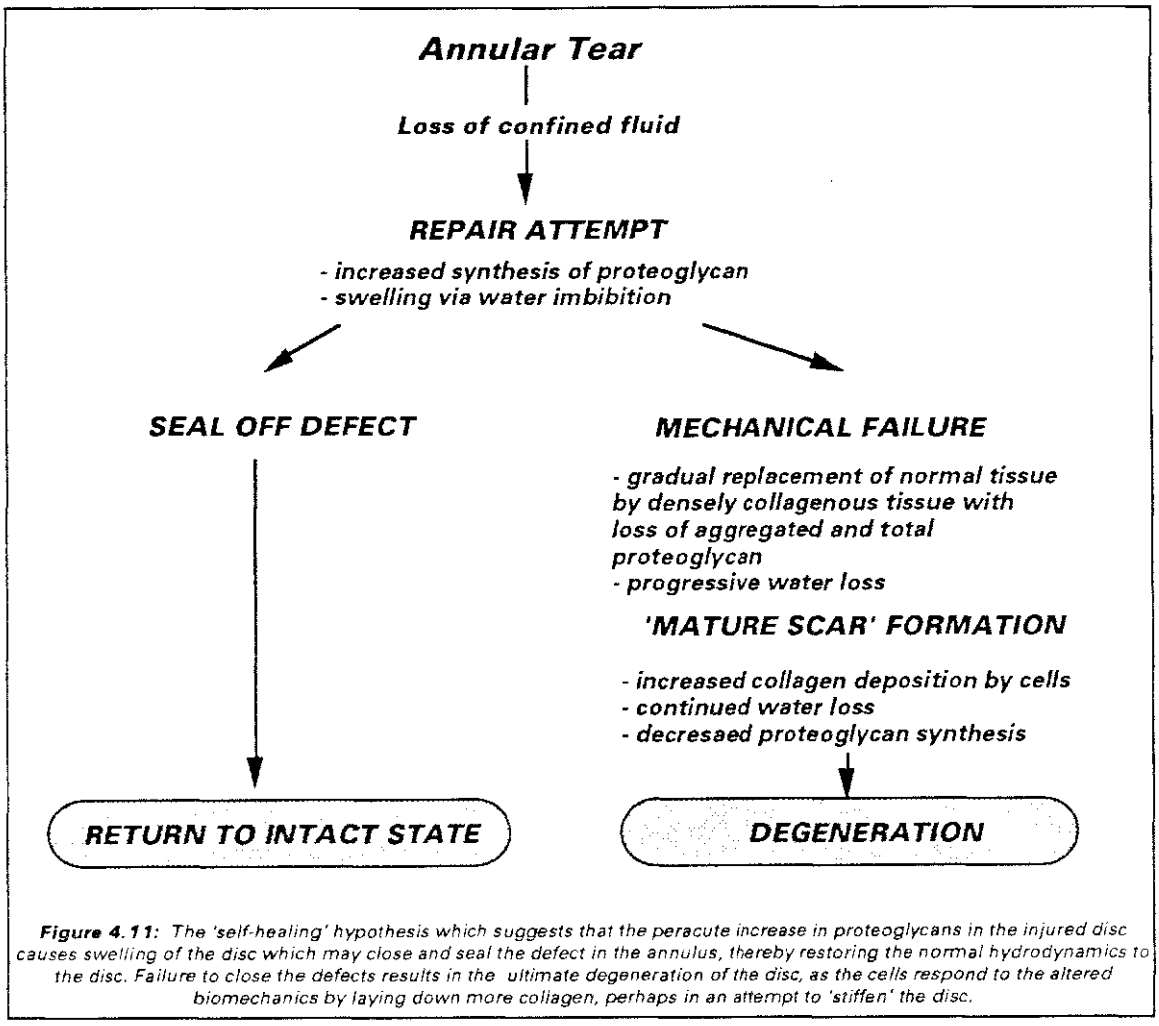


Figure 4.11: *The 'self-healing' hypothesis which suggests that the peracute increase in proteoglycans in the injured disc causes swelling of the disc which may close and seal the defect in the annulus, thereby restoring the normal hydrodynamics to the disc. Failure to close the defects results in the ultimate degeneration of the disc, as the cells respond to the altered biomechanics by laying down more collagen, perhaps in an attempt to 'stiffen' the disc.*

The importance of the cellular elements on the maintenance of the intervertebral disc is thus apparent. Malfunction of the cellular components of any organ system will result in some disruption to its performance. *Malfunction*, in this instance, could be considered to be a) a consequence of normal substrate remodelling or b) the result of cellular death. The cellular influence on disc degeneration is the focus of the next section.

4.5.2 Cellular influence on degeneration

The cells of the intervertebral disc produce and maintain collagen and other components of the intercellular matrix (ie proteoglycan, including the glycosaminoglycans). As discussed earlier (Section 4.2.3 (i)), active reorganisation of the regional distribution of Type I and Type II collagen appears to occur in the intervertebral disc, with a significant rise in the amount of Type I collagen developing in the nucleus with increasing age[35].

It is well accepted that mechanical forces can influence the type of tissue formed by mesenchymal cells. In tissues subjected to tensile forces, elongated fibrous-type cells form (fibrocytes) whereas those designed to withstand compressive forces develop ovoid cartilaginous cells (chondrocytes). One of the characteristics of connective tissue cells is the mobility of its membrane, so it is not difficult to visualise the influence a predominating force may have on this 'compliant' cell wall.

Scapinelli and Little (1970)[241] reported that mesenchymal cells of different shapes also produced differently staining matrix, and that the change in the shape of the cells preceded the formation of pericellular matrix. Frequently, at the ends of cells they reported "multiple 'onion-skin' striations". They considered these to be due to the abnormal production of matrix from the ends of the cells which occurred during the transition of predominantly tensile force to compressive forces. Hansen (1952)[126] described similar structures occurring in the perinuclear region of both the chondrodystrophoid and the non-chondrodystrophoid disc.

The observation of the above cellular changes occurring in the degenerating intervertebral disc is in accord with the concept that a greater proportion of a compressive load passes through the annular regions with increasing dysfunction of the nucleus. The alteration in collagen distribution, glycosaminoglycan concentration and proteoglycan structure can probably be attributed to this modification of cellular function. Similar transformations in the matrix components have been recorded in other tissues within the body which are subjected to changing biomechanical loading situations during their lifetime[167, 241, 243, 289, 293] and recently, Scott and Haigh (1988)[249] suggested that low oxygen tension may cause the increased synthesis of keratan sulphate rather than chondroitin sulphate[249].

The contribution of biomechanical forces to degeneration of the intervertebral disc (and the subsequent effect of degeneration on biomechanical function) is the focus of the next section.

4.5.3 Effects of Degeneration on Biomechanical Function

Disruption to the biomechnical function of the degenerating intervertebral disc can be related to ultrastuctrual changes within the disc, including the increased proportion of Type I collagen, a higher concentration of keratan sulphate and a smaller population of proteoglycan molecules, many of which have lost their ability to aggregate with hyaluronic acid. As we have seen, many of these changes can themselves be induced by abnormal biomechanical loading of the disc, which perhaps indicates that degeneration may be a self-perpetuating process.

The effect of these ultrastructural changes is apparent when a degenerate intervertebral disc is placed under static load[40,135,141,142,158,181,187,213,219,302]. The creep behaviour that is reported in the normal disc under similar loading conditions is drastically altered in the degenerate disc. Here, the immediate deformation that occurs upon application of a load is more instantaneous and of a greater degree than that observed in the normal disc, and the final equilibrium state is attained over a shorter time interval. This deterioration in creep behaviour is partly attributable to the loss in aggregating ability by the protoeglycan molecules, whose consequent smaller size renders them more likely to diffuse from the disc under compressive loading[293]. The rapid loss of proteoglycan from the disc during loading renders it unable to function effectively as a hydraulic cushion, because the degenerate nucleus cannot develop sufficient fluid pressure to resist the load[135]. The load transferring mechanism is thereby affected, which significantly reduces the development and maintenance of resistive tensile forces in the annular fibres. A greater proportion of the compressive load must therefore be borne by the annular fibres, to which they are ill-suited to respond. Type I collagen fibres, as has been mentioned, are unable to withstand compressive loads, and tend to buckle[81,135,245,302]. Experimentally, this buckling can be observed in the degenerate disc by the increased bulging of the outer circumference of the annulus fibrosus during loading[40,141,142,187,302]. Assuming similar behaviour occurs in the clinical patient with a degenerate disc, it has been suggested that the resultant impingment of spinal nerve roots emerging from the inetervertebral foramen by this bulge may explain some of the clinical signs seen[131,135].

4.5.4 Nutritional effects on degeneration

The possible role of nutrition in the development of intervertebral disc degeneration is the last influence to be investigated in this section on pathophysiology. Investigations into this aspect of intervertebral disc function have revealed that nutritive supply to the centre of the disc is probably quite tenuous[151,173,199,210,219,264,291].

The intervertebral disc is one of the largest avascular stuctures in the body[57,82,156,262]. Whilst the outer annulus is reasonably well nourished from peripheral blood vessels, supply via this route decreases rapidly within a few millimetres into the annulus[290]. The predominant route for nutrient transport into and through the disc has been shown to be largely due to diffusion[57,82,199,210,219,291]. Experimental studies show that both the cartilaginous end plate and the outer annulus are important regions for the exchange of nutrients, but the centre of the endplate, the area adjacent to the nucleus, is much more permeable than the periphery[199,210,291].

Diffusion gradients are established in the disc due to the metabolic activity of the cells in the disc creating an imbalance between the glucose, oxygen and water levels at the centre of the disc

as opposed to the outside[151, 173, 199, 264, 291]. Using an indirect estimate of the metabolic rate of the cells in the intervertebral disc, Maroudas (1975)[199] theoretically determined that, even assuming the most desirable situation of diffusion and glycolytic rate, there remains a portion of the centre of the nucleus pulposus which would be deprived of glucose. Though this is practically improbable, it does emphasise the point that, whilst articular cartilage is determined to have a 50% excess of supply over demand[199], in the intervertebral disc there is only just a balance when both routes are considered and even if the most optimistic conditions are assumed. The diffusion of other solutes into the intervertebral disc is similar to that of glucose[151, 264, 291].

The availability of oxygen is the next most critical factor in the nutrition of the intervertebral disc. In other avascular tissues, a high level of anaerobic metabolism is reported. Unfortunately, little information is available concerning disc metabolism. However, the pH of the disc is very low, and this has been correlated with an increased concentration of lactic acid[264]. This supports the suggestion of the significance of anaerobic metabolism in the intervertebral disc because lactic acid is a by-product of anaerobic glycolysis.

Diffusion of small solutes into the disc is dependant on the watery-nature of the matrix[219]. The passage of water through the disc is dependant on the effective fixed charge density of the matrix. The fixed charge density is an expression of the concentration of the negative charge prevailing in the disc at any time, which is imparted to the matrix by the glycosaminoglycan molecule[199, 210, 219, 291]. Chondroitin sulphate has a double negative charge whereas keratan sulphate has only one[77]. It has been shown that the effective fixed charge density in the nucleus pulposus of a disc statically loaded with a 30 kg weight increases to about five times higher than that of the unloaded disc[219], probably due to the relative concentrating effects of a reduced intervertebral space and the loss of water. The osmotic drive of this high negative charge carries water and dissolved solutes into the centre of the disc when the load is released[199, 210, 219, 264, 291]. With keratan sulphate becoming the predominant glycosaminoglycan in the degenerate disc (Sections 4.2.3 and 4.3.3), the fixed charge density of the nucleus pulposus will be considerably reduced and, as a consequence, a smaller osmotic gradient will develop.

Disruption to the normal diffusion of nutrients into the intervertebral disc will have significant effects on cellular homeostasis. Two perceived disruptions to normal diffusion are (i) the degeneration of the cartilaginous end plate and (ii) the fibrosis of the intervertebral disc.

(i) Degeneration of the Cartilaginous End Plate:

It has not been reliably determined whether the main route of diffusion into the centre of the disc is the cartilaginous end plate or the annulus fibrosus[199, 210, 219, 291].

Nevertheless, Pritzker (1977)[234] has reported that the cartilaginous end plate becomes progressively ossified with age, and the vascular channels seen in younger specimens (Section 2.7.4 (iii)) become obliterated. Such changes would be expected to hinder the passage of water and solutes through the cartilaginous end plate, but this has not been confirmed experimentally.

(ii) Fibrosis of the Intervertebral Disc:

The increased collagen content of the disc, which in advanced cases of degeneration progresses to calcification of the tissues, would probably reduce the passage of water through the disc. Similarly, the reduced proteoglycan content would result in the overall dehydration of the disc and with it, a decrease in dissolved solutes. Furthermore, the predominant tendency for the pericellular localisation of newly synthesised proteoglycan would serve to further isolate the cell from solute[126].

The observed degeneration of isolated pockets of nucleus pulposus following the increased lobulisation may perhaps be explained by the above processes. Once cellular degeneration is initiated, further acceleration of changes would occur due to the loss of maintenance of the collagen and proteoglycan matrix.

4.6 SUMMARY: INTERVERTEBRAL DISC DISEASE

As presented in the previous sections, two types of disc prolapse are recognised: Types I and II. Type I prolapses are typically of considerable size and at times their height may be more than half of the neural canal, extending cranially and caudally to the disc space. These prolapses are characteristically explosive and associated with significant haemorrhage and the associated inflammatory response may result in fibrinous adhesions between the degenerate material and the dura. By comparison, Type II prolapses are considerably smaller, having a more limited and regular shape. Dural adhesions are uncommon, and the surface of the prolapsed material is smooth and even.

It is clear that the aetiological development of each type is quite different. Type I protrusions are confined to the chondrodystrophoid breeds of dogs (and, interestingly, chondrodystrophoid human dwarfs) and their occurrence reflects the poor differentiation of the intervertebral disc in these animals. This congenital malformation, combined with the disproportionate nature of their skeletal anatomy, influences the rapid development of degenerative changes in the disc. Progressive failure of the surrounding annular fibres occurs and, when a critical point is reached, the gritty, degenerate nucleus may extrude through the weakened annular fibres with relatively

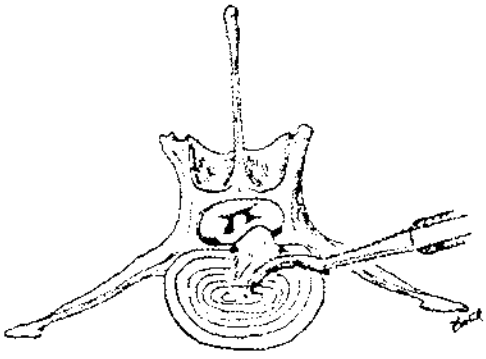
mild stress or trauma. Due to the eccentric location of the annulus and the typical ventral concavity which develops in the vertebral column during ambulation, the nucleus usually ruptures in a dorsal direction, impacting on the spinal cord with considerable force. Laceration of the vertebral sinuses may also occur, causing haemorrhage and exacerbation of the inflammatory response.

Type II protrusions may occur in (generally) geriatric individuals of any breed. They are also typical of the protrusions which develop in man, cats and most other species. The slow progression of degenerative changes in the disc gradually alters the normal distribution of mechanical loads. As the disc becomes dehydrated, its hydraulic function is lost and it is unable to develop the pre-load tension in the annular fibres which resist the compressive load.

The effect the prolapsed disc material has on the animal depends on the size of the extrusion and the duration over which the disc extruded. Type I extrusions are typically explosive resulting in severe spinal cord injury. Treatment of these cases is directed at resolving the spinal trauma, with recovery of the disc material a secondary objective. In other cases, particularly Type II protrusions, removal of partially or fully extruded disc material from the neural canal is the principal objective of therapy.

A complete discussion of the clinical aspects of intervertebral disc disease, with respect to diagnosis and treatment options is beyond the scope of this thesis. However, a review is provided in Appendix A for interested readers. The remainder of this thesis will focus on the techniques available for retrieving nuclear material from the surrounds of the annulus fibrosus.

5.0



FENESTRATION

5.1 INTRODUCTION

Fenestration is a surgical procedure which was initially described for the dog in 1951[222]. The objective of fenestration is to remove degenerate nuclear material from the annulus fibrosus. The rationale for this is a) to relieve spinal and nerve root compression due to protrusion of annulus and/or dorsal longitudinal ligament (therapeutic) and b) to prevent the potential violent herniation of the nuclear material in the future (prophylaxis). The policy of fenestration is fraught with controversy. More on this matter will be presented in a subsequent section.

5.1.1 Techniques for fenestration of thoracolumbar discs

The technique of fenestration entails making an incision into the annulus fibrosus of the disc, removal of a portion of the annulus and subsequent curettage of the nuclear material through this 'window' *(L: fenestra)*. The intervertebral disc may be accessed via ventral[23, 24], lateral[24, 71, 92, 255, 308] and dorsolateral[21, 24, 103] surgical approaches.

Ventral fenestration is accomplished through a paracostal incision with the patient in right lateral recumbency. After entering the abdomen, the left kidney and peritoneum are reflected ventrally and the abdominal viscera packed off with damp laparotomy sponges. The *iliopsoas* muscle is elevated and the short transverse process of L_1 is located for orientation. The intervertebral discs of L_{1-2} through L_{5-6} can be fenestrated through this incision by digitally depressing the aorta and sympathetic trunk.

After fenestrating the lumbar discs, the abdominal musculature is closed with an absorbable suture material. The skin incision is then advanced cranially to the tenth intercostal space, and a thoracotomy is performed. At this time, ventilation must be controlled by an anaesthetist. The intervertebral discs of T_{9-10} through T_{13}/L_1 are identified and dissected free of pleura. The sympathetic trunk, the aorta and the intercostal vessels are avoided in this dissection. These discs can then be fenestrated. The incision is closed in a routine manner.

The advantages of this approach are that only a small amount of haemorrhage is produced and spinal nerve roots are avoided. The disadvantages include the need for a thoracotomy and therefore ventilatory assistance, dissection near major blood vessels and the risk of forcing nuclear material into the spinal cord.

Lateral fenestration is performed with the animal in right lateral recumbency for right handed surgeons and left lateral recumbency for left handed surgeons. An area from the caudal aspect of the scapula to the greater trochanter and from the dorsal spinous processes to a few

inches below the transverse process is clipped and aseptically prepared for surgery. The skin incision follows an oblique line from the dorsal spinous process of T_9 to the ventral aspect of the wing of the ileum. This incision is extended through the subcutaneous fat and lumbar fascia until the fibres of the *longissimus dorsi* and the *iliocostalis lumborum* muscles are seen. At this time the tips of the transverse process are palpable.

With the index finger placed on the transverse process a curved Kelly forcep is bluntly forced through the muscle fibres of the *iliocostalis lumborum,* which lie dorsal to the transverse process. Care should be taken to avoid the blood vessels which run cranial and caudal to the transverse process. Elevation of this muscle is continued with the index finger until the lateral aspect of the vertebral lamina can be palpated. The muscle can be retracted dorsally using a deep-tongued, blunt retractor to facilitate exposure. If retraction is inadequate and vision poor, failure to fenestrate the disc without injury to the spinal nerves may result.

The intervertebral disc lies immediately cranial and slightly ventral to the junction of the transverse process and the vertebral body. The disc is covered by loose fascia containing a blood vessel and spinal nerve. These structures should be carefully retracted cranially using a blunt instrument. The lateral surface of the annulus fibrosus is identified by its white fibrous sheath. A rectangular window is then cut into the annulus with a #11 scalpel blade. The dorsal border of this incision should not extend above the junction of the transverse process and the vertebral body as this may result in penetration of the spinal cord.

The excised portion of annulus can be removed with haemostatic forceps and the nucleus pulposus removed using a curette passed in a downward, inward and outward fashion to reduce the potential for forcing nuclear material dorsally into the neural canal.

The thoracic intervertebral discs can be fenestrated in a similar fashion, but the surgical approach is more difficult due to the proximity of the rib articulation. After separating under the *iliocostalis lumborum* muscle and the rib, an index finger follows the rib to the insertion on the vertebrae. The muscle is retracted dorsally as for the lumbar region. The remaining musculature on the cranial aspect of the rib is retracted ventrally, exposing the lateral aspects of the annulus fibrosus. The thoracic pleura is immediately ventral to this dissection and should be avoided. Rupture of this pleura will result in a pneumothorax. The incision into the lateral annulus and the fenestration of the thoracic intervertebral discs should be below the arch of the rib. Dissection or fenestration above this level will penetrate the spinal cord. Because of the limited visibility on this region, a smaller window is created.

The advantages of the lateral approach include the muscle separation approach, therefore reduced soft tissue trauma and haemorrhage. It can also be easily combined with a concomitant decompressive procedure. The disadvantages are the need for a good and moveable light, good assistance for tissue retraction, the poor visualisation in muscular or obese animals, the difficulty of exposing the thoracic discs and the dissection near spinal nerve roots and vessels.

The dorsolateral approach for fenestration of the thoracolumbar intervertebral disc is similar to the approach for laminectomy procedures. The patient lies in sternal recumbency and is clipped and aseptically prepared from the mid-shoulder to mid-pelvic region down to about two inches below the transverse processes on both sides. A skin incision is made on the dorsal midline from the ninth thoracic vertebrae to the fifth or sixth lumbar vertebrae. The incision is continued through the subcutaneous fat to expose the thoracolumbar fascia. An incision in the thoracolumbar fascia is made using a scalpel blade beginning on the dorsal midline between the ninth and tenth thoracic vertebrae and continuing as near to the lateral aspect of the dorsal spinous process as possible. This scalloped incision is continued to the fifth lumbar vertebrae.

Using periosteal elevators, the thoracolumbar musculature is dissected from the lateral aspect of the dorsal spinous process of each vertebrae on the side nearest the surgeon. Tendinous attachments to the dorsal spinous processes are severed with scissors. This muscular elevation is continued to below the level of the articular facets. Dissection about the facet joints is best conducted in a caudal to cranial fashion. Scissors can then be used to sever the remaining tendinous attachment to the articular process. Cutting the tendon close to the bone minimises haemorrhage. When this muscle dissection is complete, Gelpi retractors can be used to maintain exposure.

Continued elevation of musculature from the dorsal aspect of the transverse process and the cranial surface of the ribs permits visualisation of the lateral aspect of the annulus fibrosus. The spinal nerve and vessels are located in the muscular attachments to the accessory process of the vertebrae and these structures should be retracted cranially in order to preserve them. The intervertebral disc can then be fenestrated in the manner described above.

The advantage of the dorsolateral technique is that it can be combined with laminectomy procedures. The disadvantages include extensive soft tissue dissection, frequent haemorrhage and potential damage to spinal nerves.

5.2 FENESTRATION AS A TREATMENT

The role of fenestration in the *treatment* of thoracolumbar intervertebral disc disease is the focus of considerable debate in both the veterinary and medical communities[21,72,90,101,232]. The reason for the dissension is the inability of the fenestration procedure to remove herniated disc material from within the neural canal[232]. Most surgeons accept that fenestration alone is inappropriate following an acute disc prolapse. Decompression, which allows the removal of both disc material and the constricting confines of the roof of the neural canal, is essential and must be performed within 48 hours for greatest success.

Proponents of fenestration cite the following clinical criteria as indication for surgical therapy by this technique[21,24,101,255]:

1. Back pain attributable to a degenerate intervertebral disc
2. Back pain, with or without *mild* proprioceptive and/or paraparetic deficits
3. Recurrent episodes of back pain, with or without paresis, non-responsive to conservative management

These criteria fit the clinical signs which would be expected with either a Type II, or very early Type I disc lesion.

It is generally conceded that pain syndromes related to disc degeneration are likely to arise from one of three sources[90]: local inflammation produced by the chemical irritation of prolapsed disc material; nerve root attenuation and ischaemia (radiculopathy); and 'discogenic' pain.

Inflammation of nerve root sheaths or the epidural sac is unlikely to cause neurological deficits but could conceivably be a focus of considerable discomfort[222]. The inciting factor for the inflammatory process has not been determined but an autoimmune basis has been suggested[230].

Chronic, mechanical displacement of nerve roots and spinal cord, combined with ischaemic changes within the affected structure, can contribute to the production of significant pain. There is considerable evidence in the human[23,96,131,135,158,160,166,181,202] and veterinary[28,126,127,205,222,223,232] literature to support this modality of neurogenic pain production. Prompt relief of the clinical signs of pain can be achieved by the decompression of the affected structure[100,232]. In relation to intervertebral disc degeneration, such compression may come from partially extruded disc material, bulging of annulus fibrosus due to the presence of underlying nuclear material or distortion of dorsal annulus and/or dorsal longitudinal ligament due to altered

biomechanics. It has been suggested that fenestration is ineffectual in the relief of pain attributable to this type of lesion[90, 232]. Clinical reports reveal that, whilst a positive effect is frequently seen in these patients following fenestration, the response to treatment may take several weeks[232]. Conversely, dramatic and immediate relief of clinical signs occurs within hours to days after decompressive surgery of these *relatively mild* compressive lesions.

Fenestration may however be effective in dogs with disc related pain, but without either prolapse of nuclear material nor herniation or bulging of the nucleus. Such dogs may be suffering from discogenic pain[90]. Discogenic pain implies pain resulting from the biochemical and anatomical derangement of the disc due to degeneration, without any external compression of overlying structures. The mediator of this pain may be the receptors of the sinuvertebral nerve, which penetrates the outer third of the annulus fibrosus[23, 94, 160]. The exact stimulus that excites these nociceptors has not been elucidated.

A definitive diagnosis of discogenic pain would entail negative findings of compressive disc herniation on plain and contrast radiographs, as well as positive finding on discography[90, 306]. Very few dogs with clinical symptoms would fulfil these criteria and therefore, the clinical use of fenestration as a sole therapeutic procedure cannot be generally recommended[90]. Because the vast majority of dogs with disc-related signs of pain can be demonstrated to have bulging or extrusion of disc fenestration will offer, at best, a protracted course of convalescence compared to the generally rapid and complete recovery seen after definitive decompressive surgery[232].

5.3 FENESTRATION AS A PROPHYLACTIC

Whilst the use of fenestration as a treatment for intervertebral disc protrusion is not strongly advocated, prophylactic use to prevent further episodes of disc related clinical signs has wide support[21, 24, 56, 72, 83, 90, 101, 143, 149, 179, 186, 232, 242]. Prophylactic fenestration is advocated for several reasons:

1. To prevent repeat episodes at the same intervertebral disc space
2. To prevent repeat episodes at other levels
3. To eliminate clinical signs related to mechanical derangement of disc function (discogenic pain).

A prophylactic fenestration procedure is most commonly performed at the time of a decompressive procedure, with extension of the incision and appropriate muscular dissection to allow access

to the thoracolumbar discs most prone to rupture ($T_{10/11}$ - $L_{3/4}$). Occasionally, prophylactic fenestration may be performed as an isolated procedure in a dog considered to be at high risk of developing an acute disc herniation.

The proponents of prophylactic fenestration contend that the procedure will be successful because of the removal of all degenerate and potentially extrudable nuclear material from the disc. It is also stated that fenestration will encourage prolapses, should they occur, to follow the path created by the incision into the lateral aspect of the annulus fibrosus[24, 56, 101, 221].

Though widely practised and advocated by many, the use of prophylactic fenestration is still a controversial area of intervertebral disc disease management[51, 39, 72, 90, 179, 186, 232]. The arguments for and against focus on the relative risk of the occurrence of disc prolapse, the efficacy of the procedure, and the morbidity associated with the procedure.

5.3.1 Recurrence of Disc Prolapse

The rationale for performing prophylactic fenestration is to reduce the incidence of the animal developing or redeveloping clinical signs attributable to disc degeneration. From our knowledge of disc degeneration in the non-chondrodystrophoid breeds, prophylactic fenestration would seem unwarranted in these animals due to the infrequent nature of their development. For a similar reason, fenestration of remaining thoracolumbar discs at the time of a decompressive procedure need not be performed in this breed because the chances of a second disc developing a clinically evident protrusion is extremely low[90].

The incidence of disc degeneration in the chondrodystrophoid breeds however is very high. Whilst this fact is well accepted, the true frequency of disc related clinical signs in the chondrodystrophoid population is unknown. In a retrospective study on an isolated population of Dachshunds, intervertebral disc disease developed in 79% of dogs with radiographic evidence of disc degeneration (calcification)[133]. However, other studies have shown that only 23.5% of Dachshunds in a population may have one or more calcified discs[10, 268]. On the basis of these figures, the overall incidence of intervertebral disc disease in a population of Dachshunds could be estimated to be only 19%[268].

However, it has not been established whether a individual dog, having already developed a clinically apparent disc prolapse will be at a greater risk for a future recurrence than a dog which has remained asymptomatic. Whilst a familial basis of intervertebral disc degeneration apparently occurs in Dachshunds[10], the influence of environmental effects is likely to be considerable. It is reported that 30.5 - 83% of dogs presented with an acute prolapse have had

previous episodes of 'back pain'[39,101,179,186]. These episodes may have been related to disease in other discs. More likely however is the progressive prolapse of a single disc producing recurring clinical signs until ultimate failure of the annulus causes development of more severe neurological deficits. This protracted course has been described by many authors[126,145,232].

Many surgeons recommend that a prophylactic fenestration of the intervertebral discs adjacent to the site of an acute prolapse (or in some cases, all thoracolumbar discs) be performed in all chondrodystrophic dogs which suffer an acute prolapse, due to a presumed increase risk for future clinical problems related to disc degeneration. Unfortunately, this supposition has little objective, scientific support. The development of further clinical symptoms suggestive of prolapse of either a previously treated or 'virginal' thoracolumbar disc has been addressed in several retrospective studies over the last 36 years[39,56,71,72,92,101,103,148,179,186]. Most of these studies purported that recurrent disc prolapse is common, and fenestration was successful in decreasing the incidence.

Unfortunately, most of these studies can be criticised for their inability to definitively prove that the recurrence of clinical signs was related to a new disc prolapse, and not on-going irritation from a previous disc prolapse or indeed, one of the other differential diagnosis for this condition. Several of the reports[39,71,72,92,101,179,186] based their findings on telephone reports or questionnaires from owners, an often unreliable means of measuring clinical results. Furthermore, despite advocating the use of fenestration on the basis of their figures, the investigators failed to definitively prove the involvement of a second disc in most of the animal's included in their study. In one study, plain radiographs, which provide merely presumptive evidence of a second disc prolapse[47], were available in only 13 of 33 cases.[186]. Myelography or surgery provided confirmatory evidence of a second disc prolapse in only three of these cases. In another study, documented recurrence of a second disc prolapse was available in only five of the 29 cases included in the retrospective investigation[39].

Conclusive proof of additional disc prolapses is essential if prophylactic fenestration is to be strongly advocated. Recurrence of disc-related clinical signs is just as likely to be a consequence of continued herniation of a previously prolapsed disc, or persistent nerve-root attenuation due to inadequacy of a decompressive procedure[90,232]. Reliable decompression is only achieved after complete evacuation of the extradural mass[232]. Failures may be due to poor surgical exposure, or lack of experience with the chosen technique. The recurrence of clinical signs in these cases therefore, constitutes surgical failure, rather than the reherniation of disc material *per se.*

5.3.2 Efficacy of Fenestration

The basis of fenestration is to effect removal of degenerate nuclear material to prevent its prolapse into the spinal cord[222]. It is supposed that following curettage of the majority of the disc material, intraoperative chiropractic manoeuvres would squeeze remaining material through the fenestration incision. This window would also remain as a 'weak' point in the annulus and in the unlikelihood of subsequent prolapse of this disc would present a 'path of least resistance'. Only recently have these suppositions been investigated[51, 152, 258].

One of the more significant observations that came from these studies is the apparent lack of inflammatory response which follows fenestration of the intervertebral disc[258]. Due to the avascular nature of the intervertebral disc, establishment of an inflammatory process is reliant upon the ingrowth of vessels along the wound created in the annulus during the fenestration procedure. After six months the defect in the outer regions of the annulus fibrosus is completely closed with organised scar tissue and there is a variable degree of invasion of this fibrous and vascular tissue into the central portions of the nucleus pulposus. The remaining nuclear material does not appear to incite further fibrous reaction and the overall picture following fenestration is one of 'quiescence'. Occasionally, proliferation of chondrocytes may be seen.

Based on these observations, therefore, it is apparent that the completeness of removal of nuclear material is dependant upon surgical technique. Most authors stress the importance of the thoroughness and deliberate action necessary to perform fenestration appropriately[71, 72, 83, 92, 143]. The limited exposure and poor vision associated with all fenestration techniques can frustrate even the most conscientious surgeon. Investigations conducted on human subjects has determined that, even after the most thorough fenestration performed by experienced surgeons, relatively little disc material is removed[51]. In this study, which was based on theoretical calculations of disc volume, the average percentage of nuclear material removed was only six percent. A similar investigation in the dog compared the efficacy of manual and power-assisted fenestration of thoracolumbar intervertebral discs[152]. In this study, the average amount of nucleus pulposus removed by manual curettage was only 41%. The use of a power-assisted burr introduced through the fenestration wound increased disc retrieval by a further 24%.

5.3.3 Morbidity

The intervertebral disc is located in a position which is not easily approaced surgically. Fenestration requires extensive soft tissue dissection, with elevation of the epaxial muscles from transverse and dorsal spinous processes; transection of tendinous insertions from accessory processes and articular facets; and the occlusion and laceration of spinal blood vessels and nerves. Post-operative morbidity and discomfort is high. Potential complications include laceration of

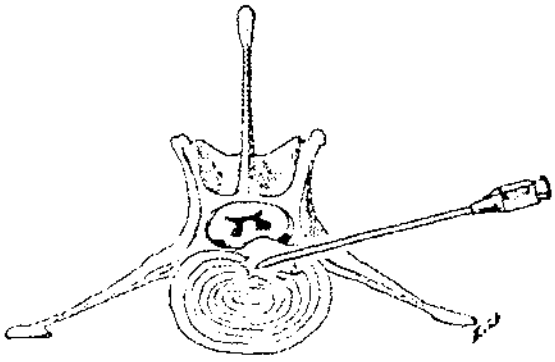
the aorta, pneumothorax, iatrogenic disc prolapse and spinal cord compression, infection and wound breakdown[21, 22, 24, 39, 71, 90, 92, 101, 103, 179, 186, 255, 300, 305, 308]. An overall complication rate of 30.7% has been reported following routine dorsolateral fenestration[308].

5.4 SUMMARY

It is apparent, therefore, that fenestration as a prophylactic procedure is fraught with criticism. It is contended that, given a *possible* recurrence rate of 20- 40%[186], fenestration is a very invasive and traumatic procedure to perform, in order to reduce the recurrence rate to only about 10 - 20%[90]. Nevertheless, it has been suggested that the inefficacy of surgical fenestration techniques to completely remove 100% of the nuclear material is the reason for continued development of clinical signs, albeit at a reduced incidence[258].

If evacuation of nuclear material could be conducted in an atraumatic and complete manner, greater acceptance of prophylaxis for intervertebral disc disease may result.

6.0



CHEMONUCLEOLYSIS

6.1 INTRODUCTION

Enzymatic dissolution of the nucleus pulposus was first proposed in 1959[141], and the term chemonucleolysis was coined to describe this process. The attraction of chemonucleolysis is that it dissolves the nuclear material of the intervertebral disc. The enzyme can be injected into the intervertebral disc via a needle introduced through the skin and guided to the centre of the disc by fluoroscopy. Invasive surgery can therefore be avoided, thus reducing the morbidity and costs associated with this procedure. A number of possible chemical agents are capable of converting the intervertebral disc to scar tissue[66, 97, 79, 133]. Two products to gain acceptance and undergo clinical use are chymopapain and collagenase. The pharmacology of these two enzymes is quite different, and is presented below.

6.2 CHYMOPAPAIN

Originally isolated in 1941[98], chymopapain is the major proteolytic component in the latex of the papaya fruit. Its effect on cartilaginous tissues was graphically demonstrated by Thomas who injected the enzyme into the ear veins of rabbits[201]. Following this injection the ears wilted and appeared to lack cartilaginous support. However, this effect was temporary and normal structure returned, indicating that the enzyme did not permanently alter the structural elements of the ear. Subsequent investigation[264] found that the proteolytic activity of chymopapain is directed at the proteoglycan molecule breaking it into its component parts: keratan sulphate, chondroitin-6-sulphate and protein. These small fragments readily dissolve out of the disc and their excretory products can be detected in the urine for several days following intradiscal injection[201].

6.2.1 Pharmacology

Since chymopapain was first injected into the intervertebral disc of rabbits, dogs and man[201], subsequent investigations have attempted to determine the effect of this enzyme on disc structure. Grossly, the most obvious effect is complete dissolution of the nucleus pulposus[27, 108, 193, 201, 265, 303]. Frequently a cavity, which includes some fibrillar material, is all that remains[27, 303]. No gross or histologic changes are seen in the annulus fibrosus, cartilaginous end plate or surrounding structures. The magnitude of nuclear digestion is dose dependant and optimal chemonucleolytic concentrations of chymopapain have been determined as 1 mg/disc and 2 mg/disc for dog[108, 303] and man[27, 65] respectively. Injection of ten times the optimal dose can result in focal defects to the cartilaginous end plate and occasionally, intradiscal haemorrhage may occur[27, 65, 303]. However, disruption of annular fibres is not observed and no adverse clinical effects are recorded at these dose levels.

Histochemical examination of the intervertebral disc two weeks after injection with a clinical dose of chymopapain confirms the loss of proteoglycan from the matrix, as indicated by the loss of staining to Safranin-O[65, 107, 108, 193, 303]. Proteoglycan loss is not confined solely to the nucleus pulposus as Safranin-O staining is absent throughout the annulus fibrosus and cartilaginous end plate[26, 193]. Biochemical analysis of the injected disc has determined that the total glycosaminoglycan content of the disc can decrease to less than 20% that of non-injected discs by two weeks post-injection[215]. The loss of proteoglycan from the matrix of the intervertebral disc reduces the water holding capacity of the disc and the disc collapses. This loss of disc width is observable radiographically within hours of disc injection[8, 9, 26, 27]. The disc width continues to decrease following injection and by two weeks may be less than one third its original measurement. The magnitude of this change is dose dependant.

Six months after injection the disc will have regained up to 66% of its normal width[9] and this progressive increase is paralleled by a return of safranin-O staining throughout the matrix of the intervertebral disc[26, 201]. Biochemical analysis of the disc during the postinjection period shows that regeneration of proteoglycan has begun by two weeks[215]. Initially these newly synthesised proteoglycan molecules are of smaller size than the pre-injection controls. Continued proteolytic digestion of these newly synthesised proteoglycans occurs, but is unclear whether this is due to residual chemonucleolytic activity of chymopapain or due to intrinsic tissue enzymes associated with tissue remodelling. By three months of age the structure of the newly synthesised proteoglycan molecules more closely resembles the untreated disc. Keratan sulphate is the major glycosaminoglycan in these new molecules[215].

Biomechanical analysis of the intervertebral disc reflect these changes in the matrix composition of the nucleus pulposus[178, 274, 297]. At three weeks post-injection, the canine disc shows significant loss in compressive stiffness and an increased 'creep rate'[297]. These findings are consistent with a reduced hydraulic performance by the dehydrated disc. However, by three months the biomechanical properties are beginning to return to normal, indicating restoration of the viscoelastic properties of the nucleus pulposus as proteoglycan, and thus water content, of the disc is restored.

The mechanism by which chymopapain relieves the symptoms of lumbar disc herniation is attributed to the release of nerve roots entrapped in herniated disc material as the disc shrinks following the loss of proteoglycans[75, 137, 201]. Shrinkage of nuclear fragments distorting annular fibres is also thought to be beneficial due to reduced stimulation of the pain receptors within the annular rings.

6.2.2 Toxicity of Chymopapain

The enzymatic activity of chymopapain is not specific to proteoglycans of the intervertebral disc and proteolytic activity may occur in other tissues[65, 201]. The predominant effect of chymopapain on other tissues is massive haemorrhage and vascular disruption due to enzymatic digestion of the bonds between endothelial cells of capillaries[27, 64, 65, 98, 201, 239]. Intravenous injection of high concentrations of chymopapain can result in massive and diffuse haemorrhages throughout the lung, liver, pericardium and peritoneum[27, 201]. Dose related peripheral nerve conduction impairment may occur following injection directly into the nerve substance[239]. The attenuated nerve conduction is associated with haemorrhage of the microvasculature of the nerve. In reality however, these systemic effects are unlikely to be a clinical concern because although potentially fatal, the median lethal dose of intravenous chymopapain is over 1000 times the optimal chemonucleolytic dose[27, 201].

The potential for leakage of the enzyme from the disc after injection, or its inadvertent placement into spinal ligaments, epidural tissue, dura, subarachnoid space, spinal cord or nerve roots necessitated investigation into the effect of chymopapain on these peridiscal tissues. Once again there is a large difference between the maximal non-lethal and the optimal chemonucleolytic dose in all locations except the subarachnoid space[27, 64, 98, 201]. Indeed, unless the dura mater is inadvertently ruptured, injection of chymopapain into the intervertebral disc has a margin of safety of over 2000 in the dog[201]. Because chymopapain has been found to bond irreversibly to the local substrate, leakage of the enzyme from the disc following accurate needle placement into the nucleus pulposus is unlikely to occur. Furthermore, protease inhibitors within serum rapidly inactivate free enzyme. Chronic toxic effects have not been observed in man or dog following intradiscal injection of chymopapain.

In contrast, injection of large doses of chymopapain into the subarachnoid space produces acute haemorrhage and elevated intrathecal pressure, leading to death[18, 64]. The source of the haemorrhage comes from rupture of the numerous thin-walled vessels of the pia-arachnoid. Death can sometimes be averted if intrathecal pressure is relieved by draining the bloody cerebrospinal fluid[201]. Vascular integrity is soon regained indicating that enzymatic activity does not persist. The margin of safety for chymopapain in the subarachnoid space is less than three[201], and therefore is of significant clinical concern. Complications can develop following chemonucleolysis with chymopapain should there be communication between the nucleus pulposus and the subarachnoid space due to unrecognised rupture of the dura mater.

Chymopapain is an allergenic substance[122, 123, 201]. Severe, and potentially fatal, anaphylactic reactions can occur in up to 1% of human patients[122]. Presensitisation to the enzyme is a significant problem in man because chymopapain, and other papaya derivatives are used extensively by the food industry in products such as meat tenderisers, toothpaste, chewing gum and beer. Cutaneous and serological testing are effective in identifying the majority of immunologically sensitive patients and these tests are performed routinely in most institutions prior to chemonucleolytic therapy[122]. Performance of chemonucleolysis under local anaesthesia not only reduces the incidence of anaphylactic reactions, but also permits rapid detection of their occurrence[121]. Rapid institution of appropriate therapy is therefore possible.

Allergic reactions have not been reported in the dog.

6.2.3 Clinical results with Chymopapain

*1) **Human:*** The use of chymopapain in the treatment of lumbar disc herniation in the human has been surrounded by controversy since its introduction by Smith in 1969[61, 75, 216, 217, 292]. Federal Drug Administration (FDA) approval of chymopapain has been revoked twice since that time, once because of fatal complications following injection, and again after a double-blind study failed to demonstrate a significant level of efficacy of chymopapain over a placebo[201]. This study was later criticised and discounted for a number of reasons, including inadequate dose levels and premature code-breaks. FDA approval has subsequently been regained.

The success of chemonucleolysis with chymopapain in the treatment of lumbar disc herniation in the man has been variably reported as 49 - 90%, though the majority of investigators report a success rate between 60 - 70%[38, 61, 69, 121, 137, 163, 193, 216, 217, 292]. However, critical analysis of these studies reveals significant inadequacies in protocol, with most studies being uncontrolled and non-randomised. Moreover, the definition of treatment 'success' is open to subjective interpretation. Patient psyche is so central to recovery that it is not surprising therefore to find that private and non-compensation patients (who have a significant financial commitment in their therapy) report significantly superior success rates to public or compensation patients[163].

In reliable double-blind studies, chemonucleolysis with chymopapain achieved successful cures in significantly more patients (72 - 80%) than a placebo injection (42 - 57%)[121, 291]. In these studies, a clinical cure was interpreted as return to normal or light work, although pain was not always relieved in these patients. (Though 'significantly' inferior, it is remarkable that placebo injections effected so many successful 'cures'!)

Post-injection back pain and muscular spasms are reported by up to 50% of human patients and this pain may persist for up to three days[38, 163]. On the other hand, some patients report immediate relief of pain. This response is attributed to direct neurolysis of the annular pain receptors.

Acute disc herniation following chemonucleolysis has been reported, but does not appear to be a common complication. Discitis, either aseptic or bacterial, is a complication seen after discography in 1 to 3.4% of cases, and is a recognised complication of chemonucleolysis[65, 163, 193, 201].

Where chemonucleolysis is deemed to have failed, subsequent surgery is not hindered by the previous enzymatic therapy[70, 201].

2) Dog: There are few reports which can be used to reliably estimate the value of chymopapain as a treatment of intervertebral disc disease in dogs. The reports that do exist lack pertinent clinical information, although most of the investigators consider it causes effective chemonucleolysis and achieves success rates comparable to surgical decompression[8, 18, 19, 20, 98, 107, 240].

The prophylactic use of chymopapain for intervertebral disc disease in the dog has been recorded in too few cases to allow an accurate assessment of its efficiency for this purpose to be made.

6.2.4 Discussion of Chymopapain

The use of chymopapain in man and dog remains controversial. The critics of chymopapain point out a variable therapeutic efficacy and state that the results are frequently equivalent to conservative treatment[75, 201]. A cynical view suggests that the success attributed to chymopapain is largely due to the propensity for the patient to follow more closely the post-operative conservative management guidelines simply because 'something has been done'.

Failures of chemonucleolysis can often be attributed to the inappropriate selection of candidates[69, 121, 201, 216, 217]. In the human patient, clinical signs must be associated with an identifiable disc lesion. Spinal stenosis, and other extra-discal lesions are unlikely to respond to chymopapain therapy, for obvious reasons. Herniated disc material, sequestered between annular fibres and nuclear material extruded into the neural canal is inaccessible to the enzyme, thus making digestion unlikely. Leakage of chymopapain into the subarachnoid space due to unrecognised rupture of the dura mater can be a serious complication, resulting in a haemorrhagic myelopathy. Dural lesions would be expected following an acute Type I herniation, due to the

explosiveness of the event. Dural tears are probably less likely with the slowly developing Type II lesion. As a consequence, pre-injection discograms are frequently performed in man to enable recognition of this potentially serious complication[201].

The strongest criticism against chymopapain is its inactivity towards collagen. As previously presented, the degenerate intervertebral disc of man, non-chondrodystrophoid and chondrodystrophoid dogs contains proportionately higher levels of collagen (up to 25%) and lower concentrations of proteoglycan than the normal disc. The ability of chymopapain to effect mass removal, and therefore provide relief from its compressive effects, is understandably questioned. Moreover, regeneration of the matrix after three months returns the disc to its pre-injection morphology[26]. It has not been elucidated why clinical signs do not always return with restoration of disc size.

A strong critic of chymopapain proposed the use of a collagenase enzyme[270], because collagen is the predominate structural element of the degenerate disc.

6.3 COLLAGENASE

Collagenase is a proteolytic enzyme extracted from a culture of *Clostridium histolyticum* and *C. welchii*. For many years it has been utilised in the debridement of dermal ulcers, necrotic tissue and burns. In the research laboratory it has been used to assist in the elucidation of the molecular structure of the collagen molecule and as a cell dispersion agent in tissue cultures. Purification of the collagenase enzyme for medical use is achieved through chromatographic techniques after its isolation from pure cultures of *C. histolyticum.*[98, 269]

Collagenase appears to be unique in its ability to attack native collagen under physiological conditions of pH and temperature. This proteolytic activity is highly specific for Types I and II collagen, breaking the molecule at several points along its length at the peptide bond. Collagenase will not attack other protein substances and has no effect on cellular membranes. It is rapidly inactivated in serum[98].

Collagenase was first injected into the intervertebral disc of dogs in 1969[270]. Since that time, experimental and clinical use of collagenase enzyme for chemonucleolysis in dog[19,37, 203, 268, 271] and man[36, 270] have provided some insight into its effect on disc structure.

6.3.1 Effect of Collagenase on the intervertebral disc

Injection of collagenase enzyme into the intervertebral disc results in a dose-related dissolution of the nucleus pulposus and inner margins of the annulus fibrosus[19, 37, 203, 269, 271]. Changes in the cartilaginous end plate and surrounding ligaments are not seen. Mild digestion of the inner margins of the annulus fibrosus occur following the administration of 500 units of collagenase into the intervertebral disc[37, 106, 269, 271]. Such changes are not seen at lower doses. Progressive digestion of the inner margins of the annulus fibrosus occurs at higher concentrations of collagenase but defects are rarely recorded in the outer annulus or cartilaginous end plates[106]. Due to the minimal effects on the annulus fibrosus, 500 units of collagenase is the recommended chemonucleolytic dose for collagenase in the dog.[106]

Gross examination of the non-chondrodystrophoid canine intervertebral disc following injection of 500 units of collagenase reveals complete solubilisation of the nucleus pulposus, leaving a cavity which may contain some white fibrous material.[19, 37, 269]. The width of the disc space collapses following injection, and this is detectable radiographically. Discs in the cynomolgus monkey treated with collagenase consistently had radiographic narrowing from 17 - 67% of its initial width[310]. Unlike discs injected with chymopapain, this disc narrowing appears to be permanent. Similar narrowing has been reported in the dog[19, 203].

Histological descriptions of the disc following collagenase injection have been made in the canine disc (both non-chondrodystrophoid[37, 269, 271] and chondrodystrophoid[203]) for periods up to three months after injection. However, these reports fail to accurately describe the structural changes which occur to the cellular elements or collagen fibres of the nucleus pulposus and annulus fibrosus, nor do they provide information on the influence of collagenase and collagen dissolution on the proteoglycan content of the disc. It is presumed that proteoglycan will be lost in association with the loss of collagen.

Adaptive changes have been reported in the canine non-chondrodystrophic intervertebral disc three months after injection[37]. Narrowing of the disc space persisted and the annulus fibrosus became flattened with inward bulging of the central annular rings, thereby reducing the size of the nuclear defect. In some discs, complete replacement of the nuclear deficit with fibrocartilage occurred.

6.3.2 Toxicity

Toxicity studies have demonstrated a wide margin of safety for collagenase, both systemically and in the local tissues[19, 37, 106, 220]. As with chymopapain, the toxic effects of collagenase are related to disruption of vascular integrity. However, haemorrhagic phenomena have not

been observed until the intravenous injection of over 4000 units of collagenase[106]. Eight times the recommended dose can therefore be injected without risk of complications. Extradiscal tissues, including peritoneum and paraspinal musculature are also tolerant to very high doses of enzyme[37]. Whilst small (1 - 2 cm), well-localised necrotic changes were observed to occur at the site of injection of high doses of collagenase into paraspinal muscles, no clinical effects were described[37]. Extradural injection enjoys the same margin of safety, with localised haemorrhage occurring in one dog following the injection of 5000 units of collagenase into the extradural space[37]. Injection of collagenase into rabbit tibial nerves caused mild perineural oedema, but effects on microvasculature and conduction velocities were not noticed[239]. Systemic effects following the intradiscal injection of clinical doses of collagenase have not been documented.

Dogs appear to tolerate subarachnoid injection of collagenase less well than other species[37, 220]. Administration of doses less than 600 units resulted in some degeneration of cellular elements of the ventral horn and varying degrees of demyelination[36]. However, no clinical changes were observed in these animals. Injection of increased doses of collagenase resulted in progressively severe changes in the ventral horn and extensive demyelination. Pial and subarchnoid haemorrhages developed at very high doses. Worsening neurological deficits accompanied these pathological changes, but were frequently only transient following injection of less than 1200 units. Permanent and progressively severe paraplegia occurs following injection of more than 1500 units. In some animals, subarachnoid injection of doses greater than 1500 units may be fatal[19, 37, 106].

Allergenicity to collagenase has not been reported in either experimental or clinical trials in dog or man.

6.3.3 Clinical use of Collagenase

Chemonucleolysis with collagenase has ridden the same wave of controversy as chymopapain since its institution[269]. Unfortunately, the few experimental and clinical trials documented suffer the same inadequacies in protocol as previously described for chymopapain.

In one retrospective study on clinical use of collagenase, complete relief from pain was achieved in 6 out of 29 (21%) patients[270]. Improvements were noted in a further 18 (63%) patients and 17 of these were able to return to work. Four patients who failed to respond to collagenase chemonucleolysis underwent surgical operation and all were found to have extruded disc fragments in the neural canal. The previous enzymatic therapy did not detract from the success of this surgery[91].

Use of collagenase in the dog is limited and clinical use has only been reported by one author[19]. This author considers chemonucleolysis with collagenase to be an effective alternative to surgery for the treatment of intervertebral disc disease and reports comparable success rates for laminectomy and chemonucleolysis (83%).

Prophylactic injection of collagenase into up to 7 thoracolumbar discs has been performed[¶] but the efficacy of this procedure has not been documented. Injection of 800 units of collagenase into a calcified disc resulted in the relief of clinical signs associated with this disc and, as determined by radiographic evaluation, its subsequent decalcification.[20]

Complications and failures associated with collagenase chemonucleolysis are similar to those identified with chymopapain[98].

6.4 CONCLUSIONS

Chemonucleolysis is a procedure which has a number of attractions. It is easily performed under fluoroscopy and can, in most cases, be completed in less than one hour[19]. Transient pain and muscle spasm is the most common post-operative complaint and in the human, this procedure can frequently be performed on an outpatient basis[201]. In experienced hands and with injection of accepted dose rates, systemic and local toxicity is negligible. Anaphylaxis with chymopapain is the most serious potential complication associated with chemonucleolysis[98,201].

The clinical success of chemonucleolysis with either chymopapain or collagenase is the focus of much debate. The treatment of human disc disease is so affected by patient psyche that objectively appraising the effectiveness of a procedure is difficult. This is demonstrated most graphically by the high level of 'cures' effected by placebo injections.

Clinical use of chemonucleolysis in the dog has mainly been for the treatment of acute disc herniations. This may be a misguided application of this procedure, particularly in the chondrodystrophoid breeds, and more appropriate employment may be reflected in improved clinical successes.

As we have learned from previous sections, treatment of acute Type I herniations in the chondrodystrophoid dog is best achieved by immediate decompression of the spinal cord and appropriate medical management of the spinal cord injury which has ensued. Numerous studies with chemonucleolysis have emphasised the inability of the enzyme to digest nuclear material within the neural canal. Rapid decompression of the spinal cord is therefore unlikely to occur.

[¶] Biggart JF. (1992) Personal Communication

Reported success following this practice may be explained by the influence of conservative management. It is probably reasonable to assume that recovery would have been more rapid and more complete had immediate surgical decompression been performed. Further to this, treatment with chymopapain is potentially hazardous following such an acute injury, due to the likelihood of dural tears being present. Leakage of enzyme through annular tears could easily result in potentially fatal subarachnoid haemorrhage.

Type II herniations would seem to be more amenable to treatment by chemonucleolysis. However, if clinical signs are related to the presence of herniated disc material within the neural canal, chemonucleolysis is probably unlikely to achieve the speed and completeness of resolution that surgical decompression may provide. This argument concerning mass removal has been presented previously for fenestration, but is equally applicable to chemonucleolysis.

7.0 RESEARCH OBJECTIVES AND HYPOTHESIS

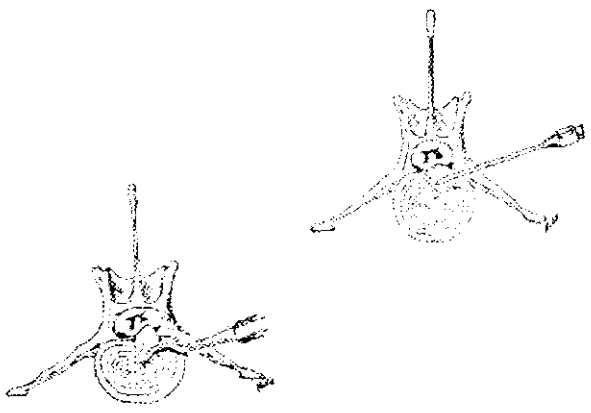
Removal of degenerate nuclear material by fenestration has been the accepted technique for both the treatment of mild clinical signs related to altered intervertebral disc function, and for the prophylaxis against massive disc hernation and, as a consequence, acute paralysis. In the latter case in particular, the use of fenestration is fraught with criticism. Fenestration is invasive, traumatic, painful and costly, and its efficacy at eliminating the occurrence of disc herniations has not been conclusively proven. It has been suggested that the reason for the continued development of clinical signs, albeit at a reduced incidence, is related to the inability of surgical fenestration techniques to completely remove all of the degenerate nuclear material from the intervertebral disc.

The use of chemonucleolysis for the prophylaxis of intervertebral disc herniations would appear to be an as yet unexplored aspect of this therapy. Collagenase enzyme is the more appropriate agent for this application, due to the permanence of its effect and the targeted proteolytic activity on the predominant component of the degenerate disc, collagen. It has been shown that the intradiscal injection of 500 units of collagenase causes the complete dissolution of the nucleus pulposus and, if used appropriately, causes no side effects. Chemonucleolysis is an attractive technique because it is atraumatic, simple and relatively painless.

This study will investigate the histologic effects of fenestration and collagenase on the intervertebral disc six months after treatment. It is hypothesised that the morphological changes in the intervertebral disc six months after injection of the nucleus pulposus with collagenase enzyme will be similar or greater than after fenestration. Were the hypothesis to be upheld, it could be reasoned that the intradiscal injection of collagenase enzyme might provide similar or greater prophylaxis against the herniation of nuclear material as fenestration. Further clinical use of the technique could then be tested.

The hypothesis will be tested on the thoracolumbar intervertebral discs of eight, non-chondrodystrophic young adult mongrel dogs. Four of the dogs will be subjected to either percutaneous, intradiscal collagenase injection or surgical fenestration. The remaining four dogs will act as controls (placebo injection and untreated).

8.0



MATERIALS AND METHOD

8.1 EXPERIMENTAL ANIMALS

Eight dogs were used in this study. Six of the dogs were similar sized, one year old mongrels weighing, on average, 25 kg (range 24.8 - 25.2 kg). They came from two litters (A and B) sired by the same animal and had been kept under similar conditions since birth. The remaining two dogs were young adult mongrels, weighing approximately 20 kg. These dogs were clinically normal, and were being euthanased for behavioural, rather than medical concerns. The spines from these dogs were used to provide a reference for the normal histological appearance of the young, non-chondrodystrophic thoracolumbar intervertebral disc.

The intervertebral discs from T_{10-11} to $L_{2/3}$ in the six related dogs were treated with one of three procedures 6 months prior to euthanasia. Only one treatment category was used in each dog.

Two of the animals (from litter A) were used for the percutaneous injection of 0.9% sterile saline into the nucleus pulposus of the thoracolumbar intervertebral disc (controls). The thoracolumbar intervertebral discs of a further two dogs (one from litter A, one from litter B) were injected with collagenase enzyme (treatment 1). The remaining two animals (one from litter A, one from litter B) were treated surgically by thoracolumbar fenestration (treatment 2).

Physical and neurological examinations were completed on all dogs at the start of the study. At this time, blood samples were submitted for haematological evaluation, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine transferase, sodium and potassium levels.

The dogs were maintained for 28 weeks after the experimental procedure and examined at periodic intervals. Between examinations the dogs were housed in private kennels. No modifications were made to the established kennel routine during the study period.

Final physical and neurological examinations were conducted in the week prior to euthanasia. The animals were euthanased using an intravenous injection of sodium pentobarbitone[a]. Within 2 hours of euthanasia, final radiographs of the thoracolumbar spine were taken.

---

a: Sodium pentobarbitone 300 mg. Chemstock Animal Health, Auckland, New Zealand

8.2 EXPERIMENTAL PROCEDURE

8.2.1 Anaesthesia

Animals were premedicated with a subcutaneous injection of acetylpromazine[b] (0.04 mg/kg), atropine[c] (0.02 mg/kg) and morphine[d] (0.4 mg/kg). Prior to induction, an intravenous fluid line[e] containing lactated ringers solution[f] was connected to a 22 g over-the-needle Jelco catheter[g] which had been inserted into the cephalic vein. Anaesthesia was induced with an intravenous injection of 2.5% thiopentone sodium[h] (8 mg/kg) and the dogs were then intubated with a No 12 endotracheal tube. Anaesthesia was maintained with a halothane[i]/oxygen mixture delivered by a vaporiser-out-of-circuit circle system. Throughout the anaesthesic period, lactated ringers solution was administered at a rate of 10 ml/kg/hr.

8.2.2 Radiographic assessment

Radiographs (two lateral and two ventrodorsal) of the thoracolumbar spine using medium rare earth screens and a grid were taken in all animals pre-operatively, immediately post-operatively, at 2 days, 2 weeks and each month after the experimental procedure[j]. An attempt was made to centre the x-ray beam over T_{12} and L_2 in each view to minimise parallax errors.

Following euthanasia of the dogs, two lateral and two ventro-dorsal radiographs were taken of each vertebral column using a cabinet x-ray machine.

8.2.3 Injection technique

With the animal in right lateral recumbency the left paravertebral region from the scapula to the ilial wing was prepared for surgery by clipping the hair and then scrubbing the skin surface with 0.5% chlorhexidine solution. After isolating with sterile drapes, the area of skin overlying the intervertebral disc of Disc $T_{10/11}$ was identified by fluoroscopic imaging. Under sterile conditions a 1 cm skin incision, 10 cm lateral to the dorsal midline, was made with a scalpel blade at this site.

---

b: *Acetylpromazine 2%. Delta Vet Labs. Australia*
c: *Atropine sulphate (0.65 mg/ml). Phoenix Pharmaceutical Distributors, Auckland, NZ*
d: *Morphine sulphate (15 mg/ml)*
e: *Travenol Laboratories, Deerfield, Illinois, USA*
f: *Abbot Hospital Products, Australia Pty Ltd, Sydney, Australia*
g: *Critikon, Tampa, Florida, USA*
h: *Intraval sodium, Pitman-Moore, Palmerston North, NZ*
i: *Fluothane, ICI, Auckland, NZ*
j: *Picker Explorer Mobile, Medical Systems Ltd, GEC, Australia.*

A 3.5" 20 g spinal needle[k] was inserted through this incision and gently passed immediately ventral to the dorsal convexity of the rib cage. When the needle was felt to contact the vertebral column, further imaging provided guidance for accurate penetration of the intervertebral disc and positioning of the needle tip in the nucleus pulposus. Preliminary investigations in cadavers with methylene blue had determined that accurate injection into the nucleus pulposus was made with the needle tip positioned slightly dorsal to the midpoint of the intervertebral disc. (Figure 8.1)

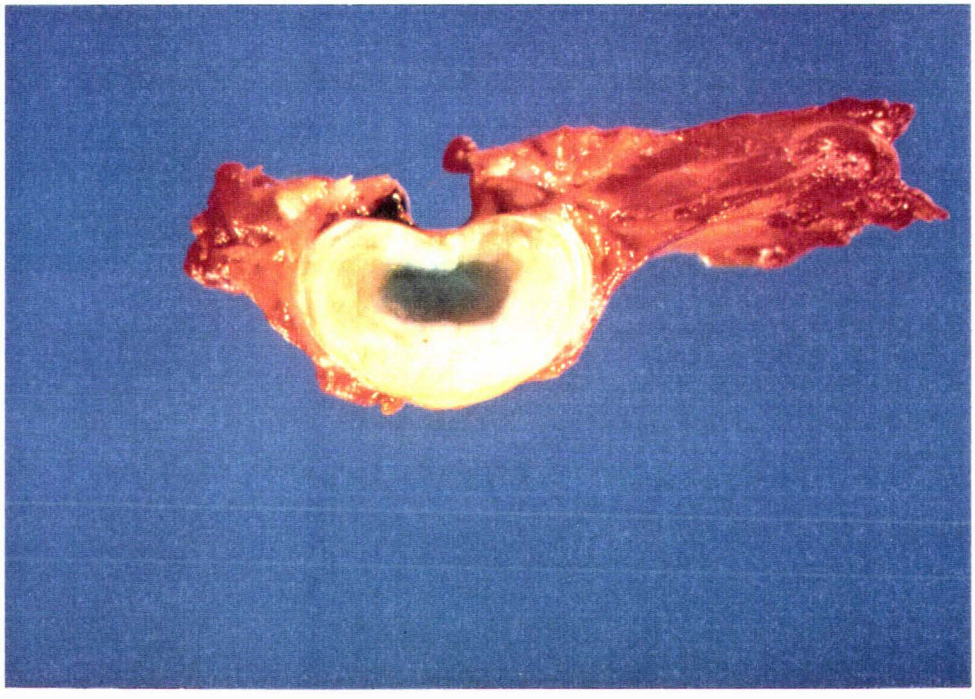


Figure 8.1: *Preliminary investigation of the injection technique in cadavers showed that consistent injection of the nucelus pulposus was possible. In this cross-section of an intervertebral disc, the nucleus pulposus is stained blue from a percutaneous injection of methylene blue.*

Once the needle was accurately positioned, the skin overlying the next caudally adjacent disc was identified and another spinal needle was inserted in a similar fashion. In the lumbar region, the needle was inserted immediately ventral to the transverse processes.

When all needles had been accurately positioned in the intervertebral discs of $T_{10/11}$, $T_{11/12}$, $T_{12/13}$, T_{13}/L_1, $L_{1/2}$ and $L_{2/3}$ the fluoroscopic unit was repositioned to allow ventrodorsal imaging. This facilitated accurate positioning of the needles within the centre of each nucleus pulposus.

k: Yale spinal needle, Becton-Dickinson & Co, Rutherford, New Jersey, USA

The high intrinsic pressures within the intervertebral disc made injection of even small volumes of fluid difficult. To enable precise delivery of fluid despite these pressures, 1.0 ml glass syringes with Leur lock adaptors were used. The volume of fluid contained in the bore of each spinal needle was 0.05 ml. Therefore, to ensure accurate delivery of 0.1 ml into the intervertebral disc, 0.15 ml of agent was aseptically drawn into the glass syringe immediately prior to injection.

8.2.4 Injection of collagenase

The glass vial containing a freeze-dried preparation of collagenase enzyme[l] was maintained at -18°C until the commencement of the study. Immediately prior to injection, the enzyme was mixed with 0.3 ml 0.9% sterile sodium chloride solution[m] providing a collagenase concentration of 500 units per ml. The latex injection port of the enzyme vial was swabbed with chlorhexidine in 70% alcohol and 0.15 ml of solution was drawn into the glass syringe and injected into the intervertebral disc. (Figure 8.2)

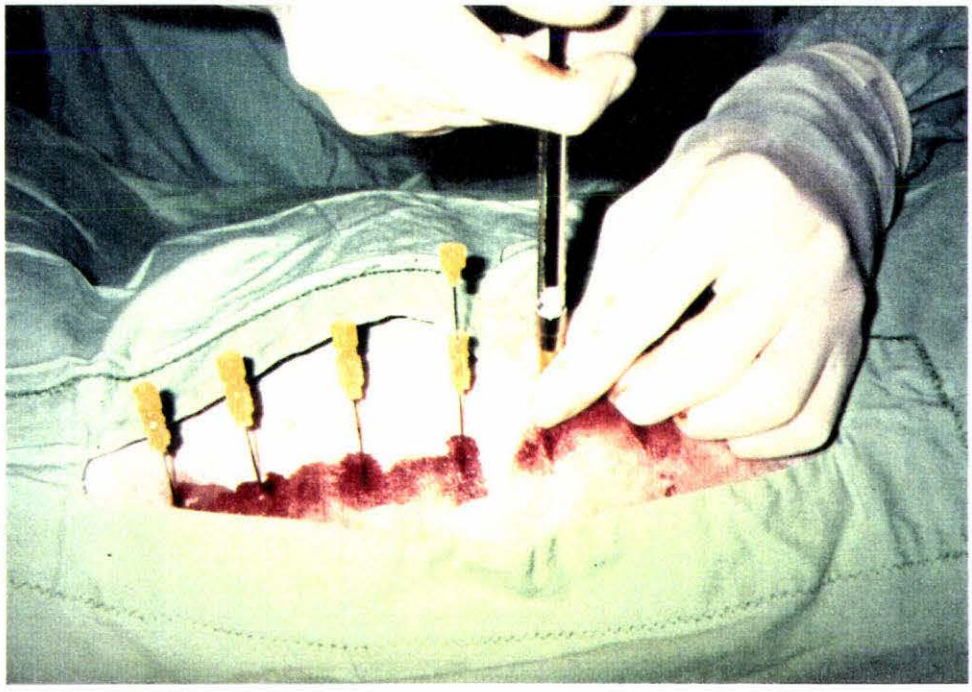


Figure 8.2: *Once all the needles were positioned within the intervertebral discs, a leur-lock glass syringe was attached and injection completed. Note the delineation of the operating site with sterile drapes.*

Following injection of fluid the needles were removed from the intervertebral discs. The skin wounds were closed with simple interrupted sutures of monofilament nylon[n].

l: *Collagenase VIIs, Sigma Laboratories, USA*
m: *Abbott Hospital Products, Sydney, Australia*
n: *Dermalon, Davis & Geck, Auckland, New Zealand*

Post-operative radiographs were obtained and the animals allowed to regain consciousness. Fluid administration was discontinued and the catheter removed from the cephalic vein. The injection site was covered with sterile gauze. Following extubation, the dogs were left to recover in heated cages. When full consciousness was regained the animals were placed in larger runs.

8.2.5 Injection of saline

Control animals were anaesthetised and prepared as described above. When spinal needles were accurately positioned in the intervertebral disc's of $T_{10/11}$, $T_{11/12}$, $T_{12/13}$, T_{13}/L_1, $L_{1/2}$ and $L_{2/3}$, 0.1 ml of a control injection of 0.9% sterile sodium chloride solution was injected into the intervertebral disc using a glass syringe as previously described.

Post-operative management of control dogs was identical to the collagenase treatment group of dogs.

8.2.6 Surgical fenestration

Following induction of anaesthesia, the animals were clipped and prepared for surgery as described above. The surgical procedure used was similar to that described for lateral fenestration of the thoracolumbar discs by Flo and Brinker (1975)[92]. Fenestration of an intervertebral disc was achieved using a small curette introduced through a scalpel incision into the lateral annular wall. The currette was twisted one quarter turn downwards several times, to remove as much nucleus pulosus as possible. Following fenestration of all discs, the separated muscle layers were closed with absorbable polydioxanone[o] and the skin with a locking loop suture of polyethylene[p]. The incision was bandaged in one dog for 3 hours but was left unbandaged in the other. The post-operative management of these dogs was similar to that described for the injected dogs.

---

o: PDS, Davis & Geck, Auckland, New Zealand

p: Dermalon, Davis & Geck, Auckland, New Zealand

8.3 POST-OPERATIVE EVALUATION

8.3.1 In vivo examinations

(i) Physical and neurological examination

Physical and neurological evaluations were repeated in each of the six related dogs at 24 and 48 hours, two weeks, and every 28 days after the procedure.

(ii) Anaesthesia

General anaesthesia was deemed necessary to permit accurate positioning of the animal for radiographic evaluation. The dogs were given acetylpromazine (0.04 mg/kg) 30 minutes before anaesthesia was induced using an intravenous injection of short-acting barbiturate[q].

8.3.2 Post-mortem analysis

(i) Gross pathology

All eight animals were submitted for post-mortem examination within 2.5 hours of death, and the vertebral spine from T_4 to L_7 was collected from each animal. The epaxial and hypaxial musculature was dissected from the vertebral column. During this dissection, the vertebral column and surrounding tissues were examined for the presence of gross lesions. Care was taken not to damage the outer fibres of the annulus fibrosus and ventral longitudinal ligament.

Following fixation of the vertebral column in 10% buffered formalin, the intervertebral disc units of $T_{10/11}$, $T_{11/12}$, $T_{12/13}$, T_{13}/L_1, $L_{1/2}$, $L_{2/3}$ were identified and isolated by cutting through the centre of the adjacent vertebral bodies with a hacksaw blade. These individual bone-disc-bone units were uniquely identified with a code number and transferred to individual jars containing 10% buffered formalin, where they were fixed for a further 7 days.

8.3.3 Histological preparation

Decalcification of the individual intervertebral disc units was for the most part achieved using 5.5% ethylene diamine tetraacetic acid (EDTA) in a 0.1M phosphate buffer. During the decalcification process, the container was gently vibrated to ensure constant mixing

---

q: Intraval sodium, Rhone-Meriuex, c/- Pitman-Moore, Palmerston North, New Zealand

of the EDTA solution. The samples were placed in fresh EDTA solution every month, with decalcification taking from three to five months. Periodic radiography of selected samples was used to monitor the decalcification process.

When decalcification was nearing completion, samples were transferred to a solution containing equal parts of 8N formic acid and 1N sodium formate. This acidic solution caused rapid decalcification of the samples within 7 days.

On completion of the decalcification process, the samples were rinsed overnight in running tap water, before being placed in Bouin's fluid for 48 hours. The samples were rinsed again in running tap water before being stored in 70% ethanol.

Within two days of being placed in ethanol, each block was cut in half along the median plane using a new razor blade. Only one half was used in subsequent processing: the other half was returned to the ethanol solution.

Each intervertebral disc half was embedded in wax using a standard extended programme. At the end of this programme, the block was placed in a vacuum oven at 28 psi and 60°C for 4 hours.

Six 5mm histological sections were cut from each block. Two sections were stained with alcian blue/haematoxylin and eosin (H&E) and two were stained with van Gieson. The remaining two slides were stored.

8.3.4 Histological analysis

Single-blind examination of all histological slides was conducted, and the observations compared and contrasted with the appearance of the intervertebral disc retrieved from the two untreated dogs. Only when an impression of all variations from the normal had been gauged was a grading system developed which enabled subjective quantification of the individual variations seen. This grading system permitted quantification of variations in 20 distinct morphological features of the intervertebral disc. Each slide was graded twice by the examiner on separate occasions.

The basis of the grading system is described on the following pages.

NUCLEUS PULPOSUS

1. Normal structure

Grade 1: Normal nuclear tissue could be readily discerned occupying the nuclear space, with little to no disruption to its structure.

Grade 2: Normal nuclear tissue was present within the nuclear region but its organisation was fragmented due to the action of some other reactive tissue.

Grade 3: Nuclear region had been replaced entirely by other tissue substance, and no evidence of normal nuclear tissue could be seen.

2. Communication with annulus fibrosus

Grade 1: The inner rings of the annulus fibrosus were intact, and there was a clear demarcation between the annulus fibrosus and peri-nuclear tissue. This observation is typical of the normal disc.

Grade 2: There was some collapse of the inner annular rings into the nuclear region, or there were some areas of nuclear herniation.

Grade 3: No clear distinction could be made between the annulus fibrosus and the nucleus pulposus.

3. Proteoglycan content

Grade 1: On the basis of the staining intensity of the alcian blue and van Gieson stains, the proteoglycan content of the nuclear region was approaching nil.

Grade 2: Intense proteoglycan staining was observed about the majority of the cellular elements of the nuclear region only, with only pale staining of the intercellular matrix. This grade was typical of the appearance of the normal disc.

Grade 3: The pericellular and transcellular regions of the tissue occupying the nuclear region of the disc stained intensely for proteoglycan. This tissue was similar to the normal histological appearance of cartilaginous tissue.

4. General matrix content

This described the nature of the acellular matrix of the nuclear region of the disc. This matrix was amorphous in nature, and stained weakly with alcian blue.

Grade 1: The nuclear region contained very little typical of this matrix tissue.

Grade 2: Occasional pockets of matrix were observed between 'nests' of cellular elements. This grade was typical of the normal structure.

Grade 3: The nuclear region contained large amounts of this poorly staining matrix.

5. Collagen fibre content

Grade 1: No evidence was seen for the presence of collagen fibres in the nuclear region of the intervertebral disc, on the basis of the collagen fibre affinity for van Geison stain.

Grade 2: Only fine, occasional collagen fibres could be seen throughout the nuclear region of the disc. These collagen fibres appeared to have no noticeable organisation to their fibre orientation. This grade was typical of the normal intervertebral disc.

Grade 3: The nuclear region of the intervertebral disc contained extensive and well-organised collagen fibre bundles. Typically, these fibre bundles were continuous with the fibres of the annulus fibrosus.

6. Cell nest formation

This described the tendency for the cellular elements of the nucleus pulposus to accumulate in 'nests', with each nest separated by a zone of acellular matrix and collagen fibres

Grade 1: The nuclear region of the disc was relatively acellular compared to the normal disc, or if the cells that were present showed no clear organisation into isolated 'nests'.

Grade 2: This grade was typical of the normal disc and was given if the cellular elements of the nuclear region of the intervertebral disc were organised into clear 'nests'.

Grade 3: The nucleus contained many cellular elements which did not show clear organisation into 'nests'. Typically, these discs contained tissue similar to the appearance of cartilaginous tissue with some columnar organisation of the cellular elements.

7. Presence of cartilaginous tissue

This observation described the appearance of tissue within the nuclear region of the intervertebral disc which resembled hyaline cartilage ie intense proteoglycan staining and round cells arranged in columns.

Grade 1: No sign of cartilaginous tissue could be seen in the nuclear region of the intervertebral disc. This grade was typical of the normal intervertebral disc.

Grade 2: Small, isolated pockets of cartilaginous tissue could be seen within the disc. Typically, this cartilaginous tissue occurred at the perimeter zones of the nuclear region, and along the cartilaginous end plate.

Grade 3: The nuclear region of the intervertebral disc was entirely replaced by cartilaginous tissue.

ANNULUS FIBROSUS

The following observations were recorded separately for both the dorsal and ventral region of the annulus fibrosus.

8. Lamellar organisation

Grade 1: The lamellae of the annulus fibrosus displayed a normal organisation.

Grade 2: The inner layers of the annulus fibrosus were collapsed inwards, but still showed some separation from the nucleus pulposus.

Grade 3: The lamellae of the annulus fibrosus were completely collapsed into the nuclear region of the intervertebral disc and there was no clear separation between the nucleus pulposus and the annulus fibrosus.

9. Lamellar thickening

Grade 1: The lamellae of the annulus fibrosus displayed a normal organisation.

Grade 2: Areas of thickening were observed at focal locations in the lamellae of the annulus fibrosus.

Grade 3: The annulus fibrosus showed large zones of destruction with reorganisation of the lamellae into ill-defined layers.

10. Interlamellar ground substance

Grade 1: The lamellae of the annulus fibrosus displayed a normal organisation of interlamellar ground substance.

Grade 2: Increased matrix staining intensely with alcian blue was present at focal zones within the annulus fibrosus. This staining was predominantly peri-cellular in location.

Grade 3: Large areas of the annulus fibrosus displayed increased amounts of matrix staining intensely with alcian blue between the lamellae.

11. Cellular morphology

Grade 1: The cells of the annulus fibrosus possessed a normal spindle-shaped morphology typical of fibroblasts.

Grade 2: The inner regions of the annulus fibrosus contained large numbers (>20 per field) of cells whose shape was rounded in character, more typical of chondrocytes.

Grade 3: The annulus fibrosus contained occasional areas of tissue with the histological characteristics of hyaline cartilage.

12. Nuclear herniation

Grade 1: The lamellae of the annulus fibrosus displayed a normal organisation with a distinctive separation from the tissue of the annulus fibrosus.

Grade 2: Focal areas of nuclear tissue could be observed within the lamellae of the annulus fibrosus.

Grade 3: The inner layers of the annulus fibrosus had collapsed and large amounts of nuclear tissue had herniated through the disrupted layers of the annulus fibrosus.

DORSAL LONGITUDINAL LIGAMENT

13. Dorsal longitudinal ligament

Grade 1: The dorsal longitudinal ligament was normal in appearance.

Grade 2: Focal areas of disruption could be observed within the dorsal longitudinal ligament. Typically, these changes consisted of thickening and chondrification of the outer lamellae of the annulus fibrosus and paravertebral regions of the ligament. These changes were presumed to represent early spondylosis deformans lesions.

Grade 3: Complete disruption of the dorsal longitudinal ligament was observed. This disruption was generally associated with dorsal herniation of nuclear tissue, resulting in disruption of the fibre organisation.

VENTRAL LONGITUDINAL LIGAMENT

14. Ventral longitudinal ligament

Grade 1: The ventral longitudinal ligament was normal in appearance.

Grade 2: Focal areas of disruption could be observed within the ventral longitudinal ligament. Typically, these changes consisted of thickening and chondrification of the outer lamellae of the annulus fibrosus and paravertebral regions of the ligament. These changes were presumed to represent early spondylosis deformans lesions.

Grade 3: Complete disruption of the ventral longitudinal ligament was observed. This disruption was generally associated with ventral herniation of nuclear tissue, resulting in the disruption of the fibre organisation.

CARTILAGINOUS END PLATE

15. Cartilaginous end plate

Grade 1: The cartilaginous end plate appeared normal.

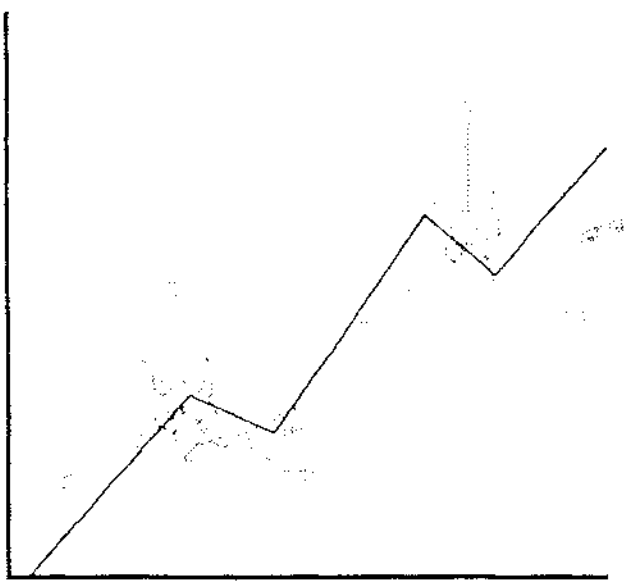
Grade 2: The cartilaginous end plate had focal areas of thickening along its length.

Grade 3: The cartilaginous end plate was completely disrupted, with herniation of nuclear material into the interstices of the vertebral trabeculae.

8.4 HISTOLOGICAL DESCRIPTION

Following the breaking of the identification code, each slide from a particular treatment group was reexamined, and the changes observed described and analysed. No changes were made to the recorded grades at this time.

9.0



RESULTS

9.1 PRE-OPERATIVE EVALUATION

All animals were apparently healthy at the time of examination.. Haematological, biochemical and electrolytic measurements were all within normal ranges.

9.2 EXPERIMENTAL PROCEDURES

9.2.1 Anaesthesia

All animals, except for one of the collagenase treated dogs, recovered uneventfully from general anaesthesia.

This one dog remained semi-comatose for up to nine hours following anaesthesia. Over the next 24 hours, this dog was responsive to touch and voice, but remained very depressed. By the third day, however, the dog was walking, but had a mild hind-limb ataxia. Intravenous fluids and supportive nursing care was given during this recovery period.

9.2.2 Pre-operative radiographic assessment

The pre-operative radiographs in all except one dog in the fenestration group revealed no abnormalities. In this dog, mild degenerative changes were observed about the articular facets of $T_{10/11}$.

9.2.3 Injection technique

In all four animals, considerable difficulty was experienced in cleanly penetrating the intervertebral discs of $T_{10/11}$, $T_{11/12}$ and $T_{12/13}$ due to the close proximity of the rib-head articulation with the intervertebral space.

9.2.4 Injection of collagenase

Collagenase was injected into the intervertebral discs of both dogs without complication. However, up to 30 minutes was spent before the needle tip was located within the centre of the nucleus pulposus of $T_{10/11}$ in one dog.

9.2.5 Injection of saline

In one of the dogs, the intervertebral discs from $T_{10/11}$ to $L_{2/3}$ were injected without complication.

In the remaining dog, all discs except $T_{10/11}$ were entered without difficulty. In this one disc, the needle could not be precisely located within the intervertebral disc and after repeated attempts, the procedure was abandoned in this disc.

9.2.6 Surgical fenestration

All intervertebral discs, except $T_{10/11}$ in one of the dogs, were fenestrated. In this dog, the proximity of the rib head to the intervertebral disc space prevented access to this site. No nuclear material was observed to extrude from any of the intervertebral discs during curettage.

The surgical wound of one dog broke down 4 days after surgery. This wound was left to close by second intention.

9.3 POST-OPERATIVE EVALUATIONS

9.3.1 In vivo examinations

(i) Physical and neurological examinations

Two of the animals (one saline injected and one collagenase injected) had no changes to their physical and neurological evaluations for the entire period of the study.

The two dogs subjected to fenestration demonstrated a mild resentment to abdominal palpation in the immediate post-operative period only. For the remainder of the study, no changes were seen in the physical or neurological examinations.

In the remaining two dogs, some variations in physical or neurological state were recorded during the study. These changes are outlined below.

One dog, who had been given a saline injection, was physically and neurologically normal at the 24 and 48 hours observation periods. However, at the one month post-treatment period, this dog displayed an exaggerated response to postural reflexes in the right hind leg and there was some resentment to cranial abdominal palpation. No other problems were discovered. By two months, the dog had again become neurologically normal although he remained sensitive to cranial abdominal palpation for the remainder of the experimental period. No clinical or behavioural changes were observed in association with this finding.

The remaining dog, who had been treated with collagenase, remained depressed at 24 hours after the procedure and was unable to support his own weight. The forelimbs were held in rigid extension, and there was a slight crossed extensor response in both forelegs. A normal withdrawal and flexor response was present in the hind legs and pain sensation was normal. The animal occasionally vocalised when lifted and frequently turned his head to his back region.

By 48 hours, the dog was improved and able to walk when supported. The forelimbs were neurologically normal, but the dog remained weak in the hind quarters, with no voluntary motor function evident. By 72 hours the dog was walking unaided, though he remained ataxic in the hind-quarters and walked with a crouched stance. Normal movement returned over the remaining 24 hours and no further problems were recorded through the remainder of the experimental period.

(ii) Radiographic examination

No radiographic changes were observed in the thoracolumbar spine or treated intervertebral discs of three dogs (two fenestrated and one saline injected) during the entire study. Radiographic changes were observed at some stage during the study in the thoracolumbar spine of both collagenase treated and one saline injected dogs.

In one saline injected dog, a lytic defect was observed within the disc space of $L_{1/2}$ at one month following injection. This lesion included destruction of the central zones of the cartilaginous end plates of the adjacent vertebral bodies. This lytic zone was bounded by a small zone of increased radiographic density, or sclerosis, within the vertebral body. During the remainder of the study, this lesion did not progress and by 6 months the lytic zone had increased in radiodensity.

In one of the collagenase dogs, osteophyte deposition was evident about the ventral border of disc space $T_{12/13}$ at 6 months This osteophyte build up was especially prominent on the cranial border of T_{13}. In the high definition radiographs taken at post-mortem, ventral osteophyte deposition could also be seen about the disc spaces of $T_{10/11}$, $T_{11/12}$ and $T_{12/13}$.

In the remaining collagenase dog, a small lytic region was observed within the central zone of the cranial cartilaginous end plate and extending about 1 cm into the body of L_1 two months after injection. At three months this lytic area was surrounded by a small zone of sclerosis but by six months the lesion was resolving, with increased radiodensity of the previously lytic zone.

In only one dog, treated with collagenase, all treated intervertebral disc spaces were observed to be narrowed at two days post-injection. By six months, the observed disc width had returned to pre-operative levels in this dog.

9.3.2 Post-mortem examination

(i) Gross pathology

No gross findings were observed during post-mortem examination of the animals.

(ii) Histological grading and description

The histological grades, and a description of the histological findings within each disc, are outlined on the following pages. It was clearly apparent that each treatment caused visually identifiable changes in the disc architecture and this was confirmed by the repeatability of the grading system, whose results were identical for each disc on both occasions.

NUCLEUS PULPOSUS:

1. NORMAL STRUCTURE

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	2	3	3	3	3	3	3	3	3	3	3	3
Fenestration	1	2	2	1	2	2	2	2	1	2	2	2
Saline	1	2	2	2	1	2	2	2	2	3	2	2
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY: Normal structure

Grade 1: Normal nuclear tissue could be readily discerned occupying the nuclear space, with little to no disruption to its structure.

Grade 2: Normal nuclear tissue was present within the nuclear region, but its organisation was fragmented due to the action of some other reactive tissue.

Grade 3: Nuclear region had been replaced entirely by another tissue substance, and no evidence of normal nuclear tissue could be seen.

In only one of the twelve discs injected with collagenase could normal nuclear tissue be seen remaining within the disc. In this one disc, the nuclear material remaining was confined to one focal area, surrounded by the replacement tissue. (Figure 9.2)

In most of the fenestrated and saline injected discs, tissue resembling that seen in the normal disc was visible throughout the nuclear region. In five discs (three fenestrated and two saline injected), the normal architecture of the nucleus pulposus remained, although there was a noticeably increased fibrosis of the intercellular matrix. (Figure 9.4) In the remaining majority of discs, islands of tissue resembling normal nuclear material were distributed throughout an amorphous matrix which stained moderately with alcian blue. This tissue was, in most discs, undergoing a transformation to fibrocartilage. (Figure 9.3)

In one of the saline injected discs ($L_{1/2}$) the nuclear tissue had been almost completely replaced by well-organised fibrocartilage. Only a small pocket of tissue resembling that seen in the normal disc was present, and this tissue was also showing some evidence of transformation to fibrocartilage.

2. COMMUNICATION WITH ANNULUS FIBROSUS

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	3	3	3	3	3	2	3	3	2	3	3	3
Fenestration	3	1	1	1	1	1	1	1	1	2	2	1
Saline	1	1	1	1	1	1	1	1	1	3	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

In all of the collagenase injected discs, a clear distinction between the annulus fibrosus and the nuclear region was lost, with the inner lamellae of the annulus being almost continuous with the replacement tissue in these discs.

KEY:	**Communication with annulus fibrosus**
Grade 1:	The inner rings of the annulus fibrosus were intact, and there was a clear demarcation between the annulus fibrosus and peri-nuclear tissue. This observation is typical of the normal disc.
Grade 2:	There was some collapse of the inner annular rings into the nuclear region, or there were some areas of nuclear herniation.
Grade 3:	No clear distinction could be made between the annulus fibrosus and the nucleus pulposus.

In most of the fenestrated discs, normal architecture of the perinuclear region of the disc was preserved. In three discs, however, slight infolding of the inner lamellae of the annulus fibrosus was seen, resulting in some disruption of the disc structure at this point.

In all but one of the saline injected discs, the perinuclear region and inner layers of the annulus fibrosus did not differ from the normal discs. In one disc however, the fibrocartilage replacing the nuclear region was continuous with the annulus fibrosus.

3. PROTEOGLYCAN CONTENT

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	2	2	2	2	2	2	3	2	3	3	2	2
Fenestration	2	1	2	1	1	1	2	1	1	2	2	2
Saline	2	1	1	1	1	1	1	1	1	2	1	1
Normal	2	2	2	1	2	1	2	1	2	2	2	2

In all of the collagenase injected discs, the matrix of the nuclear region stained more intensely with alcian blue than in the normal discs. In many discs, intense matrix staining was limited to aggregations of proliferating chondrocytes within the fibrocartilage. In some of these discs, lakes of matrix substance appeared to persist within cystic structures within the fibrocartilage. (Figure 9.2 c) In three discs, there was an intensely basophilic matrix surrounding clusters of plump chondrocytes arranged in lacunae more characteristic of hyaline cartilage than nucleus pulposus.

KEY: Proteoglycan content

Grade 1: On the basis of the staining intensity of the alcian blue and van Gieson stains, the proteoglycan content of the nuclear region was approaching nil.

Grade 2: Intense proteoglycan staining was observed about the majority of the cellular elements of the nuclear region only, with only pale staining of the intercellular matrix. This grade was typical of the appearance of the normal disc.

Grade 3: The pericellular and transcellular regions of the tissue occupying the nuclear region of the disc stained intensely for proteoglycan. This tissue was similar to the normal histological appearance of cartilaginous tissue.

In nine fenestrated and nine saline injected discs, the proteoglycan content, on the basis of the staining affinity for alcian blue, was greater than that of normal discs. This increased staining affinity was limited to the amorphous matrix present between the cellular aggregations. (Figure 9.3)

In the remaining discs, the staining affinity for alcian blue within the nuclear region did not differ markedly from the normal discs, although an increased localisation to the immediate cellular region was occurring. (Figure 9.4)

4. GENERAL MATRIX CONTENT

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	1	1	1	1	1	1	1	1	1	1
Fenestration	2	3	3	2	3	3	3	3	2	3	3	3
Saline	2	3	3	3	2	3	3	3	3	1	3	3
Normal	2	2	2	2	2	2	3	3	2	2	2	2

KEY: **General matrix content**

This described the nature of the acellular matrix of the nuclear region of the disc. This matrix was amorphous in nature, and stained weakly with alcian blue.

Grade 1: The nuclear region contained very little typical of this matrix tissue.

Grade 2: Occasional pockets of matrix were observed between 'nests' of cellular elements. This grade was typical of the normal structure.

Grade 3: The nuclear region contained large amounts of this poorly staining matrix.

In all of the collagenase treated discs, the matrix was abnormal, the nuclear region in these discs being replaced almost completely by either fibrocartilage or hyaline cartilage. (Figure 9.2)

In the fenestrated and saline injected discs, amorphous matrix material remained between the nests of chondrocytes. In most discs (nine fenestrated and nine saline injected), the proportion of matrix, and its distribution within the nuclear region, appeared increased over that observed in the normal discs. This matrix stained more strongly with alcian blue than that observed in the normal discs. (Figure 9.3)

5. COLLAGEN FIBRE CONTENT

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	3	3	3	3	3	3	2	3	2	3	3	3
Fenestration	2	1	1	2	1	1	1	1	2	1	1	1
Saline	2	1	1	1	2	1	1	1	1	3	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

In all of the collagenase injected discs, the collagen content of the nuclear region was considerably greater than that seen in the normal discs. In six discs (four from one dog, two from the other), this was largely in the form of fibrocartilage which completely filled the nuclear region. In the remaining six discs, the nuclear region had been replaced completely with well-organised hyaline cartilage. (Figure 9.2)

KEY: Collagen fibre content

Grade 1: No evidence was seen for the presence of collagen fibres in the nuclear region of the intervertebral disc, on the basis of the collagen fibre affinity for van Geison stain.

Grade 2: Only fine, occasional collagen fibres could be seen throughout the nuclear region of the disc. These collagen fibres appeared to have no noticeable organisation to their fibre orientation. This grade was typical of the normal intervertebral disc.

Grade 3: The nuclear region of the intervertebral disc contained extensive and well-organised collagen fibre bundles. Typically, these fibre bundles were continuous with the fibres of the annulus fibrosus.

In most of the fenestrated and saline injected discs, the nuclear region showed an increased transformation to fibrocartilage within localised cellular aggregations. However, no collagen fibres were observed passing between these localised regions. (Figure 9.3)

In the remaining discs, an increased fibrosis of the matrix surrounding the cell nests was evident (but not of sufficient quantity to warrant a Grade 3). (Figure 9.4)

In one saline injected disc, the entire nuclear region was replaced with fibrocartilage which was continuous with the lamellae of the annulus fibrosus and trabeculae of the vertebral body.

6. CELL NEST FORMATION

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	3	3	3	3	3	3	3	3	3	3	3	3
Fenestration	1	2	2	1	2	2	2	2	1	2	2	2
Saline	1	2	2	2	1	2	2	2	2	3	2	2
Normal	2	2	2	2	2	2	2	2	2	2	2	2

KEY: **Cell nest formation**
This described the tendency for the cellular elements of the nucleus pulposus to accumulate in 'nests', with each nest separated by a zone of acellular matrix and collagen fibres

Grade 1: The nuclear region of the disc was relatively acellular compared to the normal disc, or if the cells that were present showed no clear organisation into isolated 'nests'.

Grade 2: This grade was typical of the normal disc and was given if the cellular elements of the nuclear region of the intervertebral disc were organised into clear 'nests'.

Grade 3: The nucleus contained many cellular elements which did not show clear organisation into 'nests'. Typically, these discs contained tissue similar to the appearance of cartilaginous tissue with some columnar organisation of the cellular elements.

No normal cell nests remained in the nuclear region of the collagenase injected dogs. The cells that remained however, were typical of chondrocytes seen in either fibrocartilage or hyaline cartilage. (Figure 9.2)

In nine of the fenestrated and nine of the saline injected discs, cellular aggregations remained but these lacked the distinctive fibrous capsule seen in the normal discs. In most places, cellular proliferation with the formation of fibrocartilage was occurring. In some places, this fibrocartilage was developing a more laminated arrangement, with the long axis of these thin cellular sheets passing parallel to the annular lamellae. (Figure 9.3)

In the remaining discs (except one saline injected disc) the cellular aggregation typical of the normal discs remained a distinctive feature of the nuclear region. (Figure 9.4) In all of the discs, chondrocyte proliferation within these cell nests appeared to be occurring, with the tissue around these sites beginning a transformation to fibrocartilage.

In one saline injected disc, the cellular arrangment was typical of fibrocartilage.

7. PRESENCE OF CARTILAGINOUS TISSUE

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	2	2	3	2	2	2	3	2	2	3	2	2
Fenestration	1	1	1	1	1	2	1	1	1	2	1	2
Saline	1	1	2	1	1	2	2	2	1	1	2	2
Normal	1	1	1	1	2	2	2	2	1	1	1	1

KEY: **Presence of cartilaginous tissue**

This observation described the appearance of tissue within the nuclear region of the intervertebral disc which resembled hyaline cartilage ie intense proteoglycan staining and round cells arranged in columns.

Grade 1: No sign of cartilaginous tissue could be seen in the nuclear region of the intervertebral disc. This grade was typical of the normal intervertebral disc.

Grade 2: Small, isolated pockets of cartilaginous tissue could be seen within the disc. Typically, this cartilaginous tissue occurred at the perimeter zones of the nuclear region, and along the cartilaginous end plate.

Grade 3: The nuclear region of the intervertebral disc was entirely replaced by cartilaginous tissue.

In the collagenase injected dogs, hyaline cartilage was well formed in the nuclear region of three discs, with a further three showing variable quantities of hyaline cartilage throughout the fibrocartilage tissue. Focal areas of hyaline cartilage formation occurred to a lesser degree within the remaining six discs. (Figure 9.2)

In all of the fenestrated and saline injected discs, the formation of hyaline cartilage showed no clear difference from the normal discs.

ANNULUS FIBROSUS

8A. LAMELLAR ORGANISATION (DORSAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	2	2	1	2	2	1	2	1	1	2	3	1
Fenestration	2	1	1	1	1	1	1	1	1	1	3	1
Saline	1	1	1	1	1	1	1	1	1	2	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Lamellar organisation
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation.
Grade 2:	The inner layers of the annulus fibrosus were collapsed inwards, but still showed some separation from the nucleus pulposus.
Grade 3:	The lamellae of the annulus fibrosus were completely collapsed into the nuclear region of the intervertebral disc and there was no clear separation between the nucleus pulposus and the annulus fibrosus.

In almost all treated discs, the changes to the dorsal annulus fibrosus consisted of slight inward collapse of the inner lamellae of the annulus fibrosus. The remaining structure of the outer annulus fibrosus showed no difference from normal.

In one collagenase injected disc, annulus disruption was considerable although the outer few lamellae of the annulus fibrosus, as well as the dorsal longitudinal ligament remained unchanged. The cause of this disruption was not evident.

8B. LAMELLAR ORGANISATION (VENTRAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	1	1	1	1	1	1	1	1	1	1
Fenestration	2	1	1	1	1	1	1	1	1	2	1	1
Saline	1	1	1	1	1	1	1	1	1	2	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Lamellar organisation
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation.
Grade 2:	The inner layers of the annulus fibrosus were collapsed inwards, but still showed some separation from the nucleus pulposus.
Grade 3:	The lamellae of the annulus fibrosus were completely collapsed into the nuclear region of the intervertebral disc and there was no clear separation between the nucleus pulposus and the annulus fibrosus.

Apart from a slight infolding of the inner lamellae seen in three discs (one fenestrated and two saline injected) the ventral annulus fibrosus of all treated discs showed no difference from normal.

9A. LAMELLAR THICKENING (DORSAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	1	1	1	1	1	3	1	1	3	2
Fenestration	1	1	1	1	1	1	1	1	1	1	1	1
Saline	1	1	1	1	1	1	1	1	1	1	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Lamellar thickening
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation.
Grade 2:	Areas of thickening were observed at focal locations in the lamellae of the annulus fibrosus.
Grade 3:	The annulus fibrosus showed large zones of destruction with reorganisation of the lamellae into ill-defined layers.

The lamellae of the dorsal annulus fibrosus in all treated discs showed little to no difference from the normal structure.

9B. LAMELLAR THICKENING (VENTRAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	1	1	2	1	3	3	1	2	3	2
Fenestration	1	1	1	1	1	1	1	1	1	1	2	2
Saline	1	1	1	1	1	1	1	1	1	2	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Lamellar thickening
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation.
Grade 2:	Areas of thickening were observed at focal locations in the lamellae of the annulus fibrosus.
Grade 3:	The annulus fibrosus showed large zones of destruction with reorganisation of the lamellae into ill-defined layers.

Focal thickening of the ventral annulus fibrosus was recorded in six collagenase injected, one saline injected and two fenestrated discs. In all nine affected discs, the focal thickening occurred at the junction of the outer zones of the annulus fibrosus and the cranial and caudal borders of the vertebral body. Proliferation of chondrocytes within the fibrocartilage was occurring in most discs and, in three of the collagenase injected discs, small pockets of hyaline cartilage had developed. These changes were indicative of an early spondylytic lesion.

No changes in the ventral annulus fibrosus were seen in the remaining discs.

10A. INTERLAMELLAR GROUND SUBSTANCE (DORSAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	3	2	3	3	3	2	2	2	3	3	2
Fenestration	1	1	1	1	1	1	1	1	1	2	2	1
Saline	1	2	1	1	1	1	1	1	1	1	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Interlamellar ground substance
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation of interlamellar ground substance.
Grade 2:	Increased matrix staining intensely with alcian blue was present at focal zones within the annulus fibrosus. This staining was predominantly peri-cellular in location.
Grade 3:	Large areas of the annulus fibrosus displayed increased amounts of matrix staining intensely with alcian blue between the lamellae.

In all of the collagenase injected discs, the staining affinity of the interlamellar ground substance to alcian blue was increased.

No significant difference from the normal discs was recorded in the interlamellar ground substance of the fenestrated or saline injected discs.

10B. INTERLAMELLAR GROUND SUBSTANCE (VENTRAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	1	3	1	2	1	1	1	1	1	1
Fenestration	1	1	1	1	1	1	1	1	1	2	1	1
Saline	1	1	1	1	1	1	1	1	1	1	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Interlamellar ground substance
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation of interlamellar ground substance.
Grade 2:	Increased matrix staining intensely with alcian blue was present at focal zones within the annulus fibrosus. This staining was predominantly peri-cellular in location.
Grade 3:	Large areas of the annulus fibrosus displayed increased amounts of matrix staining intensely with alcian blue between the lamellae.

In only one collagenase injected disc was the interlamellar ground substance found to be increased markedly compared to the normal discs. Focal increases were recorded in one saline injected and one fenestrated disc.

No obvious differences were recorded in the other treated discs.

11A. CELLULAR MORPHOLOGY (DORSAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	2	2	2	1	2	2	3	3	2	2	2	2
Fenestration	1	1	1	1	1	1	1	1	1	1	1	1
Saline	1	1	1	1	1	1	1	1	1	2	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY: Cellular morphology

Grade 1: The cells of the annulus fibrosus possessed a normal spindle-shaped morphology typical of fibroblasts.

Grade 2: The inner regions of the annulus fibrosus contained large numbers (>20 per field) of cells whose shape was rounded in character, more typical of chondrocytes.

Grade 3: The annulus fibrosus contained occasional areas of tissue with the histological characteristics of hyaline cartilage.

The cells in the inner margins of the annulus fibrosus in all of the collagenase injected discs showed some transformation to the rounder chondrocytes more typical of the nuclear region. In two of these discs, some proliferation of these chondrocytes was occurring, with the formation of hyaline cartilage.

Similar cellular changes were recorded in one of the saline injected dogs, though the formation of hyaline cartilage was not apparent.

In all other fenestrated and saline injected dogs, the cells of the annulus fibrosus showed no difference from the normal discs.

11B. CELLULAR MORPHOLOGY (VENTRAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	2	1	1	1	2	1	2	2	2	1	2	2
Fenestration	1	1	1	1	1	1	1	1	1	1	1	1
Saline	2	1	1	1	1	1	1	1	1	2	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY: Cellular morphology

Grade 1: The cells of the annulus fibrosus possessed a normal spindle-shaped morphology typical of fibroblasts.

Grade 2: The inner regions of the annulus fibrosus contained large numbers (>20 per field) of cells whose shape was rounded in character, more typical of chondrocytes.

Grade 3: The annulus fibrosus contained occasional areas of tissue with the histological characteristics of hyaline cartilage.

Mild cellular transformation to chondrocytes was seen in many of the collagenase injected discs, and one saline injected disc.

No other changes were recorded in the other treated discs.

12A. NUCLEAR HERNIATION (DORSAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	2	1	1	1	3	1	1	1	2	1
Fenestration	1	1	1	1	1	1	1	1	1	2	1	1
Saline	1	1	2	1	1	1	2	1	2	1	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Nuclear herniation
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation with a distinctive separation from the tissue of the annulus fibrosus.
Grade 2:	Focal areas of nuclear tissue could be observed within the lamellae of the annulus fibrosus.
Grade 3:	The inner layers of the annulus fibrosus had collapsed and large amounts of nuclear tissue had herniated through the disrupted layers of the annulus fibrosus.

In one of the collagenase injected discs, massive herniation of nuclear material through the dorsal annulus was apparent with disruption of the annular lamellae and dorsal longitudinal ligament (see below). Nuclear tissue was present in focal areas throughout the dorsal annulus, with a considerable amount remaining in the dorsal longitudinal ligament. (F igure 9.5)

In two of the collagenase injected and three of the saline injected discs, focal pockets of nuclear material could be seen between lamellae of the dorsal annulus fibrosus.

In one of the fenestrated discs, a slight bulging of the dorsal nuclear tissue was beginning to push through the inner layers of the annulus fibrosus. However, no separation of the lamellar fibres was visible. (Figure 9.3d)

12B. NUCLEAR HERNIATION (VENTRAL ANNULUS)

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	1	1	1	1	1	1	1	1	1	1
Fenestration	1	1	1	1	1	1	1	1	1	1	1	1
Saline	1	1	1	1	1	1	1	1	1	1	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY:	Nuclear herniation
Grade 1:	The lamellae of the annulus fibrosus displayed a normal organisation with a distinctive separation from the tissue of the annulus fibrosus.
Grade 2:	Focal areas of nuclear tissue could be observed within the lamellae of the annulus fibrosus.
Grade 3:	The inner layers of the annulus fibrosus had collapsed and large amounts of nuclear tissue had herniated through the disrupted layers of the annulus fibrosus.

In all discs studied, the ventral annulus fibrosus remained intact, with no evidence of nuclear herniation occurring.

13. DORSAL LONGITUDINAL LIGAMENT

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	2	1	2	1	3	1	1	2	2	1
Fenestration	1	1	1	1	1	1	1	1	1	1	1	1
Saline	1	1	1	1	1	1	1	1	1	1	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY: Dorsal longitudinal ligament

Grade 1: The dorsal longitudinal ligament was unchanged.

Grade 2: Focal areas of disruption would be observed within the dorsal longitudinal ligament. Typically, these changes consisted of thickening and chondrification of the outer lamellae of the annulus fibrosus and paravertebral regions of the ligament. These changes were presumed to represent early spondylotic defects.

Grade 3: Complete disruption of the dorsal longitudinal ligament was observed. This disruption was generally due to massive dorsal herniation of nuclear tissue, resulting in the disruption of the fibre organisation.

In five of the collagenase injected discs, the normal structure of the dorsal longitudinal ligament had been disrupted. In one of these discs, this disruption was due to the presence of a large pocket of nuclear material between the fibre bundles of the ligament. (Figure 9.5) The reaction of the dorsal longitudinal ligament was characterised by thickening of the lamellar fibres, chondrocyte proliferation, and disruption of the normal longitudinal orientation of the collagen fibres, which had become thrown into loops and whorls. Formation of hyaline cartilage at the cranial and caudal border of the vertebral bodies adjacent to this disc space, together with an increased staining affinity to alcian blue by the surrounding fibrocartilage was also seen.

In the remaining four collagenase injected discs, disruption of the dorsal longitudinal ligament was caused by small, focal herniations of nuclear material. Thickening and proliferation of the fibrocartilage, together with slight disruption of their longitudinal orientation was seen. In one of these dogs, chondrocyte proliferation was occurring at the border of the vertebral body, suggesting the development of an early spondylytic lesion. (Figure 9.6)

No changes were recorded in the dorsal longitudinal ligament of either fenestrated or saline injected discs.

14. VENTRAL LONGITUDINAL LIGAMENT

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	1	1	2	1	1	1	1	1	1	1	1	1
Fenestration	2	1	1	1	1	1	1	1	1	1	1	1
Saline	1	1	1	1	1	1	1	1	1	1	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY: Ventral longitudinal ligament

Grade 1: The ventral longitudinal ligament was unchanged.

Grade 2: Focal areas of disruption would be observed within the ventral longitudinal ligament. Typically, these changes consisted of thickening and chondrification of the outer lamellae of the annulus fibrosus and paravertebral regions of the ligament. These changes were presumed to represent early spondylotic defects.

Grade 3: Complete disruption of the ventral longitudinal ligament was observed. This disruption was generally due to massive ventral herniation of nuclear tissue, resulting in the disruption of the fibre organisation.

In all but one of the collagenase injected discs, the ventral longitudinal ligament was normal. Nevertheless, spondylytic lesions were developing in the adjacent outer lamellae of the ventral annulus fibrosus. In the one disc where disruption was recorded, the ligament had become thickened in focal areas due to an increased number of collagen fibres, and the pericellular region about the dividing chondrocytes showed an increased staining affinity for alcian blue. These changes were similar to those observed around herniated nuclear material in the dorsal longitudinal ligament. (Figure 9.6)

Differences in the structure of the ventral longitudinal ligament was recorded in only one other disc, which had been injected with saline. In this disc, pockets of tissue resembling nuclear material was seen between disrupted fibre bundles. The collagen fibres in this area showed the proliferation and disorganised orientation described in other discs. Chondrocyte proliferation was also occurring at the border of this disc with the vertebral body.

15. CARTILAGINOUS END PLATE

Treatment group	Disc Number											
	T_{10-11}		T_{11-12}		T_{12-13}		T_{13}-L_1		L_{1-2}		L_{2-3}	
A/B	A	B	A	B	A	B	A	B	A	B	A	B
Collagenase	2	2	2	2	2	2	2	2	2	2	2	2
Fenestration	2	1	1	1	1	1	1	1	1	1	1	1
Saline	1	1	1	1	1	1	1	1	1	3	1	1
Normal	1	1	1	1	1	1	1	1	1	1	1	1

KEY: Cartilaginous End Plate

Grade 1: The cartilaginous end plate was unchanged from normal.

Grade 2: The cartilaginous end plate had focal areas of thickening along its length.

Grade 3: The cartilaginous end plate was completely disrupted, and the nuclear region was continuous with the trabecular framework of the vertebral body.

In all of the collagenase injected discs, disruption of the normal architecture of the cartilaginous end plate had occurred. This disruption was mostly limited to thickening along its length, due to increased quantities of hyaline cartilage or fibrocartilage. In seven discs, tissue resembling normal nuclear tissue was seen between the subchondral trabeculae of the vertebral body, although in none of the slides examined were breaches seen in the cartilaginous end plate This herniated tissue was, in most cases, undergoing transformation to fibrocartilage through chondrocyte proliferation. (Figure 9.7)

In one of the fenestrated discs, the cartilaginous end plate was thickened, and a layer of hyaline cartilage about 5 - 6 lacunae thick had formed along its length. No other changes were recorded in the cartilaginous end plate of any other discs subjected to fenestration.

In only one of the saline injected discs were defects in the cartilaginous end plate seen. In this disc, the central portion of the cartilaginous end plate had been completely obliterated by the proliferating fibrocartilage which now filled the nuclear region. This fibrocartilage was continuous with the trabecular bone of the vertebral bodies.

FIGURE 9.1: NORMAL NUCLEUS PULPOSUS

a) and b): Photomicrograph (40 x) of the normal nucleus pulposus showing the organisation of the cells into characteristic 'nests' [arrow], with each nest surrounded by a fibrous capsule. The staining for proteoglycan (blue) is fairly evenly distributed throughout the nucleus, but is strongest in the region between the cell nests.

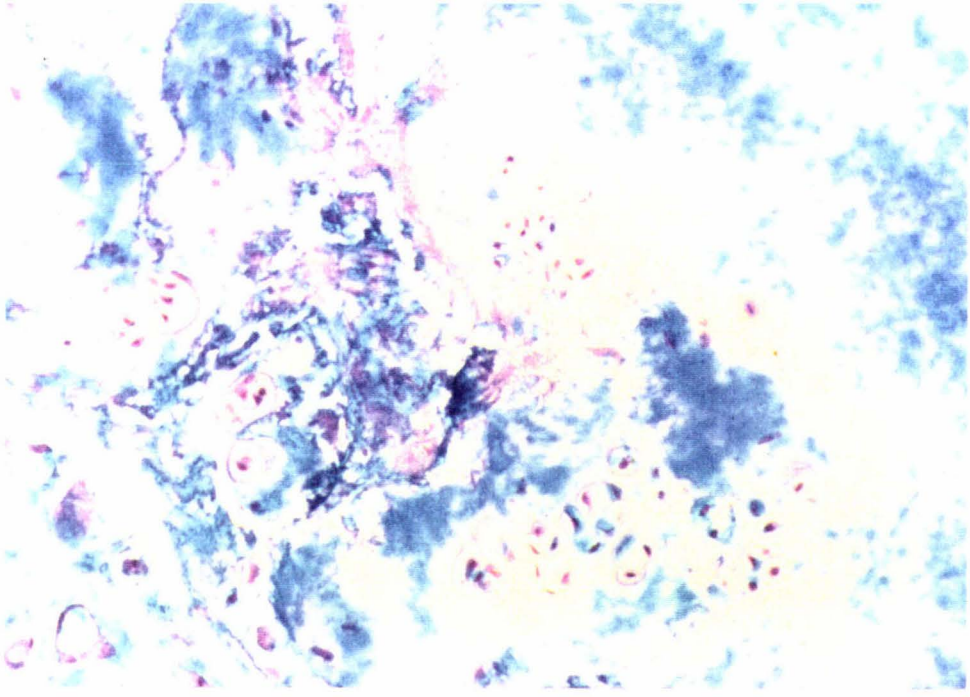
Key to Photos

Figure 9.1a:

Normal dog 1
Disc $L_{1/2}$

Figure 9.1b:

Normal dog 2
Disc $T_{12/13}$



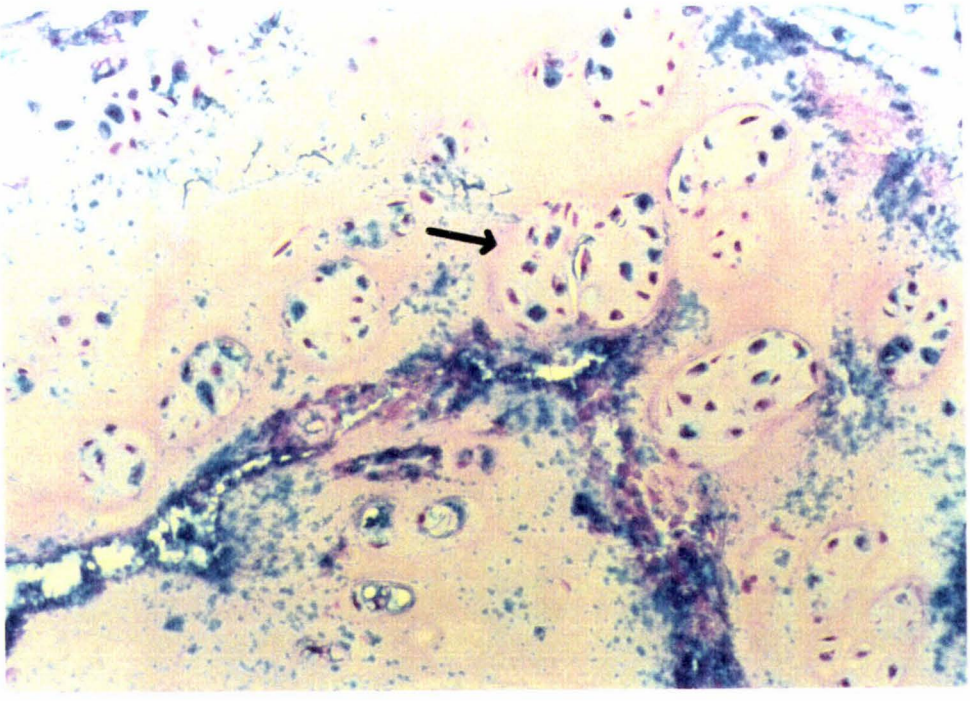
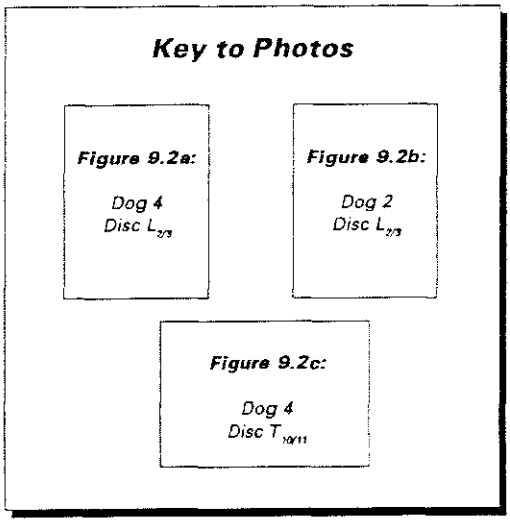
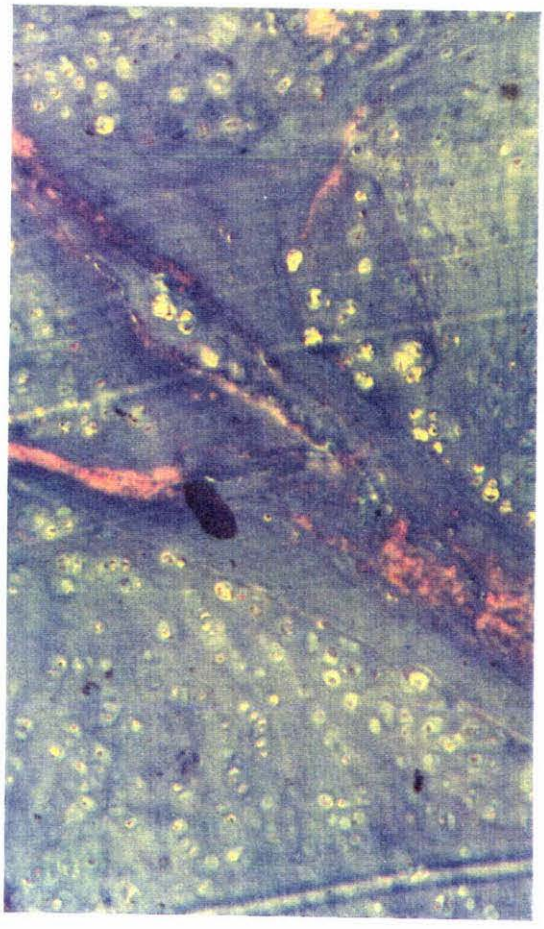


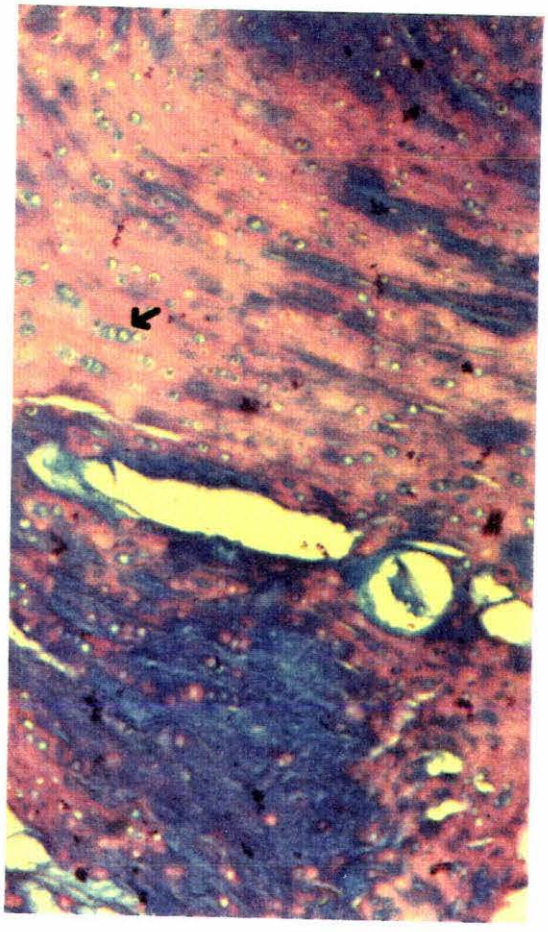
FIGURE 9.2 COLLAGENASE INJECTED DISCS

a) and b): These two photomicrographs (40 x) are representative of the resultant tissue which formed within the nuclear region of all twelve collagenase injected discs. In all cases this tissue was continuous with the collapsed inner lamellar layers of the annulus fibrosus. In three discs ($T_{11/12}$, T_{13}/L_1 - Dog A; $L_{1/2}$ - Dog B), the nuclear region had been entirely replaced by hyaline cartilage *(Fig 9.2a)*. In the remaining discs, the nuclear region contained fibrocartilage predominantly, although in focal areas the cells were showing the characteristic palisading typical of hyaline cartilage *(Fig 9.2 b - arrow)*. In only one disc was tissue resembling normal nuclear tissue seen in a focal region. In the remaining discs, complete obliteration of the normal nuclear tissue appeared to have occurred.

c): In several discs, 'lakes' of matrix material staining strongly with alcian blue had formed within the fibrocartilage of the nuclear region (double headed arrow). In many cases the content of these lakes had been lost during histological preparation (X). (40 x)







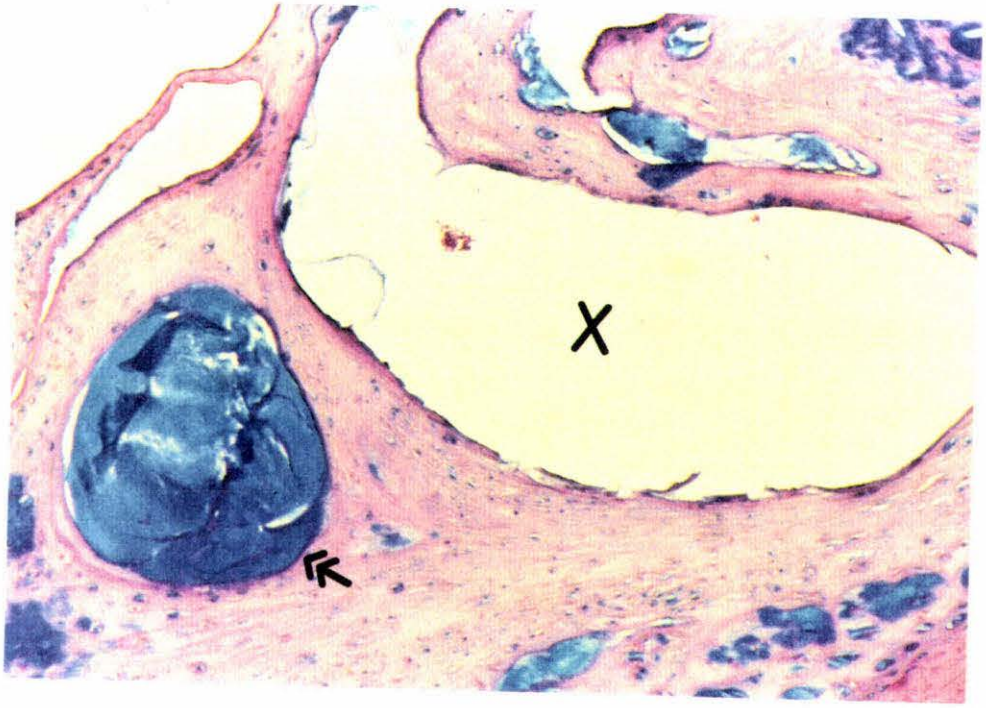
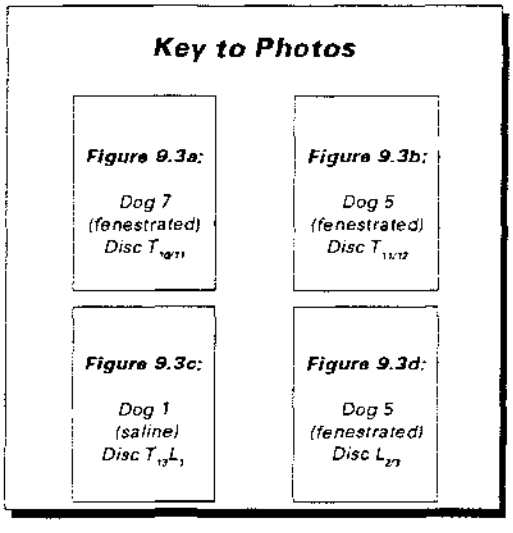

X

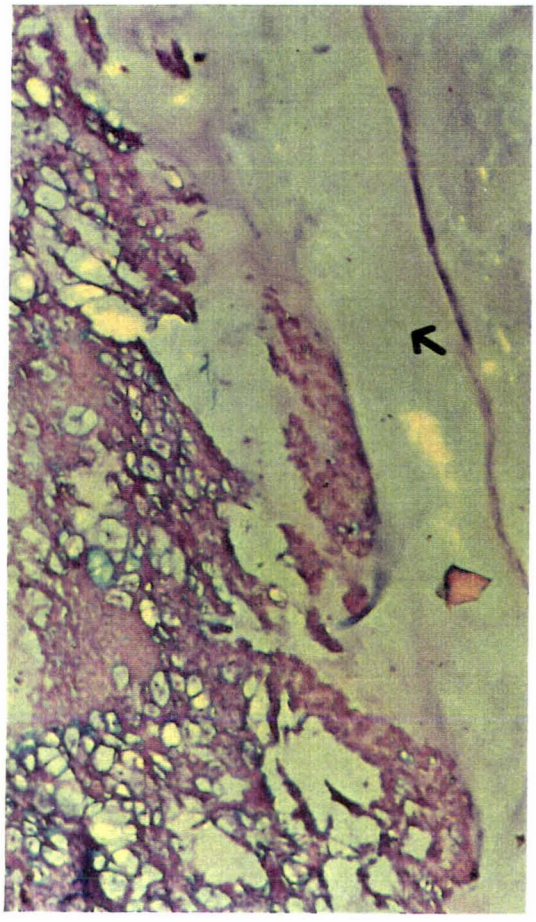
FIGURE 9.3 FENESTRATED AND SALINE INJECTED DISCS (I)

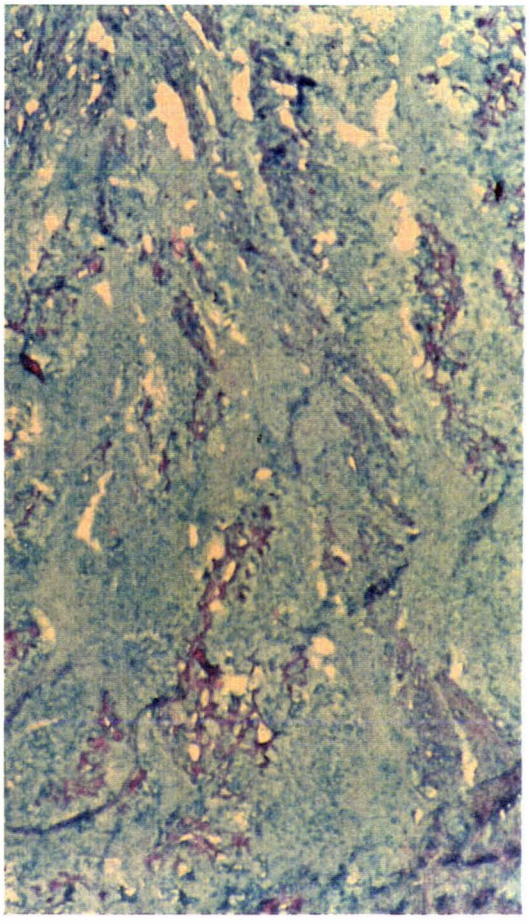
a), b) and c): These three photomicrographs (40 x) are representative of the majority of the fenestrated (9/12) and saline injected (9/12) discs.

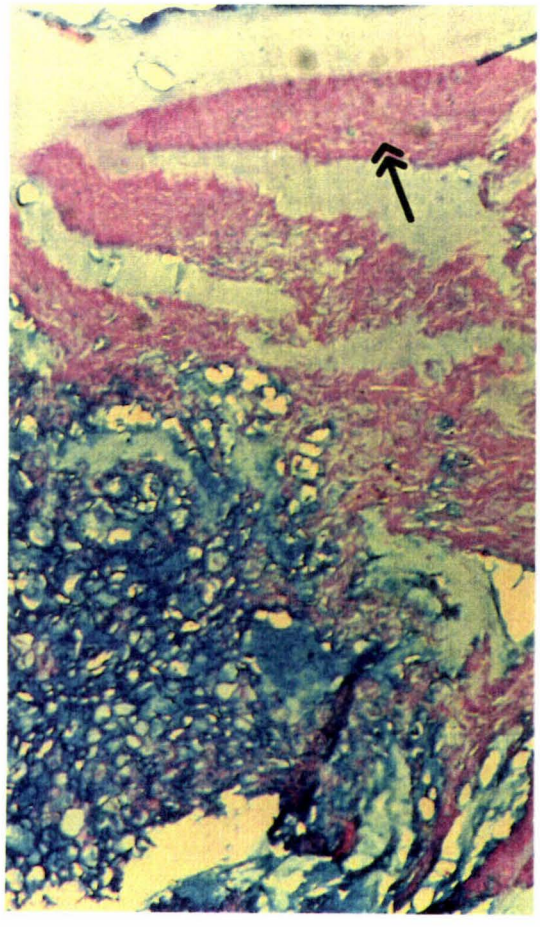
Note how the normal architecture of the nucleus pulposus has been disrupted, and the presence of a large amount of amorphous, moderately staining matrix between the fibrocartilage (arrows). Although the impression of cell 'nests' was still evident in several discs, proliferation of the tissue was occurring, resulting in the formation of fibrocartilage. This fibrocartilage was, in most cases, beginning to show sagittal orientation between the vertebral bodies (double headed arrow).

d) In all of the fenestrated and saline injected discs, the annulus fibrosus was intact, although in three fenestrated discs, the slight infolding of the inner lamellar layers had occurred. In one fenestrated disc ($L_{2/3}$ - Dog B), a small bulge of nuclear tissue was pushing outwards into the dorsal annulus. No disruption to the integrity of the annular fibres had occurred.









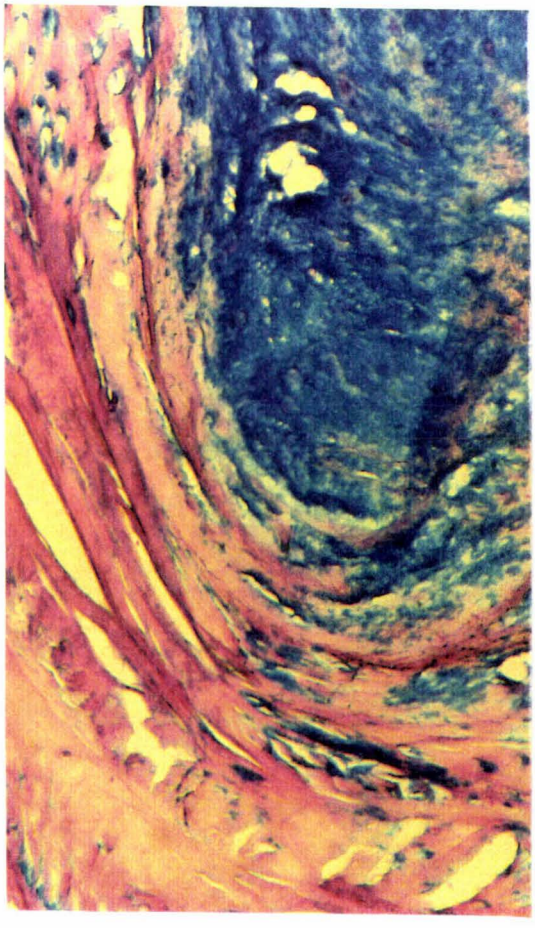
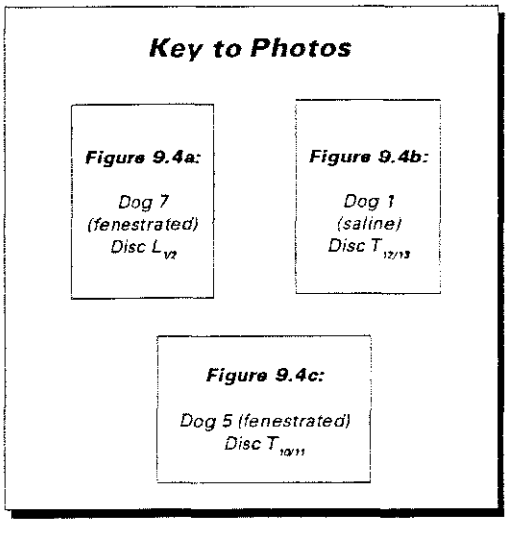


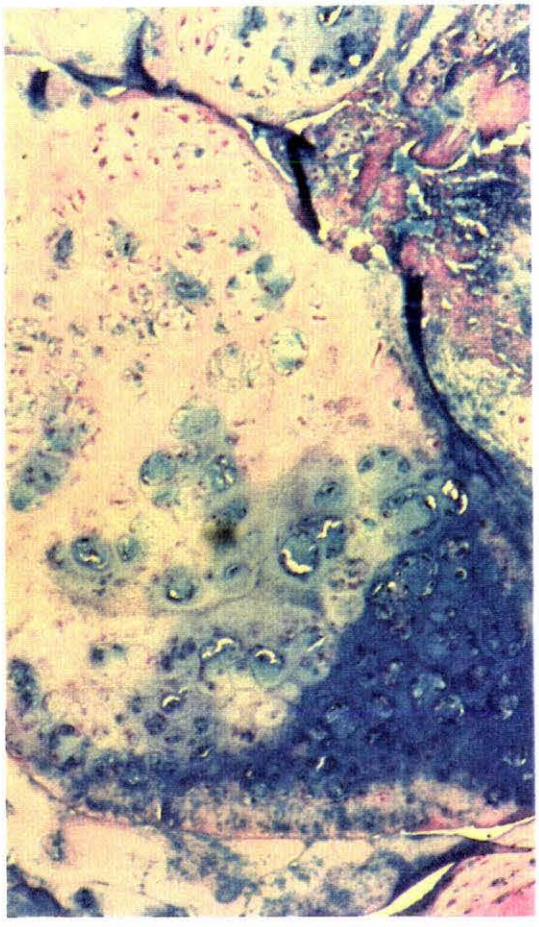
FIGURE 9.4: FENESTRATED AND SALINE INJECTED DISCS (II)

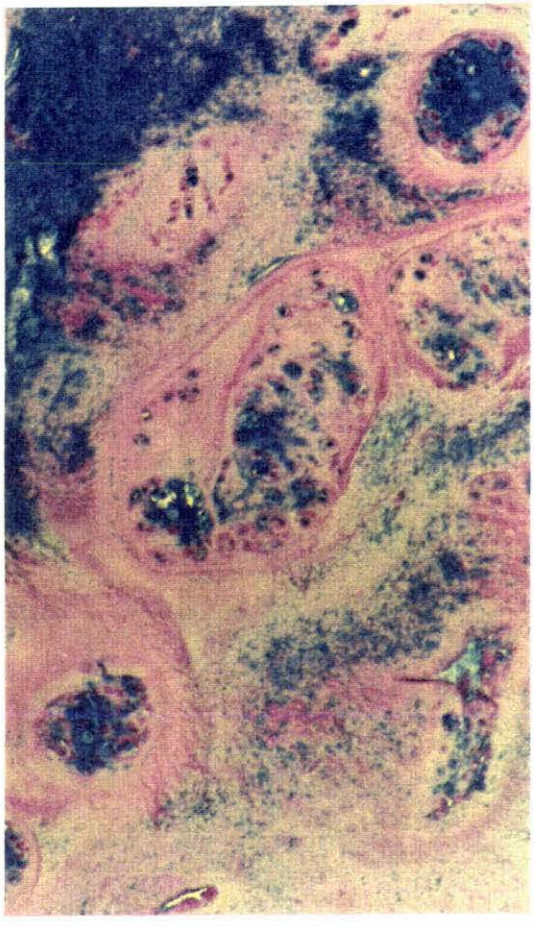
a), b) and c): These three photomicrographs (40 x) show the structure of the nucleus pulposus in five intervertebral discs (3 fenestrated and 2 saline injected). The treatment in two of these discs (one from each treatment group) had been abandoned due to the inability to gain access to the disc due to the close proximity of the rib-head articulation.

These discs show only slight alteration from the normal discs with the cells still showing their characteristic organisation into 'nests' *{see figure 9.1}*. However, in all discs, the fibrous capsule surrounding the cell nests was well-formed (arrow). This increased fibrosus of the disc resulted in the formation of distinct lobules within the nuclear region.

The staining intensity of all discs to alcian blue was similar to the normal discs. However, the localisation of proteoglycan appeared to have become more localised to the cell nests, perhaps indicating increased synthesis of matrix by the chondrocytes.







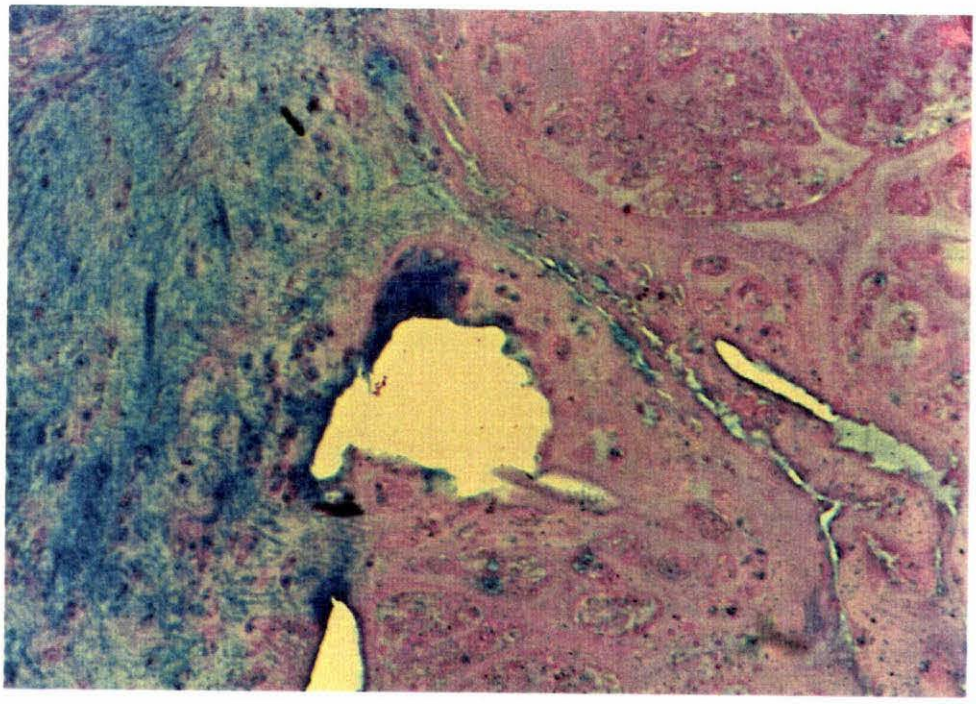


FIGURE 9.5: DORSAL LONGITUDINAL LIGAMENT

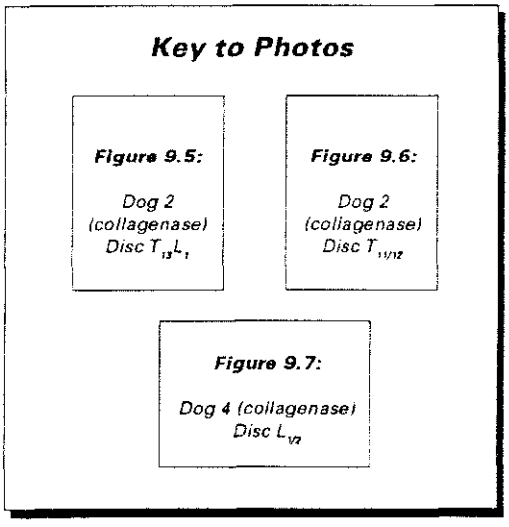
Photomicrograph (40 x) of the dorsal longitudinal ligament in one collagenase injected disc (T_{13}/L_1) in which a massive dorsal herniation of nuclear material had occurred. This nuclear material (solid arrow) had sequestered between the fibres of the outer annulus and dorsal longitudinal ligament, and these latter structures were showing some transformation to hyaline cartilage (open arrow). No obvious inflammatory response is evident about the nuclear material.

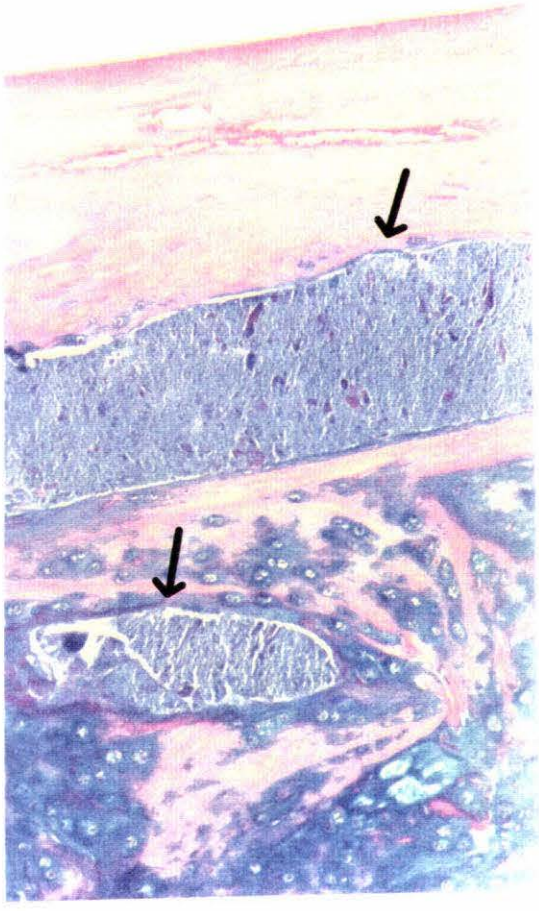
FIGURE 9.6: VENTRAL SPONDYLOSIS DEFORMANS

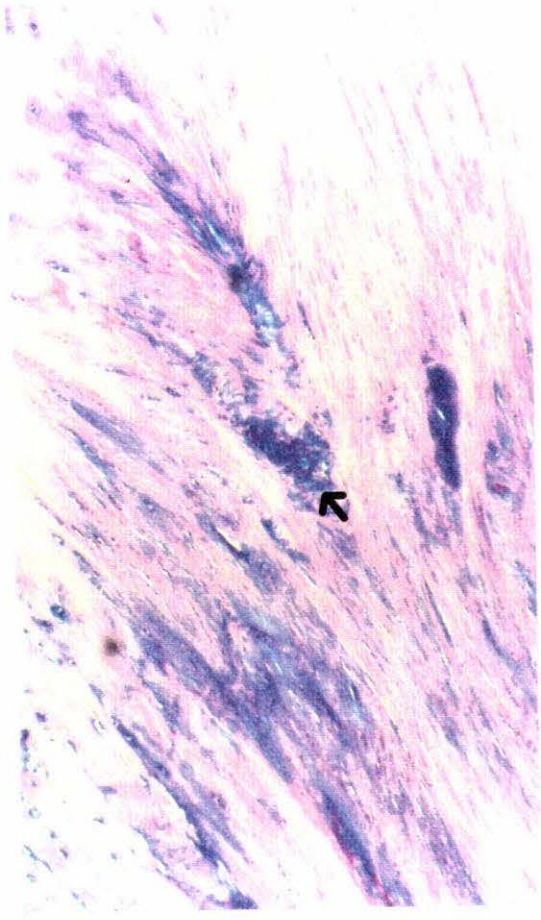
This photomicrograph (40 x) shows the junction between the outer annulus fibrosus (lower right) and the ventral longitudinal ligament. Increased fibrocartilage proliferation is occuring, representing the early development of a spondylosis lesion. A sequestered pocket of normal nucleus pulposus is visible in this disc (arrow).

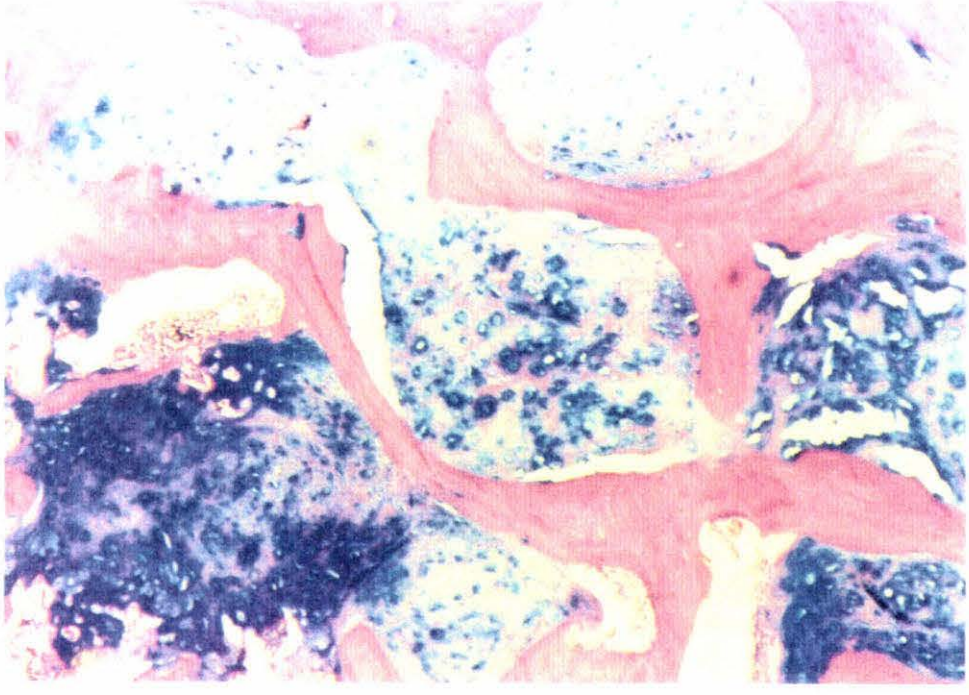
FIGURE 9.7: TRABECULAE OF THE VERTEBRAE

In this photomicrograph (40 x), the interstices between the trabeculae of the vertebral body are filled with fibrocartilage. This was seen in seven collagenase injected discs.

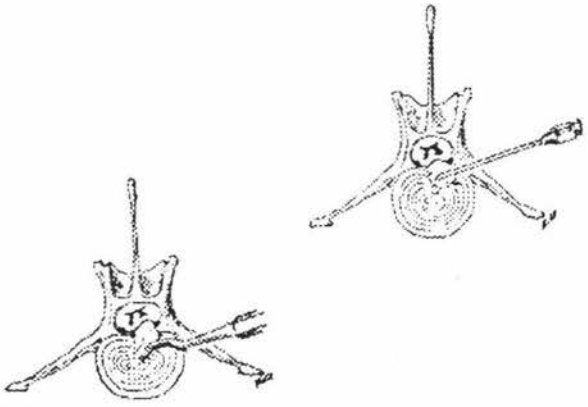








10.0



DISCUSSION

10.0 DISCUSSION

The objective of this investigation was to compare the histological effects of two clinical procedures: surgical fenestration and the percutaneous injection of collagenase enzyme, on the canine thoracolumbar intervertebral disc. The effectiveness and clinical suitability of these procedures in the clinical patient may be determined by examining the proportion of nuclear tissue removed from the disc and the consequent alteration to disc structure. Furthermore, clinical safety can be inferred by the absence of clinical complications associated with the technique.

This is the first study to directly compare these two, commonly used clinical procedures. Indeed, very few objective investigations into either procedure have been reported. This is surprising, considering the controversy surrounding their use.

In this study, intradiscal collagenase injection was found to be a considerably more effective technique than fenestration at disrupting the normal morphology of the nuclear region of the intervertebral disc. In fact, collagenase injection caused complete obliteration of all nuclear tissue in all of the discs in which it was used. This agrees with previous investigations on the canine non-chondrodystrophic disc[19,37,106,269,271]. In these studies, when the discs were examined two weeks after collagenase injection, it was found that only a variably-sized cavity remained within the nuclear region of the disc. Although a similar cavity was not observed in the present study, it is plausible that a cavity may have formed, which later was filled by the collapse of the inner layers of the annulus fibrosus. The collapsed lamellar fibres have subsequently proliferated and, to a variable degree in all the discs studied, undergone some metaplastic transformation to cartilage. It is probable that this reorganisation is a consequence of biomechanical disruption and reorganisation of the intervertebral disc with continued loading. Certainly the fibrous metaplasia observed in the cells within the collapsed annular fibres is known to occur with prolonged compressive loading in an avascular environment[241]. Similar adaptive changes can be intimated by the findings of Bromley et al (1980)[38], who described a "narrowing of the disc space and flattening of the annulus fibrosus" three months after collagenase injection. Unfortunately, he provided no detailed histological descriptions.

By comparison to collagenase, lateral fenestration was only variably effective at removing nuclear material from the confines of the annulus fibrosus. The present study showed that the histological consequences of fenestration were no different to those following injection of the disc with sterile saline. These observations are, however, consistent with the findings of Shores et al (1985)[258] who reported similar histological appearances in the intervertebral disc six

months after fenestration or the passage of either a 20 or 14 g needle through the intervertebral disc.

The similarity of results between fenestration and saline injection in this present study may be due to disruption of the integrity of the annular rings by the simple penetrance of a needle and/or the pressurised injection of saline into the confines of the intervertebral disc. This hypothesis is given credence by the observation that experimentally-induced trauma to the outer layers of the annulus fibrosus (eg either the simple passage of a 14 gauge hypodermic needle or a scalpel incision) induces a self-perpetuating cycle of destructive changes within the disc structure, resulting in significant nuclear degeneration over time[86, 125, 158, 189, 190, 223, 262]. Agreement with these experimental models of disc trauma is seen in the present study by the finding of a generally increased matrix, with evidence of an elevated proteoglycan content, in the nuclear region of both fenestrated and saline injected discs. An increased proteoglycan content would result in an elevated water content of the nucleus, and this fact formed the basis of a self-sealing hypothesis[190] (Section 4.5.1) which proposed that the defect induced in the annular wall may be closed by the swollen nucleus pulposus, thereby restoring normal biomechanical functioning to the intervertebral disc. Biochemical analysis of the nuclear tissue would be required to accurately substantiate the supposed elevation in proteoglycan content observed in this study.

The observed changes in the cellular morphology of the nuclear region of both fenestrated and saline injected discs are consistent with the reaction of mesenchymal cells to changing biomechanical forces[241]. Moreover, the alignment of the cellular aggregations along the median plane observed in many discs would permit the production of collagen fibres whose orientation within the fibrocartilage was best suited to withstand the prevailing stresses within the altered intervertebral disc[81]. In the present study, early chondroid changes were observed in occasional focal areas about the nuclear region of several discs. It could be speculated that, in time, more mature fibrocartilage and/or hyaline cartilage could develop, similar to that described eighteen months following experimental disc injury[189, 223, 262].

Similar to the observations in the collagenase treated discs, collapse of the inner lamellae was found in some saline injected and fenestrated discs in the present study. This annular collapse has been reported in experimental models[189, 203, 223, 262] and may be caused by failure of the self-sealing phenomenon, resulting in an increased compressive load passing through the annulus fibrosus.

All treatments used in the present study caused the extrusion of variable amounts of nuclear material from the confines of the annulus fibrosus. Similar herniation has been previously

reported in histological descriptions of fenestration and collagenase injection[203,258]. The lack of an obvious inflammatory response to the extruded material in this studies provides further evidence against the hypothesis that exposed nucleus pulposus evokes an inflammatory reaction that ultimately causes its complete dissolution[221].

Herniation of nuclear material into the trabecular bone of the vertebral bodies was observed only in collagenase injected discs. Similar lesions have been created *in vitro* during compressive loading of intervertebral disc segments. Miyabashi et al (1992)[203] reported similar extrusions in his investigation of collagenase injection in chondrodystrophoid dogs. He proposed that pre-existing degeneration of the discs in the middle-aged chondrodystrophic dogs used in his study may have predisposed the end-plates to non-discriminate effects of collagenase. Since, in the present study, the extrusions were found in young, non-chondrodystrophic digs this explanation is unlikely to be credible. Perhaps a better hypothesis is that defects may have been created in the cartilaginous end plate by collagenase digestion, thereby providing an avenue through which nuclear material could pass into the vertebral body. It could be speculated that injection of a reduced concentration of collagenase may minimise the possibility of such herniation occurring.

The more common finding of extruded material outside the nuclear region of injected rather than fenestrated discs, perhaps indicates that the act of forced injection of even small amounts of fluid into the confined nuclear region can bring about the forcible extrusion of nuclear material from the disc. Injection of smaller, more concentrated enzymatic solutions or ensuring that forceful injection does not occur may provide some protection against this pressurised herniation of nuclear material. This fact may be important in limiting potentially serious complications associated with collagenase injection.

The safety of a clinical procedure is fundamental to its acceptability as a treatment modality. The only short term clinical complication occurring in any of the three treatment groups occurred in one collagenase injected dogs which developed a transient hind-limb paralysis. The massive dorsal extrusion of nuclear material observed at histology in this animal was presumed to be the cause and probably occurred at the time of injection. Fortunately, the disc material extruded in this normal non-chondrodystrophic dog would have consisted mostly of water, therefore the sudden impact and residual tissue remaining within the neural canal following herniation would have been slight. If a similar herniation had occurred following injection of a degenerate non-chondrodystrophic or chondrodystrophic disc, the neurological consequences of spinal injury with the hardened, less yielding nuclear tissue of these discs would be potentially

more catastrophic. Unfortunately, the spinal cord immediately associated with this herniated disc was not available for histological evaluation.

Of the twelve fenestrated discs, focal defects in the lamellae of the dorsal longitudinal ligament were present in only one disc. The defects in this disc contained nuclear material, which may have been forced into the dorsal longitudinal ligament at the time of fenestration. This finding, together with the knowledge that fenestration both fails to remove all the nuclear material and induces degenerative changes in the disc (through disruption of the annulus fibrosus) does raise concerns about its effectiveness in providing reliable protection against subsequent herniation.

Why then, should fenestration be an effective therapeutic and prophylactic procedure given these short-comings? Some clinicians believe that the apparent efficacy of fenestration is simply a result of the creation of a weakened zone (the fenestration window) in the lateral annular wall[221]. They consider that subsequent herniations are more likely to follow this weakened path, rather than create a new one. The apparent lack of healing in the transected annulus, reported in other studies[125], would add some support to this hypothesis.

In the current investigation, enzymatic digestion appeared to be limited to the nucleus pulposus in all of the discs studied. This finding is consistent with the dose-related digestion described by other authors[19, 37, 106, 269, 271]. The immature fibrocartilage or well organised cartilaginous tissue which formed in the nuclear region would clearly lack the hydraulic properties of the normal disc architecture and is therefore likely to react very differently to biomechanical loading. The nature of this alteration is unclear and should be the basis of further *in vitro* and *in vivo* investigation. From the current knowledge on the biomechanical properties of the vertebral column and intervertebral discs, the annulus fibrosus of the collagenase injected disc would certainly be expected to withstand a greater compressive load which, as has been discussed previously, it is poorly designed to resist (Sections 3.4 and 4.5.3). Moreover, the biomechanical load acting on the disc will be of greater magnitude and less dynamic in effect, due to the loss of hysteresis and shock attenuation normally provided by the now-digested viscoelastic nucleus pulposus.

In this study, disruption of annular structure was observed in five of the twelve collagenase injected discs. Disruption to the dorsal longitudinal ligament in one disc was quite extensive, and was caused by the massive nuclear herniation mentioned earlier. The remaining dorsal annular defects were focal in nature and again appeared to be attributable to minor extrusions of nuclear material. The surrounding lamellar structure of the dorsal annulus and longitudinal ligament continued to show no difference from its normal architecture. Whether these

structures will continue to maintain this integrity, in the face of the altered biomechanical loading was not definitively answered in this investigation. The lack of significant variation in almost all of the treated discs at six months after treatment provides some assurance that the disc has adjusted adequately to its new state. Further investigation of annular changes, at intervals greater than six months following treatment, is required to completely ensure the safety of collagenase injection in the clinical patient.

Some evidence for increased instability about the intervertebral space, particularly about the diaphragmatic region, was provided by the presence of ventral spondylosis deformans in three of the collagenase injected discs by six months after treatment. The development of spondylosis deformans is believed to be due to stretching and tearing of the Sharpey's fibres at the insertion of the outer annular fibres into the vertebral body[237]. Such stretching is likely to develop with increased bulging of the outer circumference of the annulus fibrosus due to the loss of hydraulic resistance following destruction of the nucleus pulposus (Section 3.4). Development of spondylosis deformans has also been reported following the herniation of nuclear material into the outer margins of the disc[126, 191]. This herniated material supposedly results in mechanical disruption of the fibre organisation. Chondrification and, ultimately, calcification of these fibres produces an osteophyte. This latter form of pathogenesis may explain some of the spondylosis observed in the treated discs.

In two of the five collagenase injected discs which developed spondylosis, lesions occurred in both the dorsal and ventral longitudinal ligaments. These occurred in the one dog, and occupied adjacent locations in the vertebral column ($T_{10/11}$ and $T_{11/12}$). In this dog, massive dorsal extrusion of nuclear material had occurred (at T_{13}/L_1) and it is possible that this acute loss introduced a greater degree of instability into the diaphragmatic region of the vertebral column, resulting in the more rapid development of radiographically evident spondylosis lesions in this dog.

Descriptions of thoracolumbar fenestration in major surgical references make only passing referral to the difficulties in gaining access to the intervertebral discs of $T_{10/11}$, $T_{11/12}$ and $T_{12/13}$[21, 24, 71, 92, 103, 255, 308]. The proximity of the rib head articulation to these intervertebral disc spaces can, in some dogs, hinder access to the lateral annulus fibrosus for fenestration. Difficulties in accessing the intervertebral space of these discs were experienced all six dogs in this study. In two discs (one fenestration and one saline injected), treatment of disc $T_{10/11}$ had to be abandoned. Surprisingly, mild histological changes were recorded in these untreated discs, and these alterations were similar to those observed in the nuclear architecture of some treated discs in the same and other (saline or fenestrated) dogs. It is possible that some trauma to the annulus fibrosus may have occurred during the aborted attempt, thereby initiating degenerative changes

in the disc. Furthermore, slight alterations in biomechanical loading of this disc, due to the disruption of the other intervertebral discs in the vertebral column following treatment, would also produce some indeterminable effects on histological structure. This domino-like effect has been recorded by several authors, particularly following fusion of an intervertebral disc space in humans[99, 166, 174, 180, 185].

10.1 EXPERIMENTAL CRITICISMS

A number of criticisms can be levelled at the experimental design of this study. These are: (1) the limited number of dogs used, (2) the histological nature of the discs involved and (3) the lack of randomisation of the procedures within individual dogs.

(1) Although only eight dogs could be used in this study, allowing two dogs to be included in each experimental category, it did provide twelve discs per treatment group. The magnitude of the differences between the collagenase injected and the others was such that, despite the low number of discs investigated, reasonably acceptable conclusions could be made.

(2) By using young, non-chondrodystrophoid dogs, in which previous investigations have shown there is little histological variation in structure[126], the effects of individual variation could be minimised between groups. Similar histological uniformity would not be true for similar-aged chondrodystrophic dogs.

However, one of the disadvantages of this was that it provided no definitive information on the effectiveness of these treatments in the discs which they are most commonly applied, the degenerate chondrodystrophic intervertebral disc. Despite the apparent agreement of the present study with another recent investigation into collagenase injection of the chondrodystrophic disc[203], directly relating the conclusions of this study to the clinical situation must be approached with caution. Further investigation of collagenase injection, saline injection and fenestration in the chondrodystrophoid breeds is required.

(3) Each of the three experimental procedures could not be performed in an individual animal since the long skin incision and muscular dissection required for surgical fenestration would cause indeterminate effects on the adjacent injected intervertebral discs. Furthermore, by restricting the use of one treatment to a dog, the immeasurable influence of domino-like biomechanical effects on other discs would be included in the

overall structural alteration observed. These effects, resulting from alteration in the load-transferring mechanism in other treated discs, would occur with clinical use of the treatment, so it is important to account for their influence on the intervertebral disc structure.

10.3 CONCLUSIONS

The following conclusions can be drawn from the results of this investigation:

1. Percutaneous injection of the thoracolumbar intervertebral disc is a minimally traumatic and relatively problem-free procedure. In skilled hands, injection of most thoracolumbar intervertebral discs can be conducted in less than 30 minutes, with few post-treatment complications developing in the patient.

2. There are few histological differences in the structure of the intervertebral disc six months after injection of the nucleus pulposus with 0.1 ml of physiologic saline and surgical fenestration of the intervertebral disc. These results suggest that the simple percutaneous injection of saline into the disc could be as effective in disrupting the nucleus pulposus as the more painful, costly and invasive clinical technique of fenestration. In spite of the clear visual evidence, the lack of statistical proof means this conclusion should be treated with caution.

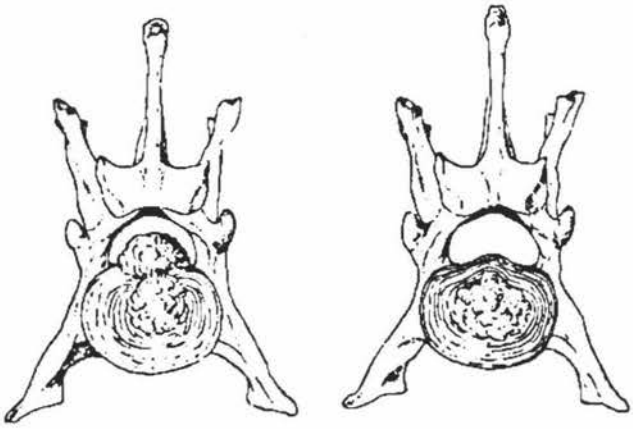
3. Percutaneous injection of collagenase enzyme is a clearly superior technique to thoracolumbar fenestration based on its simplicity, lack of long-term post-treatment morbidity, totality of removal of normal nuclear material and the apparent short and medium term maintenance of disc integrity after injection. It is not clear, however, whether the cartilaginous tissue that develops in the collagenase injected disc is any stronger, or less likely to herniate, than the disc material it replaced.

Based on the findings of the present study, and on the basis of previous clinical and experimental trials, the investigators would support the use of collagenase enzyme in the prophylactic dissolution of the thoracolumbar intervertebral disc of both chondrodystrophic and non-chondrodystrophic dogs. However, a well-designed prospective clinical trial is still needed to determine:

1. The true incidence of herniation in dogs not subjected to a prophylactic procedure, and

2. The relative effectiveness of fenestration, saline injection and collagenase injection at preventing the occurrence of herniation in treated dogs.

A



APPENDIX A

A 1.0 DIAGNOSIS OF INTERVERTEBRAL DISC DISEASE

The diagnosis of intervertebral disc disease involves the identification of that disease process as the cause of the presenting signs and the exclusion of other diseases that can cause a similar presentation. Other causes of acute onset transverse myelopathy typical of Type I disc injuries include vascular accidents (fibrocartilaginous emboli), trauma, granulomatous meningoencephalitis, suppurative meningitis, discospondylitis, toxoplasmosis, cryptococcosis, rabies and canine distemper virus[30, 33, 53, 70, 145, 184, 254]. Progressive neurological lesions, as characterised by Type II lesions need to be distinguished from neoplasia, hypoglycaemia, caudal cervical spondylomyelopathy, degenerative myelopathy and diffuse myelomalacia[30, 33, 53, 70, 145, 184, 254].

Differentiation of these diseases from intervertebral disc herniation can be made on the basis of the history, clinical, neurological and radiological evaluation. Other special diagnostic aids may be used to facilitate diagnostic and prognostic abilities.

A 1.1 NEUROLOGICAL EXAMINATION

The basis of the neurological examination is to identify the location of the lesion as well as determine the severity of the spinal cord injury. As discussed previously, spinal deficits are gained in a predictable fashion with compressive spinal cord disease; proprioceptive and sensory information are lost initially, followed by loss of motor function and finally, deep pain perception.

The basic techniques to identify the status of the sensory and motor tracts are described below[14, 30, 33, 53, 70, 145].

A 1.1.1 General gait and strength

Minor deficiencies in the proprioceptive and voluntary and involuntary motor function of the animal can be detected during normal ambulation. The animal is observed whilst walking and trotting along flat ground, up and down steps and turning in a tight circle to the left and right. In partially or non-ambulatory animals the tail can be lifted, or the abdomen supported with a towel to allow subtle, purposeful movements to be revealed. Deficiencies are detected by observing the position of the limbs and trunk during these activities. It is also possible to detect lateralising signs, where one side or one leg is more greatly affected than the other.

A wide-based stance, or standing with one or more limbs at abnormal angles may indicate a proprioceptive deficit. Dragging the toes, or standing on the dorsum of the paw also indicates a proprioceptive deficit.

Muscular weakness affecting one side of the body can be accentuated by lifting and supporting the limbs on one side of the animal while it 'hemistands' or 'hemiwalks' on the opposite limbs. Similarly, hopping on one limb accentuates deficits in that leg.

A 1.1.2 Motor deficits

The pathway for cerebral control of voluntary (flexor) and involuntary (extensor) muscles can be classified into two distinct systems: the upper motor neurone (UMN) and the lower motor neurone (LMN). The lower motor neurone system consists of the peripheral nerve, neuromuscular junctions and dorsal nerve root. The cell bodies are located in the grey matter of the spinal cord. Reflex arcs act through the lower motor neurone system. The upper motor neurone system is a multisynaptic series of neurones arising in the cerebral cortex and cerebellum and descends in the white matter of the spinal cord. Through synaptic connections with the cell bodies of the lower motor neurones, the upper motor neurone system provides cortical control of muscular activity.

The upper motor neurone is inhibitory to the reflex arc, dampening and coordinating movements of the fore and hind limbs. This factor can be used to isolate and identify the affected section. Loss of upper motor neurone input to the peripheral nerves will result in increased extensor tone and exaggerated reflexes. Thus the gait will be 'jerky' and the limbs will be held quite rigid. Deficiencies in the lower motor neurone function results in loss of neural input to the muscles of the affected area. Depending upon the degree of involvement there will be variable degree of loss in muscle tone, with weakness and reduced reflex activity. Neurogenic atrophy of the muscle occurs rapidly and may be clinically apparent within a week.

Lower motor neurone output to the forelimbs occurs at the cervical enlargement of the spinal cord, between C_6 and T_2. Output to the hindlimbs occurs from L_4 to S_3. Sections of the spinal cord between these regions contain upper motor neurone fibres. By examining the gait strength, coordination and reflexes of each limb the lesion can usually be isolated to one of four regions: above C_6, between C_6 and T_2, between T_2 and L_4 and lastly, below L_4.

1. *Above C_6* : Upper motor neuron lesions to both fore- and hind-limbs.
2. *Between C_6 and T_2* : Lower motor neuron lesion to foreleg, with upper motor neurone effects to hind limbs.
3. *Between T_2 and L_4* : Upper motor neuron lesion to hindlegs. Forelegs should be normal except in very severe cases, where loss of inhibitory innervation results in increased extensor tone in the fore-legs (Schiff-Sherrington syndrome). This is a grave indicator of serious thoracolumbar spinal cord injury.
4. *Below L_4* : Lower motor neuron lesion to hindlegs. Forelegs should be normal. With sacral lesions there may also be urinary and faecal incontinence.

Testing of the reflex arc of individual spinal segments provides information on the integrity of that segment and, through the inhibitory effect of the upper motor neurone it provides some information on the status of higher regions of the spinal cord. The responses of the spinal reflexes can be graded on the following scale: (o) = absent; (+) = depressed (hyporeflexic); (++) = normal; (+++) = exaggerated (or hyperreflexic). Clonus is said to occur when the reflex is brisk and repeats itself, or when the reflex is extremely exaggerated.

All reflexes should be conducted in lateral recumbency to enable relaxation of the limbs to occur. A tense, stiff limb can override the normal reflex activity.

i) Forelimb reflexes:

The nerves that innervate the muscles of the forelimb include the radial, musculocutaneous, median, ulna and axillary nerves. They leave the spinal cord and innervate the muscles of the forelimb in a roughly cranial to caudal pattern.

Withdrawal reflex: The withdrawal reflex is elicited by pinching the skin between the toes. In the forelimb this action is mediated through all nerves of the forelimb and whilst providing information on the status of the cervical enlargement it does not indicate the integrity of specific peripheral nerves.

Biceps reflex: The biceps reflex can be elicited by tapping a finger resting on the distal end of the biceps brachii and brachialis muscles with a neurologic hammer. A positive sign is slight contraction of these muscles which may result in flexion of the elbow. An exaggerated reflex is indicative of an upper motor neurone lesion but an absence of the reflex is inconclusive, as this

may frequently occur in otherwise normal animals. The biceps reflex tests the integrity of the musculocutaneous nerve (spinal segments C_6 - C_8, or intervertebral disc spaces $C_{5/6}$ - C_7/T_1).

Triceps reflex: The triceps reflex is elicited by tapping the triceps tendon just proximal to the olecranon with a neurologic hammer. Again a negative reflex is inconclusive evidence for dysfunction of the radial nerve (spinal segments C_7 - T_2, or the intervertebral disc spaces $C_{6/7}$ - $T_{1/2}$).

ii) Hindlimb reflexes:

The sciatic and femoral nerves innervate the muscles of the hindlimb. The perineal nerve innervates the structures of the anus, rectum and genitalia. Isolation of individual nerve dysfunction is therefore more reliable in the hindlimb.

Withdrawal reflex: In the hindlimb the withdrawal reflex is mediated entirely by the sciatic nerve. It can be elicited by pinching the toes and all major joints of the hindlimb should flex. This reflex tests the integrity of spinal segments L_6 - S_1 (intervertebral disc spaces $L_{4/5}$ - $L_{5/6}$).

Patellar reflex: The patellar reflex is the only reliable tendon reflex in the pelvic limb and is the only tendon reflex present in all normal animals. It can be elicited by tapping the straight patellar ligament in the relaxed limb. This should produce a brisk extension of the stifle. Clonus may be observed in severely injured animals. The left and right side should always be examined and compared in the same animal, as the animal lies on each side. The patellar reflex tests the integrity of the femoral nerve (spinal segments L_4 - L_6 , or intervertebral disc spaces $L_{3/4}$ - $L_{4/5}$).

Note: Lesions of the sciatic nerve may cause flaccidity in the flexor muscles normally antagonistic to the patellar reflex. In these animals, the reflex may appear exaggerated, falsely leading to a diagnosis of an upper motor neuron lesion.

Perineal reflex: The presence of sphincter tone in the anus and bladder provides some information on the status of the sacral segments of the spinal cord. The anal tone can be checked by pinching the skin ventral to the anus, which should cause sharp contraction of the sphincter.

Bladder reflex: Palpation of the bladder may also allows its separation into a 'lower' or 'upper' motor neuron state. Control of bladder tone and reflex urination occurs through the combined action of the pelvic and pudendal nerve, both of which originate in the S_1 - S_3 spinal segments. The pelvic nerve carries sensory information from and parasympathetic innervation to the detrusor muscle of the bladder wall. Increased parasympathetic tone stimulates contraction of

the bladder. The pudendal nerve provides motor innervation to the external urinary sphincter. When the pelvic nerve stimulates urination, the pudendal nerve is inhibited, which relaxes the sphincter tone. Conscious urination occurs through cortical modulation of the same reflex arc.

A lower motor neuron bladder can develop when a lesion between S_1 and S_3 interferes with this reflex arc. Innervation of the bladder through the pelvic and pudendal nerves is disrupted and the bladder becomes flaccid, dilated and easily expressed due to the lack of sphincter tone. There may be frequent dribbling of urine. Lesions above S_1 result in an upper motor neuron bladder, which may have a variable presentation. Reflex urination is possible but its effectiveness is dependent upon the extent of the sympathetic innervation to the bladder wall which increases the threshold of the reflex arc and permits the detrusor muscle to stretch and increase the bladder volume before contraction occurs. Loss of sympathetic innervation results in a small, tight bladder with a hyperactive reflex arc - the bladder empties when filled with small amounts of urine, resulting in frequent spurting of small amounts of urine. Sympathetic fibres to the bladder originate in the upper lumbar spinal cord, and exit through the intervertebral foramen at $L_{1/2}$ and $L_{2/3}$.

iii) Other reflexes:

Crossed Extensor: This reflex is indicative of an upper motor neuron lesion when elicited during testing of the withdrawal reflex in any fore or hindlimb. If the reflex is present, the opposite limb will extend when the opposite limb withdraws. The toe-pinch given should be as light as possible to avoid the voluntary extension which can occur in response to a painful stimuli. Testing both limbs with the animal lying on both sides will give the most reliable results.

Panniculus reflex: Contraction of the cutaneous trunci muscle following stimulation by a light prick is a segmental reflex mediated by the sensory nerve roots. These nerves ascend in the spinal cord and exit at C_8 - T_1 to innervate the cutaneous trunci muscle. Normal action of the reflex therefore indicates that the spinal cord is intact from the level of stimulation to T_2. In normal dogs the reflex may be absent caudally to the level of the mid-lumbar spine. In dogs with severe thoracolumbar spinal cord compression the reflex may be absent caudal to the lesion. Hyperactivity of the response may occasionally be seen in the spinal segment immediately cranial to the lesion due to the loss of caudal inhibition.

More forceful pinching of the skin allows determination of the sensory level. Animals with a severe spinal lesion may be analgesic distal to the lesion. Due to the stoic nature of some animals, this test can be difficult to interpret.

Babinski reflex: The Babinski sign is best elicited in animals by a medial to lateral upwards stroking of the metacarpal or metatarsal bones with the metal end of a neurologic hammer. In normal animals, the toes flex slightly in response to the stroking. With a positive Babinski sign, the toes extend. The presence of the Babinski sign in man indicates corticospinal tract damage. By extrapolation it is suspected to indicate a similar lesion in dogs and cats. A Babinski sign is more common in upper motor neuron lesions to the hindlimbs.

Tail wag: Tail wagging may be reflex in origin and may be intact even when the spinal cord is completely severed, if it is above the level of the reflex arc (Co_1). Voluntary tail wagging, however, in response to an appropriate stimuli (owner's voice etc) is a sign of spinal cord integrity.

A1.1.3 Sensory deficits

Deficits in pain perception are the last to occur and imply severe spinal cord injury. A diminished ability to perceive pain occurs caudal to the lesion. The ability to recognise this deficiency is critical because it is one of the few tests which provides prognostic information.

Examination of an animals response to painful stimuli can be difficult to interpret. The patient should be as relaxed as possible. A nervous, frightened animal will frequently respond inappropriately. If necessary, repeat testing after the animal has been placed quietly in cage to allow it to become more comfortable with its surroundings.

The animal should be tested for the perception of superficial and deep pain stimuli. The reaction to superficial painful stimuli, such as a pin prick, may frequently be inconsistent. The animal may also become tolerant to repetitive testing. Deep pain perception is more consistent and can be tested by squeezing the base of the toenails with forceps. Conscious perception of the stimuli must be observed and the withdrawal of the limb should be separated from the simple, unconscious reflex arc. The animal may cry out, turn and look at the source of pain, or actively pull away from the stimulus.

The accuracy of the neurological evaluation at determining the site of spinal cord injury is good. Identification of the lesion as either an upper or lower motor neurone syndrome permits its localisation to one of four regions along the spinal cord. Further evaluation of the neurological

examination should permit localisation to within two intervertebral spaces 75% of the time. Localisation to a single intervertebral disc space based on neurological evaluation only is 40% accurate.

A 1.2 RADIOLOGICAL EXAMINATION

A1.2.1 Plain radiographs

Whilst the previous evaluations will identify and localise a spinal deficit, radiology is necessary to confirm a diagnosis of intervertebral disc protrusion[14, 35, 46, 87, 148, 184, 254]. Standard radiographic techniques are utilised and general anaesthesia should be used to facilitate proper positioning. Lateral and ventrodorsal views should be taken in all cases. Use of sand bags and foam padding enable the vertebral column to be aligned parallel to the table top for lateral projections. Rotation of the vertebral bodies due to poor positioning can cause normal structures (eg articular facets, transverse processes) to become superimposed over the intervertebral foramens, making evaluation difficult[14]. Due to the smallness of the intervertebral space, radiographic studies should be limited to areas no greater than 20 cm in length. This eliminates the problems associated with parallax which, due to the divergent nature of the X-ray beam, make interpretation of the peripheral disc spaces difficult.

The intervertebral disc, neural canal, vertebral bodies and surrounding structures should be examined on the radiograph. As described previously (Section 1.1), calcification may frequently be seen in chondrodystrophoid dogs. This calcification may vary in density, ranging from a slight haziness to that equal to the vertebral body. Although indicative of degeneration, a calcified disc need not be clinically significant[14, 126, 145].

Radiographic signs indicative of Type I disc extrusion include narrowing or wedging of the intervertebral disc space, presence of calcified material within the neural canal and/or narrowing of the joint space of the articular facets. Narrowing of the disc space can be determined by comparing the width of the questionable space with those immediately adjacent to it. The disc spaces are usually constant within the cranial thoracic spine but normally become narrower about the anticlinal space ($T_{9/10}$ - $T_{10/11}$). The disc spaces then tend to widen caudal to this point and stabilise in size in the caudal lumbar spine. Wedging of the disc space is seen as a narrower dorsal portion and wider ventral portion of the intervertebral disc.

Recognition of calcified material within the neural canal may frequently be possible on the lateral projection. The intervertebral foramen, which usually appears as a 'horse's head' on the lateral projection, may become fogged (increased density). Occasionally, a more classical 'amphora' shape composed of strongly calcified material may be seen.

Type II disc protrusions may be associated with narrowing of the disc space, osteophyte production about the ventral borders of the vertebral bodies (ventral spondylosis) and end-plate sclerosis. Arthritic changes associated with the articular facets may also be apparent. Calcification of the intervertebral disc is rarely seen.

In some animals the degree of ventral spondylosis is considerable and there may be complete bridging of an intervertebral space. Though indicative of disc degeneration the clinical significance of these changes, despite their severity, has not been elucidated[237]. In man, arthritic changes associated with the articular facet joints have never been identified without considerable degeneration of the intervertebral disc being identified[50,131,166]. Similar investigations have not been performed in the dog.

A1.2.2 Contrast radiography

In situations where calcified disc material is not visible on the plain radiograph, or when it is necessary to confirm the site (or sites) of disc herniation, or when the disc material is suspected to have extruded in a predominantly lateral (or dorsolateral) direction, a myelogram should be performed[14, 30, 33, 35, 46, 53, 70, 87, 145, 147, 148, 184, 254]. If surgical decompression is not anticipated, and previous investigations are highly suggestive of disc extrusion, a myelogram is unnecessary.

A myelogram involves the injection of a radiopaque contrast agent into the subarachnoid space surrounding the spinal cord. The contrast material most commonly used for this procedure is iohexol[a]. Iohexol is a water-soluble, non-ionic compound which has good flow characteristics, good radiographic density and is relatively safe to use[301, 304]. One of the complications of myelography is seizure upon recovery due to the relative hypertonicity of the compounds. The reported incidence of seizures with the water-soluble, ionic compounds used prior to the availability of iohexol was as high as 32%[302]. The use of iohexol has significantly reduced the complications associated with myelograms and seizure activity is minimal.

Several precautionary measures are advised prior to anaesthesia and during the performance of myelography[14, 35, 53, 304]. Pre-medication with acetylpromazine should be avoided due to its epileptogenic potential. Morphine and neuroleptanalgesics are also contraindicated. The head should be held elevated relative to the rest of the vertebral column to prevent flow of contrast

---

a: *'Omnipaque 300μg', Sterling-Winthrop NZ Ltd, Auckland, New Zealand*

into the ventricles of the brain. Should this occur, anaesthesia should be prolonged as this has been shown to reduce the incidence of seizures.

Diazepam[b] has been found to decrease the incidence of seizures in man but this has not been well substantiated in the dog. The serum half-life of diazepam in the dog is about 3 hours, which is considerably shorter than the 20 - 50 hours recorded for man. Maximum benefit will therefore be gained if given immediately prior to recovery, rather than at the start of the procedure.

The injection of contrast agent into the subarachnoid space can be performed at the cisterna magna or at the caudal lumbar cul-de-sac[14, 35, 53]. At both these sites the subarachnoid space is comparatively large, thus minimising the risk of penetrating the spinal cord. Though no rule applies, it is common to perform a cervical puncture for cervical lesions and a lumbar puncture for thoracolumbar lesions. If either site fails to completely delineate the lesion, the other should also be performed.

(i) Cervical puncture (cisterna magna)

Proper positioning is essential for the penetration of the cisterna magna in the cervival region. Cervical punctures are performed more commonly in lateral recumbancy. Sandbags or foam pads should be used to ensure the vertebral column is parallel to the table-top. A large area, extending cranially from the mid-point of the cranium to the mid-cervical region, bounded laterally by the ears should be clipped and aseptically prepared for surgery. The head should be held by an assistant in a slightly flexed position and the nose should be parallel to the table.

The landmarks to identify prior to insertion of the needle include the occipital protuberance and the wings of C_1 (Axis). The needle should be inserted on the midline at a point halfway between these two prominences. Frequently a small depression can be observed in the skin at this site. The spinal needle should be inserted slowly until it is felt to pop through the epidural sac. At this point the stylet can be removed and cerebrospinal fluid collected. Some authors describe removing the stylet immediately after penetrating the skin. Because cerebrospinal fluid can be observed in the hub immediately upon penetrating the subarachnoid space this technique may minimise overpenetrance of the needle into the spinal cord. This technique is preferred by the author.

Once the needle is within the subarachnoid space the contrast agent should be slowly injected. The recommended dose rates for iohexol depend on the site of administration, the area of interest

---

b: 'Valium 2mg', Pitman-Moore, Palmerston North, New Zealand

and the size of the dog. Dosages should be based on the ideal body weight because in obese animals the neural canal is not proportionately larger. The following dose rates are considered suitable:

Large breeds:	2,000 mgI
Medium breeds:	1,500 mgI
Small breeds:	650 mgI

The patient should be closely monitored during injection of the contrast agent. Tachycardia and mild hypotension are frequently recorded. Though not specifically reported with Iohexol, cardiac arrhythmias may be induced owing to endogenous release of catecholamines casued by nerve root irritation.

(ii) Lumbar puncture

The lumbar puncture is technically more difficult and cerebrospinal fluid is rarely collected. Lateral recumbancy is again preferred as this allows the hind legs to be drawn forwards, increasing the dorsal flexion of the vertebral column which opens the dorsal intervertebral space. The most common site is $L_{6/7}$, but L_7/S_1 or $L_{5/6}$ may be used. The likelihood of developing post-procedure neurological complications increases when using sites cranial to L5/6 and therefore should be avoided if possible.

The back should be flexed maximally. The skin over the proposed injection site should be clipped and aseptically prepared. The needle is inserted on the midline immediately over the most cranial aspect of the dorsal spinous. The needle should be advanced slowly, passing parallel to the cranial ridge of the dorsal spinous process. Should the needle contact bone the needle should be redirected. If difficulties are encountered, further advancement of the needle can be conducted using fluoroscopy, or survey radiographs may facilitate accurate needle placement. Successful penetrance into the ventral subarachnoid space is evidenced by a slight 'kick' from the hind legs as the needle passes through the nerve roots of the cauda equina. Passage through these nerve roots does not appear to cause any clinically evident trauma. Once the needle is accurately positioned the contrast media can be slowly injected. Slightly reduced dose rates from those presented above are used in this area.

Interpretation of the myelogram

The contrast agent outlines the subarachnoid space and the alterations in the spinal cord and epidural space can be assessed by inference to the changes they may induce in the subarachnoid anatomy[14, 46]. In the dog, the subarachnoid space is relatively constant in width

throughout the thoracic and lumbar spine, but thins over the intervertebral disc spaces ventrally. It is fairly wide at the craniocervical and cervicothoracic junctions.

Myelographic lesions may be classified as intramedullary, extramedullary-intradural and extradural. Intramedullary lesions represent swelling of the spinal cord itself and are characterised by a thinning of the contrast column in all projections. Lesions within the subarachnoid space (extramedullary-intradural) show deviation of the column in one projection, with widening in the other. Occasionally they may become delineated by surrounding contrast media. An extradural lesion is typified by a narrowing or compression of the spinal cord in both projections. Intervertebral disc lesions typically causes extradural compression of the spinal cord with classical myelographic signs over the involved interspace. Occasionally, the resultant inflammation causes swelling of the spinal cord which may develop over several vertebrae. The myelogram in these cases will show thinning of the contrast column, as would be expected for an intramedullary swelling. In severe cases the swollen cord will completely fill the neural canal, preventing its delineation by contrast media. In these cases, clinical judgement must be used to identify the involved disc space.

Extruded disc material is most commonly seen on the midline of the vertebral column. Occasionally the material is extruded in a dorsolateral location. Because only one side of the spinal cord will be compressed, a twin extradural line may seen on the myelogram. True lateral extrusions will cause deviation of the spinal cord visible only on the ventrodorsal view. Rarely, laceration of the vertebral sinuses at the time of herniation will result in severe extradural haemorrhage. This will appear as a large filling defect which may extend over several vertebral segments.

Myelography is often essential for the precise diagnosis of Type II lesions. These will appear as extradural masses over the involved intervertebral space.

(iii) Special imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) have only been used to a limited degree in the diagnosis of intervertebral disc disease in the dog, despite their widespread use in the human.

A 1.3 CEREBROSPINAL FLUID EXAMINATION

Cerebrospinal fluid should be collected prior to myelography to rule out inflammatory or infectious diseases of the spinal cord or meninges. It should be analysed for colour,

turbidity, protein content, total and differential cell counts. Theoretically, this analysis should be conducted prior to myelography but this is usually practically difficult to achieve.

Alterations in the cerebrospinal fluid are most pronounced in samples taken near or caudal to the lesion[282]. Therefore, the spinal fluid from the lumbar cistern is more frequently altered by lesions anywhere along the vertebral column and changes in the cervical cerebrospinal fluid samples occur more frequently with cervical lesions than thoracolumbar lesions.

The most frequent alterations detected in the cerebrospinal fluid is a mild elevation in protein and white cell count[282]. The degree of protein elevation appears to be proportional to the severity and acuteness of the neurological signs. This elevation is the result of exudation of serum proteins across the blood:cerebrospinal fluid barrier due initially to acute rupture of vessels. Subsequent loss is related to vessel wall necrosis caused by ischaemia.

White cell elevation occurs less commonly and has a poorer correlation with the severity of the spinal trauma[282]. The differential white cell count does not appear to differ dramatically from normal and tends to represent a typical inflammatory pattern. Mononuclear cells tend to predominate and depending on the chronicity of the injury may show slight degenerative changes as they phagocytose nuclear material.

A 2.0 TREATMENT OF INTERVERTEBRAL DISC DISEASE

A 2.0.1 Introduction

There is no true 'treatment' for intervertebral disc degeneration. The objective of the following therapy's is to minimise the spinal cord injury which results following the extrusion of nuclear material into the neural canal, thereby relieving the clinical signs related to disc herniation.

Depending on the nature of the herniation, the clinical presentation can be quite variable. The spinal cord appears to be able to accomodate slowly developing compressive lesions and clinical signs tend to be slight compared to a similar degree of compression following an acute injury. A variety of treatment options are therefore available following intervertebral disc herniation and their use depends on the clinical presentation and the experience of the attending clinician.

A review of the pathophysiology of spinal cord injury is appropriate because it facilitates an understanding of the development of clinical signs following a traumatic incidence (eg disc

herniation) and it enables comprehension of the treatment options available and when their use is appropriate.

A 2.1 REVIEW OF SPINAL CORD INJURY

The response of the spinal cord to trauma is characterised by a self-perpetuating cycle of events which result in the exacerbation of the initial injury[15,29,32,55,65,88,124,162,182,238]. These events can be separated into three separate categories which influence each other to a variable degree. Recognition of the separate states enables recognition of reversible and irreversible changes, and thus directs the logical application of treatment modalities. These categories are: direct morphological changes; vascular changes; biochemical and metabolic changes.

A 2.1.1 Direct Morphological changes

The explosive impact of the calcified disc material onto the spinal cord results in laceration of the fibre tracts of the white matter and microvasculature of the pia mater and deeper regions of the spinal cord. Within an hour the entire grey matter is haemorrhagic and the initiation of the inflammatory response, due to cellular damage and leakage of vascular products, results in the development of oedema of the immediate and surrounding regions of the spinal cord. The spinal cord begins to swell, but this expansion is limited by the surrounding inelastic meningeal membrane and the bony surrounds of the neural canal. Disruption of the substance of the spinal cord follows, generally spreading along radial and longitudinal planes. This disruption tends to be greatest in the grey matter of the cord probably because its metabolic demands are greater, continued swelling of the cord tends to concentrate pressures centrally and the neuropil of the grey matter is more easily separated compared to the tightly packed fibres of the white matter.

These primary mechanical events result in irreversible damage to fibres tracts and cellular elements of the spinal cord. Most significantly however, these events initiate a self-perpetuating cycle of vascular and biochemical changes which result in continued disruption of the spinal cord. These autolytic changes, which are potentially reversible in the early stages, are responsible for the subsequent aggravation of the clinical presentation following spinal cord injury. It is probable that permanent disability following spinal cord injury is the result of exuberant autolysis rather than the result of direct mechanical trauma.

A 2.1.2 Vascular changes

As presented previously (Section 2.5.3), the spinal cord is supplied by one ventral and two dorsal spinal arteries. These vessels send a variable number of branches deeper into the

cord parenchyma: the distribution of these vessels shows some abatement in the thoracic region of the spinal cord. In most mammals, two thirds of the spinal cord is nourished by blodd from the ventral spinal artery. The remaining third is supplied by the dorsal spinal arteries.

Immediately following injury there is frequently a dramatic decrease in spinal cord blood flow. One of the reasons for this is the loss of autoregulation, probably due to extreme systemic hypotension mediated by endogenous opiates (endorphins) which are released from the spinal cord due to an as yet undetermined triggering factor. Vascular occlusion of the small perforating vessels may also develop due to vasoconstriction, thrombosis and extra-luminal compression due to the developing oedema.

Thrombosis is a consequence of the accumulation of platelets which adhere to the damaged endothelial lining of the vessel. This platelet adhesion can develop within 90 seconds following injury, promoting further biochemical alterations within the substance of the spinal cord because of activation of the inflammatory cascade.

This acute reduction in spinal cord blood flow results in an ischaemic hypoxia of the spinal cord. This necessitates a shift from aerobic to anaerobic metabolism by the cells. Anaerobic metabolism is an inefficient means of generating energy and results in the accumulation of lactic acid. These two factors combine to disrupt neuronal transmission through inactivation of the energy-dependent Na/K pump and acidification of the extracellular space. The Na/K pump restores the normal sodium and potassium balance following nerve transmission. When not functioning, potassium accumulates outside the cell, sodium inside. The high intracellular concentration of sodium causes swelling of the cell as water is attracted to it. Cell disruption can follow.

A 2.1.3 Biochemical and Metabolic changes

Disruption of cellular membranes results in release of membrane-bound phospholipids and the activation of the arachidonic acid inflammatory cascade. The products of this cascade result in further development of tissue oedema by increasing vessel permeability, further promotion of platelet aggregation and the attraction of inflammatory cells to the injured area. Initially these cells are neutrophils, but eventually monocytes and macrophages arrive. These cells release destructive enzymes (lysosomal enzymes). The inflammatory cascade tends to be self-perpetuating once established.

Lysosomal enzymes are also released from other cells within the spinal cord. These proteolytic enzymes are an important factor in the continuing autolysis of the spinal cord parenchyma.

Disruption to the normal metabolic processes of the cell can result in the release of free oxygen radicals. In the normal cell these molecules are incorporated into water and oxygen by the electron transport chain. Ascorbic acid, Vitamin E and glutathione peroxidase can function as effective scavengers when free radicals are formed outside this chain, but this protective mechanism quickly becomes overwhelmed during spinal injury. Free oxygen radicals are significant because they produce peroxidative damage to membrane lipids resulting in the alteration of membrane fluidity, loss of their barrier function and selective permeability and the inactivation of membrane-bound enzymes. Because of the high lipid content of the nerve fibres in the white matter these free radicals can cause extensive disruption.

The white matter of the spinal cord can therefore be damaged by the initial traumatic episode which lacerates the individual fibres and the ensuing biochemical alterations. Free oxygen radical-induced disruption of cellular membranes in turn leads to swelling of the fibres and surrounding myelin cells due to breakdown of the Na/K pump and loss of the selective barrier function of the cell membrane. Further destruction results from the influence of the lysosomal enzymes released by inflammatory cells and local necrotic cells.

The grey matter suffers most from the direct and indirect vascular disturbance. The high metabolic demand of this tissue necessitates a switch to anaerobic metabolism making energy production inefficient. Oedema of the parenchyma, mediated by the developing inflammatory process, causes the spinal cord to swell within the confines of the meningeal membrane, concentrating pressures within the central portion of the cord. Cellular death, due to hypoxia, causes release of lysosomal enzymes which induce further disruption of the parenchyma. Breakdown of Na/K pumps, the increased lactic acid content of the extracellular tissue and the alteration to the membrane barriers cause swelling of other cells, which may ultimately burst, releasing further lysosomal enzymes into the inflammatory milieu.

A 2.2 NON-SURGICAL (CONSERVATIVE) MANAGEMENT

The non-surgical management of intervertebral disc disease describes a) the use of pharmacological agents to control and limit the inflammatory process within the spinal cord and b) the nursing care aimed at restoring the animals systemic well-being, thereby ensuring that fundamental processes continue whilst the animal recovers from the trauma. The intensity of this nursing management will depend upon the severity of the injury.

The principles of non-surgical management are utilised even in surgical candidates[34, 238]. The importance of nursing care in the rehabilitation of these animals cannot be overstressed.

2.2.1 *Pharmacological agents*

The role of the pharmacological agents is to control the inflammatory response, thereby reducing the autolytic changes which may develop in the spinal cord following injury[15, 29, 32, 55, 88, 238]. A number of drugs have been investigated on an experimental basis[68, 124, 162]. The theoretical basis for using these agents is based on their perceived effect on the model of spinal cord injury presented previously. However, in most cases, the effect of these drugs does not provide a clinical advantage and therefore their use has not become widely accepted.

The beneficial effect of corticosteroids, however, is well accepted in the veterinary profession. Most experimental work strongly supports the ability of corticosteroids to significantly improve the speed and completeness of the functional recovery following spinal cord injury in the dog[15, 29, 32, 55, 68, 88, 124, 162, 182]. Improvement can be noted even when the corticosteroid is given up to 24 hours after the injury.

Corticosteroids appear to limit spinal cord damage by suppressing the development of oedema, enhancing the spinal cord blood flow, inhibiting the inflammatory response and stabilising cell membranes, thereby limiting the development of free oxygen radicals and preventing the release of lysosomal enzymes.

Recommended dose rates for corticosteroid therapy vary widely between institutions. However, it is well accepted that to be effective, the corticosteroid must be administered as soon after trauma as possible and therapeutic blood levels must remain during the period of most activity by inflammatory mediators and free oxygen radicals. This period may last at least two days.

The most commonly reported corticosteroid dose to relieve cerebrospinal oedema is 2 mg/kg of Dexamethasone[184]. Dexamethasone is a synthetic corticosteroid with a potency roughly 30 times that of hydrocortisone. It has no mineralocorticoid effects. Although other corticosteroids are effective at equivalent dose rates, dexamethasone is recommended because of its high potency, low cost and lack of sodium retention effects. A recommended dosage regime is presented over the page.

First 24 hours after injury	Dexamethasone 2 mg/kg given intravenously every eight hours
Second day after injury:	Dexamethasone 1 mg/kg given intravenously, once
Third day after injury:	Dexamethasone 0.5 mg/kg given intravenously, once
Fourth day after injury:	Prednisone 2 mg/kg given per os
Fifth day after injury:	Prednisone 1 mg/kg given per os
Sixth day after injury:	Prednisone 0.5 mg/kg given per os
Seventh day after injury:	No treatment
Eighth day after injury:	Prednisone 0.5 mg/kg given per os
Ninth day after injury:	No treatment
Tenth day after injury:	Prednisone 0.5 mg/kg given per os

The clinically beneficial period of this regime is during the first three days of treatment. The remaining period of gradually reducing dose rate is to minimise the adverse systemic effects which may develop following the use of high doses of corticosteroids.

Corticosteroids affect virtually every cell in the body and the physiological role of endogenous corticosteroids are diverse. The clinical use of corticosteroid is therefore not without potential complications. Digestive system dysfunction, immunosuppression, anaemia, exacerbation of neurological dysfunction, pancreatitis, iatrogenic hyperadrenocorticism, iatrogenic hypoadrenocorticism and direct neuronal toxicity have all been reported following systemic use of corticosteroids. One of the more frequent clinical complications to develop following the use of corticosteroid for spinal trauma is gastrointestinal ulceration and haemorrhage[34, 62, 78, 119, 204, 284]. Ulceration appears to be due to the influence of steroid on increasing the serum gastrin level, increasing the hydrochloric acid secretion in the stomach, decreasing the quantity and quality of gastric mucus and reduction in the gastric epithelial cell turnover.

Ulceration of the colon and duodenum is also commonly recognised in paralysed patients[62, 78, 119, 204, 284]. These ulcers, which may progress to complete perforation of the intestine, are believed to be caused by ischaemic necorsis of the intestinal wall, following chronic vasoconstriction of the splanchic vasculature. Inadequate post-surgical fluid therapy resulting in hypovolaemia and the influence of a pain-induced increase in sympathetic tone are believed to important in the development of intestinal ulceration.

The clinical signs of gastrointestinal ulceration and haemorrhage include vomiting, anorexia or malaena. If this occurs steroid administration should be discontinued immediately. Treatment with H2 agonists (to decrease acid production), intestinal protectants and antibiotics should be

initiated and the patient evaluated carefully for signs indicative of gastric perforation. Diagnostic peritoneal lavage may be required to rule out completely intestinal perforation.

2.2.2 Nursing care

Pre- and post-operative nursing care is vital and probably plays an important role in the speed and completeness of recovery by the animal[34,242,305]. Maintaining a positive attitude in the patient is essential for ensuring success. Some of the more important factors include nutrition and hydration, urination and defecation, decubital ulcers and physical therapy.

Nutrition: The normal nutritive requirements should be met at all times. The energy requirements of the animal should be roughly 25 - 50% more than the basal metabolic rate.

Hydration: Animals on corticosteroid will have a glucocorticoid induced polyuria, and a compensatory polydipsia. Water should therefore be available at all times. If the animal is unable to drink, hydration should be maintained with intravenous fluids.

Urination: It is important to establish whether the patient has voluntary control over urination. This will depend on the location and severity of the disc lesion. Those animals who are urinary continent should be given the opportunity to urinate three to fours times daily to prevent bladder distension and urinary retention. Failure to do this can promote infection.

Incontinent animals will need to have their bladders emptied every four hours. In most cases the bladder can be easily expressed by gentle manual external pressure. When the external sphincter is spastic, bladder expression may be difficult. Catheterisation is required in these cases to empty the bladder safely and completely . The catheter should be placed using proper sterile technique to minimise development of infection. Repeated, aseptic, atraumatic catheterisation is recommended over long term indwelling catheterisation.

Urine should be collected for culture at least once during the hospitalisation period. Further analysis may be indicated if infection, due to retention or repeated catheterisation, is suspected.

Defecation: The ability of the animal to defecate following a disc herniation may be affected either due to direct neurogenic influence, or due the inability of the animal to posture appropriately. Dogs with upper motor neuron injuries generally have an intact anal sphincter. Anal continence is therefore intact and the animals can generally be stimulated to defecate using warm water enemas. Dogs with lower motor neuron injuries will be prone to faecal incontinence

due to the lack of anal tone. These animals should be checked and the perirenal area kept clean and dry to prevent soiling and chaffing.

Decubital ulcers: The non-ambulatory animal may develop decubital ulcers over bony prominences. This is particularly a problem when analgesia persists beyond the disc lesion. Decubital ulcers are prevented by providing good bedding, frequently changing the position of the recumbent animal and keeping him clean, dry and free of urine or faecal soiling.

Physical therapy: Physical therapy is used to increase physical strength, prevent disability by maintaining the normal range of motion in joints and preventing the atrophy of muscles. Encouragement of the use of paralysed limbs, thereby facilitating functional recovery is also important. If properly used, physical therapy may also improve the patient's mental status and hasten the recovery period.

Physical therapy consists of massage, passive and active exercise. The most effective form of active exercise is swimming because this enables the paretic or paralysed hind limbs to perform voluntary movements without their requirement to bear weight, due to the buoyancy provided by the water.

A 2.4 SURGICAL MANAGEMENT

The basis of surgical intervention following acute or chronic intervertebral disc herniation is to relieve excessive spinal cord compression and/or oedema. The surgical management includes decompressive procedures (laminectomy)[21, 56, 100, 153] and the removal of degenerated disc material from within the confines of the intervertebral disc (fenestration)[21, 24, 71, 92, 103, 255, 308]. The indications for decompressive surgery include evidence of spinal cord compression on myelogram, spinal cord oedema sufficient to cause constriction by the neural canal, a lack of response to corticosteroid after 48 hours or a sudden worsening in condition despite appropriate medical treatment. Fenestration will not remove extruded nuclear material from the spinal cord and is therefore only indicated in cases of incomplete extrusion (eg Type II) or as a preventative procedure (prophylaxis).

2.4.1 Decompression (laminectomy)

It is generally accepted that removal of herniated disc material should be the objective of all decompressive surgical procedures. Extruded discs are not completely resorbed by the body and may remain as a fibrotic mass which continues to cause spinal cord compression. Where disc removal is not performed recovery is attributable to the decompressive effects of surgery, the subsequent restoration of spinal cord blood flow and the resolution of hypoxia and oedema. The

remaining disc material continues to cause spinal cord compression and may limit the degree of functional recovery.

Decompression of the thoracolumbar region of the spinal cord is achieved by the variable removal of dorsal aspects of the neural canal. A variety of techniques have been described aimed at increasing the spinal cord compression and improving the removal of disc material[21,56,100,153,254,300]. These include: dorsal laminectomy (deep dorsal, Funquist Type A and Funquist Type B); hemilaminectomy; and foraminotomy. The surgical approach is similar for all these procedures and they differ obstensibly in the amount of bone which is removed. In all cases the length of bone removed, or the laminectomy window, is dependent on the visualisation of dural fat at the cranial and caudal margins. This dictates the extent of spinal cord swelling. At the very least, the laminectomy window should extend at least one vertebral body cranial and caudal to the affected disc.

The **dorsal laminectomy** involves removal of the dorsal spinous process and bilateral articular facets over the involved intervertebral disc. The lamina of the dorsal neural arch is removed with rongeurs or high speed drill with care taken not to injure the spinal cord. The extent of bone removal between the Funkquist Type A, B and deep dorsal are shown in Figure ?. Type B has been further modified to improve retrieval of disc material.

The disadvantages of the dorsal laminectomy include extensive soft tissue trauma, loss of bony protection to considerable portions of the spinal canal, destabilisation of the vertebral column, development of a cosmetic defect due to loss of dorsal spinous processes and the relatively poor retrieval of disc material. Post-operative contraction of scar tissue over the exposed spinal cord can result in neurological deficits. The advantages include good exposure of spinal cord and good decompression, and it also permits a durotomy or myelotomy to be performed for prognostic purposes.

The **hemi-laminectomy** involves removal of the articular facets and lamina on one side of the vertebral column. A myelogram will determine the appropriate side on which to perform the procedure based on the potential lateralisation of the disc material. If necessary, the hemilaminectomy may be converted to a dorsal laminectomy by removal of the dorsal spinous process. Alternatively, a hemilaminectomy may be performed bilaterally, thereby preserving the dorsal spinous process.

The advantages of the hemilaminectomy include reduced tissue dissection, maintenance of spinal stability, no cosmetic defect due to preservation of the dorsal spinous processes, reduced

incidence of laminectomy scar formation. However, unless disc material is extruded in a particularly lateral direction, retrieval may be poor without extensive manipulation of the spinal cord.

A **foraminotomy** is only suitable where the disc material is located in a lateral position. This approach differs to the hemilaminectomy in that it preserves the articular facets, further minimising the post-operative instability of the vertebral column. The reduced amount of bone removal further minimises potential complications.

BIBLIOGRAPHY

1. Adams MA, Dolan P, Hutton WC (1987): Diurnal variations in the stresses on the lumbar spine. *Spine* 12: 130-137

2. Adams MA. Dolan P, Hutton WC (1986): The stages of disc degeneration as revealed by discograms. *Journal of Bone and Joint Surgery* 68B: 36-41

3. Adams MA, Hutton WC (1985): The effect of posture on the lumbar spine. *Journal of Bone and Joint Surgery* 67B: 625-629

4. Adams P, Eyre DR , Muir H (1977): Biochemical aspects of development and ageing of human intervertebral discs. *Rheumatology and Rehabilitation* 16: 22-29

5. Alexander R McN, Goldspink G. (1977): *Mechanics and energetics of animal locomotion* London. Chapman & Hall.

6. Altman RD, Pita JC , Howell DS (1973): Degradation of proteoglycans in human osteoarthritic cartilage. *Arthr Rheum* 16: 179-185

7. Andersson G, Ortengran R, Nachemson A, Elfstrom G (1974): Lumbar disc pressure and myoelectric back muscle activity during sitting. *Scand J Rehab Med* 6: 104-144

8. Atilola MAO, Bailey CS , Morgan JP (1988): Cervical chemonucleolysis in the Dog. A surgical technique. *Vet Surg* 17: 135-140

9. Atilola MAO, Morgan JP , Bailey CS, Miyabayashi T (1988): Canine chemonucleolysis. An experimental radiographic study. *Veterinary Radiology* 29: 168-175

10. Ball MU, McGuire JA , Swaim SF, Hoerlein BF (1982): Patterns of occurrence of disc disease among registered Dachshunds. *JAVMA* 180: 519-522

11. Bayliss MT (1990): Proteoglycan structure and metabolism during maturation and ageing of human articular cartilage. *Biochemical Society Transactions* 18: 799-802

12. Bayliss MT, Johnstone B, O'Brien JP (1988): Proteoglycan synthesis in the human intervertebral disc. Variation with age, region and pathology. *Spine* 13: 972-981

13. Beard HK, Roberts S, O'Brien JP (1981): Immunofluorescent staining for collagen and proteoglycan in normal and scoliotic intervertebral discs. *Journal of Bone and Joint Surgery* 63B: 529-534

14. Berg J (1989): Problems in neurolocalisation. *Problems in Veterinary Medicine* 1: 359-380

15. Berg RJ, Rucker NC (1985): Pathophysiology and medical management of acute spinal cord injury. *Comp Cont Ed* 7: 646-653

16. Bernardi G, Happey F, Naylor A (1957): Mucopolysaccharides from cartilage and nucleus pulposus. *Nature* 180: 1341-1342

17. Bernick S, Walker JM , Paule WJ (1991): Age changes to the annulus fibrosus in human intervertebral discs. *Spine* 16: 520-524

18. Biggart JF (1985): Letter to practitioners, Berkely, California.

19. Biggart JF, Gill GR (1984): Discolysis: An introduction. *Califonia Veterinarian* 10: 10-11, 25

20. Biggart JF, Rodrigo JJ , Scheck M, Caruthers E (1985): Chemonucleolysis of canine disc by purified collagenase: experimental, clinical and toxicity studies. *Abbot Proceedings* 16: 56-65

21. Bitetto WV, Brown NO (1989): Selection of the appropriate surgical approach for intervertebral disc disease. *Problems in Veterinary Medicine* 1: 415-433

22. Bitetto WV, Kapatkin AS (1989): Intraoperative problems associated with intervertebral disc disease. *Problems in Veterinary Medicine* 1: 434-444

23. Bogduk N (1989): *The Innervation of the Disc. In: The Biology of the Disc. Ghosh P (ed).* Boca Raton. CRC Press.

24. Bogduk N, Tynan W, Wilson AS (1981): The nerve supply to the human lumbar intervertebral discs. *J Anatomy* 132: 39-56

25. Bojrab MJ (1983): *Prophylactic thoracolumbar disc fenestration. In: Current techniques in small animal surgery. Second edition. Bojrab MJ (ed)* Philadelphia. Lea & Febiger.

26. Bollet AJ, Nance JL (1966): Biochemical findings in normal and osteoarthritic articular cartilage. II: Chondroitin sulphate concentration and chain length, ash and water content. *J Clin Invest* 45: 1170-1177

27. Bradford DS, Cooper KM , Oegema TR (1983): Chymopapain, chemonucleolysis and nucleus pulposus regeneration. *Journal of Bone and Joint Surgery* 65A: 1220-1231

28. Branemark P, Ekholm R, Lundskog J (1969): Tissue response to chymopapain in different concentrations. *Clinical Orthopaedics and related research* 67: 52-65

29. Braund KG (1981): *Acute spinal cord traumatic compression. In: Pathophysiology in small animal surgery. Bojrab MJ (ed)* Philadelphia. Lea & Febiger.

30. Braund KG (1985): Localising lesions using neurologic syndromes. Part 2: Spinal cord syndromes. *Vet Med* 72: 54-59

31. Braund KG, Ghosh P, Taylor TKF, Larsen LH (1975): Morphological studies of the canine intervertebral disc. The assignment of the beagle to the achondroplastic classification. *Research in Veterinary Science* 19: 167-172

32. Braund KG, Shores A, Brawner WR (1990): The etiology, pathology, and pathophysiology of acute spinal cord trauma. *Vet Med* 77: 683-691

33. Braund KG, Shores A, Brawner WR (1990): Localising spinal cord lesions through recognition of neurologic syndromes. *Vet Med* 77: 692-702

34. Braund KG, Shores A, Brawner WR (1990): Recovering from spinal cord trauma: the rehabilitative steps, complications, and prognosis. *Vet Med* 77: 740-743

35. Brawner WR, Braund KG , Shores A, (1990): Radiographic evaluation of dogs and cats with acute spinal trauma. *Vet Med* 77: 703-723

36. Brickley-Parsons D, Glimcher MJ (1984): Is the chemistry of collagen in intervertebral discs an expression of Wolff's Law? A study of the human lumbar spine. *Spine* 9: 148-163

37. Bromley JW, Gomez JG (1983): Lumbar intervertebral discolysis with collagenase. *Spine* 6: 322-324

38. Bromley JW, Hirst JW , Osman M, Steinlauf P, Gennace RE, Stern H (1980): Collagenase. An experimental study of intervertebral disc dissolution. *Spine* 5: 126-132

39. Brown MD, Tompkins JS (1989): Pain response post-chemonucleolysis or disc excision. *Spine* 14: 321-326

40. Brown NO, Helphrey ML , Prata RG (1977): Thoracolumbar disc disease in the dog: A retrospective analysis of 187 cases. *JAAHA* 13: 665-672

41. Brown T, Hansen RJ , Yorra AJ (1957): Some mechanical tests on the lumbosacral spine. *Journal of Bone and Joint Surgery* 39A: 1135-1164

42. Buckwalter JA, Cooper RR , Maynard JA (1976): Elastic fibres in human intervertebral discs. *Journal of Bone and Joint Surgery* 58A: 73-76

43. Buckwalter JA, Maynard JA , Cooper RR (1979): Banded structures in human nucleus pulposus. *Clinical Orthopaedics and related research* 139: 259-266

44. Buckwalter JA, Maynard JA , Cooper RR, Gaddis S (1978): Sheathing of collagen fibrils in human intervertebral discs. *J Anatomy* 125: 615-618

45. Buckwalter JA, Pedrini-Mille A, Pedrini V, Tudisco C (1985): Proteoglycans of human infant intervertebral disc. *Journal of Bone and Joint Surgery* 67A: 284-294

46. Buckwalter JA, Pedrini-Mille A, Pedrini V, Tudisco C (1985): Proteoglycans of human infant intervertebral disc. *Journal of Bone and Joint Surgery* 67A: 284-294

47. Burk RL (1989): Problems in the radiographic interpretation of intervertebral disc disease in the dog. *Problems in Veterinary Medicine* 1: 381-401

48. Burke MJ, Banks WJ , Nelson AW, Seim HB (1986): Histochemical study of the annulus fibrosus in normal canine caudal cervical intervertebral discs. *Research in Veterinary Science* 40: 18-23

49. Bushell GR, Ghosh P, Taylor TKF, Akeson WH (1977): Proteoglycan chemistry of the intervertebral discs. *Clinical Orthopaedics and related research* 129: 115-123

50. Butler D, Trafimow JH , Andersson GBJ, McNeill TW, Huckman MS (1990): Discs degenerate before facets. *Spine* 15: 111-113

51. Capanna AH, Williams RW , Austin DC, Darmondy WR, Thomas LM (1981): Lumbar discectomy - percentage of disc removal and detection of anterior annulus perforation. *Spine* 6: 610-614

52. Cassidy JJ, Hiltner A, Baer E (1989): Hierarchical structure of the intervertebral disc. *Connective Tissue Research* 23: 75-88

53. Chrisman CL (1982): *Problems in small animal neurology* Philadelphia. Lea & Febiger.

54. Cole T, Melrose J, Ghosh P (1988): Cysteine proteinase inhibitors of the canine intervertebral disc. *Biochimica et Biophysica Acta* 952: 201-207

55. Colter S, Rucker NC (1988): Acute injury to the central nervous system. *Vet Clinics of North America* 18: 545-563

56. Colter SB (1978): Fenestration, Decompression or both? *Vet Clinics of Nth America (Small Animal)* 8: 379-383

57. Coventry MB (1969): Anatomy of the intervertebral disc. *Clinical Orthopaedics and related research* 67: 9-15

58. Coventry MB, Ghormley RK , Kernohan JW (1945): The intervertebral disc: It's microscopic anatomy and physiology. Part II: Changes in the IVD concomitant with age. *Journal of Bone and Joint Surgery* 27: 233-474

59. Coventry MB, Ghormley RK , Kernohan JW (1945): The intervertebral disc. Its microscopic anatomy and pathology. Part III: Pathological changes in the IVD. *Journal of Bone and Joint Surgery* 27A: 460-464

60. Coventry MB, Ghormley RK , Kernohan JW (1945): The intervertebral disc: Part I. Its microscopic anatomy and pathology. *Journal of Bone and Joint Surgery* 27: 105-112

61. Cozad J (1984): Chemonucleolysis: An alternative to laminectomy. *Point of View* 21: 4-6

62. Crawford LM, Wilson RC (1982): Malaena associated with dexamethasone therapy in the dog. *J Small Anim Pract* 23: 91-97

63. Crock HV, Goldwasser M. (1984): Anatomic studies of the circulation in the region of the vertebral end-plate of adult greyhounds. *Spine* 9: 702-706

64. Cusick JF, Ho KC , Schamberg JF (1987): Subarachnoid haemorrhage following chymopapain chemonucleolysis: a case report. *J Neurosurg* 66: 775-778

65. Dabezies EJ, Langford K, MOrris J, Shields CB, Wilkinson HA (1988): Safety and efficacy of chymopapain (Discase) in the treatment of sciatica due to herniated nucleus pulposus. Results of a randomised, double-blind study. *Spine* 13: 561-565

66. Dando PM, Morton DB , Buttle DJ, Barrett AJ (1988): Quantitative assessment of human proteinases as agents for chemonucleolysis. *Spine* 13: 188-192

67. Davies AS (1982): Quadrupedal Mechanics. Anatomic principles of the musculoskeletal system. Dissertation. New Zealand. Massey University.

68. de la Toore JC (1981): Spinal Cord Injury. A Review of Basic and Applied research. *Spine* 6: 315-335

69. deBurge A, Rocolle J, Benoist M (1985): Surgical findings and results after surgery after failure of chemonucleolysis. *Spine* 10: 812-815

70. deLahunta A (1983): *Veterinary Neuroanatomy and Clinical Neurology. 2nd Edition* Philadelphia. WB Saunders.

71. Denny HR (1978): The lateral fenestration of thoracolumbar disc protrusions in the dog. *Journal of Small Animal Practice* 19: 256-266

72. dePalma AF, Gillespy T. (1962): Long-term results of herniated nucleus pulposus treated by excision of disc only. *Clinical Orthopaedics and related research* 22: 139-144

73. Dias JJ, Grindulis KA , Finlay DBL, Nichol FE (1988): Letter: Chemonucleolysis of calcified prolapsed intervertebral discs. *British Journal of Rheumatology* 27: 493-500

74. Donohue PJ, Jahnke MR , Blaha JD, Caterson B (1988): Characterisation of link protein(s) from human intervertebral disc tissues. *Biochem J* 251: 739-747

75. Editorial (1986): Chymopapain and the intervertebral disc. *Lancet* Oct 11: 843-845

76. Ehrlich MG, Mankin HJ , Jones H, Grossman A, Crispen C, Ancona D (1975): Biochemical confirmation of an experimental osteoarthritis model. *JBJS* 57A: 392-396

77. Emes JH, Pearce RH (1975): The proteoglycans of the human intervertebral disc. *Biochem J* 145: 549-556

78. Epstein N, Hood DC , Ransohoff J (1981): Gastrointestinal bleeding in patients with spinal cord trauma. Effects of steroids, cimetidine and mini-dose heparin. *J Neurosurg* 54: 16-29

79. Eurell JC, Brown MD , Ramos M (1990): The effects of chondroitinase ABC on the rabbit IVD. *Clinical Orthopaedics and related research* 256: 238-243

80. Evans HE, Christensen GC (1979): *Miller's Anatomy of the Dog. Second Edition* Philadelphia. WB Saunders.

81. Evans JM, Barbenel JC (1975): Structural and mechanical properties of tendon in relation to function. *Equine Vet Journal* 7: 1-8

82. Eyring EJ (1969): The biochemistry and physiology of the intervertebral disc. *Clinical Orthopaedics and related research* 67: 16-25

83. Falconer MA, McGeorge M, Begg AC (1948): Surgery of lumbar intervertebral disc protrusions. A study of principles and results based upon one hundred consecutive cases submitted to operation. *British Journal of Surgery* 35: 15-249

84. Farfan HF (1977): A reorientation in the surgical approach to degenerative lumbar intervertebral disc disease. *Orthopaedic Clinics of North America* 8: 9-21

85. Farfan HF (1985): The use of mechanical aetiology to determine the efficacy of active intervention in single joint lumbar intervertebral joint problems. Surgery and chemonucleolysis compared: a prospective study. *Spine* 10: 350-358

86. Farfan HF, Cossette JW , Robertson GH, Wells RV, Kraus H (1970): The effects of torsion on the lumbar intervertebral joints. The role of torsion in the production of disc degeneration. *Journal of Bone and Joint Surgery* 52A: 468-497

87. Feeney DA, Oliver JE (1980): Blunt spinal trauma in the dog and cat: Insight into radiographic lesions. *JAAHA* 16: 885-890

88. Feeney DA, Oliver JE (1980): Blunt spinal trauma in the dog and cat: Neurologic, radiological, and therapeutic correlations. *JAAHA* 16: 664-668

89. Feinberg J, Boachie-Adjei O, Bullough PG, Boskey AL (1990): The distribution of calcific deposits in IVDs of the lumbosacral spine. *Clinical Orthopaedics and related research* 254:

90. Fingeroth JM (1989): Fenestration. Pros and Cons. *Problems in Veterinary Medicine* 1: 445-466

91. Fisher RG, Bromley JW , Becker GL, Brown M, Mooney V (1986): Surgical experience following intervertebral discolysis with collagenase. *J Neurosurg* 64: 613-616

92. Flo GL, Brinker WO (1975): Lateral fenestration of thoracolumbar discs. *JAAHA* 11: 619-626

93. Flynn J, Rudert MJ , Olson E, Baratz M, Hanley E (1990): The effects of freezing or freeze-drying on the biomechanical properties of the canine intervertebral disc. *Spine* 15: 567-570

94. Forsythe WB, Ghoshal NG (1984): Innervation of the canine thoracolumbar vertebral column. *The Anatomical Record* 208: 57-63

95. Friberg S (1948): Anatomical studies on lumbar disc degeneration. *Acta Orthop Scandinavica* 17: 224-230

96. Friberg S, Hirsch C. (1949): Anatomical and clinical studies on lumbar disc degeneration. *Acta Orthop Scandinavica* 19: 222-242

97. Fry TR, Eurell JC , Johnson AL, Brown MD, Losonsky JM, Schaeffer DJ (1991): Radiographic and histologic effects of chondroitinase ABC on normal canine lumbar IVD. *Spine* 16: 816-819

98. Fry TR, Johnson AL (1991): Chemonucleolysis for treatment of intervertebral disc disease. *JAVMA* 199: 622-627

99. Frymoyer JW (1981): The role of spine fusion. Question 3. *Spine* 6: 284-290

100. Funkquist B (1962): Decompressive laminectomy for cervical disk protrusion in the dog. *Acta Vet Scandinavica* 3: 88-101

101. Funkquist B (1978): Investigation of the therapeutic and prophylactic effects of disc evacuation in cases of thoracolumbar herniated discs in dogs. *Acat Vet Scandinavica* 19: 441-457

102. Gage ED (1975): Incidence of clinical disc disease in the dog. *JAAHA* 11: 135-138

103. Gage ED (1975): Modifications in dorsolateral hemilaminectomy and disc fenestration in the dog. *JAAHA* 11: 407-411

104. Galante JO (1967): Tensile properties of the human lumbar annulus fibrosus. *Acta Orthop Scandinavica*

105. Gambaryan PP (1974): *How animals run. Anatomy and adaptations.* New York. Wiley.

106. Garvin PJ (1974): Toxicity of collagenase. The relation to enzyme therapy of disc herniation. *Clinical Orthopaedics and related research* 101: 286-291

107. Garvin PJ, Jennings RB , Stern IJ (1977): Enzymatic digestion of the nucleus pulposus: A review of experimental studies with chymopapain. *Orthopaedic Clinics of North America* 8: 27-35

108. Garvin PJ, Jennings RB (1973): Long-term effects of chymopapain on intervertebral discs of dogs. *Clinical Orthopaedics and related research* 92: 281-295

109. Gertzbein S, Holtby R, Tile M, Kapsouri A, Chan K, Cruickshank B (1984): Determination of a locus of instantaneous centres of rotation of the lumbar disc by moire fringes: A new technique. *Spine* 9: 409-413

110. Ghosh P, Bushell GR , Taylor TKF, Akeson WH (1977): Collagens, Elastin and non-collagenous protein of the intervertebral disc. *Clinical Orthopaedics and related research* 129: 124-129

111. Ghosh P, Taylor TKF , Braund KG, Larsen LH (1976): The collagenous and non-collagenous protein of the canine intervertebral disc and their variation with age, spinal level and breed. *Gerontology* 22: 124-134

112. Ghosh P, Taylor TKF , Braund KG, Larsen LH (1976): A comparative chemical and histochemical study of the chondrodystrophoid and non-chondrodystrophoid canine intervertebral disc. *Vet Pathol* 13: 414-427

113. Ghosh P, Taylor TKF , Braund KG (1977): Variation of the GAG's of the intervertebral disc with ageing. II: the non-chondrodystrophic breed. *Gerontology* 23: 99-109

114. Ghosh P, Taylor TKF , Braund KG (1977): The variation of the GAG's of the canine intervertebral disc with ageing. I: Chondrodystrophoid breed. *Gerontology* 23: 87-98

115. Gibson MJ, Buckley J, Mulholland RC, Worthington BS (1986): The changes in the intervertebral disc after chemonucleolysis demonstrated by magnetic resonance imaging. *Journal of Bone and Joint Surgery* 68B: 719-723

116. Gibson MJ, Karpinski MRK , Slack RCB, Cowlishaw WA, Webb JK (1987): The penetrattion of antibiotics into the normal intervertebral disc. *Journal of Bone and Joint Surgery* 69B: 784-786

117. Gillett NA, Gerlach R, Cassidy JJ, Brown SA (1988): Age-related changes in the beagle spine. *Acta Orthop Scandinavica* 59: 503-507

118. Goggin JE, Ann-si Li , Franti CE (1970): Canine intervertebral disc disease. Charcterisation by age, sex. breed and anatomic site of involvement. *Am J Vet Res* 31: 1687-1692

119. Gore RM, Mintzer RA , Calenoff L (1981): Gastrointestinal complications of spinal cord injury. *Spine* 6: 538-544

120. Gower WE, Pedrini V. (1969): Age-related variations in protein polysaccharides from human nucleus pulposus, annulus fibrosus and costal cartilage. *Journal of Bone and Joint Surgery* 51A: 1154-1169

121. Graham CE (1975): Chemonucleolysis. A preliminary report on a double blind study comparing chemonucleolyis and intradiscal administration of hydrocortisone in the treatment of backache and sciatica. *Orthopaedic Clinics of North America* 6: 259-263

122. Grammer LC, Schafer M, Bernstein D (1987): Prevention of chymopapain anaphylaxis by screening chemonucleolysis candidates with cutaneous chymopapain testing. A preliminary report. *Clinical Orthopaedics and related research* 221: 202-206

123. Hall BB, McCulloch JA (1983): Anaphylactic reactions following the intra-discal injection of chympopapain under local anaesthesia. *Journal of Bone and Joint Surgery* 65A: 1215-1219

124. Hall ED, Baker T. (1979): Acute effects of methylprednisolone sodium succinate on spinal reflexes. *Experimental Neurology* 63: 476-484

125. Hamptom D, Laron G, McCarron R, Franks D (1989): Healing potential of the annulus fibrosus. *Spine* 14: 398-401

126. Hansen HJ (1952): A pathologic-anatomical study on disc degeneration in the dog. *Acta Orthop Scandinavica* Suppl 11:

127. Hansen HJ (1966): *Pathogenesis of disc degeneration and rupture. In: Pettit GD (ed) Intervertebral disc protrusion in the dog.* New York. Appleton-Century-Crofts.

128. Happey F, Johnson AG , Naylor A, Turner RL (1964): Preliminary observations concerning the fine structure of the intervertebral disc. *Journal of Bone and Joint Surgery* 46B: 563-567

129. Hardingham TE, Muir H. (1972): The specific interaction of hyaluronic acid with cartilage proteoglycans. *Bioch Biophys Acta* 279: 401-405

130. Hardingham TE, Muir H. (1975): Structure and stability of proteoglycan aggregation. *Ann Rheum Dis* 34: 26-28

131. Harris RI, MacNab I. (1954): Structural changes in the lumbar intervertebral discs. Their relationship to low back pain and sciatica. *Journal of Bone and Joint Surgery* 36B: 304-322

132. Hascall VC, Heinegard D. (1974): Aggregation of cartilage proteoglycans. I: The role of hyaluronic acid. *J Biol Chem* 249: 4232-4241

133. Henderson N, Stanescu V, Cauchoix J. (1991): Nucleolysis of the rabbit intervertebral disc using Chondroitinase ABC. *Spine* 16: 203-208

134. Henderson N, Stanescu V, Cauhoix J (1991): Nucleolysis of the rabbit intervertebral disc using chondroitinase ABC. *Spine* 16: 203-208

135. Hendry NGC (1958): The hydration of the nucleus pulposus and its relation to intervertebral disc derangement. *Journal of Bone and Joint Surgery* 40B: 132-143

136. Henry WB (1975): Dorsal decompressive laminectomy in the treatment of thoracolumbar disc disease. *JAAHA* 11: 627-635

137. Herkowitz HN (1991): Current status of percutaneous discetomy and chmonucleolysis. *Orthopaedic Clinics of North America* 22: 327-332

138. Hickey DS, Hukins DWL (1981): Collagen fibril diameters and elastic fibres in the annulus fibrosus of human fetal intervertebral disc. *J Anatomy* 133: 351-357

139. Hildebrand M (1974): *Analysis of vertebrate structure.* New York. Wiley.

140. Hirano N, Tsuji H, Ohshima H, Kitano S, Sano A (1988): Analysis of rabbit intervertebral disc physiology based on water metabolism. I: Factors influencing metabolism of the normal IVDs. *Spine* 13: 1291-1302

141. Hirsch C (1955): The reaction of the intervertebral disc to compressive forces. *Journal of Bone and Joint Surgery* 37A: 1188-1196

142. Hirsch C, Nachemson A. (1954): New observations on the mechanical behaviour of lumbar discs. *Acta Orthop Scandinavica* 23: 254-283

143. Hirsch C, Nachemson A. (1963): The reliability of lumbar disc surgery. *Clinical Orthopaedics and related research* 29: 189-195

144. Hirsch C, Schajowicz F. (1952): Structural changes in the lumbar annulus fibrosus. *Acta Orthop Scandinavica* 22: 184-231

145. Hoerlein BF (1953): Intervertebral disc protrusions in the dog. I: Incidence and pathological lesions. *American Journal of Vet Research* 14: 260-269

146: Hoerlein BF (1953): Intervertebral disc protrusions in the dog. Part II: Symptomatology and clinical diagnosis. *Am J Vet Res* 14: 270-274

147. Hoerlein BF (1953): Intervertebral disc protrusions in the dog. Part III: Radiological diagnosis. *Am J Vet Res* 14: 275-283

148. Hoerlein BF (1978): The status of the various intervertebral disc surgeries for the dog in 1978. *JAAHA* 14: 563-570

149. Hoerlein B.F. (1978): *Canine Neurology. Diagnosis and Treatment. 3rd Edition.* Philadelphia. WB Saunders.

150. Hoerlien BF, Redding RW , Hoff EJ (1983): Evaluation of dexamethasone, DMSO, mannitol and solcoseryl in acute spinal cord injuy. *JAAHA* 19: 216

151. Holm S, Selstam G, Nachemson A (1982): Carbohydrate metabolism and concentration profiles of solutes in the canine lumbar intervertebral disc. *Acta Physiol Scand* 145:

152. Holmberg DL, Palmer NC , Venpelt D, Willan AR (1990): A comparison of manual and power-assisted thoracolumbar disc fenestration in dogs. *Vet Surg* 19: 323-327

153. Horne TR, Powers RD , Swaim SF (1977): Dorsal laminectomy techniques in the dog. *JAVMA* 171: 742-749

154. Horton WG (1958): Further observations on the elastic mechanism of the intervertebral disc. *Journal of Bone and Joint Surgery* 40B: 552-557

155. Hosgood G (1992): Wound complications following thoracolumbar laminectomy in the dog. A retrospective study of 264 procedures. *JAAHA* 28: 47-52

156. Humzah MD, Soames RW (1988): Human intervertebral disc. Structure and Function. *The Anatomical Record* 220: 337-356

157. Hwang WS, Tock EPC , Tan KL, Tan LKA (1979): The pathology of cartilage in chondrodysplasias. *J Pathology* 127: 11-18

158. Inman VT, Saunders CM (1947): Anatomicophysiological aspects of injuries to the intervertebral disc. *Journal of Bone and Joint Surgery* 29: 461-534

159. Inoue H (1981): Three-dimensional architecture of lumbar intervertebral discs. *Spine* 6: 139-146

160. Jackson HC, Winkelmann RK , Bickel WH (1966): Nerve endings in the human lumbar spinal column and related structures. *Journal of Bone and Joint Surgery* 48B: 1272-1281

161. Jahnke MR, McDevitt CA (1988): Proteoglycans of the human intervertebral disc. Electrophoretic heterogeneity of the aggregating proteoglycans of the nucleus pulposus. *Biochem J* 251: 347-356

162. Janssen L, Hansebout RR (1989): Pathogenesis of spinal cord injury and newer treatments. A review. *Spine* 14: 23-32

163. Javid MJ (1988): Signs and symptoms after chemonucleolysis. A detailed evaluation of 214 worker's compensation and noncompensation patients. *Spine* 13: 1428-1437

164. Johnson EF, Chetty K, Moore IM, Stewart A, Jones W (1982): The distribution and arrangement of elastic fibres in the intervertebral disc of the adult human. *J Anat* 135: 301-309

165. Johnson EF, Mitchell R, Berryman H, Cardaso S, Ueal O, Patterson D (1986): Secretory cells in the nucleus pulposus of the adult human intervertebral disc. *Acta Anat* 125: 161-164

166. Jonck LM (1961): The mechanical disturbances resulting from lumbar disc narrowing. *Journal of Bone and Joint Surgery* 43B: 362-375

167. Jones IL, Klamfeldt A, Sandstrom T (1982): The effect of continuous mechnical pressure upon the turnover of articular cartilage proteoglycans *in vitro*. *Clinical Orthopaedics and related research 165: 283-289*

168. Kahmann RD, Butterman GR , Lewis JL, Bradford DS (1990): Facet loads in the canine lumbar spine before and after disc alteration. *Spine* 15: 971-978

169. Kapandji LA (1974): *The Physiology of the Joints Vol. 3* Edinburgh. Churchill-Livingston.

170. Keller TS, Holm SH , Hansson TH, Spengler DM (1990): The dependence of intervertebral disc mechanical properties on physiologic properties. *Spine* 15: 751-761

171. Keller TS, Spengler DM , Hansson TH (1987): Mechnical behaviour of the human lumbar spine. I Creep analysis during static compressive loading. *J Orthop Res* 5: 467-478

172. Kewalramani LS (1979): Neurogenic gastroduodenal ulceration and bleeding associated with spinal cord injuries. *Journal of Trauma* 19: 259-265

173. Keyes DC, Compere EL (1932): The normal and pathological physiology of the nucleus pulposus of the intervertebral disc. An anatomical, clinical and experimental study. *Journal of Bone and Joint Surgery* 14: 897-938

174. Kim YE, Goel VK , Weinstein JN, Lim T (1991): Effect of disc degeneration at one level on the adjacent level in axial mode. *Spine* 16: 331-335

175. King AS, Smith RN (1960): Disc Protrusions in the cat. Distribution of dorsal protrusions along the veterbral column. *Vet Record* 72: 335-380

176. King AS, Smith RN (1960): Disc Protrusions in the Cat. Age incidence of dorsal protrusions. *Vet Record* 72: 381-382

177. King AS, Smith RN , Kon VM (1958): Protrusion of the intervertebral disc in the cat. *Vet Record* 70: 509-515

178. Kitano S, Tsuji H, Hirano N, Sano A, Terahata N (1989): Water, fixed charge density, protein contents, and lysine incorporation into protein in chymopapain-digested intervertebral disc of rabbit. *Spine* 14: 1226-1233

179. Knapp DW, Pope ER , Hewett JE, Bojrab MJ (1990): A retrospective study of thoracolumbar disc fenestration in dogs using a ventral approach: 160 cases (1976 to 1986). *JAAHA* 26: 543-549

180. Knutsson F (1944): The instability associated with disc degeneration in the lumbar spine. *Acta Radiology* 25: 593-609

181. Koreska J, Robertson D, Mills RH, Gibson DA, Albisser AM (1977): Biomechanics of the lumbar spine and its clinical significance. *Orthopaedic Clinics of North America* 8: 121-133

182. Koyanagi I, Iwasaki Y, Isu T, Akino M, Abe H (1989): Significance of spinal cord swelling in the prognosis of acute cervical spinal cord injury. *Paraplegia* 27: 190-197

183. Kulak RF, Belytschko TB , Schultz AB, Galanate JO (1976): Non-linear behaviour of the human intervertebral disc under axial load. *J Biomech* 9: 377-386

184. LeCouteur R, Child G. (1989): *Diseases of the spinal cord. In: Textbook of Veterinary Internal Medicine. Ettinger SJ (ed)* Philadelphia. WB Saunders.

185. Lee CK (1988): Accelerated degeneration of the segment adjacent to a lumber fusion. *Spine* 13: 375-377

186. Levine SH, Caywood DD (1984): Recurrence of neurological deficits in dogs treated for thoracolumbar disc disease. *JAAHA* 20: 889-894

187. Lin HS, Liu YK , Adams KH (1978): Mechanical response of the lumbar intervertebral joint under physiological (complex) loading. *Journal of Bone and Joint Surgery* 60A: 41-50

188. Lipson SJ (1988): Metaplastic proliferative fibrocartilage as an alternative concept to herniated intervertebral disc. *Spine* 13: 1055-1060

189. Lipson SJ, Muir H. (1981): Experimental intervertebral disc degeneration. Morphologic and proteoglycan changes over time. *Arthritis and Rheumatism* 24: 12-21

190. Lipson SJ, Muir H. (1981): Proteoglycans in experimental intervertebral disc degeneration. *Spine* 6: 194-210

191. Lipson SJ, Muir H. (1980): Vertebral osteophyte formation in experimental disc degeneration. Morphologic and proteoglycan changes over time. *Arthritis and Rheumatism* 23: 319-324

192. Littlewood JD, Herrtage ME , Palmer AC (1984): Intervertebral disc protrusion in the cat. *J Small Anim Pract* 25: 119-127

193. Machunas RJ, Onofrio BM (1986): The long-term results of chymopapain chemonucleolysis for lumbar disc disease. Ten-year follow-up results in 268 patients injected at the Mayo clinic. *J Neurosurg* 65: 1-8

194. Maly P, Almen T, Golman K, Olivecrona H (1984): Excitative effects of anaesthetised rabbits from subarachnoidally injected iso- and hyper-osmolar solutions of iohexol and metrizamide. *Neuroradiology 26: 131-136*

195. Mankin HJ (1971): Biochemical and metabolic aspects of osteoarthritis. *Orthopaedic Clinics of North America* 2: 19-31

196. Mankin HJ (1974): The reaction of articular cartilage to injury and osteoarthritis. *New England Jounral of Medicine* 291: 1285-1292

197. Marchand F, Ahmed AM (1990): Investigation of the laminate structure of lumbar disc annulus fibrosus. *Spine* 15: 402-410

198. Markolf KL, Morris JM (1974): The structural components of the intervertebral disc. *Journal of Bone and Joint Surgery* 56A: 675-687

199. Maroudas A, Stockwell RA , Nachemson A, Urban J (1975): Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose *in vitro*. *J Anat* 120: 113-130

200. Maynard Smith J, Savage RJG (1956): Some locomotory adaptation in mammals. *Linnean Society Journal* 42: 603-622

201. McCulloch JA (1977): Chemonucleolysis. *Journal of Bone and Joint Surgery* 59B: 45-52

202. McNab I (1977): *Backache. 1st Edition.* Baltimore. Williams and Wilkins.

203. Miyabayashi T, Lord PF , Dubielzig RR, Biller DS, Manley PA (1992): Chemonucleolysis with collagenase. A radiographic and pathologic study in dogs. *Vet Surg* 21: 189-194

204. Moore RW, Withrow SJ (1982): Gastrointestinal haemorrhage and pancreatitis associated with intervertebral disc disease in the dog. *JAVMA* 180: 1443-1447

205. Morgan JP, Miyabayashi T. (1988): Degenerative changes in the vertebral column of the dog. A review of radiographic findings. *Olsson Seminar Proceedings* 29: 72-77

206. Morris JM, Lucas DB , Bresler B (1961): Role of the trunk in stability of the spine. *Journal of Bone and Joint Surgery* 43A: 327-351

207. Nachemson A (1963): The influence of spinal movements on the lumbar intradiscal prssure and on the tensile stresses of the annulus fibrosus. *Acta Orthop Scandinavica* 33: 183-207

208. Nachemson A (1966): The load of lumbar discs in different positions of the body. *Clin Orthop* 45: 107-122

209. Nachemson A (1960): Lumbar intradiscal pressure. Experimental studies on post-mortem material. *Acta Orthop Scandinavica* 43:

210. Nachemson A, Lewin T, Maroudas A, Freeman MAR (1970): *In vitro* diffusion of dye through the end-plates and the annulus fibrosus of human lumbar intervertebral discs. *Acta Orthop Scandinavica* 41: 589-607

211. Nachemson A, Morris JM (1964): *In vivo* measurements of intradiscal pressure. Discometry, a method for the determination of pressure in the lower lumbar discs. *Journal of Bone and Joint Surgery* 46A: 1077-1091

212. Naylor A (1962): The biophysical and biochemical aspects of intervertebral disc herniation and degeneration. *Ann R Coll Surg Engl* 31: 91-114

213. Naylor A, Happey F, MacRae T (1954): The collagenous changes in the intervertebral disc with age. *British Medical Journal* ii: 570

214. Naylor A, Horton WG (1955): The hydrophilic properties of the nucleus pulposus of the intervertebral disc. *Rheumatism* 11: 32-35

215. Nitobe T, Harata S, Okamoto Y, Nakamura T, Endo M (1988): Degradation and biosynthesis of proteoglycans in the nucleus pulposus of canine intervertebral disc after chymopapain treatment. *Spine* 13: 1332-1339

216. Nordby EJ (1985): A comparison of discectomy and chemonucleolysis. *Clinical Orthopaedica and related research* 200: 279-283

217. Nordby EJ, Lucas GL (1973): A comparative analysis of lumbar disc disease treated by laminectomy or chemonucleolysis. *Clinical Orthopaedics and related research* 90: 119-129

218. Oda J, Tanaka H, Tsuzuki N (1988): Intervertebral disc changes with ageing of human cervical vertebra. From the neonate to the eighties. *Spine* 13: 1205-1211

219. Ohshima H, Tsuji H, Hirano N, Ishihara H, Katoh Y, Yamada H (1989): Water diffusion pathway, swelling pressure, and biomechnical properties of the intervertebral disc during compressive loading. *Spine* 14: 1234-1244

220. Olmarker K, Rydevik B, Dahlin LB (1987): Effects of epidural and intrathecal application of collagenase in the lumbar spine. An experimental study in rabbits. *Spine* 12: 477-482

221. Olsson E (1951): On disc protrusion in the Dog (*enchondrosis intervertebralis*). A study with special reference to roentgen diagnosis and to the value of disc fenestration. *Acta Orthop Scandinavica* 21:

222. Olsson SE (1958): The dynamic factor in spinal cord compression. *J Neurosurg* 15: 308

223. Osti OL, Vernon-Roberts B, Fraser RD (1990): Annulus tears and intervertebral disc degeneration. An experimental study using animal models. *Spine* 15: 762-767

224. Paastsama S, Rissanen P, Rokkanen P (1969): Effect of estrodiol, testosterone, cortisone acetate, somatotropin, thyrotropin and parathyroid hormone on the lumbar intervertebral disc in growing dogs. *J Sm Anim Pract* 10: 351-354

225. Panjabi M, Brown M, Lindahl S, Irstam L, Hermans M (1988): Intrinsic disc pressure as a measure of integrity of the lumbar spine. *Spine* 13: 913-917

226. Panjabi M, Goel V, Takata K (1982): Physiological strains in lumbar spinal ligaments. An *in vitro* biomechanical study. *Spine* 7: 192-203

227. Parker AJ (1975): Durotomy and saline perfusion in spinal cord trauma. *JAAHA* 11: 412-413

228. Pearce RH, Grimmer BJ , Adams ME (1987): Degeneration and chemical composition of the human lumbar intervertebral disc. *Journal of Orthopaedic Research* 5: 198-205

229. Pedrini VA, Pedrini-Milne A. (1977): Aggregation of proteoglycans of human intervertebral disc. *Orthop Trans* 1: 201

230. Pennington JB, McCarron RF , Laros GS (1988): Identification of IgG in the canine intervertebral disc. *Spine* 13: 909-911

231. Petter CK (June 18, 1932.): Methods of measuring the pressure of the intervertebral disc. *Proceedings of the Annual Meeting of the American Orthopaedic Association* 365-368

232. Prata RG (1981): Neurosurgical treatment of thoracolumbar discs. The rationale and value of concomitant disc removal. *JAAHA* 17: 17-26

233. Priester WA (1976): Canine intervertebral disc disease. Occurrence by age, breed and sex among 117 cases. *Theriogenology* 6: 293-301

234. Pritzker KPH (1977): Aging and degeneration in the lumbar IVD. *Orthopaedic Clinics of North America* 8: 65-77

235. Puglisi TA, Green RW , Hall CL, Read WK, Green RA, Tangner CH, Mann FA, Hobson HP (1986): Comparison of metrizaminde and iohexol for cisternal myelographic examination of dogs. *Am J Vet Res* 47: 1863-1869

236. Riser WH, Haskins ME , Jezyk PF, Patterson DF (1980): Pseudoachondroplastic dysplasia in miniature poodles. Clinical, radiographic and pathologic features. *JAVMA* 176: 335-342

237. Romatowski J (1986): Spondylosis deformans in the dog. *Comp Cont Ed* 8: 531-534

238. Rucker NC, Lumb WV , Scott RJ (1981): Combined pharmacologic and surgical treatments for acute spinal cord trauma. *Am J Vet Res* 42: 1138-1142

239. Rydevik B, Branemark P, Nordberg C (1976): Effects of chymopapain on nerve tissues. *Spine* 1: 137

240. Saunders EC (1964): Treatment of the canine intervertebral disc syndrome with chymopapain. *JAVMA* 145: 893-896

241. Scapinelli R, Little K. (1970): Observations on the mechanically induced differentiation of cartilage from fibrous connective tissue. *J Path* 101: 85-91

242. Scavelli TD, Schoen A. (1989): Problems and complications associated with the non-surgical management of intervertebral disc disease. *Problems in Veterinary Medicine* 1: 402-414

243. Schneiderman R, Keret D, Maroudas A (1986): Effects of mechanical and osmotic pressure on the rate of glycosaminoglycan synthesis in the human adult femoral head cartilage. An *in vitro* study. *Journal of Orthopaedic Research* 4: 393-408

244. Schulte K, Clark CR , Goel VK (1989): Kinematics of the cervical spine following discectomy and stabilisation. *Spine* 14: 1116-1121

245. Scott JE (1990): Proteoglycan-collagen interactions and sub-fibrillar structure in collagen fibrils: implications in the development and remodelling of connective tissues. *Biochem Soc Trans* 18: 489-490

246. Scott JE (1990): Proteoglycan-collagen interactions and subfibrillar structure in collagen fibrils. Implications in the development and ageing of connective tissues. *J Anat* 169: 23-35

247. Scott JE (1988): Proteoglycan-fibrillar collagen interactions. *Biochem J* 252: 313-323

248. Scott JE, Bosworth TR (1990): A comparative biochemical and ultrastructural study of proteoglycan-collagen interactions in corneal stroma. *Biochem J* 270: 491-497

249. Scott JE, Haigh M. (1988): Keratan sulphate and the ultrastructure of cornea and cartilage: a 'stand-in' for chondroitin sulphate in conditions of oxygen lack? *J Anat* 158: 95-108

250. Shah JS, Hampson WGJ , Jayson MIV (1978): The distribution of surface strain in the cadaveric lumbar spine. *Journal of Bone and Joint Surgery* 60B: 246-251

251. Shepperd JAN, James SE , Leach AB (1989): Percutaneous disc surgery. *Clinical Orthopaedics and related research* 238: 43-49

252. Shields CB, Arnin EJ (1985): Update on chymopapain. *Neurol Clin* 3: 395-403

253. Shires PK, Waldron DR , Hedlund CS, Blass CE, Massoudi L (1991): A biomechanical study of rotational instability in unaltered and surgically altered canine thoracolumbar vertebral motion units. *Progress in Veterinary Neurology* 2: 6-14

254. Shores A (1981): Intervertebral disc syndrome in the dog. Part 1: Pathophysiology and management. *Comp Cont Ed* 3: 639

255. Shores A (1982): Intervertebral disc syndrome in the dog. Part III: Thoracolumbar disc surgery. *Comp Cont Ed* 4: 24-31

256. Shores A (1985): *Intervertebral Disc Disease. In: Testbook of Small Animal Orthopaedics. Newton CD, Nunamaker DN (eds)* Philadelphia. JP Lippincott Co.

257. Shores A, Braund KG , Brawner WR (1990): Management of acute spinal cord trauma. *Vet Med* $$$: 724-739

258. Shores A, Cechner PE , Cantwell HD, Wheaton LG, Carlton WW (1985): Structural changes in thoracolumbar discs following lateral fenestration. A study of the radiographic, histologic, and histochemical changes in the chondrodystrophoid dog. *Vet Surg* 14: 117-123

259. Sikes R (1989): Postoperative management of the neurosurgical patient. *Problems in Veterinary Medicine* 1: 467-477

260. Sillence DO, Horton WA , Rimoin DL (1979): Morphologic studies in the skeletal dysplasias. *American Journal of Pathology* 96: 813-835

261. Slijper EJ (1946): Comparative biologic-anatomical investigations on the vertebral column and spinal musculature of mammals. *Proc K ned Adak wet Verh (Tweed Sectie)* 47: 1-128

262. Smith JW, Walmsley R. (1951): Experimental incision in the intervertebral disc. *Journal of Bone and Joint Surgery* 33B: 612-625

263. Smith L (1975): Failures with chemonucleolysis. *Orthopaedic Clinics of North America* 6: 255-258

264. Stairmand JW, Holm S, Urban JPG (1991): Factors influencing oxygen concentration gradients in the intervertebral disc. A theoretical analysis. *Spine* 16: 444-449

265. Stern IJ, Smith L. (1967): Dissolution by chymopapain *in vitro* of tissue from normal or prolapsed intervertebral discs. *Clinical Orthopaedics and related research* 50: 269-277

266. Stevens RL, Dondi PG , Muir H (1979): Proteoglycans of the intervertebral disc. Abscence of degradation during the isolation of proteoglycans from the IVD. *Biochem J* 179: 573-578

267. Stevens RL, Ewins RJF , Revell PA, Muir H (1979): Proteoglycans of the intervertebral disc: Homology of structure with laryngeal proteoglycans. *Biochem J* 179: 561-572

268. Stigen O (1991): Calcification of intervertebral discs in the Dachshund. A radiographic study of 327 young dogs. *Acta Vet Scand* 32: 197-203

269. Sussman BJ (1971): Experimental intervertebral discolysis. A critique of collagenase and chymopapain applications. *Clinical Orthopaedics and related research* 80: 181-190

270. Sussman BJ, Bromley JW , Gomez JC (1981): Injection of collagenase in the treatment of herniated lumbar disc. Initial clinical report. *JAMA* 245: 730-732

271. Sussman BJ, Mann M. (1969): Experimental intervertebral discolysis with collagenase. *J Neurosurg* 31: 628-635

272. Swaim SF, Vandevelde M. (1977): Clinical and histologic evaluation of bilateral hemilaminectomy and deep dorsal laminectomy for extensive spinal cord decompression in the dog. *JAVMA* 170: 407-413

273. Sylven B, Paulson S, Hirsch C, Snellman O (1951): Biophysical and physiological investigations on cartilage and other mesenchymal tissues. II: the ultrastructure of bovine and human nucleui pulposi. *Journal of Bone and Joint Surgery* 33A: 333-340

274. Takahashi K, Inoue S, Takada S, Nishiyama H, Mimura M, Wade Y (1986): Experimental study on chemonucleolysis with special reference to the changes of intradiscal pressure. *Spine* 11: 617-620

275. Taylor JR (1975): Growth of human intervertebral discs and vertebral bodies. *J Anat* 120: 49-68

276. Taylor TKF, Ghosh P, Bushell GR (1981): The contribution of the intervertebral disc to the scoliotic deformity. *Clinical Orthopaedics and related research* 156: 79-90

277. Terpin T, Roach MR (1981): Chondrodysplasia in the Alaskan Malamute: Involvement of arteries, as well as bone and blood. *Am J Vet Res* 42: 1865-1873

278. Thacher C (1989): Neuroanatomic and pathophysiologic aspects of intervertebral disc disease in the dog. *Problems in Veterinary Medicine* 1: 337-357

279. Thacher C (1989): Problems with owner communication. *Problems in Veterinary Medicine* 1: 478-490

280. Thompson JP, Oegema TR , Bradford DS (1991): Stimulation of mature canine intervertebral disc by growth factors. *Spine* 16: 253-260

281. Thompson JP, Pearce RH , Schechter MT, Adams ME, Tsang IKY, Bishop PB (1990): Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. *Spine* 15: 411-415

282. Thomson CE, Kornegay JN , Stevens JB (1989): Canine intervertebral disc: Changes in the cerebrospinal fluid. *J Small Anim Pract* 30: 685-688

283. Toombs JP, Carwood DD , Lipowitz AJ, Stevens JB (1980): Colonic perforation following neurosurgical procedures and corticosteroid therapy in four dogs. *JAVMA* 177: 68-72

284 Townsend HGG, Leach DH (1984): Relationship between intervertebral joint morphology and mobility in the equine thoracolumbar spine. *Equine Vet Journal* 16: 461-465

285. Townshend HGG, Leach DH , Doige CE, Kirkaldy-Willis WH (1986): Relationship between spinal biomechanics and pathological changes in the equine thoracolumbar spine. *Equine Vet Journal* 18: 107-112

286. Troisier O (1990): An accurate method for lumbar disc puncture using a single channel intensifier. *Spine* 15: 222-228

287. Trout JJ, Buckwalter JA , Moore KC (1982): Ultrastructure of the human intervertebral disc. II. Cells of the nucleus pulposus. *Anatomical Record* 204: 307-314

288. Trout JJ, Buckwalter JA , Moore KC, Landa SK (1982): Ultrastructure of the human intervertebral disc. I. Changes in notochordal cells with age. *Tissue Cell* 14: 359-369

289. Twomey L, Taylor J. (1985): Age changes in lumbar intervertebral discs. *Acta Orthop Scandinavica* 56: 496-499

290. Tyler JA (1985): Chondrocyte-mediated depletion of articular cartilage proteoglycans *in vitro*. *Biochem J* 225: 493-507

291. Urban JPG, Holm S, Maroudas A, Nachemson A (1977): Nutrition of the intervertebral disc. An *in vivo* study of solute transport. *Clinical Orthopaedics and related research* 129: 101-114

292. van Alphen HAM, Braakman R, Bezemer PD, Broere G, Berfelo MW (1989): Chemonucleolysis versus discectomy: a randomised multicentre trial. *J Neurosurg* 70: 869-875

293. Vasan NS, Gutteling EW , Lee CK, Cibischino M, Parsons JR (1991): A preliminary study of mechanically stress-induced changes in the extracellular matrix of the canine intervertebral disc. *Spine* 16: 317-320

294. Venn G, Mason RM (1986): Changes in mouse intervertebral disc proteoglycan synthesis with age. *Biochem J* 234: 475-479

295. Verheijen J, Bouw J. (1982): Canine intervertebral disc disease. A review of etiologic and predisposing factors. *Vet Quarterly* 4: 125-134

296. Virgin WJ (1951): Experimental investigations into the physical properties of the intervertebral disc. *Journal of Bone and Joint Surgery* 33B: 607-611

297. Wakano K, Kasman R, Chao EY, Bradford DS, Oegema TR (1983): Biomechanical analysis of canine intervertebral discs after chymopapain injection. A preliminary report. *Spine* 8: 59-68

298. Walker TL. Gage DE, Selcer RR (1981): Disorders of the spinal cord and spine of the geriatric patient. *Vet Clinics of North America: Small Animal Practice* 11: 765-786

399. Wheeler SJ (1985): The approach to spinal disease in dogs. *British Vet Journal* 141: 222-232

300. Wheeler SJ (1988): Thoracolumbar disc surgery. *In Practice* 3: 231-239

301. Wheeler SJ, Davies JV (1985): Iohexol myelography in the dog and cat: a series of one hundred cases, and a comparison with metrizamide and iopamidol. *J Small Anim Pract* 26: 247-256

302. White and Panjabi (1978): *Clinical Biomechanics of the Spine* USA. JB Lippincott Company.

303. Widdowson WL (1967): Effects of chymopapain in the intervertebral disc of the dog. *JAVMA* 150: 608-617

304. Widmer WR (1989): Iohexol and iopamidol: New contrast media for veterinary myelography. *JAVMA* 194: 1714

305. Wilcox KR (1965): Conservative treatment of thoracolumbar intervertebral disc disc disease in the dog. *JAVMA* 147: 1458-1460

306. Wrigley RH, Reuter RE (1984): Canine cervical discography. *Veterinary Radiology* 25: 274-279

307. Yasuma T, Makino E, Saito S, Inui M (1986): Histological Development of intervertebral disc herniation. *Journal of Bone and Joint Surgery* 68A: 1066-1072

308. Yturraspe DJ, Lumb WV (1973): A dorsolateral muscle-separating approach for thoracolumbar intervertebral disc fenestration in ths dog. *JAVMA* 162: 1037-1040

309. Zaki FA, Prata RG (1976): Necrotising myelopathy secondary to embolisation of herniated disc material in the dog. *JAVMA* 169: 222-228

310. Zook BC, Kobrine AI (1986): Effects of collagenase and chymopapain on spinal nerves and intervertebral discs of *Cynomolgus* monkeys. *J Neurosurgery* 64: 474-483