DISCITIS AFTER DISCOGRAPHY AND CHEMONUCLEOLYSIS

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SIGNED STATEMENTS

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

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ABSTRACT

Whilst infection following intradiscal injections has been recognized as a distinct entity, discitis after discography with contrast material and after chemonucleolysis with chymopapain has usually been attributed to an aseptic process or a chemical reaction to the agent injected. The aims of the study were to:- 1. review the incidence of discitis following discography and to describe the pathological findings in seven patients with discitis who came to open biopsy; 2. to test the hypothesis that discitis following discography is always due to infection and not to a chemical reaction from the contrast material; 3. to measure the effect of both chymopapain and Conray 280 on a wide range of bacteria in vitro; and 4. to test the hypothesis that discitis following intradiscal chymopapain is due to infection and not to a chemical reaction.

PART I The case records and radiographs of 432 patients who had undergone lumbar discography were reviewed. When an 18-gauge needle without a stilette had been used, discitis was diagnosed in 2.7% of 222 patients but stiletted needles and a two-needle technique at each level reduced the incidence to 0.7%. Seven patients with discitis after discography had undergone anterior discectomy and fusion; in them the histopathological findings were of a chronic inflammatory response. Bacteria were isolated from the discs of three of the four patients who had open biopsy less than six weeks from the time of discography. These findings suggest that bacteria were initiators rather than promoters of the response.

PART II Multiple level lumbar discography was carried out in mature sheep, injecting contrast material with or without various concentrations of bacteria. Radiographs were taken and the discs and end-plates were examined histologically and cultured for bacteria at intervals after injection. None of the controls showed any evidence of discitis but all sheep injected with bacteria had typical radiological and histopathological changes by six weeks, though cultures were almost all negative. However, at one and two weeks after injection bacteria could be isolated, but usually not after three weeks.

PART III Chymopapain was found to have a bactericidal effect on all bacteria tested which was more pronounced with gram positive organisms whereas Conray 280 showed very little if any antibacterial effect after 48 hours.

PART IV Multiple level lumbar intradiscal injections of chymopapain were carried out in eight mature sheep. Sixteen discs in four sheep were injected with a mixture of reconstituted chymopapain and a staphylococcus epidermidis suspension and sixteen discs in another four sheep were injected with reconstituted chymopapain only. All sheep were sacrificed at six weeks and the discs and end-plates were examined by radiology and histopathology and nuclear material was cultured for bacteria. None of the controls showed any evidence of discitis whereas all sheep injected with bacteria had typical radiological and histopathological changes of discitis. However, in most cases where end-plate lesions were well established there was no evidence of bacteria at sacrifice. These findings support the opinion that discitis following intradiscal injection is always due to infection introduced by the needle tip.

INTRODUCTION

Intradiscal injections are widely used in the investigation and management of patients with spinal disorders. The substances used include injections of chymopapain, collagenase, cortico-steroid, contrast material and saline. Discography was introduced in 1948 by Lindblom as an investigation to confirm the diagnosis of intervertebral disc prolapse. With the advent of water soluble myelography and high resolution C.T. scanning, the use of discography for the diagnosis of intervertebral disc prolapse has become less popular. However, discography remains the principal investigation to confirm the diagnosis of internal disc disruption and is widely used to demonstrate the presence of normal discs adjacent to the level of an intended spinal fusion in either the cervical or lumbar spine.

Chymopapain is a proteolytic enzyme from the latex of the papaya fruit. It breaks down the proteoglycan component of the intervertebral disc which is concentrated in the nucleus pulposus. This reduces its osmotic fluid absorption and therefore the size of the nucleus pulposus. The intradiscal injection of chymopapain for the treatment of lumbar intervertebral disc prolapse was first reported by Lyman Smith (1964) and since his description of "chemonucleolysis", there has been considerable opposition to this treatment. Chymopapain would have been accepted many years ago but for two factors: the overdiagnosis of intervertebral disc prolapse, and the inadequate methods of confirming the diagnosis before the introduction of water-soluble myelography and C.T. scanning. Intervertebral disc prolapse was first described as an entity by Mixter and Barr (1934) and unfortunately this has remained the popular diagnosis to explain most cases of back pain and sciatica.

In recent years it has been increasingly realized that disc prolapse is the cause of symptoms in fewer than 10% of patients with back pain and sciatica. The overdiagnosis of disc prolapse resulted in poor selection of patients in the early double-blind studies carried out in the United States and in Australia (Graham CE 1976, Schwetzchenau PR, Ramirez A, Johnston J et al 1976: Martins AN, Ramirez A, Johnston J, Schwetzchenau PR 1978). The large number of patients who had only back pain and the administration of chymopapain in two or three adjacent intervertebral discs in a large proportion of the patients in these studies suggested the diagnosis was incorrect in many of these patients. In addition, other aspects of the methodology were criticized particularly by some of the participants (Brown MD, Daroff RB 1977). While both studies demonstrated that chymopapain therapy had a slightly higher success rate than the placebos used, this was not statistically significant and chymopapain was withdrawn from clinical use both in the United States and in Australia in 1975.

After a great deal of lobbying both by supporters and by opponents of chymopapain the Food and Drug Administration (F.D.A.) agreed to allow further double-blind studies to be carried out in the United States (Morris J, Stromberg L 1983; Nordby E J 1983). At the same time a double-blind study was carried out at the Flinders Medical Centre, Adelaide, in which chymopapain was tested against saline solution (Fraser RD 1982; 1984). All these studies had strict criteria for patient inclusion, and the diagnosis was confirmed either by water-soluble myelography or by high-resolution C.T. scanning.

The three studies demonstrated that chymopapain had a significant therapeutic effect in the treatment of patients with sciatica caused by lumbar intervertebral disc herniation; this led to its approval both in Australia and in the United States.

In the first four months after the approval of chymopapain in the United States, nearly one half of the 12,800 members of the American Academy of Orthopedic Surgeons and the American Association of Neurological Surgeons attended one-day training courses on chemonucleolysis (Medical News 1983). This was followed by a wave of popularity in this form of treatment and unfortunately serious adverse reactions, including anaphylaxis, paraplegia (due to technical errors in needle placement) and discitis.

The lateral approach avoiding the thecal sac is an essential part of the technique of chemonucleolysis and this approach is also the preferred technique for lumbar discography (McCulloch and Waddell 1978). The increased use of chymopapain for the treatment of intervertebral disc prolapse has emphasized the use of discography as an investigation since many physicians have advocated the injection of contrast material prior to chemonucleolysis to confirm correct needle placement.

There have been very few reports describing possible local complications of intradiscal injection. Discitis following discography with contrast material has been considered a rare complication (Brodsky and Binder 1979; Collis and Gardner 1962; McCulloch 1977; McCulloch 1980; McCulloch and Waddell 1978; Massie and Stevens 1967; Simmon and Segil 1975; Wiltse, Widell and Yuan 1975) with some investigators attributing it to a chemical or aseptic reaction (Brodsky and Binder 1979; Crock 1983; McCulloch 1977; Wiltse, Widell and Yuan 1975). Recently it has been suggested that discitis following discography is more common than previously recognized and may occur as often as one in every thirty patients (Crock 1983; Fraser 1984).

The term "discitis" has been more commonly associated with chemonucleolysis because of the exacerbation of back pain which frequently follows this procedure. Discitis following chemonucleolysis has been reported in as high as 2% (McCulloch - unpublished data 1985) of patients and has been generally regarded as due to a chemical or aseptic process. More recent studies have described infective discitis following chemonucleolysis but this seems to have been regarded as a separate entity (Agre, Wilson, Brim et al 1984). McCulloch and McNab (1983) reported no cases of infective discitis following chemonucleolysis in over six thousand patients and explained this apparently low incidence of discitis on the bactericidal effect of chymopapain.

The evidence to support a non-infective aetiology for discitis following discography and chemonucleolysis has been the following: the failure to isolate bacteria from biopsy material in almost all cases, the associated raised erythrocyte sedimentation rate in the presence of a normal white cell count, the histopathological findings of a chronic inflammatory process with a small round cell response; and the tendency toward natural resolution.

The aims of this project were:-

1. To review the incidence of discitis following discography.
2. To describe the pathological findings in seven patients with discitis who came to open biopsy.
3. To test the hypothesis that discitis following discography is initiated by infection and not by a chemical reaction from the contrast material.
4. To measure the bactericidal effect of chymopapain in vitro.
5. To test the hypothesis that discitis following intradiscal chymopapain is due to infection and not to a chemical reaction.

DISCITIS AFTER DISCOGRAPHY I : INCIDENCE AND PATHOLOGICAL FINDINGS

MATERIALS AND METHODS:

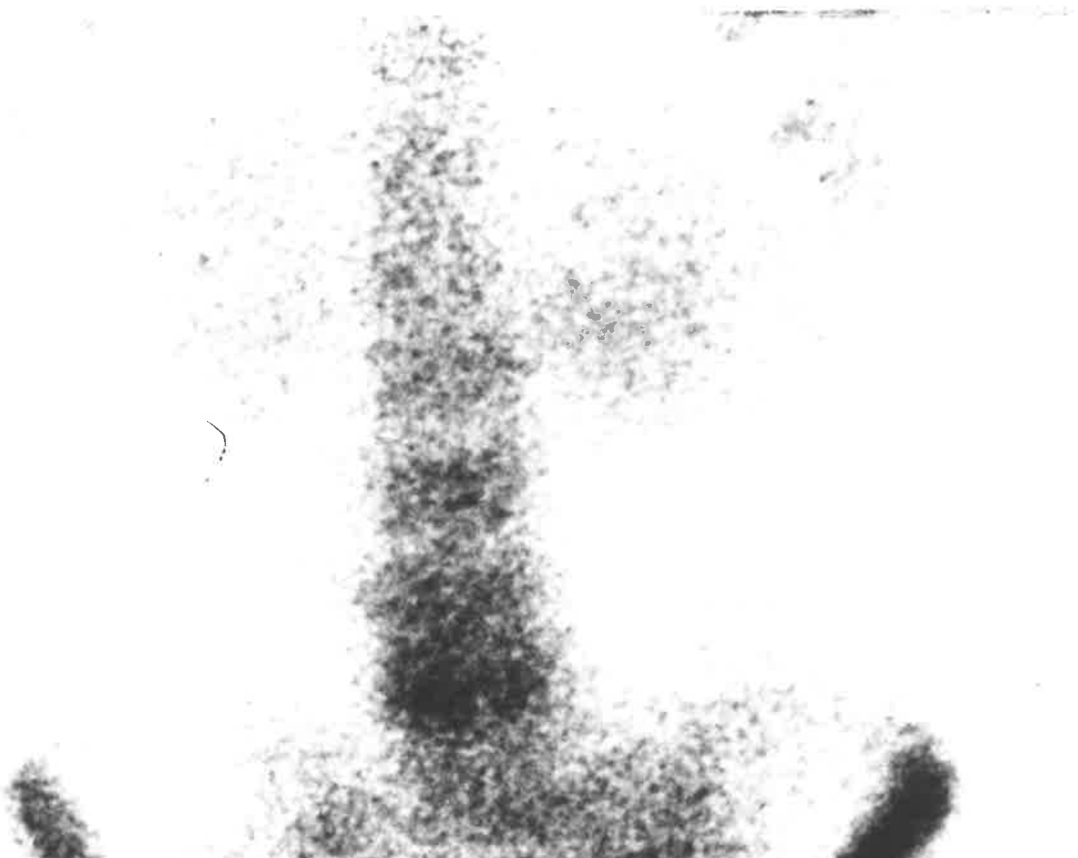
The case records and radiographs of 432 patients who had undergone lumbar discography for the investigation of low back pain between 1978 and 1984 were reviewed.

All procedures had been carried out with the patient lying in the left lateral position. The skin had been prepared with Betadine only except in the series carried out by radiologists where the skin had been prepared with Cetavlon followed by Betadine. The operator was gloved and gowned and the patient was draped with a sterile area towel. The patient was premedicated and the procedure was carried out under local anaesthesia using the lateral technique described by McCulloch and Waddell (1978).

From 1978 to 1980 the procedure was carried out using an 18 gauge needle without a stilette at all levels except at the L5-S1 level where a two-needle technique was employed using 18 gauge and 22 gauge needles without stilettes. After 1980 the procedure was always carried out with stiletted needles and a two-needle technique was employed at all levels. Conray 280 was used as the contrast material. From 1978 to 1983 the procedures were carried out by an Orthopaedic Surgeon (RDF) and from August 1983 until August 1984 the procedures were carried out by three radiologists using the same stiletted two-needle technique.

Fig. 1 Lateral tomogram of lumbar spine three months after two level discography showing the erosions of the end-plates at both levels, classical of post discogram discitis. The sclerotic margins of the erosions are typical of late lesions.

Fig. 2 Technetium bone scan showing increased uptake at the L4-5 level three weeks after discography.



A patient was diagnosed as having discitis when there had been a sustained episode of acute back symptoms following discography with a raised erythrocyte sedimentation rate and radiological changes of end-plate erosion on plain radiographs or on tomography (Fig. 1), or a positive technetium bone scan (Fig. 2).

From 1982 to 1984 seven patients with discitis following discography had undergone anterior discectomy and fusion at the affected level. Four of these patients were referred from other orthopaedic surgeons. The disc and adjacent end-plates were removed en bloc using Crock (1983) dowel cutting instruments. Swabs were taken from the disc space and were sent for microbiological examination.

Excised disc material was immersed in formalin for a minimum of 24 hours before being transferred to decalcifying fluid. The progression of decalcification was checked daily by radiography. After decalcification, selected blocks of bone were processed, embedded in wax, and 5 micrometre sections were stained with haematoxylin and eosin.

TABLE I.

DATA OF INCIDENCE OF DISCITIS IN 432 PATIENTS

SERIES (DATES)	PATIENTS	LEVELS (AVERAGE PER PATIENT)	PATIENTS WITH DISCITIS (INCIDENCE)	NO. OF DISCS INVOLVED (INCIDENCE)
Open needle technique (RDF) (1978-80	222	463 (2.0)	6 (2.7%)	6 (1.4%)
Closed needle technique (RDF) (1981-July 83)	149	283 (1.9)	1 (0.7%)	1 (0.4%)
Radiologists Aug 83-Aug 84	61	134 (2.2)	3 (4.9%)	4 (3.0%)
TOTAL	432	880 (2.0)	10 (2.3%)	11 (1.3%)

TABLE II - Details of seven patients with discitis following discography who underwent open biopsy

CASE	AGE	DIAGNOSIS PRIOR TO DISCOGRAPHY	ESR BETWEEN DISCOGRAPHY AND SURGERY	XRAY FINDINGS AFTER DISCO-GRAPHY	BONE SCAN FINDINGS	INTERVAL BETWEEN DISCOGRAPHY AND BIOPSY	HISTOLOGICAL EVIDENCE OF DISCITIS	CULTURE FOR BACTERIA
1	28	L5-S1 internal disc disruption	24	large end plate disruption (operative discography)	-	5 weeks	YES	Pseudomonas Aeruginosa (light growth)
2	21	Grade I Isthmic spondylolisthesis L5-S1	2	Large end plate erosion L4-5 (Tomo- & operative discography)	-	3 months	YES	No growth
3	47	L5-S1 Discogenic L.B.P.	50	End Plate erosion at L4-5	patchy uptake Non spec.	6 months	YES	No growth
4	38	L.B.P. following L4 compression fracture	30	End Plate erosion at L5-S1	positive	4 weeks	YES	Staph. Epidermidis
5	40	GRADE II traumatic spondylolisthesis L4-5	99	-	positive L4-5	3 weeks	YES	No growth
6	18	Grade II Isthmic Spondylolisthesis	–	–	–	4 weeks	NO	Klebsiella Pneumoniae
7	46	Grade II degenerative spondylolisthesis L4-5	105	End Plate erosion L4-5 (plain xrays)	positive L4-5	3 months	YES	No growth

TABLE III - SUMMARY AND GRADING OF HISTOPATHOLOGICAL FEATURES

		DISC						VERTEBRAL BODY				
					Inflammatory cells						Inflammatory cells	
Case	Disc	Vascular-ization	Granulation Tissue	Mature fibrous tissues	Acute	Chronic	End-Plate Defects	Nuclear Herniation	Granulation Tissue	Mature fibrous tissues	Acute	Chronic
1	L5/S1	++	+	−	−	−	++	−	++	++	+	++
2	L4/5	+++	+++	++++	−	+	+++	++	++	+++	−	+
3	L4/5	+++	+++	+++	−	+	++++	++	+	++	−	++
4	L5/S1	++	−	−	++	−	+	−	++	++	++	++
5	L4/5	++	++++	++++	++	+++	++++	++	+++	++	++	+++
6	L4/5	++	+	−	+	+	+	−	+	−	+	−
7	L4/5	+++	+++	+++	−	+	+++	++	+++	++	−	+

RESULTS AND CASE REPORTS

From March 1978 to December, 1980, 222 patients had lumbar discography carried out at 465 levels using an open needle technique: Six of these patients (incidence 2.7%) developed discitis. Subsequently, a closed two-needle technique was employed. From January 1981 to July 1983, 149 patients were injected at 283 levels, and one patient developed discitis (incidence 0.7%). From August 1983 to September 1984, 61 patients were referred for lumbar discography and this was carried out at 134 levels by three radiologists: 3 patients developed discitis (incidence 4.9%) during this period. The results overall demonstrated that 2.3% of patients had developed discitis and this complication had occurred at 1.3% of levels injected (Table I).

The case reports of the seven patients who underwent anterior discectomy and fusion are summarized in Table II, and the histopathological findings in Table III. Two illustrative cases are reported in detail.

CASE ONE

A 28 year old soldier presented with an eight month history of increasing low back pain following repeated falls during his work as an Army physical instructor. Examination revealed bilateral restriction of straight leg raising to 45 degrees by back pain and there were no abnormal neurological signs in the lower limbs.

Fig. 3 Normal L4-5 discogram (Case 1).

Fig. 4 a. Lateral discograms 5 weeks later showing end-plate disruption at L4-5 and L5-S1 (Case 1).

Fig. 4 b. AP discograms showing end-plate disruption (Case 1).

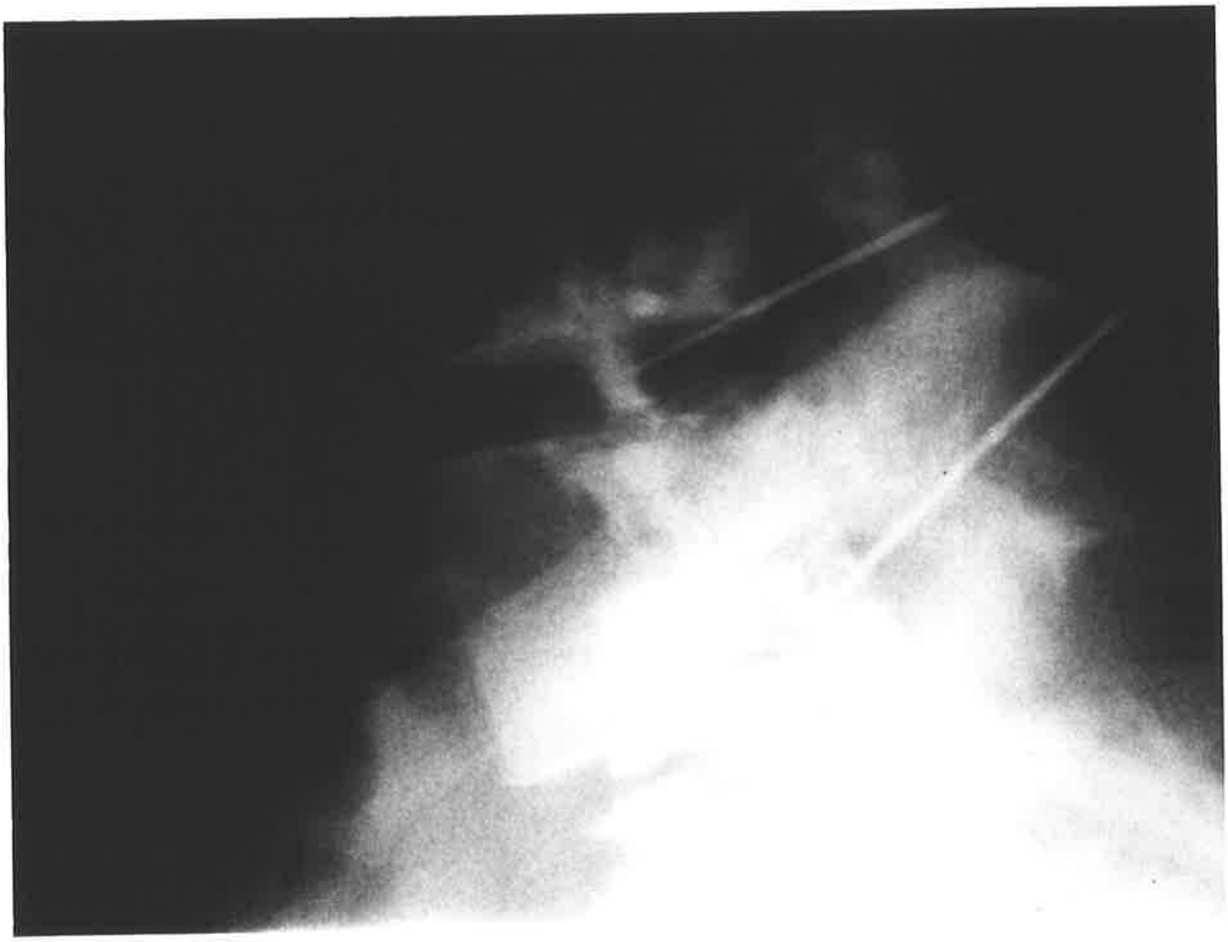
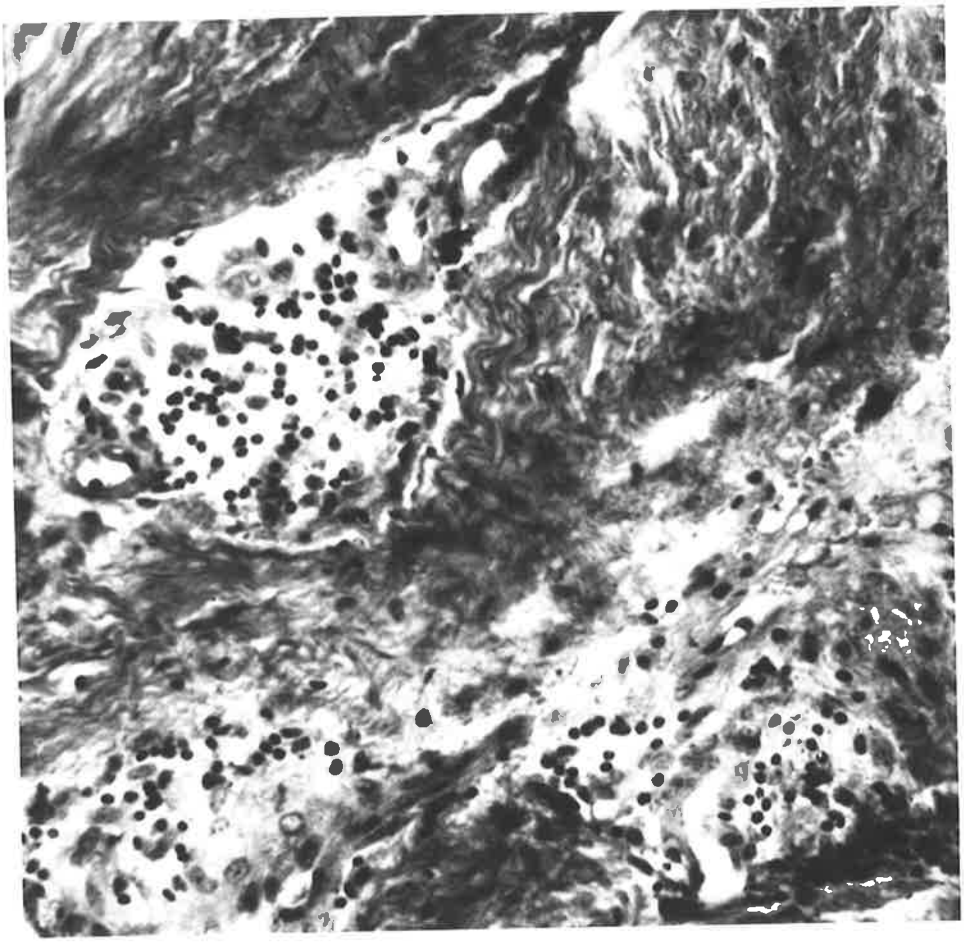
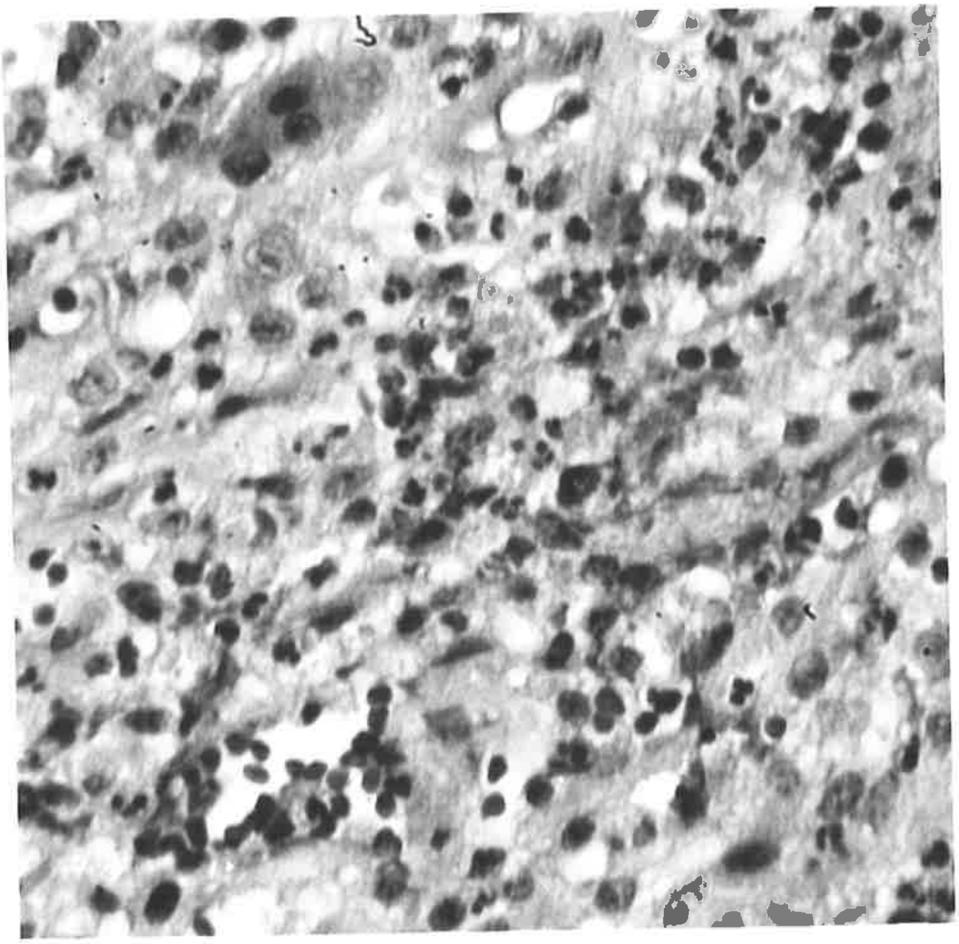


Fig. 5 Chronic inflammation in the mature fibrous scar tissue within the disc showing clusters of lymphocytes grouped around small blood vessels (Case 1). Haematoxylin-eosin X100.

Fig. 6 Active chronic inflammation within granulation tissue in the disc showing many neutrophil polymorphs in addition to chronic inflammatory cells (Case 1). Haematoxylin-eosin X250.





Discography at the L4-5 level demonstrated a normal disc with no pain reproduction (Fig. 3). At the L5-S1 level the injection was into a false cavity in the annulus and was reported to reproduce the patient's typical symptoms. Immediately following discography there was worsening of symptoms. Six weeks later the patient's ESR was 24. Under general anaesthesia discography was repeated, and major contrast extravasation was observed through the upper and lower end-plates at the L4-5 level (Fig. 4 a,b). At the L5-S1 level a more limited end-plate lesion was demonstrated posteriorly corresponding to the position of the false cavity injection. The patient underwent anterior disc clearance and interbody fusion at L4-5 and L5-S1 using a muscle splitting retroperitoneal approach (Fraser 1982). Histological examination of L5-S1 material showed heavily inflamed granulation tissue present in both the hyaline laminae and the bone of the end-plate region, with acute inflammatory cells present in addition to chronic inflammation in disc material (Figs. 5 and 6). Culture of this material demonstrated a light growth of Pseudomonas aeruginosa. The post operative course was uneventful and nine months following the procedure the patient was assessed as pain free with x-rays demonstrating consolidation occurring at both levels.

Fig. 7 Lateral discograms demonstrating minimal nuclear degeneration at L4-5 and a degenerate disc at L5-S1 (Case 2).

Fig. 8 Lateral radiograph six weeks after discography and two weeks after anterior interbody fusion at L5-S1 (Case 2).

Fig. 9 Lateral radiograph three months after interbody fusion demonstrating disc space narrowing at L4-5 and a massive erosion of L5 vertebral body (Case 2).

CASE TWO

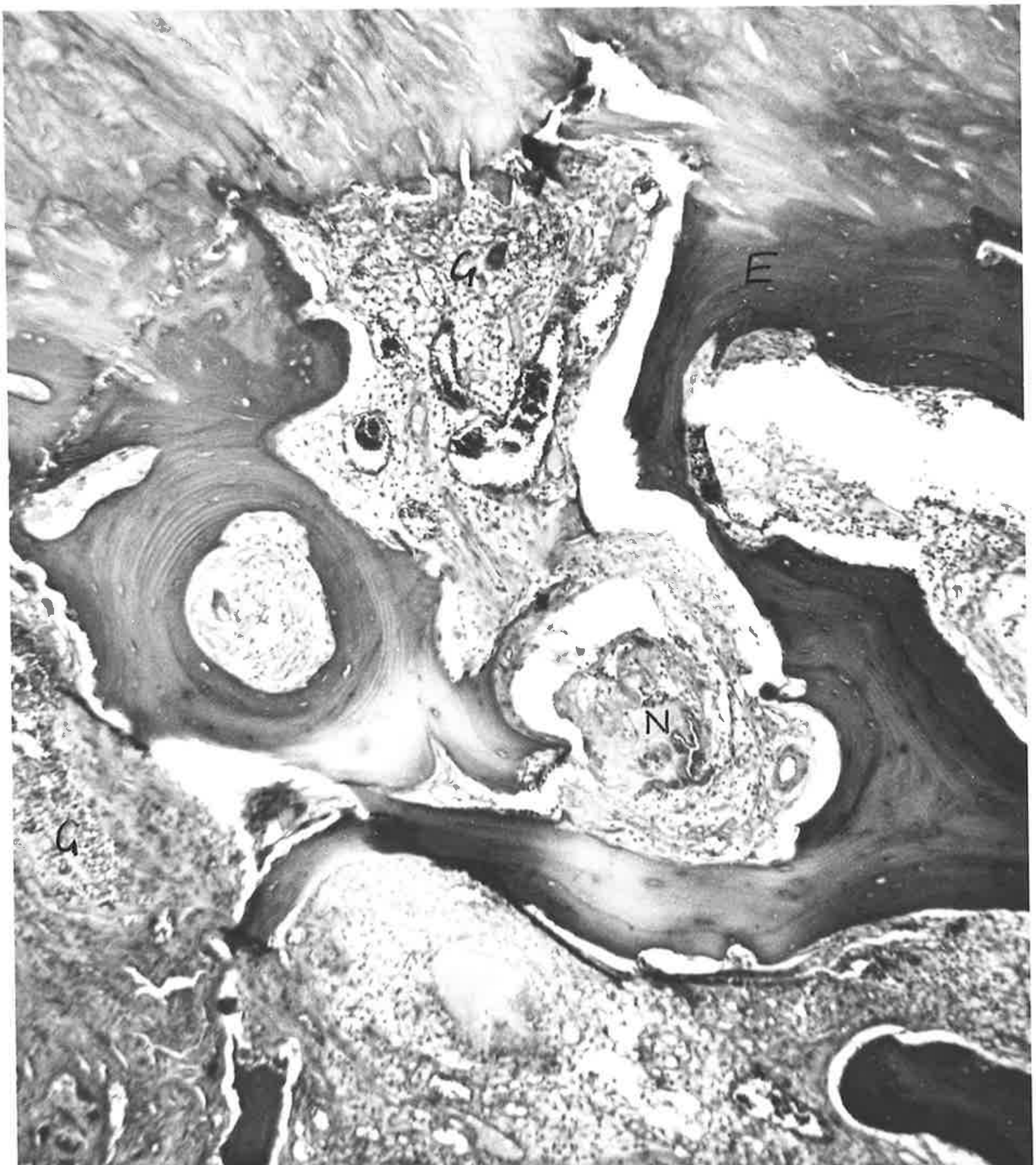
A 21 year old naval rating presented with a 12 month history of disabling low back pain after lifting heavy drums along a gangway. He had been unable to work and his condition had failed to respond to conservative treatment. His plain x-rays demonstrated a Grade I spondylolisthesis. He was investigated by lumbar discography which demonstrated minimal nuclear degeneration at the L4-5 level, and a degenerate disc at L5-S1 with reproduction of his typical back pain (Fig. 7). His symptoms progressively worsened following discography, and arrangements were made to carry out an anterior interbody fusion at the L5-S1 level. A diagnosis of discitis was considered at that time, but was thought to have been excluded by an ESR of only 2mm. in one hour, and by a plain lateral x-ray one month after discography which showed no evidence of disc space narrowing or erosion.

Two weeks after anterior interbody fusion at L5-S1 (Fig. 8), he was still complaining of severe pain requiring parenteral narcotics. He continued to require large amounts of analgesics and three months after his interbody fusion, plain x-rays demonstrated that disc space narrowing had occurred at the L4-5 level together with a massive erosion in the L5 vertebral body (Fig. 9).

Fig. 10 a. Lateral discogram demonstrating end-plate disruption four months after discography and three months after anterior interbody fusion at L5-S1 (Case 2).

Fig. 10 b. AP discogram demonstrating end-plate disruption (Case 2).

Fig. 11 Low-power view showing a breach in the end-plate (e) with herniation of nuclear material (N) and granulation tissue (G) extending into the disc and marrow spaces (Case 2). Haematoxylin-eosin X50.


G
E
N
G

Under general anaesthesia, aspiration biopsy of the L4-5 disc space was carried out but culture of this failed to demonstrate any organisms. Contrast material was injected to outline the defect (Fig. 10 a,b), and he was treated with intradiscal cortico-steroids. This failed to result in any relief of his symptoms, and he was not improved by six weeks immobilization in a brace. Seven months after the initial discogram had been carried out, he underwent anterior disc clearance and interbody fusion at the L4-5 level, and this was followed by dramatic relief of pain which has been sustained. Histological examination of the L4-5 intervertebral disc and adjacent end-plates showed extensive destruction of the end-plate region (Fig. 11). There was abundant anterior granulation and fibrous tissue extending into the nucleus but chronic inflammatory cells were few in number.

DISCUSSION

Exacerbation of back pain is common following intradiscal injections, possibly due to a mechanical or chemical effect. Whilst discitis following discography has been recognized as a distinct entity, until recently the reported incidence was in the region of 0.1% (Brodsky and Binder 1979; Collis and Gardner 1962; Gresham and Miller 1969; McCulloch 1977 and 1980; McCulloch and Waddell 1978; Massie and Stevens 1967; Simmons and Segil 1975; Wiltse, Widell and Yuan 1975). More recent reports have recorded a higher incidence of discitis following discography and chemonucleolysis, in the order of 1-2% (Crock 1983; Fraser 1984; McCulloch and McNab 1983). On this basis it seems likely that this condition has often gone unrecognized despite the fact that the patient with discitis usually experiences intense pain.

Although an infective aetiology has been recognized in some cases, numerous authors have considered that discitis following chemonucleolysis and following discography is due to a chemical or aseptic process (Brodsky and Binder 1979; Crock 1983; McCulloch and McNab 1983; Wiltse, Widell and Yuan 1975). Conclusions of a non-infective etiology have been based upon the failure to identify organisms from biopsy material in the majority of cases, the failure of the white cell count to become elevated in the presence of a raised erythrocyte sedimentation rate, the histopathological findings of a chronic inflammatory process with a small round cell infiltrate and the tendency towards natural resolution. McCulloch and McNab (1983) reported that there had not been a single known bacterial disc space infection in over 6,000 chemonucleolysis procedures. They felt the incidence of chemical discitis was probably less than 1% and they suggested that the explanation for the lack of bacterial discitis was chymopapain dissolving the cell wall of bacteria. In a review of 29,075 patients treated in the United States with chymopapain, Agre et al (1984) reported that 22 patients had developed discitis. These patients with discitis had developed severe back pain and spasm a few days to many weeks following the injection. In nine of these patients, bacterial infection had been confirmed by culture. A number of the remaining patients were considered to have aseptic discitis on the basis of negative cultures. The authors did not state the time intervals between discography and needle biopsy.

A recent experimental study of discitis following discography has suggested that all cases of discitis following intradiscal injections are due to infection introduced by the needle tip (Fraser, Osti and Vernon-Roberts 1985). Using adult sheep, typical radiographic and pathological evidence of discitis was produced by the introduction of a suspension of staphylococcus epidermidis and contrast material into the disc. The process did not seem to be related to the number of organisms injected, and only five colony forming units of bacteria were required to reproduce discitis. The study demonstrated that the bacteria disappeared rapidly once the end-plate lesion was established.

The results of the present study support the findings of our recent experimental study which suggested that all cases of discitis following intradiscal injection are due to infection introduced by the needle tip (Fraser, Osti and Vernon-Roberts 1986 b). When a single operator was carrying out the technique of discography the incidence of discitis was reduced considerably by the use of a stiletted two needle technique at each level, thus eliminating the risk of injecting a core of soft tissue including skin. In the seven cases presented, bacteria were not isolated in three patients who had open biopsies carried out more than six weeks after discography. Moreover, the histological findings of disc vascularization, mature granulation tissue formation in both discs and vertebral bodies, the association of the more marked histological changes with vertical disc protrusions, and the occasional presence of acute inflammatory cells in addition to chronic inflammation were features in common with the pathological changes observed in the sheep study. While in the sheep discitis was induced in previously normal discs, clearly this prior state of normality could not be claimed for the patients included in the present study who underwent discography as part of the clinical investigation of low back pain. It would be expected, therefore, that the majority of these patients would have age-related disc abnormalities or more advanced pathology (Vernon-Roberts 1980; Vernon-Roberts and Pirie 1977) in some instances. In this study there was no significant difference in the incidence of discitis after discography with normal morphology compared with that which occurred following discography with abnormal morphology. However discitis after normal discography produced larger discrete protrusions while the end-plate lesions following abnormal discography tended to be more diffuse.

Experimentally and conceptually, vascularized tissues are equipped to mount an inflammatory response to rapidly deal with bacterial invasion whereas avascular tissue (such as the normal disc) do not have this facility but are normally resistant to bacterial invasion. The observations in the sheep model of discitis (Fraser, Osti and Vernon-Roberts 1985) are consistent with the concept that, while bacteria can initiate the early lesion, it is possible that they play no further role in the destructive chronic inflammatory process which follows. These experimental observations have also suggested that the principal initial pathological process affecting the tissues in discitis in the sheep may be granulation tissue formation and bone resorption in the end-plate region.

The pathological features offer an explanation for the observation by Crock (1983) that discitis following discography can be successfully treated with intradiscal hydrocortisone. However, on the basis of our findings in the sheep study it would be unwise to use steroid therapy in patients before the end-plate lesion is radiologically established with a smooth sclerotic margin demonstrated by lateral tomography.

On the basis of the present study there is some doubt as to the role antibiotics play in the treatment of discitis following intradiscal injection. Although the bacterial response seems to be self limited in most cases, appropriate antibiotic treatment may hasten recovery but this is yet to be proven. One of the difficulties in treating discitis following multiple level discography is to identify the level or levels involved early enough to isolate the bacteria by needle biopsy. Technetium bone scanning seems to be the most reliable investigation. Plain x-ray changes may take several weeks to develop, although end-plate erosions may be demonstrated by lateral tomography as early as two weeks following the intradiscal injection.

The 4.9% incidence of discitis following discography by three operators in 61 patients over a 12 month period is of particular concern. In view of the lack of awareness of this complication in the past it seems likely that this complication is still unnoticed by many surgeons and radiologists. In a radiological review of 60 patients two years following intradiscal injections, spontaneous anterior ossification found to have occurred in two patients at the level injected was attributed to previous unrecognized discitis (Fraser 1984).

The results of the present study support the conclusions of our recent experimental study (Fraser, Osti and Vernon-Roberts 1985), and suggest that all cases of discitis following intradiscal injections are initiated by infection. On this basis in order to reduce the incidence of discitis following intradiscal injection it is recommended that: the procedure should only be carried out in a clean room with full aseptic techniques; the skin should be prepared over a wide area to allow for movement of drapes; care should be taken to avoid contamination of the drapes by x-ray equipment; only stiletted needles should be used, and a two-needle technique for each level is recommended and the technician should avoid handling the shaft of the needle, particularly near the needle tip; and separate needles should be used for each skin puncture.

DISCITIS AFTER DISCOGRAPHY II : AN EXPERIMENTAL STUDY

MATERIALS AND METHODS

Eight adult sheep (merino wethers) were entered into the study. Under general anaesthesia, three adjacent lumbar intervertebral discs were exposed by a retroperitoneal approach using a vertical skin incision anterior to the transverse processes. Each disc was injected under direct vision using 27.5 gauge needles. In four sheep, meglumine iothalamate 60% (Conray 280) only was injected to a maximum volume of 0.1ml. per disc. In the other four sheep meglumine iothalamate 60% and a bacterial suspension comprising different dilutions to a maximum volume of 0.1ml. per disc was injected. Immediately following the intradiscal injection plain radiographs were taken to confirm that the needle tip was in the nucleus.

The bacterial suspension used for injection was prepared from a strain of staphylococcus epidermidis isolated from a patient with an infected total hip replacement who subsequently developed sub-acute bacterial endocarditis. The strain was chosen for the study because of its unusual sensitivity pattern. It was sensitive to Erythromycin, Clindamycin and Sulphamethoxazole and resistant to Penicillin, Tetracycline, Gentamycin and Methicillin. The isolate was grown in brain heart infusion broth for forty eight hours. Plate counts were carried out on different dilutions prepared in snap freeze broths to determine the number of organisms at each dilution and the dilutions then stored at -170 degrees centigrade in liquid nitrogen. For the preparation of the intradiscal injection, appropriate dilutions were thawed and an aliquot added to meglumine iothalamate 60% such that in 0.1 millilitres of suspension a known number of organisms were present.

In two sheep the following approximate concentrations of organisms were injected into the three adjacent discs: 20,000 organisms per 0.1 ml, 2,000 organisms per 0.1 ml, and 200 organisms per 0.1 ml. Another two sheep were injected with approximately three organisms per 0.1 ml. into each disc. Plate counts were repeated at the time of the intradiscal injections to check again the concentration of organisms.

At the time of surgery and at weekly intervals thereafter, plain lateral radiographs of the lumbar spine were taken and specimens of venous blood obtained from each animal for ESR, white cell count and viscosity (measured according to the method described by Archer and Allen in 1970).

Six weeks following the intradiscal injection all animals wre sacrificed and a needle biopsy of nuclear material was obtained from each of three discs injected and inoculated to the following culture media:

(1) blood agar incubated in 5% carbon dioxide.

(2) anaerobic agar incubated in 7% carbon dioxide, 83% nitrogen, and 10% hydrogen.

(3) glucose cooked meat broth.

The media (1) and (2) were incubated for seven days and inspected each day for growth. The medium (3) was incubated for seven days and then cultured if turbid or clear for two days on media (1) and (2).

The lumbar spine was removed by transecting the vertebral column through T12 above and the mid sacrum below. Following fixation in formaline, spines were divided in the sagittal plane and examined macroscopically. Blocks for histological processing were made comprising half of each vertebral body above and below the interposed intervertebral disc. Blocks were immersed in decalcifying fluid and the progression of decalcification checked daily by radiography. After decalcification the blocks were processed, embedded in wax, and five micrometre sections were stained with haematoxylin and eosin. In some instances, following the making of a sagittal slab for histology, some discs of the residual portions were divided through their centre parallel to the end-plates to determine macroscopically the extent of the lesions within the discs.

An additional three sheep were entered into the study. Using the methods already described, meglumine iothalamate 60% and the following concentrations of staphylococcus epidermidis were injected into three adjacent intervertebral discs: 2,000 organisms per 0.1 ml, 200 organisms per 0.1 ml, and twenty organisms per 0.1 ml. Each sheep was then sacrificed at one, two and three weeks, respectively. Needle biopsies were taken from the intervertebral discs and the whole spine processed according to the procedure described above.

TABLE IV

DATA ON EIGHT SHEEP SACRIFICED SIX WEEKS AFTER INTRADISCAL INJECTIONS OF CONRAY 280 ± STAPHYLOCOCCUS EPIDERMIDIS

Sheep	Levels	Volume (mls.)	Number of Bacteria	Radiographic & Macroscopic Discitis	Histological Discitis	Culture
	L1-2	0.1	32,000	Yes	Yes	Negative
1	L2-3	0.08	2,560	Yes	Yes	Negative
	L3-4	0.09	301	Yes	Yes	Negative
	L1-2	0.06	16,800	Yes	Yes	Negative
2	L2-3	0.06	1,680	Yes	Yes	Negative
	L3-4	0.06	206	Yes	Yes	Negative
	L2-3	0.03	3	Yes	Yes	Negative
3	L3-4	0.05	2	No	No	Negative
	L4-5	0.07	7	No	No	Negative
	L1-2	0.02	1	No	No	Positive
4	L2-3	0.02	1	Yes	Yes	Negative
	L3-4	0.02	1	No	Yes	Positive
	L1-2	0.07	0			
5	L2-3	0.07	0			
	L3-4	0.06	0			
	T12-L1	0.09	0			
6	L1-2	0.1	0			
	L2-3	0.09	0			
	L1-2	0.1	0	No	No	Negative
7	L2-3	0.1	0			
	L3-4	0.01	0			
	L1-2	0.09				
8	L2-3	0.09	0			
	L3-4	0.08	0			

Fig. 12 a. Operative discograms at three levels.

Fig. 12 b. Lateral radiograph 5 weeks after discography at three levels with end-plate erosions at the middle level. Each level had been injected with approximately one organism only.

Fig. 13 a. Three level end-plate erosions at 3 weeks. The upper level had been injected with 32,000 organisms and the lower level with approximately 300 organisms.

Fig. 13 b. Appearance of gross erosions in the same spine at 5 weeks.

RESULTS

(a) Sheep sacrificed at six weeks (eight sheep)

Radiology

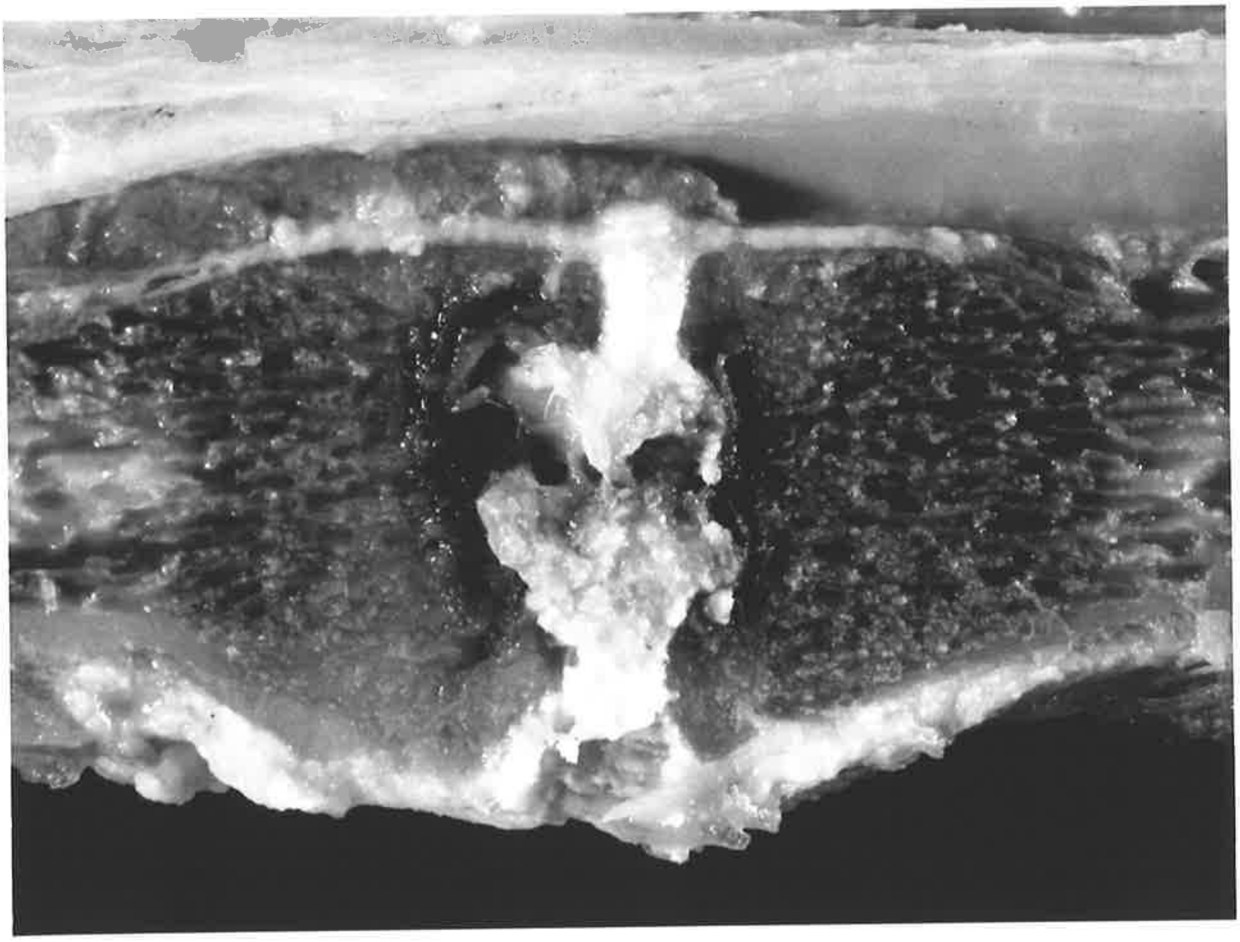
Narrowing of the disc space height and erosion of the bony end-plates occurred at all of the six levels in two sheep (numbers one and two) where more than 200 bacteria were injected (Table IV). Two sheep (numbers three and four) with an estimated seven or less bacteria injected at each level developed the same radiographic findings at one level only in each sheep (Fig. 12). The extent of end-plate erosion did not seem to be related to the number of organisms injected (Fig. 13).

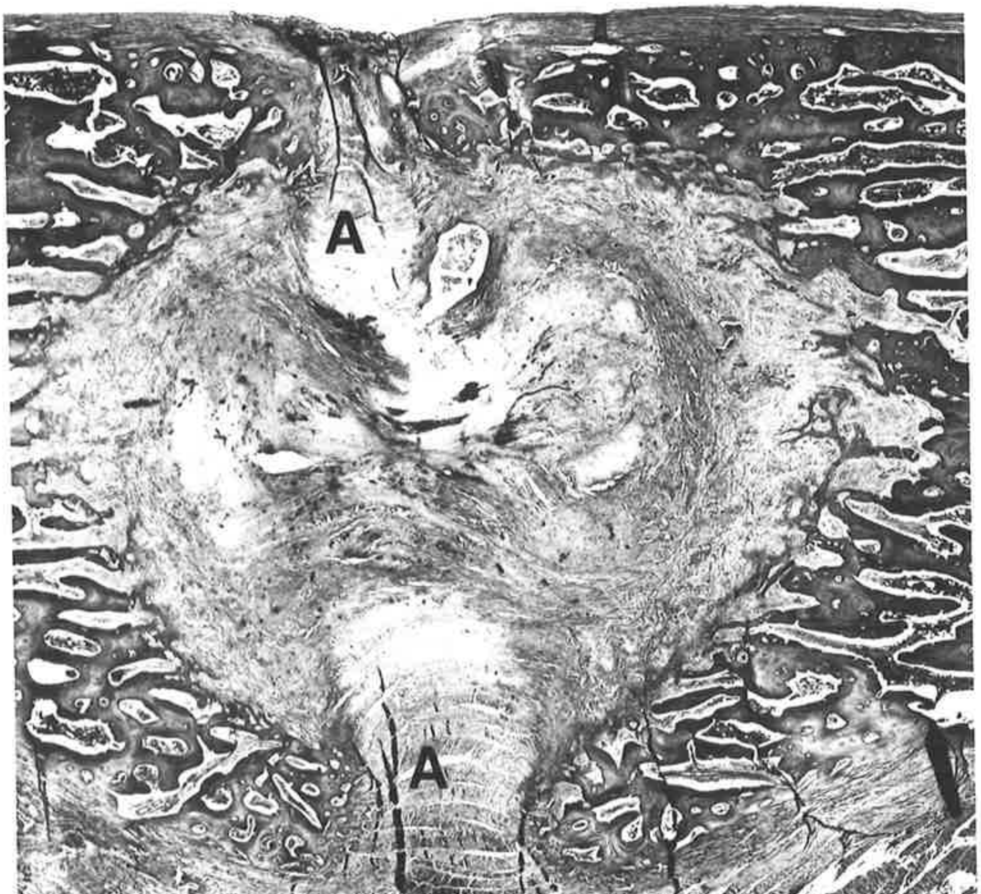
The earliest radiographic change was a reduction in the height of the disc space posteriorly which occurred as early as two weeks after injection at one level. Erosion of the bony end-plate had occurred at three levels by three weeks and at eight of the twelve levels injected with bacteria by five weeks.

There was no radiographic evidence of discitis at any of the twelve levels in the four control sheep where Conray 280 alone were injected (Table IV). Thus the presence of bacteria in the contrast material increased the incidence of discitis ($p = .00016$, Fishers Exact Test).

Fig. 14 Macroscopic view of L2-3 disc in sheep No. 4 six weeks after injection. It shows disorganisation of the nucleus and inner annulus, protrusions of nuclear material into both vertebral bodies, and dark haemorrhagic zones surrounding the protrusions and involving the central area of the disc.

Fig. 15 Low-power micrograph of L3-4 disc in sheep No. 1 six weeks after injection. It shows the disorganisation of the nucleus with preservation of the outer annulus (A), disappearance of end-plates and adjoining vertebral cancellous bone, herniation of nuclear material into both vertebral bodies, granulation tissue and fibrous tissue extending into the central region of the disc with (dark) intradiscal haemorrhages, and new bone forming at the junction of fibrous tissues with the residual cancellous bone of the vertebral bodies. Haematoxylin-eosin X8.




A
A

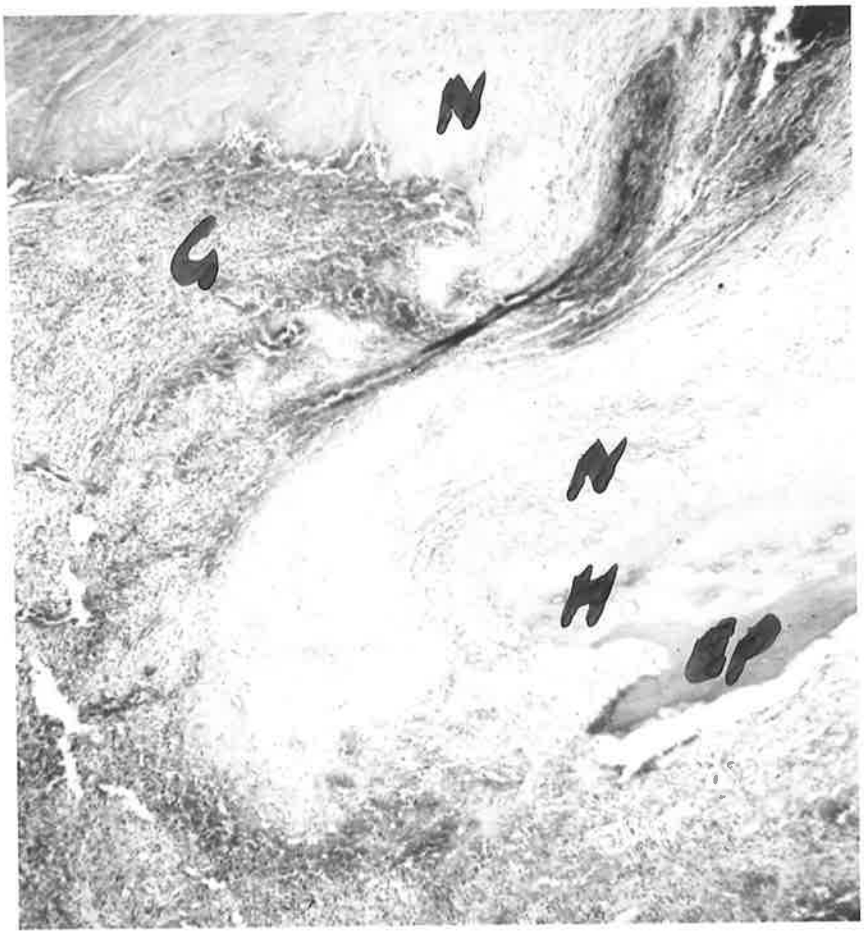
Pathology

At the time of sacrifice (six weeks) macroscopic end-plate lesions and histological changes were found at all levels where radiographic changes had been noted, but there was no macroscopic or microscopic abnormality at any of the remaining levels except for the lower level in sheep number four where there was histological evidence alone of discitis (Table IV).

Macroscopically, the lesions involved the central zones of each abnormal disc affecting predominantly the nucleus pulposus and, in some instances. the inner layers of the annulus fibrosus. That the lesions did not extend through the outer annulus was confirmed by close examination of each disc. Without exception, lesions involved also adjoining vertebral bodies usually affecting both vertebral bodies (Fig. 14). Characteristically, the lesions (Fig. 15) were similar in size and comprised extensive destruction and disorganization of the nucleus pulposus; occasional focal haemorrhages in the central nuclear area; destruction of nuclear material into the vertebral bodies; haemorrhagic areas surrounding extruded nuclear material and extending into the central area of the disc and an outer pale zone surrounding the haemorrhagic areas. Commonly, the lesions in the lower vertebral bodies were larger than in the upper body adjoining the affected disc. Whilst the majority of affected discs showed these features, in a few instances the lesions involved only one (usually lower) vertebral body.

Fig. 16 Low-power micrograph of L3-4 disc in sheep No. 4 six weeks after injection. It shows breach in the end-plate (EP) and hyaline lamina (H), granulation tissue (G) extending into the nucleus. Haematoxylin-eosin X50.

Fig. 17 Medium-power micrograph of L3-4 disc in sheep No. 1 six weeks after injection. It shows vascular channels enlarging by osteoclastic resorption (R) in the end-plate (EP) and extending into the hyaline lamina (H). Young granulation tissue (G) has extended into the adjoining marrow space. Haematoxyling-eosin X100.


N
G
N
H
EP

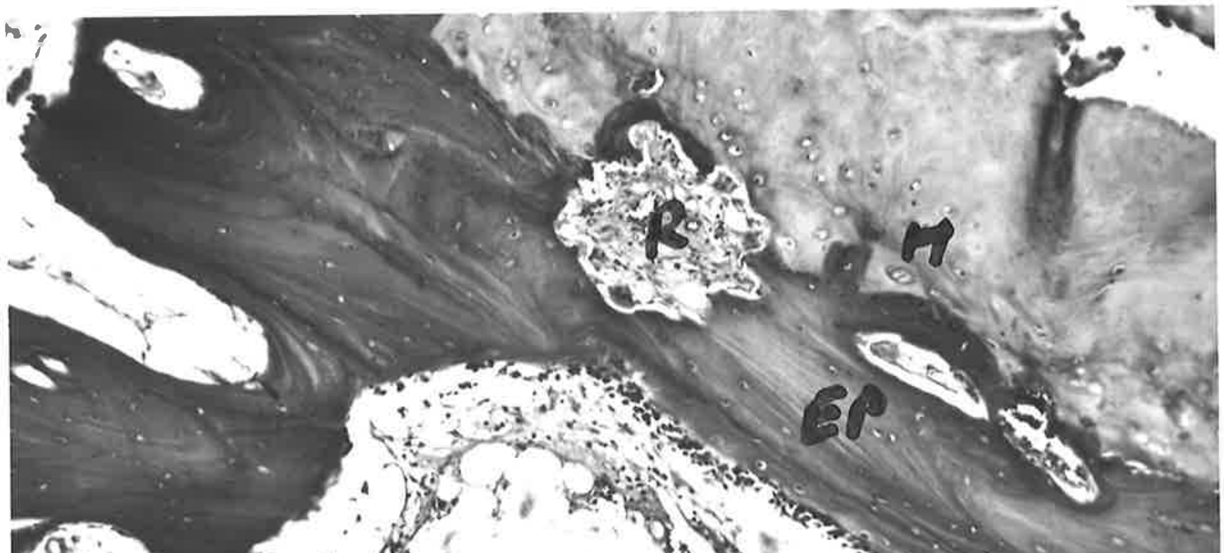
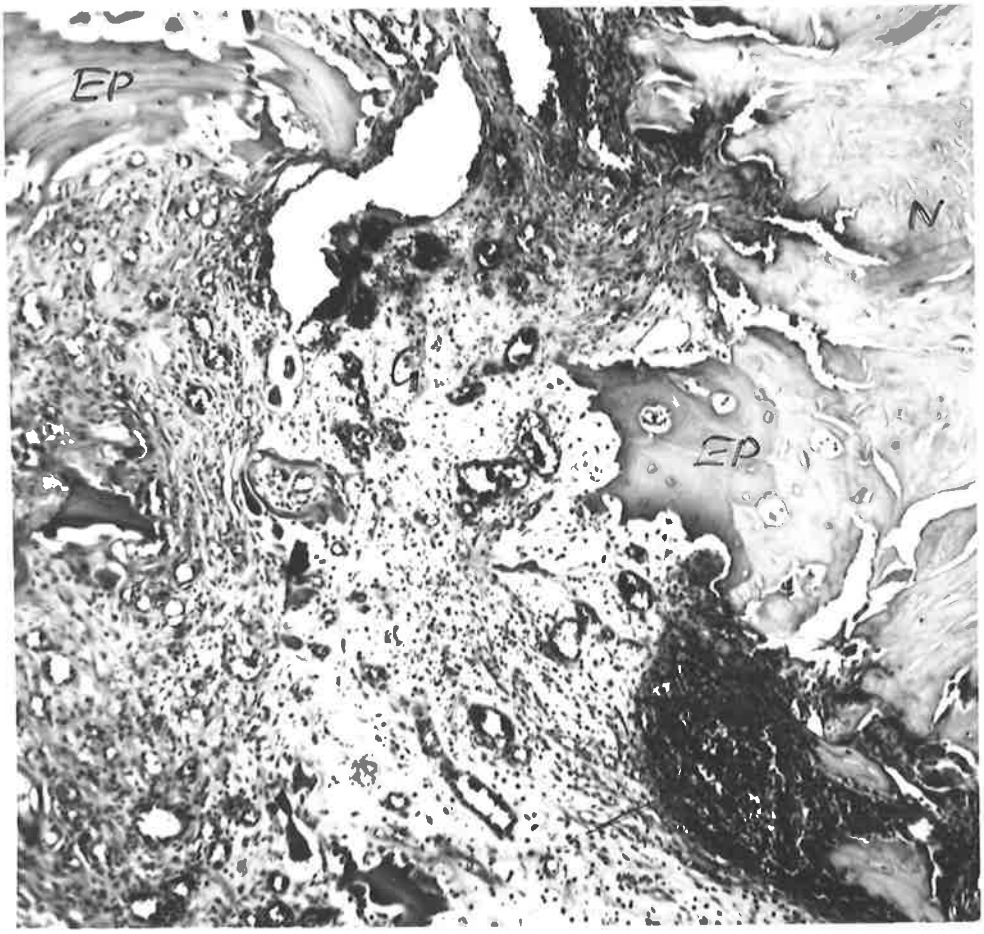

R
M
EP

Fig. 18 Low-power micrograph of L2-3 in sheep No. 3 six weeks after injection. It shows the formation of new woven bone (WB) and appositional bone (AB) at the junction of granulation tissue (G) with residual lamellar bone (LB) in the vertebral body. Haematoxylin-eosin X50.

Fig. 19 Low-power micrograph of L2-3 disc in sheep No. 1 six weeks after injection. It shows that the end-plate (EP) has been breached by granulation tissue (G) extending into the nucleus (N), and the presence of a microabscess (arrow). Haematoxylin-eosin X50.


G
AB
WB
LB


EP
N
G
EP

Microscopically, the discs showing macroscopic abnormalities showed generally similar histological features. Characteristically, there was extensive disorganization of the disc with partial or total replacement of the normally avascular nucleus by granulation tissue of varying maturity (Fig. 15, 16). The granulation tissue contained abundant mature fibrous tissue with occasional scattered small groups of chronic inflammatory cells comprising mainly lymphocytes and plasma cells. In some instances, focal areas of haemorrhage were present in the granulation tissue replacing the nucleus. Granulation tissue also replaced the hyaline laminae, bony end-plates, and variable portions of the adjoining cancellous bone (Fig. 16). Where portions of the hyaline laminae and end-plates remained intact at the margins of the granulation tissue, enlargement of vascular channels by osteoclastic resorption in the end-plate zone (Fig. 17) and vascular invasion through the hyaline laminae often were observed. In the vertebral bodies, new woven bone had formed at the junction of granulation tissue with pre-existing bone and the latter showed appositional new bone formation resulting in sclerosis (Fig. 18). In the majority of lesions, the granulation tissue contained moderate to few inflammatory cells and these were mainly lymphocytes and plasma cells forming small aggregates. However, one or two microabscesses were present in the granulation tissue in three discs (Fig. 19).

The histological findings, therefore, suggested that the earliest change was the disorganization of the end-plate region associated with the presence of granulation tissue and herniation of the nucleus pulposus. By six weeks, there had been extensive replacement of extruded and residual nuclear material by relatively avascular fibrous tissue and with evidence of new bone forming at the periphery of the lesions in the vertebral bodies.

TABLE V

DATA ON THREE SHEEP SACRIFICED ONE, TWO AND THREE WEEKS AFTER INTRA-DISCAL INJECTIONS OF CONRAY 280 AND STAPHYLOCOCCUS EPIDERMIDIS

Sheep (Time of sacrifice)	Levels	Volume (mls.)	Number of Bacteria	Radiographic Discitis	Histological Discitis	Culture
9 (1 week)	L1-2	0.09	1,800	No	No	Positive
	L2-3	0.1	200	No	No	Positive
	L3-4	0.1	20	No	No	Positive
10 (2 weeks)	L1-2	0.09	1,800	No	No	Positive
	L2-3	0.09	180	No	No	Positive
	L3-4	0.09	18	No	No	Positive
11 (3 weeks)	T12-L1	0.09	1,800	Yes	Yes	Negative
	L1-2	0.09	180	Yes	Yes	Negative
	L2-3	0.09	18	Yes	Yes	Negative

Bacterial Culture

A positive culture for staphylococcus epidermidis was obtained at only two levels (Table IV) and the unusual sensitivity pattern confirmed that this was the bacterial strain originally injected. The two level from which a positive culture had been obtained were both injected with an estimated one organism only. Negative cultures were obtained in the control group and from all levels where radiographic and macroscopic evidence of discitis was found.

Haematology

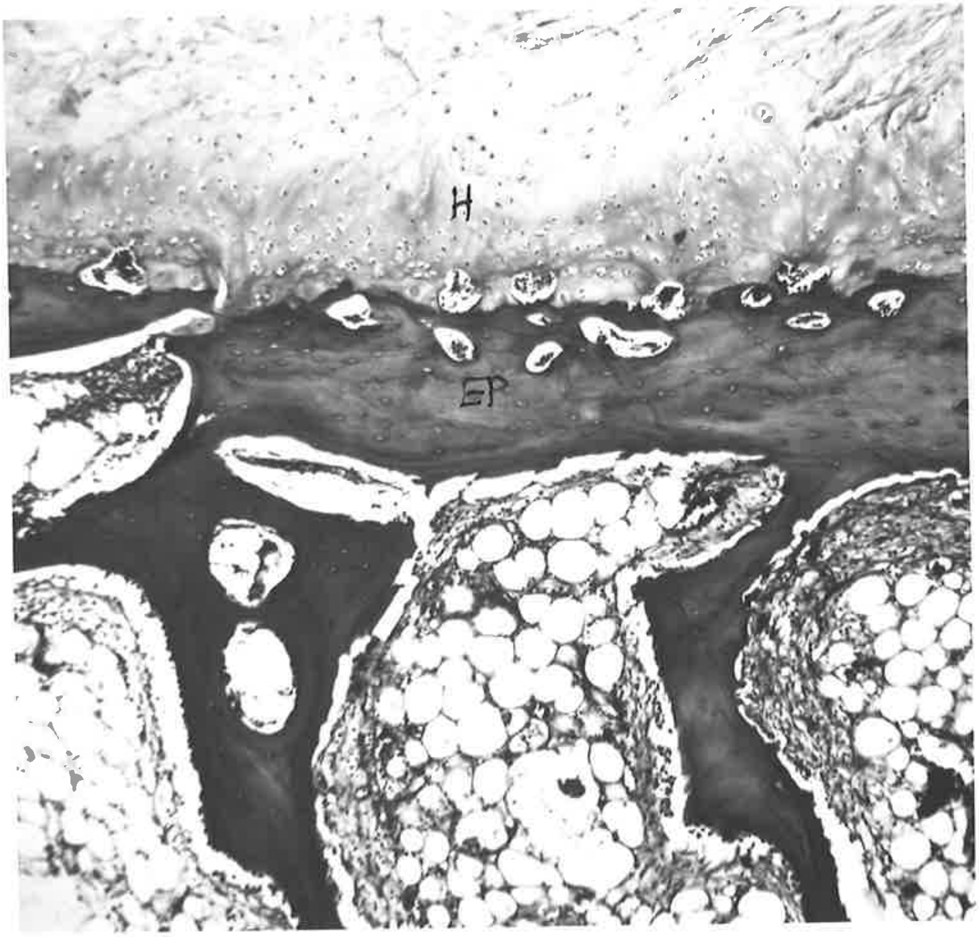
There was no significant change in either the erythrocyte sedimentation rate or the white cell count throughout the study period. Three of the four sheep injected with bacteria developed a raised blood viscosity level from a reading of zero to approximately two. This elevation was first noted at two weeks in each animal. The remaining sheep did not have an increase in the viscosity level although the fourth sheep injected with bacteria was noted to have a raised blood viscosity level of approximately two at the time of surgery.

(b) Sheep sacrificed at weekly intervals (three sheep)

At one week there was no radiographic, macroscopic or histological evidence of discitis, but staphylococcus epidermidis was cultured from all three discs (Table V). At two weeks, the radiographs were normal but the affected discs showed small erosions of the bony end-plate with an associated inflammatory reaction. Staphylococcus epidermidis was cultured from all three levels. The remaining sheep sacrificed at three weeks had developed radiographic and gross macroscopic and histological evidence of discitis at all levels injected. However, staphylococcus epidermidis was not isolated from any of these discs.

Fig. 20 Low-power micrography of L1-2 disc in sheep No. 10 two weeks after injection. It shows intact region of the end-plate (EP) with prominent vascular channels extending into the hyaline lamina (H). The marrow spaces in the adjoining bone show young granulation tissue (G) extending along the trabecular surfaces which are undergoing resorption. Haematoxylin-eosin X50.

Fig. 21 Low -power micrograph of L2-3 disc in sheep No. 11 three weeks after injection. It shows herniations (H) of material from the nucleus pulposus (N) through the end-plate (EP). The dark areas (arrows) capping the herniations represent an acute inflammatory reaction with numerous neutrophil polymorphs. Haematoxylin-eosin X50.


H
EP

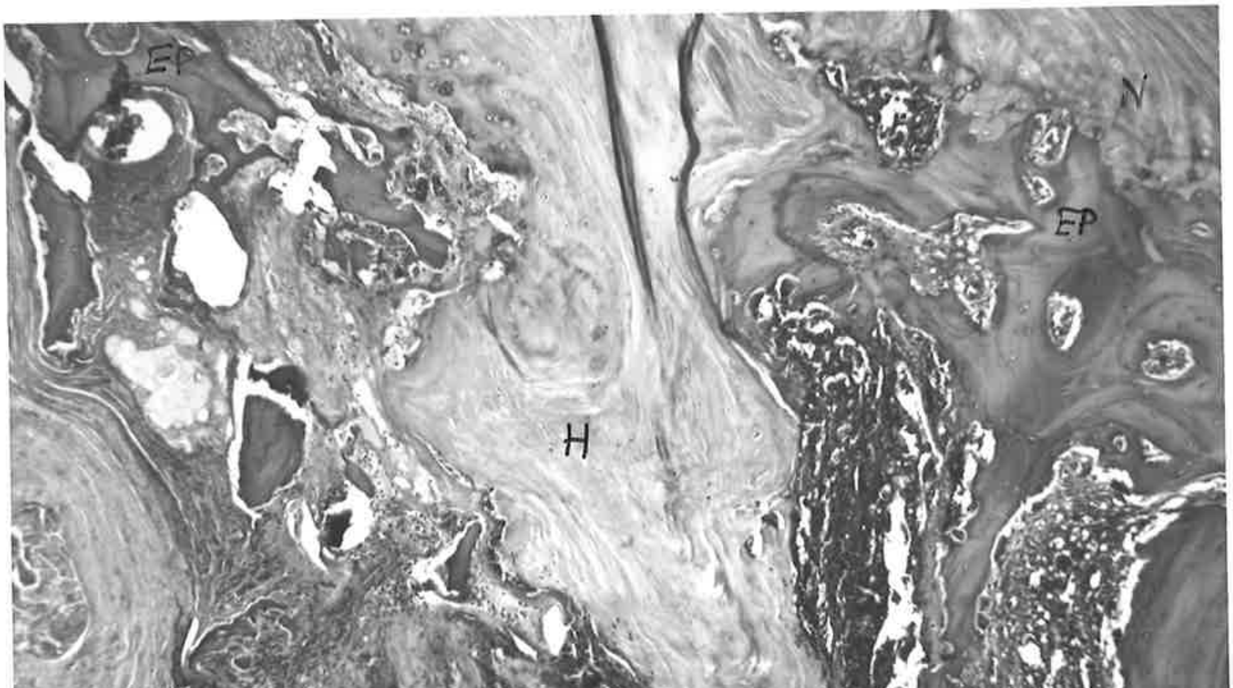
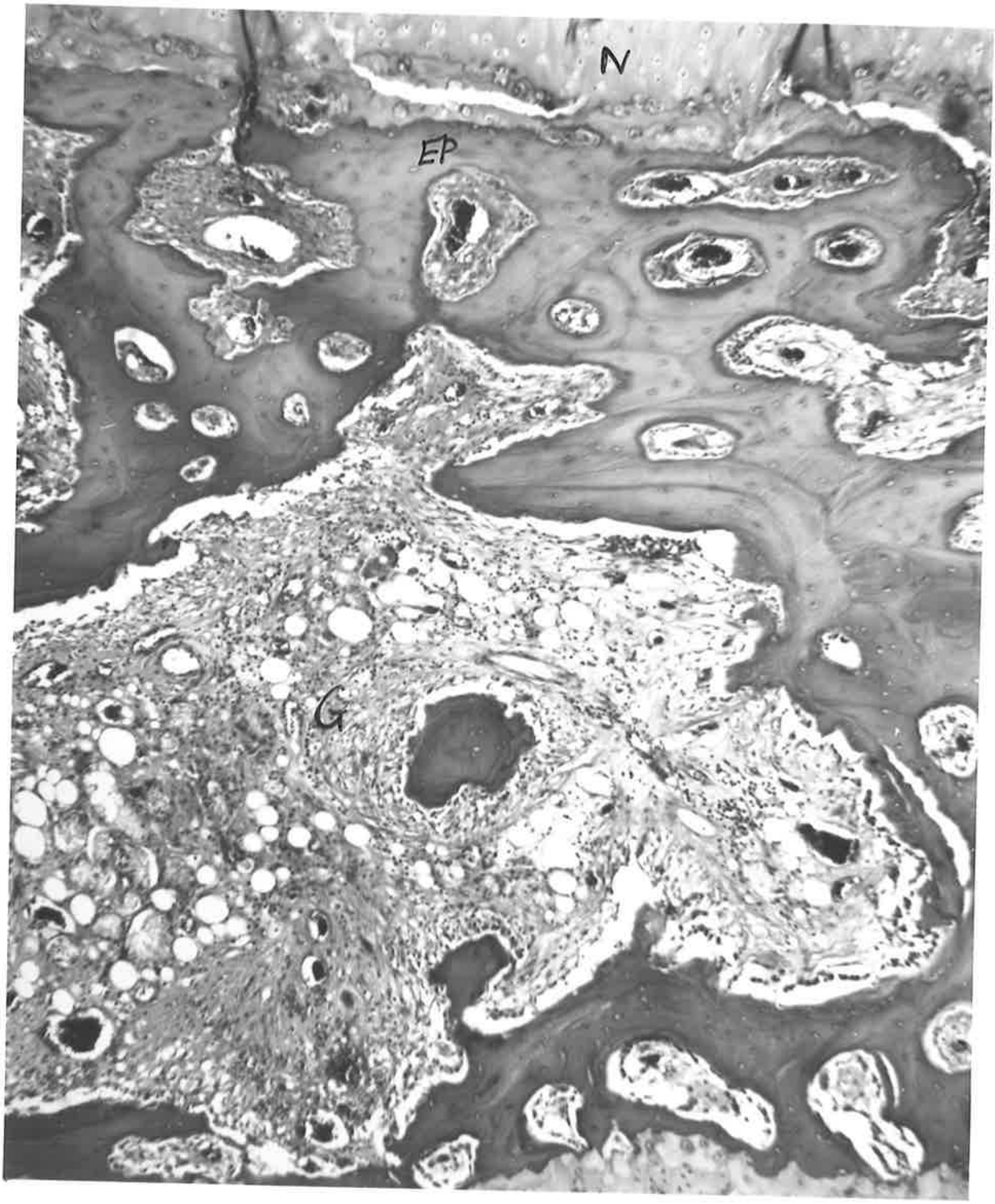

EP
N
EP
H

Fig. 22 Low-power micrograph of L2-3 disc in sheep No. 11 three weeks after injection. It shows extensive replacement of vertebral cancellous bone by granulation tissue (G) adjoining the intact end-plate (EP) and normal nucleus (N). Haematoxylin-eosin X50.


N
EP
G

Pathology

Macroscopically, whilst lesions were not visible at one week after injection, at two weeks the affected discs showed small herniations of nuclear material with an inflammatory reaction confined to the zone close to the end-plate. At three weeks, macroscopic examination showed that there were moderate to large herniations of nuclear material with an extensive surrounding reaction. Microscopically, at two weeks, the bony end-plates and hyaline laminae had been breached in several places by granulation tissue and by the herniation of nuclear material. These changes were associated also with more extensive young granulation tissue occupying marrow spaces adjoining intact zones of the end-plate, early bone resorption and with enlargement of vascular channels and their extension into the hyaline laminae (Fig. 20). In some instances, haemorrhage into the central nucleus had taken place. In all cases, neutrophil polymorphs were restricted to the zone immediately surrounding recently extruded nuclear material.

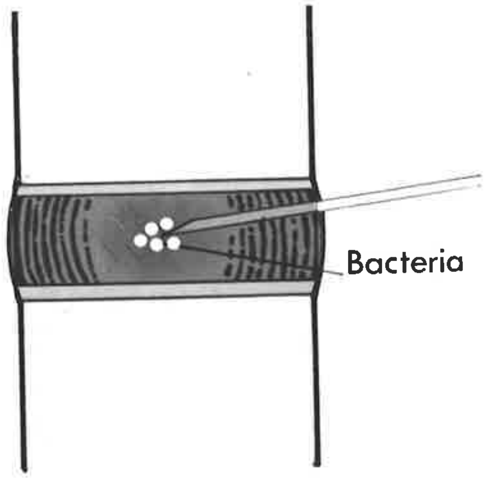
At three weeks, there was much more extensive loss of end-plates and hyaline laminae associated with larger protrusions of nuclear material into the vertebral bodies. In some instances, there had been extensive haemorrhage into the residual and extruded nuclear material. The extruded nuclear material showed marked "capping" by neutrophil polymorphs in places (Fig. 21), but the granulation tissue generally showed a paucity of acute inflammatory cells. A striking feature was the extensive replacement of vertebral bone by granulation tissue even in regions where the end-plate and nucleus were normal (Fig. 22).

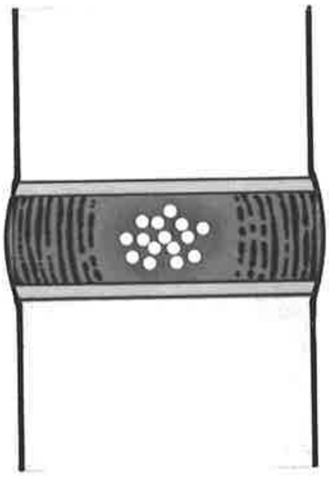
Fig. 23 The stages in the development of discitis after discography.

a. Bacteria introduced on tip of needle.

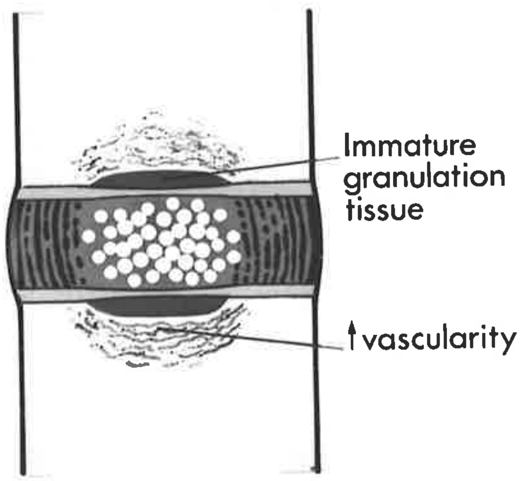
b. At 1 week the bacteria have multiplied but there are no other abnormalities.

c. At 2 weeks the end-plate is thinned but still intact. Immature granulation tissue has formed on the vertebral side of the end-plate and this is surrounded by a vascular response.





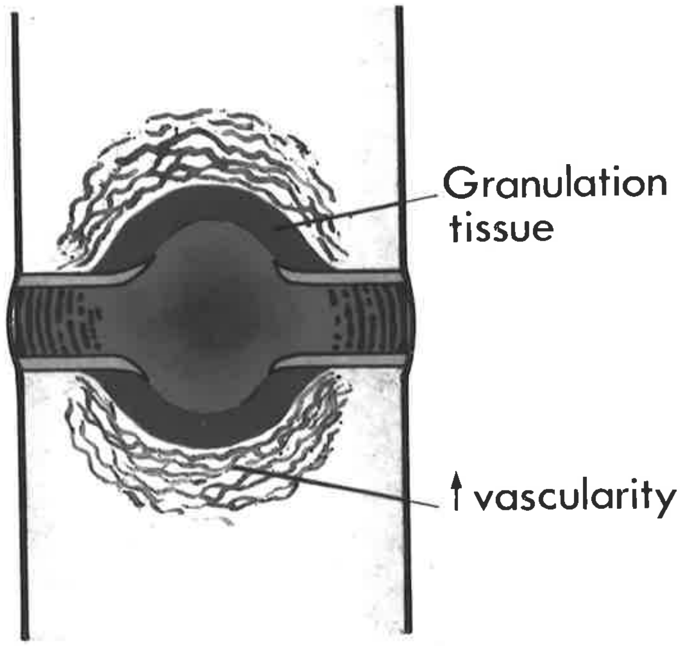
1 WEEK



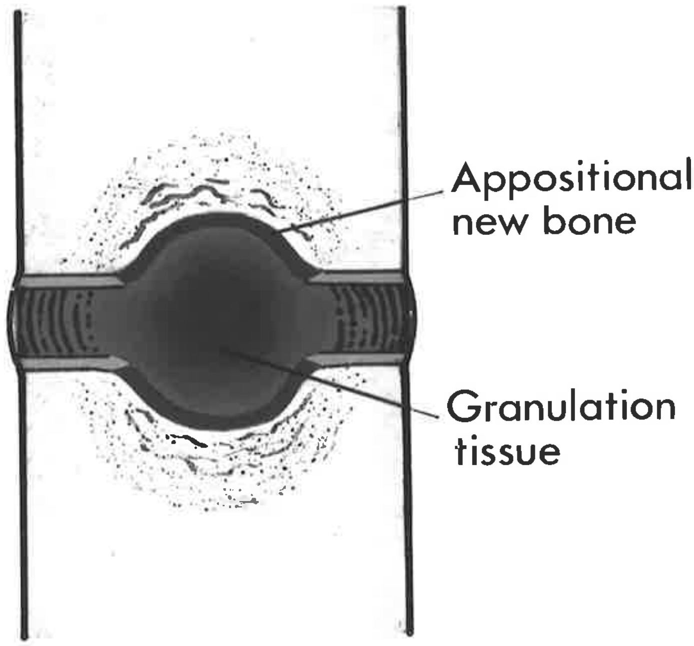
2 WEEKS

Fig. 23 d. Three weeks after inoculation the end-plates have been ruptured by the herniation of nuclear material into the vertebral bodies. Granulation tissue lies against the herniated nucleus and a marked vascular response is present. At this stage the bacteria have usually been destroyed.

Fig. 23 e. By six weeks the lesion is approaching maturity with appositional new bone against the nuclear material which has been largely replaced by granulation tissue.



3 WEEKS



6 WEEKS

DISCUSSION

The results of this experimental study offer an explanation for the changes previously attributed to a chemical reaction and indicate that discitis following intradiscal injection is probably always initiated by infection. The sheep is renowned for its ability to resist infection and yet in this study an estimated single staphylococcus epidermidis injected into the intervertebral disc under aseptic conditions was able to produce radiographic, macroscopic and histological discitis. The reaction produced by a single organism was the same as that produced by 32,000 organisms. In both cases organisms could not be isolated at sacrifice. This observation and the general absence or paucity of neutrophil polymorphs is not consistent with bacterial inflammation, and suggests that bacteria are initiators but not promoters of the response.

The normal adult intervertebral disc is a largely avascular structure which obtains its nutrition by diffusion across the central end-plate and from the outer annulus. Because of its normal isolation from vascular tissue, the nucleus pulposus may be considered to be an ideal culture medium. The earliest change of discitis is the appearance of granulation tissue on the vertebral side of the bony end-plate associated with small defects in the hyaline laminae and the bone end-plate. This is followed by protrusion of nuclear material and an increase in vascularity of the adjacent vertebral bone. This study has demonstrated that once the bony end-plate is breached the bacteria are rapidly removed (Fig. 23). The chronic inflammatory response with lymphoid cell infiltration may be associated with an immunological reaction, and offers an explanation for the observation that many patients with established radiographic signs of discitis gain improvement in their symptoms with treatment by intradiscal cortico-steroids (Crock 1983). The identification of bacteria six weeks after inoculation in two discs with no macroscopic end-plate destruction is in keeping with the late presentation of radiographic changes in occasional patients.

The late (six weeks) lesions were similar in structure to the human Schmorl's nodes and this raises the possibility of an infective etiology for the nodes in some instances.

The cause of intradiscal haemorrhage noted in the sheep is obscure as this is not a feature of material obtained from humans with discitis (Fraser, Osti and Vernon-Roberts 1985).

The erythrocyte of the sheep is quite different in shape and size to the human red blood cell. Whereas the erythrocyte sedimentation rate is a useful indicator of inflammation in the human, in the sheep is rarely raised (Greenwood 1977). Blood viscosity seems a more reliable indicator of inflammation in some animals (Archer and Allen 1970) and this distinction was confirmed by the results of this study. The white blood cell count in sheep with infective discitis did not alter significantly and this finding was similar to that seen with discitis following discography in the human.

Clinical experience has led us to believe that discitis is a common and important complication of intradiscal injection (Fraser, Osti and Vernon-Roberts 1986). The incidence has possibly been overlooked because of the latent period between the injection procedure and the onset of symptoms with the erythrocyte sedimentation rate often not being elevated for 10 days or more, the lack of clinical contact between the patient and the radiologist carrying out the procedure, and a lack of awareness by the clinician.

The conclusion from this experimental study is that probably all cases of discitis following intradiscal injections are due to bacteria introduced by the needle tip and this highlights the need for strict aseptic technique with any intradiscal procedure.

DISCITIS FOLLOWING CHEMONUCLEOLYSIS - AN EXPERIMENTAL STUDY

MATERIALS AND METHODS:

In Vitro Study: Two 1ml samples of reconstituted chymopapain were inoculated together with a small number of E. Coli and staphylococcus epidermidis. At 1, 2, 4, and 8 hours after inoculation a 100ul sample was removed from each of the two samples and plated on to sheep blood agar and spread over the surface of the plate using a sterile glass rod. All plates were incubated at 35^{O}C for 24 hours and the number of colonies (organisms recovered) counted.

Aliquots of meglumine iothalamate 60% (Conray 280) and chymopapain (immediately after reconstitution with distilled water) were dispensed into tubes. Dilutions of the organisms to be tested were added to both the Conray and chymopapain, such that the final number of organisms in each preparation was approximately 100 organisms per 100ul. Immediately after the addition of the organisms to each preparation, a 100ml. aliquot was removed from each sample and transferred onto sheep blood agar plates. The samples were then spread over the surface of the plates, and the plates incubated at 35^{O}C for 18-24 hours. After incubation the number of organisms recovered was determined. Samples were removed at 4, 8, 24, and 48 hours, and processed in a similar manner. The results of the count were expressed as colony-forming units with each unit being approximately equal to one organism. The following organisms were tested in this manner:-

Pseudomonas Aeruginosa, Staphylococcus Aureus, Beta Haemolytic Streptococcus, Klebsiella Pneumoniae, Streptococcus Faecalis, and Proteus Mirabilis.

In Vivo Study: Eight adult sheep (Merino wethers) were entered into this study. Each animal was premedicated with 1.5mg of Acepromazine, anaesthetised with 0.5gm of intravenous Sodium Pentothal, intubated using a 9mm endotracheal tube, and anaesthesia was maintained with 1.5% Halothane in two litres of oxygen and two litres of Nitrous Oxide per minute. The lumbar spine was then exposed through a left retroperitoneal muscle splitting approach using a vertical skin incision anterior to the transverse processes to expose up to five adjacent lumbar intervertebral discs.

Using 27.5 gauge needles a total of 32 discs in eight sheep were injected under direct vision. In four sheep reconstituted chymopapain (Discase) only was injected to a maximum volume of 0.12ml per disc. In the other four, reconstituted chymopapain and a bacterial suspension comprising different dilutions to a maximum volume of 0.15ml per disc was injected. During the procedure plain radiographs were taken to record the levels injected.

The bacterial suspension used for injection was prepared from a strain of staphylococcus epidermidis which was sensitive to Erythromycin, Clinomycin, Sulpha Methoxazole, Penicillin, Tetracycline, Gentamycin, and Methicillin. This fully sensitive strain was chosen for the study as almost all contaminants in our hospital environment are multiresistant. The isolate was grown in brain heart infusion broth for forty eight hours. Plate counts were carried out on different dilutions prepared in snap freeze broths to determine the number of organisms at each dilution and the dilutions then stored at -170 degrees centigrade in liquid nitrogen. For the preparation of the intradiscal injection, appropriate dilutions were thawed and an aliquot added to meglumine iothalamate 60% such that in 0.1 millilitres of suspension a known number of organisms were present.

In two sheep the following approximate concentrations of organisms were injected into three adjacent discs: 20,000 organisms per 0.1ml, 200 organisms per 0.1ml, and 10 organisms per 0.1ml. Another two sheep were injected with approximately ten organisms per 0.1ml into five adjacent discs. Plate counts were repeated at the time of the intradiscal injections to check again the concentration of organisms.

Following the intradiscal injection the wound was closed using resorbable suture material and the sheep housed in pens. At the time of surgery and at weekly intervals thereafter, plain lateral radiographs of the lumbar spine were taken. Six weeks following the intradiscal injection all animals were sacrificed with intravenous barbiturate and, using aseptic technique, a needle biopsy of nuclear material was obtained from each of three discs injected and sent for culture and sensitivity. The nuclear material biopsy was then inoculated to the following culture media: (1) blood agar incubated in 5% carbon dioxide; (2) anaerobic agar incubated in 7% carbon dioxide, 83% nitrogen and 10% hydrogen; (3) glucose cooked meat broth. The media (1) and

(2) were incubated for seven days and inspected each day for growth. The medium (3) was incubated for seven days and then cultured if turbid or clear for two days on media (1) and (2).

The lumbar spine was removed by transecting the vertebral column through T12 above and the mid sacrum below. Following fixation in 10% formal-saline, spines were divided in the sagittal plane using a fine tooth bandsaw. The surfaces were then gently cleaned with a soft brush under a stream of cold water to remove surface debris and were examined macroscopically. Following photography of selected features of the cut surfaces a three millimetre thick sagittal slab was made using a bandsaw and the pedicles were divided to provide a slab composed of all the lumbar vertebral bodies and intervertebral discs. By cutting across the mid point of each vertebral body in the slab, blocks for histological processing were made comprising half of each vertebral body above and below the interposed intervertebral disc. Blocks were immersed in decalcifying fluid and the progression of decalcification checked daily by radiography. After decalcification the blocks were processed, embedded in wax, and five micrometre sections were stained with haematoxylin and eosin. In some instances, following the making of a sagittal slab for histology, some discs of the residual portions were divided through their centre parallel to the end plates to determine macroscopically the extent of the lesions within the discs.

TABLE VI

EFFECT OF CHYMOPAPAIN ON STAPH. EPIDERMIDIS AND E. COLI IN VITRO

Staph. Epidermidis		E. Coli	
T = 0	120 organisms	T = 0	150 organisms
T = 1 hr.	60 organisms	T = 1 hr.	150 organisms
T = 2 hr.	7 organisms	T = 2 hr.	50 organisms
T = 4 hr.	1 organism	T = 4 hr.	40 organisms
T = 8 hr.	0 organism	T = 8 hr.	20 organisms

TABLE VII

ORGANISM INHIBITION BY CONRAY 280 AND CHYMOPAPAIN IN VITRO

ORGANISM	PREP	TIME				
		OHR	4HR	8HR	24HR	48HR
PS.AERUGINOSA	CON	76	73	65	65	60
	PAP	80	10	5	0	0
S. AUREUS	CON	47	46	42	42	38
	PAP	43	11	0	0	0
B HAEMOLYTIC	CON	49	45	32	18	4
STREPTOCOCCUS	PAP	55	0	0	0	0
K.PNEUMONIAE	CON	76	74	72	68	59
	PAP	73	58	20	0	0
S.FAECALIS	CON	56	59	55	53	34
	PAP	54	0	0	0	0
P. MIRABILIS	CON	98	94	94	90	70
	PAP	92	73	44	20	4

T A B L E VIII

DATA ON 8 SHEEP SACRIFICED 6 WEEKS AFTER INTRADISCAL INJECTION OF CHYMOPAPAIN + STAPHYLOCOCCUS EPIDERMIDIS

SHEEP	LEVELS	VOLUME (MLS)	NUMBER OF BACTERIA	RADIOGRAPHIC & MACROSCOPIC	HISTOLOGICAL DISCITIS	CULTURE FOR STAPH.EPIDERMIDIS
1.	L1-2	0.13	2,500	YES	YES	+ (BROTH CULTURE)
	L2-3	0.13	250	YES	YES	-
	L3-4	0.09	20	YES (Macro. only)	YES	+ (MOD.GROWTH)
2.	L1-2	0.15	2,500	YES	YES	-
	L2-3	0.13	250	YES	YES	-
	L3-4	0.09	20	YES	YES	-
3.	T12-L1	0.10	100	YES (Macro. only)	YES	+ (SCANT GROWTH)
	L1-2	0.10	40	YES	YES	-
	L2-3	0.10	10	YES	YES	-
	L3-4	0.10	10	YES	YES	-
	L4-5	0.10	10	YES	YES	-
4.	T12-L1	0.10	10	YES	YES	+ (SCANT GROWTH)
	L1-2	0.10	10	YES	YES	+ (SCANT GROWTH)
	L2-3	0.10	10	YES	YES	+ (BROTH CULTURE)
	L3-4	0.10	10	YES	YES	-
	L4-5	0.10	10	YES	YES	-
5.	L1-2	0.11	0	NO	NO	-
	L2-3	0.10	0	NO	NO	-
	L3-4	0.10	0	NO	NO	-
6.	L1-2	0.09	0	NO	NO	-
	L2-3	0.11	0	NO	NO	-
	L3-4	0.12	0	NO	NO	-
7.	T12-L1	0.10	0	NO	NO	-
	L1-2	0.10	0	NO	NO	-
	L2-3	0.10	0	NO	NO	-
	L3-4	0.10	0	NO	NO	-
	L4-5	0.10	0	NO	NO	-
8.	T12-L1	0.10	0	NO	NO	-
	L1-2	0.10	0	NO	NO	-
	L2-3	0.10	0	NO	NO	-
	L3-4	0.10	0	NO	NO	-
	L4-5	0.10	0	NO	NO	-

RESULTS:

A. IN VITRO STUDY:

Chymopapain had a bactericidal effect on all the organisms tested (Tables VI and VII). The effect was more pronounced with gram positive organisms which have a higher concentration of protein in their cell walls than gram negative organisms whose cell wall is composed of lipopolysacaride. Conray, however, showed very little if any antibacterial activity, even after 48 hours incubation. The organism counts remained reasonably constant during the time the tests were run, indicating that Conray was not able to act as a growth medium for any of the test organisms (Table VII).

B. IN VIVO STUDIES:

Radiology

Narrowing of the disc space height occurred in all of the levels injected in both groups of sheep (Table VIII). Erosion of the bony end-plates occurred at fourteen of the sixteen levels where bacteria were injected, but did not occur at any level where chymopapain only was injected. The extent of end-plate erosion did not seem to be related to the number of organisms injected (Fig. 24).

Disc space narrowing was not detected one week after the injection, but by two weeks a reduction in the height of the disc space was noted in 26 of the 32 levels injected. Three weeks after the injections, radiographs demonstrated end-plate lesions in eight of the sixteen levels injected with bacteria. At the time of sacrifice (six weeks) fourteen of the sixteen levels injected with bacteria demonstrated end-plate erosion. The majority of these lesions corresponded to the nucleus pulposus but in 7 cases the end-plate lesions were anteriorly placed (Fig. 24).

Fig. 24 Radiograph showing the lower three discs of sheep No. 3 injected with chymopapain and staphylococcus epidermidis. Anterior end-plate destruction is demonstrated at the upper level, disc space narrowing with no end-plate destruction at the middle level and central and anterior end-plate destruction at the lower level.

Fig. 25 Lateral radiograph of sheep No. 5 demonstrating disc space narrowing with an associated lumbar kyphosis from three level intradiscal chymopapain.

TABLE IX

SUMMARY AND GRADING OF HISTOLOGICAL FEATURES

SHEEP	LEVELS	NUCLEAR DEGENERATION/ CAVITATION	DEGENERATION OF ANTERIOR ANNULUS	FORWARD PROTRUSION OF NUCLEUS	CENTRAL END-PLATE DESTRUCTION & DISCITIS	ANTERIOR END-PLATE DESTRUCTION DISCITIS	NEW BONE ON ANTERIOR VERTEBRAL BODY
1	L1-2	N.A.	N.A.	N.A.	++++	+++	+++
	L2-3	++	++	N.A.	++++	++++	+++
	L3-4	+++	++	+	–	+++	+++
2	L1-2	++	++	++	–	++++	+++
	L2-3	++	+	++	–	++++	++++
	L3-4	++	N.A.	N.A.	+++	++++	+++
3	T12-L1	++	++	–	++++	+	–
	L1-2	+	++	++	–	++++	++++
	L2-3	++++	+++	–	+++	–	–
	L3-4	N.A.	N.A.	N.A.	++++	++++	++++
	L4-5	+++	++	++	–	++++	++++
4	T12-L1	+++	+++	+++	–	+++	++
	L1-2	+	+++	+++	–	+++	++
	L2-3	N.A.	N.A.	N.A.	++++	+++	++++
	L3-4	N.A.	++++	N.A.	++++	+++	++++
	L4-5	++++	+++	N.A.	++++	++	++++
5	L1-2	++++	+++	–	–	–	–
	L2-3	++++	+++	–	–	–	–
	L3-4	+++	++++	–	–	–	–
6	L1-2	+++	+++	–	–	–	–
	L2-3	++++	+++	–	–	–	–
	L3-4	++++	+++	–	–	–	–
7	T12-L1	+++	++	–	–	–	–
	L1-2	+	+	–	–	–	–
	L2-3	++	–	–	–	–	–
	L3-4	+++	++	–	–	–	–
	L4-5	++	–	–	–	–	–
8	T12-L1	++	+++	–	–	–	–
	L1-2	+	–	–	–	–	–
	L2-3	+	–	–	–	–	–
	L3-4	+	–	–	–	–	–
	L4-5	+	–	–	–	–	–

N.A. = not assessable due to changes of discitis.
– = absent

There was no radiographic evidence of end-plate destruction at any of the sixteen levels in the four control sheep where chymopapain alone winjected (Fig. 25).

Pathology

At the time of sacrifice (6 weeks) macroscopic and histological evidence of discitis was found at all levels where radiographic evidence of end-plate destruction had been noted. In addition, histological evidence of discitis was found at the two levels in sheep injected with bacteria where end-plate destruction was not observed with radiographs (Table VIII).

All non-injected discs were macroscopically and microscopically normal. The pathological findings are summarized in Table IX.

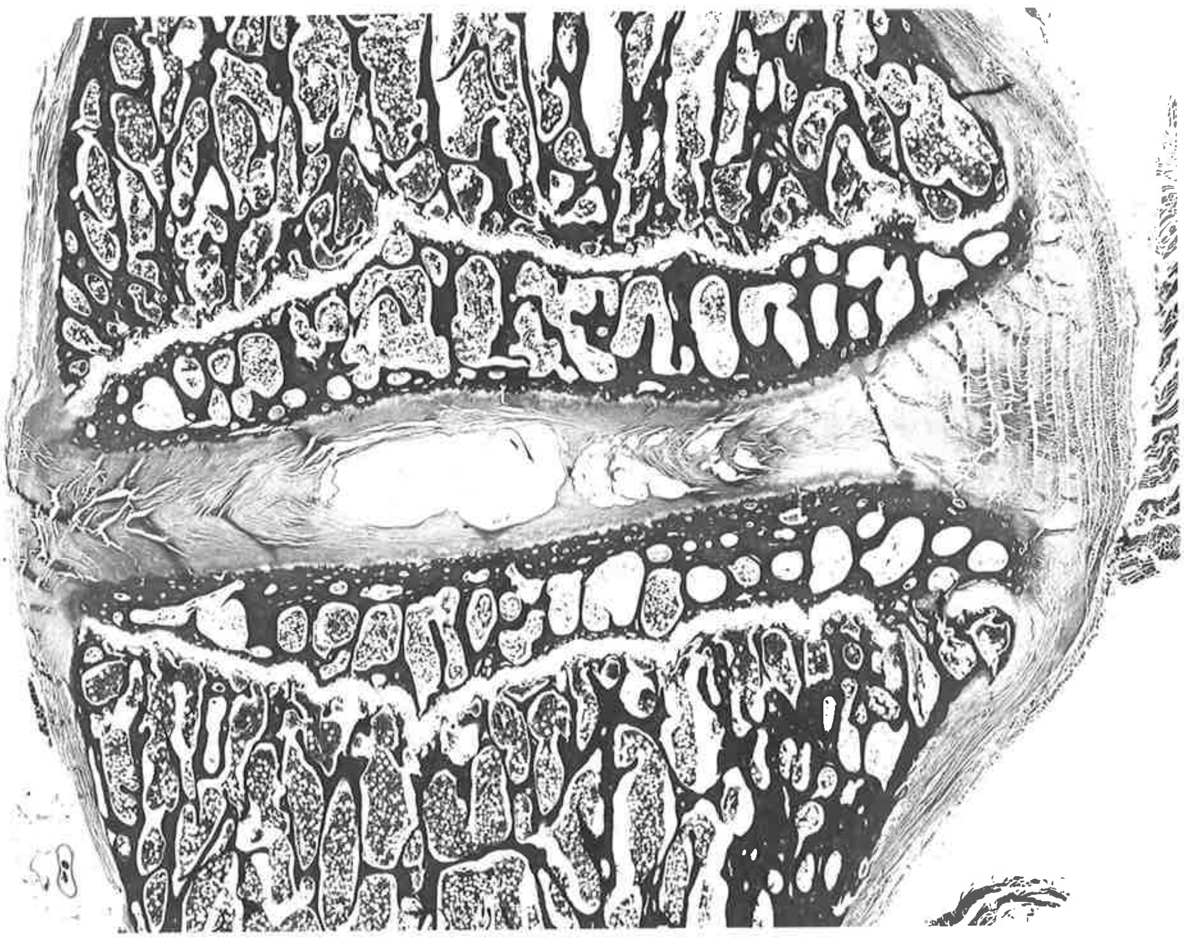
In the discs which had been injected with chymopapain alone, macroscopically there was marked narrowing compared with normal non-injected discs (Fig. 26), but the end-plates were intact. Frequently, the area previously occupied by the nucleus pulposus exhibited the formation of cyst cavities (Fig. 27), and clefts occupying the nuclear zone and arranged parallel to the end-plates invariably were present and frequently extended into the fibres of the posterior annulus (Fig. 26). Microscopically, the nuclear region invariably showed degenerative changes which were accompanied frequently by the formation of cystic cavities (Fig. 28). The degenerative changes always observed in the nucleus sometimes

Fig. 26 Macroscopic view of portion of spine in sheep No. 6. The T12-L1 disc (left) is normal. The L1-2 disc (right) injected with chymopapain shows marked narrowing and clefts present in the nuclear region and in the posterior annulus.

Fig. 27 Macroscopic view of L3-4 disc in sheep No. 5. Shows marked narrowing of the disc with cyst formation in the nuclear area following chymopapain injection.

Fig. 28 Lower power micrograph of L3-4 in sheep No. 5. Following chymopapain injection there has been degeneration and cystic cavitation of the nucleus. Haematoxylin-eosin X8.

Fig. 29 Lower power micrograph of L4-5 disc in sheep No. 5. Cystic degeneration of the nucleus and marked degeneration of the inner annulus has taken place following chymopapain injection. Haematoxylin-eosin X8.



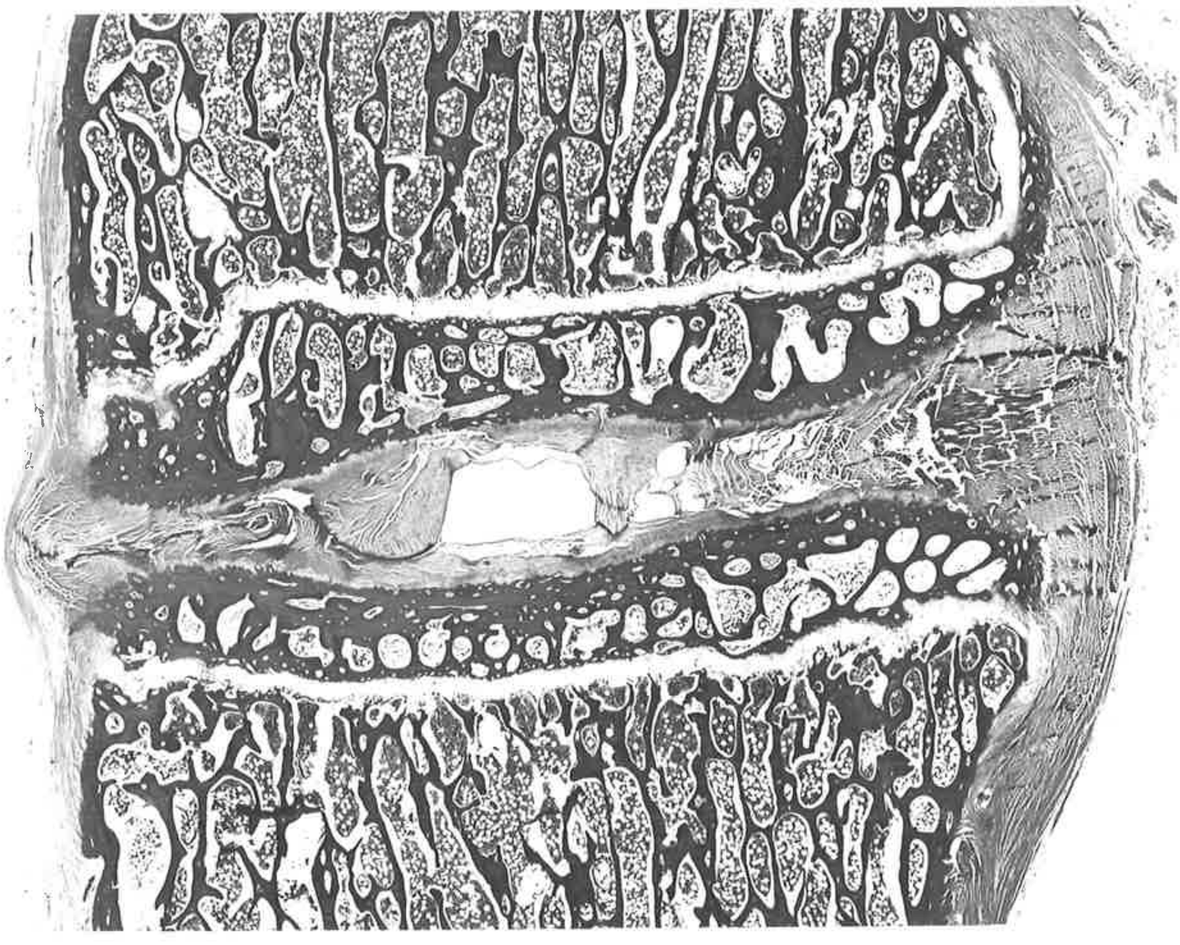


Fig. 30 Medium power micrograph of L4-5 disc in sheep No. 5. Shows cystic degeneration of nucleus (left) and marked degeneration of annular fibres (A) following chymopapain injection. Small amounts of granulation tissue (arrows) are present in the marrow spaces adjoining the bone plate. Haematoxylin-eosin X50.

Fig. 31 Low power micrograph of T12-L1 disc in sheep No. 7. Shows degeneration of nucleus following chymopapain injection with cleft (arrow) extending through the posterior annulus. Haematoxylin-eosin X8.


A
A

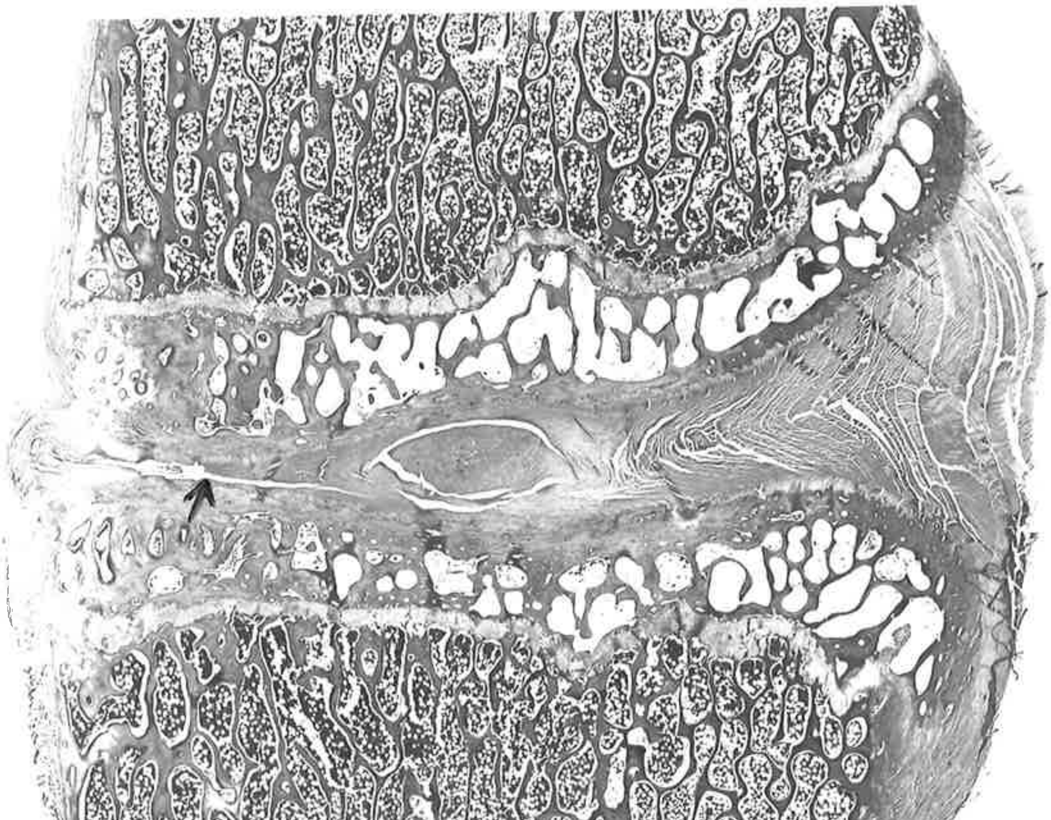
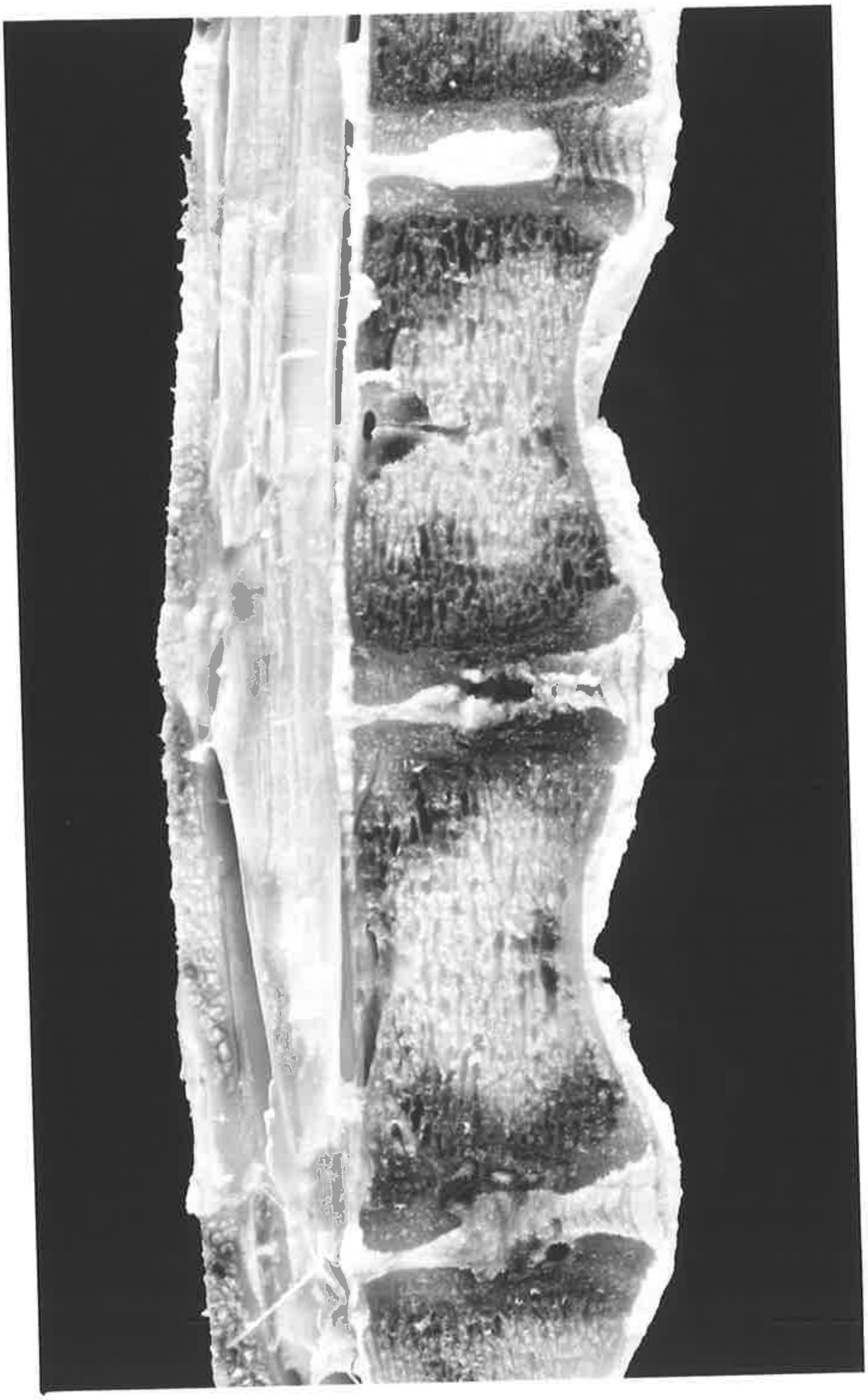


Fig. 32 Macroscopic view of portion of spine in sheep No. 4 following injection of chymopapain and bacteria at all three levels illustrated. L3-4 disc (left) shows largely central discitis; L2-3 disc (centre) shows combination of central and anterior discitis; and L1-2 disc (right) shows anterior discitis alone.

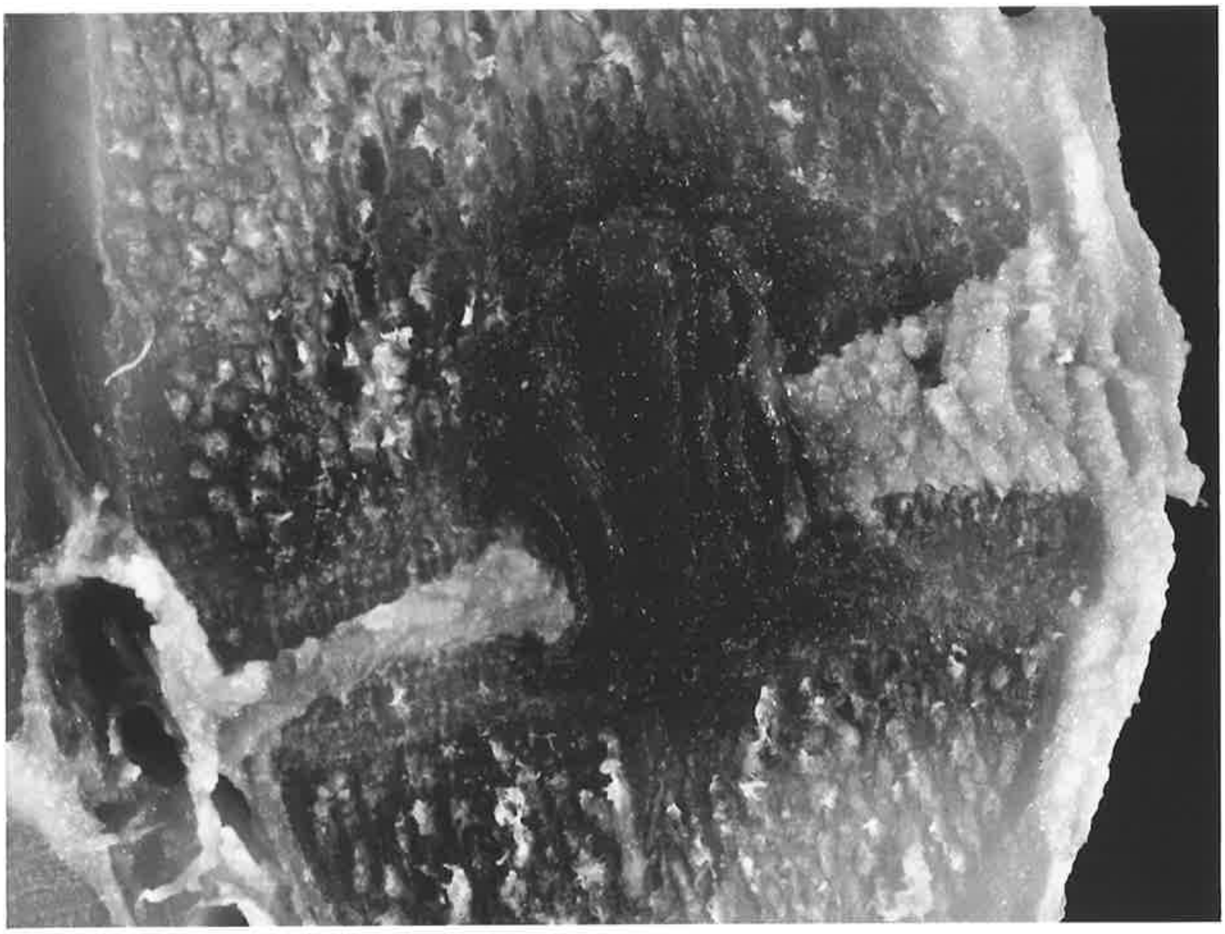


extended to involve the anterior annulus to a varying degree with occasional cystic cavitation having taken place in the inner annulus (Fig. 29, 30). While the posterior annulus did not show significant degenerative changes or cystic cavitation, clefts present in the nuclear region were observed rarely to extend through the posterior annulus (Fig. 31). Microscopy confirmed that the end-plates were intact, and that no nuclear herniations were present. There was no cellular evidence of inflammation within the disc, but small amounts of granulation tissue and small groups of inflammatory cells were observed occasionally in the marrow spaces immediately adjoining the intact end-plates (Fig. 30).

In all discs which had been injected with bacteria, evidence of discitis was present. Macroscopically, discitis was observed as involving the central region of the disc (Fig. 32 left), the anterior annulus (Fig. 32 right), or a combination of both regions (Fig. 32 centre). Central discitis was associated with destruction of both end-plates with herniation of nuclear material into the vertebral bodies (Fig. 33): and, frequently, extensive haemorrhage had occurred within (Fig. 33) and around (Fig. 33) nuclear material in the region of end-plate destruction. Anterior discitis usually was associated with haemorrhage among the layers of the anterior annulus and anterior internal protrusion of the nucleus (Fig. 34), destruction of both anterior end-plates (Fig. 34), and new bone formation at the anterior vertebral rims (Fig. 34).

Fig. 33 Macroscopic view of L3-4 disc in sheep No. 4 following injection of chymopapain and bacteria. Shows marked central discitis with destruction of end-plates and extensive haemorrhage.

Fig. 34 Macroscopic view of L1-2 disc in sheep No. 4 following injection of chymopapain with bacteria. Shows anterior discitis with destruction of end-plates, anterior displacement of nucleus, haemorrhage among annular fibres, and new bone formation (arrows) on vertebral bodies.



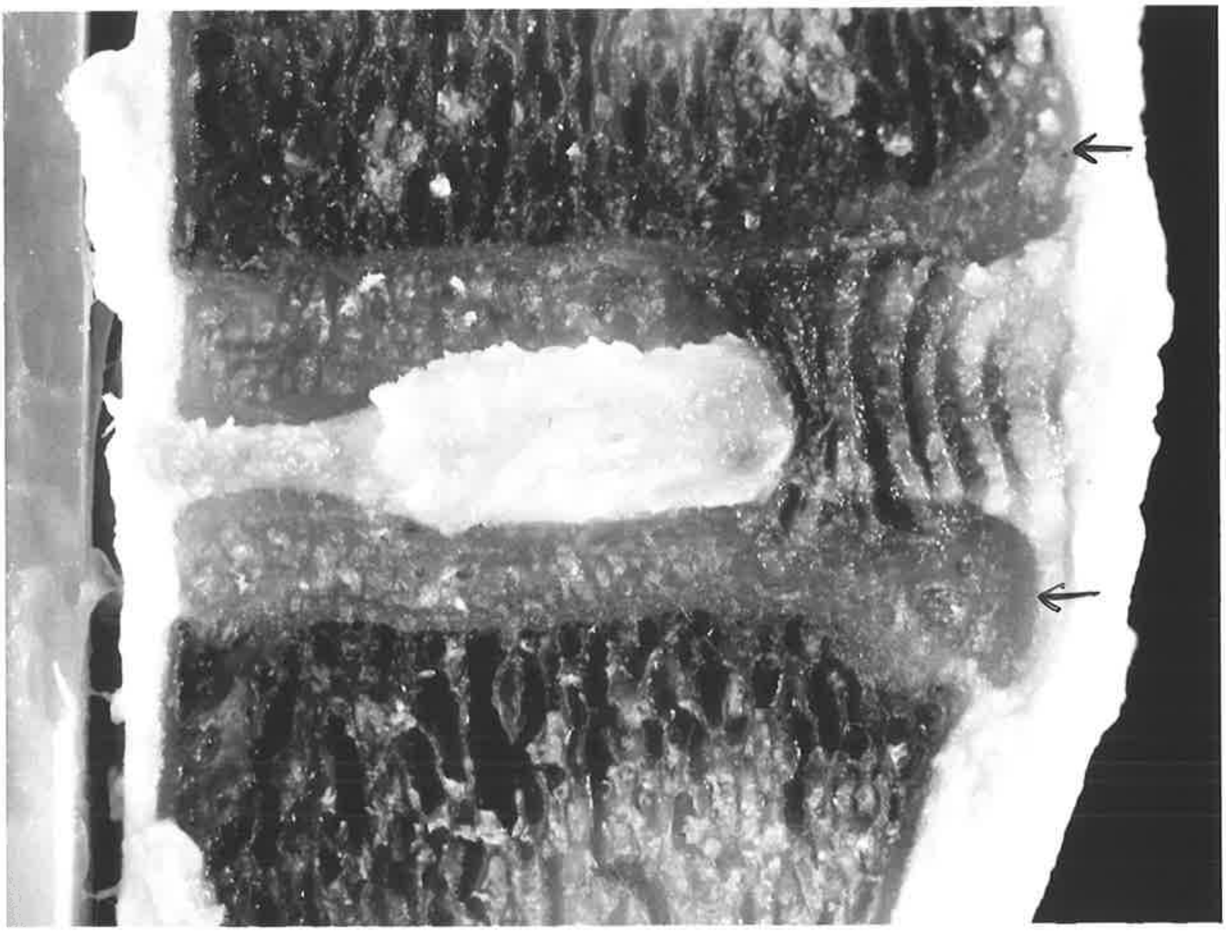
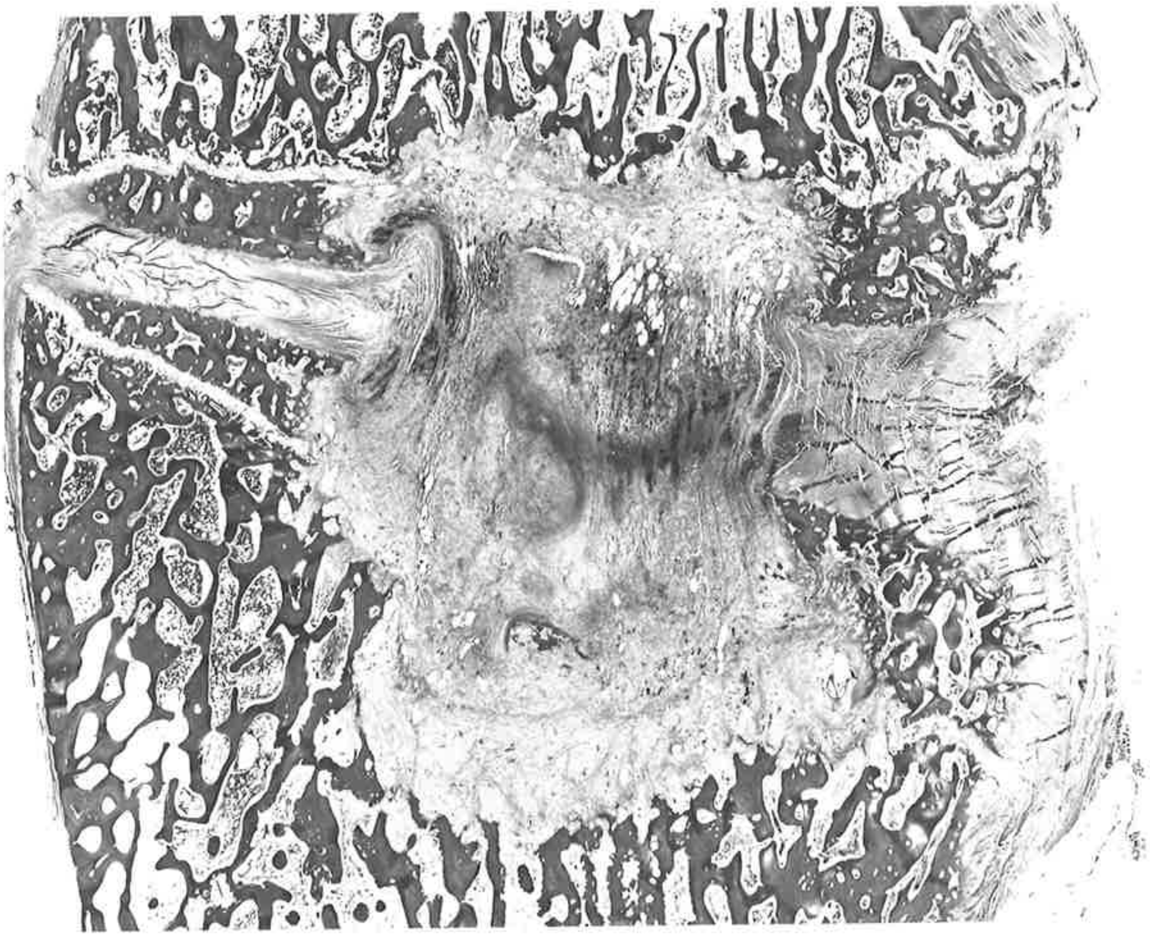


Fig. 35 Low power micrograph of L3-4 disc in sheep No. 4 following injection of chymopapain and bacteria. Shows marked central discitis, also involving inner annulus, with extensive replacement of bone by granulation tissue. Haematoxylin-eosin X8.

Fig. 36 Low power micrograph of L2-3 in sheep No. 2 following injection with chymopapain and bacteria. Shows anterior discitis with disruption of annulus, loss of end-plates, replacement of vertebral bone with granulation tissue, and new bone formation (arrow) on anterior vertebral body. Haematoxylin-eosin X8.



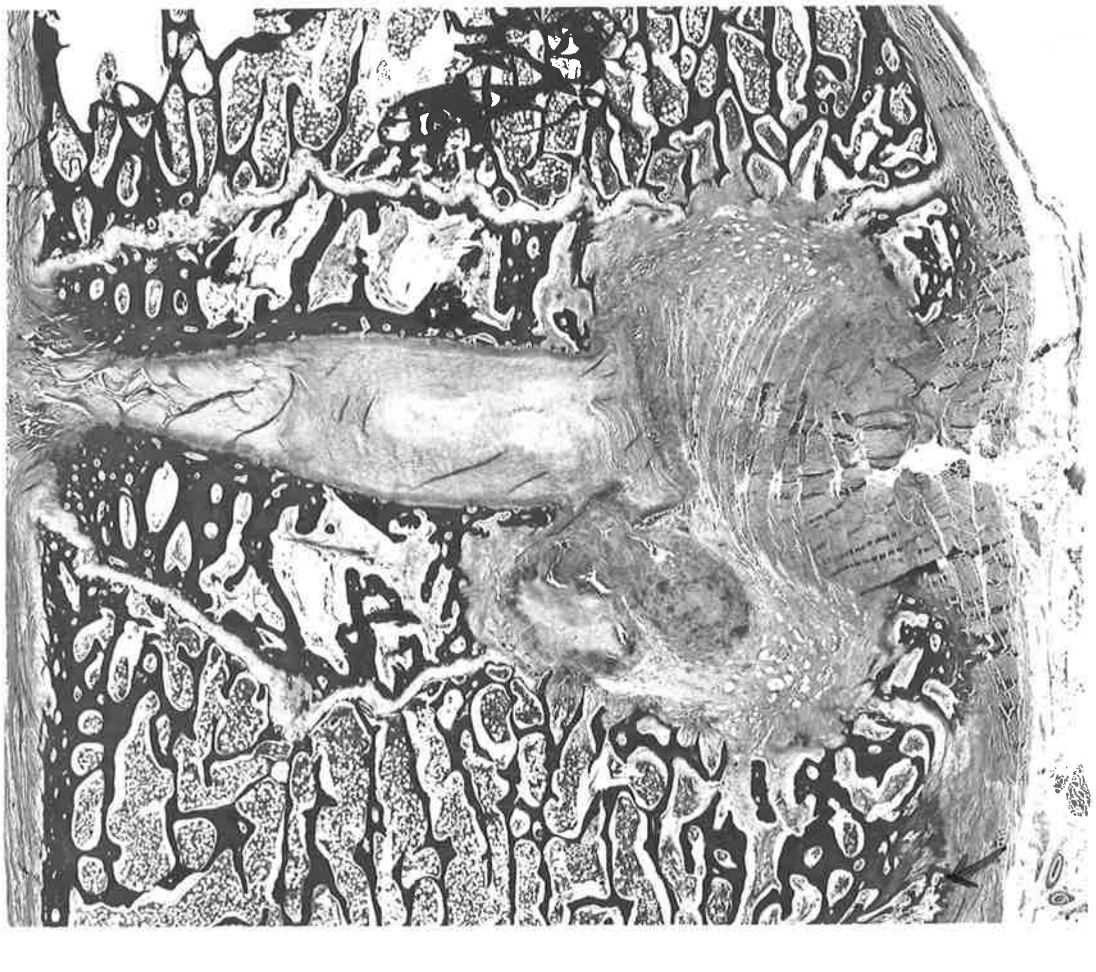
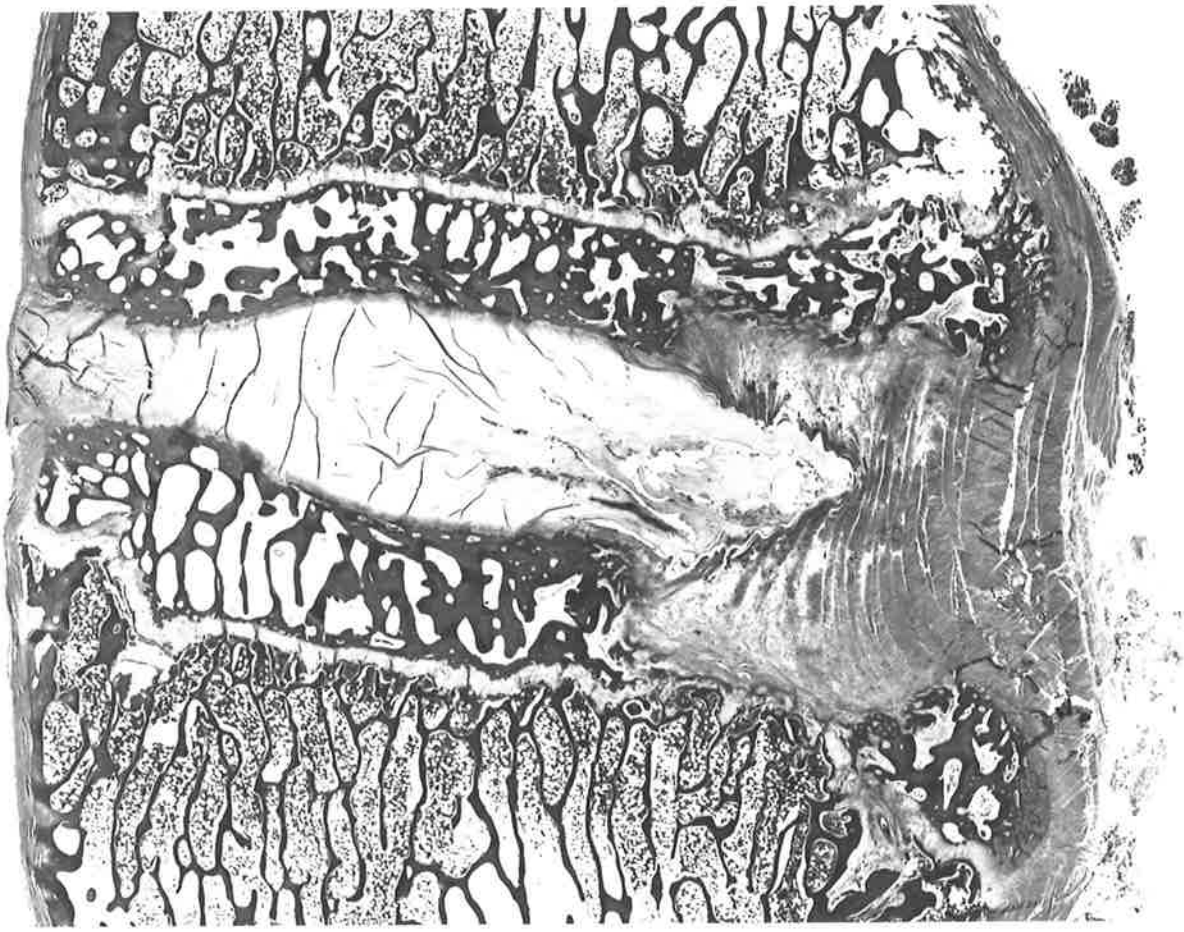


Fig. 37 Low power micrograph of T12-L1 disc in sheep No. 4 following injection of chymopapain and bacteria. Shows anterior discitis with forward protrusion and nuclear material through degenerate region of inner annulus. Haematoxylin-eosin X8.

Fig. 38 Medium power micrograph of L2-3 disc in sheep No. 2 following injection of chymopapain and bacteria. Shows changes of anterior discitis with destruction of attachment of annulus (A) to end-plate, abundant vascular granulation tissue (G), and portion of nucleus (N). Haematoxylin-eosin X50.



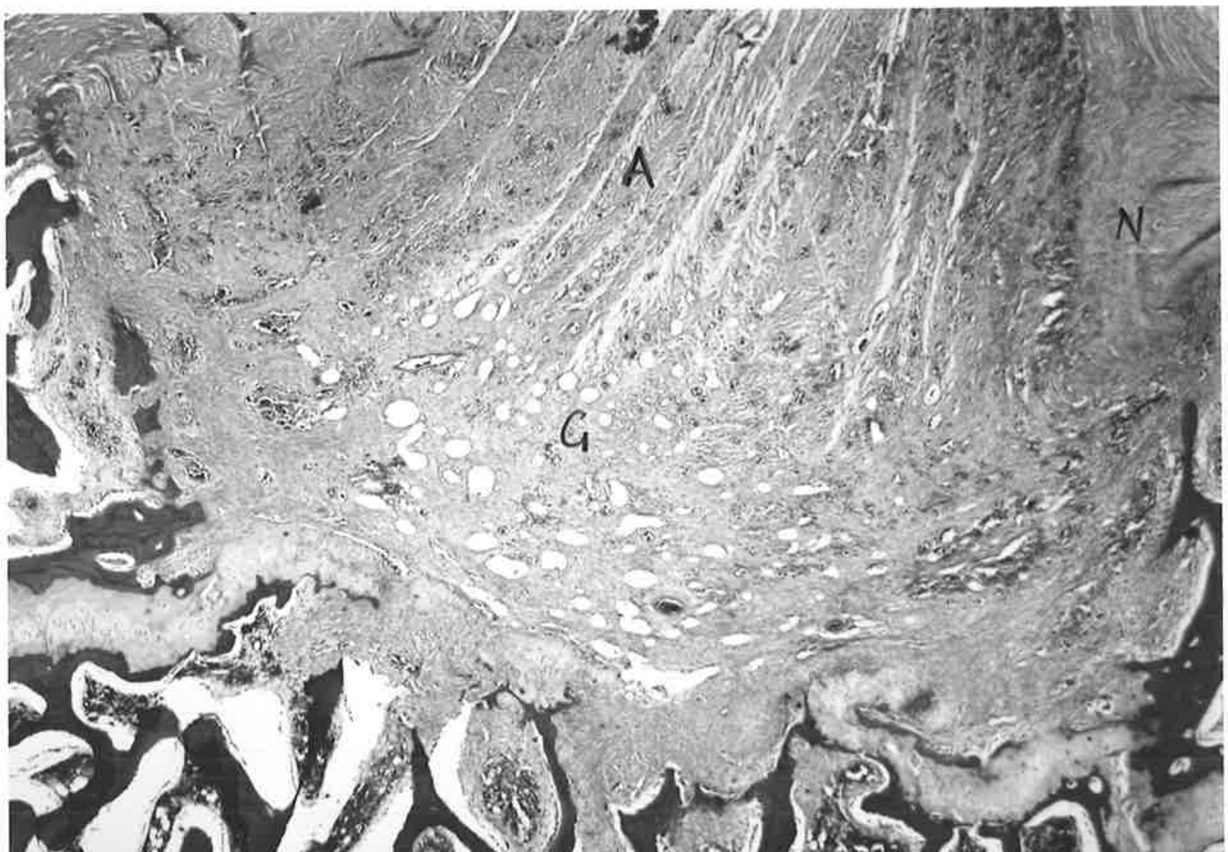
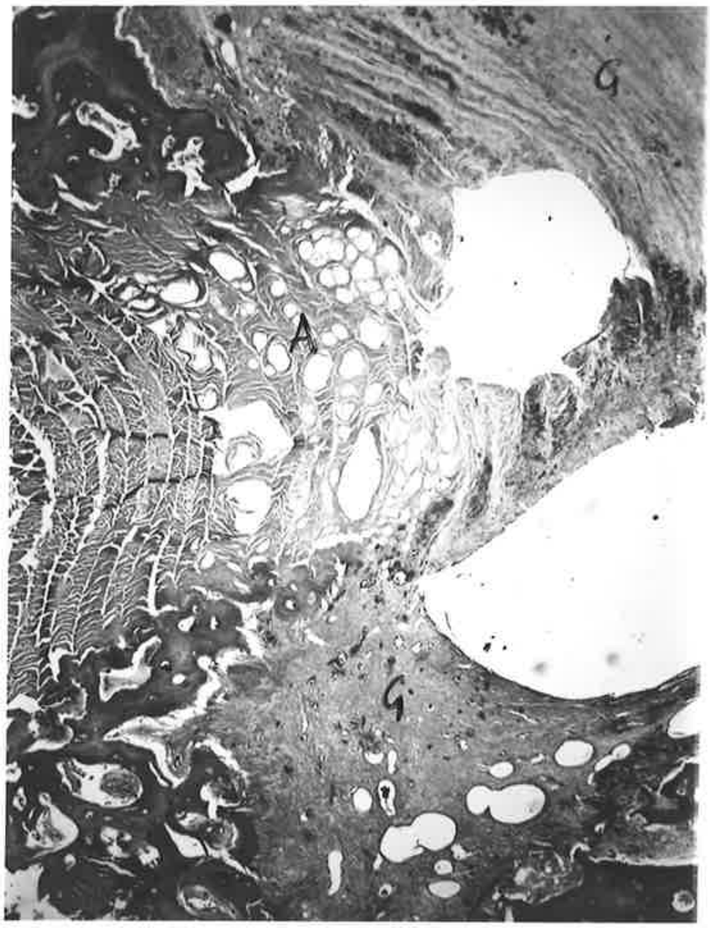

A
N
G

Fig. 39 Medium power micrograph of L4-5 disc in sheep No. 4 following injection of chymopapain and bacteria. Shows extensive cystic degeneration of inner annulus (A), and central discitis with granulation tissue (G) within vertebral bodies. Haematoxylin-eosin X50.

Fig. 40 High power micrograph of centre of nucleus in L4-5 disc in sheep No. 4 following injection of chymopapain and bacteria. Shows microabscess containing abundant neutrophil polymorphs. Haematoxylin-eosin X100.


G
A
G

Microscopically, central and anterior discitis accompanied by extensive replacement of the disc and adjoining vertebral body bone by granulation tissue; blood vessels extending into the disc with frequent haemorrhage into the tissues; diffuse and focal aggregates of chronic inflammmatory cells; a zone of new bone formation at the junction of granulation tissue and vertebral bone; and clefts within residual nuclear tissue (Fig. 35-39). Acute inflammatory cells were present at only one level in one sheep, and microabscesses were present in the nucleus pulposus (Fig. 40).

Bacterial Culture

A positive culture for staphylococcus epidermidis was obtained at six levels, and the sensitivity pattern suggested that this was the bacterial strain originally injected. A moderate growth of staphylococcus epidermidis was obtained at one level only. This was the L3-4 level of sheep No. 1 where an estimated 20 bacteria only was injected and where at sacrifice there were no radiographic features of end-plate erosion. A scanty growth only of staphylococcus epidermidis was obtained at another three levels, one of which also did not demonstrate radiographic end-plate destruction. In the remaining two levels the staphylococcus epidermidis was not grown on primary culture and was obtained only by broth culture. Negative cultures were obtained from the remaining levels.

DISCUSSION:

Until recently the complication of discitis following intradiscal injections was considered to be a rare occurrence with an incidence in the order of 1:1000 (Brodsky and Binder 1979; Collis and Gardner 1962; Massie and Stevens 1967; McCulloch 1977; McCulloch 1980; McCulloch and Waddell 1978; Simmon and Segil 1975; Wiltse, Widell and Yuan 1975). Recent studies have reported a much higher occurrence of discitis following discography (Crock 1983; Fraser 1984; Fraser, Osti and Vernon-Roberts 1975; McCulloch and McNab 1983) with an incidence as high as 3.2% (Fraser 1984). An incidence of "chemical" discitis following chemonucleolysis of 2% has been reported (McCulloch 1985). The argument for a non-infective aetiology has been : the inability to isolate organisms from biopsy material in almost all cases; the failure of the white cell count to become elevated in the presence of a raised erythrocyte sedimentation rate; and the histological findings of a chronic inflammatory process with a small round cell response. Occasional cases of proven infective discitis following intradiscal injections have been reported but this has been regarded as a separate entity by some authors (Agre, Wilson Brim et al 1984; Crock 1983, McCulloch and McNab 1983).

A recent experimental study indicated that discitis following discography with contrast material is probably always initiated by infection (Fraser, Osti and Vernon-Roberts 1985). A clinical review suggested that the incidence of discitis following discography could be reduced by meticulous asepsis using a two-needle stiletted technique (Fraser, Osti and Vernon-Roberts 1985).

The results of this experimental study support the hypothesis that discitis following intradiscal injection is probably always initiated by infection. As with our previous experimental study, we found the occurrence of discitis did not seem to be related to the number of bacteria injected (Table VIII). Whereas in the earlier experimental study (Fraser, Osti and Vernon-Roberts 1985) bacteria would not be isolated from those levels where established discitis had occurred, in the present study staphylococcus epidermidis was recovered from six of sixteen levels injected. However, in all but one level the growth was either scanty or obtained only after broth culture, suggesting that the infective process had almost been eradicated. The one level which produced a moderate growth of bacteria had not developed radiographic discitis. It seems likely that for some reason the infective process had been delayed at this level and that with time radiographic end-plate destruction would have occurred with subsequent removal of the organisms. This variation in the time from inoculation to the development of established radiographic discitis has been observed in clinical practice.

The in vitro study demonstrated that staphylococcus epidermidis is rapidly destroyed by chymopapain. However, the high concentration of proteoglycan in the nucleus pulposus would be expected to markedly reduce the antibacterial activity of chymopapain by acting as a substrate. In the present experimental study end-plate destruction occurred at most levels within two weeks of the injection, whereas in the experimental study of discitis following the injection of contrast and bacteria alone, it was not until three weeks following the injection that a similar incidence of radiographic end-plate erosion was observed.

It therefore seems likely that there is little if any bactericidal effect of chymopapain in vivo. The delay in the eradication of the organism is more likely to be due to the effect of chymopapain on the annulus enabling a more extensive spread of bacteria throughout the disc.

Our earlier study (Fraser, Osti and Vernon-Roberts 1985) demonstrated that bacteria were eradicated following the injection of contrast and staphylococcus epidermidis by the time bony end-plate erosions were demonstrated on plain radiographs. The present study suggests that bacteria are removed at a slightly later stage following discitis with chymopapain.

Whereas the end-plate lesions produced by contrast material and bacteria in the earlier study were central corresponding to the nucleus pulposus, the end-plate lesions produced with chymopapain and bacteria frequently tended to involve the anterior part of the disc. Although contrast was not used in the present study to confirm needle placement, the same technique of injection was used as for the earlier study (Fraser, Osti and Vernon-Roberts 1985). While anterior annular injection of bacteria is likely to be the cause for this effect it is possible that the increased kyphosis noted with chymopapain injections in the sheep (Fig. 22) may have been responsible for the bacteria causing more anterior end-plate destruction in some cases. In the previous experimental study (Fraser, Osti and Vernon-Roberts 1985) where chymopapain was not used, the earliest sign of discitis was posterior narrowing of the disc space and the tendency for an increase in the lumbar kyphosis was not observed. The pathological finding that chymopapain alone frequently caused degeneration of the anterior annulus, and that anterior discitis was associated with anterior protrusion of the nucleus through the inner annulus, suggests that the destructive effects of chymopapain in the annulus may have played a significant role in producing the changes observed when bacteria were injected. This contrasts with the absence of anterior discitis when bacteria are injected without chymopapain (Fraser, Osti and Vernon-Roberts 1985).

The observation that chymopapain alone was associated with the presence of minor chronic inflammatory features in the marrow spaces adjoining intact end-plates is consistent with our observations in human material, and also is consistent with evidence that diffusion normally takes place across the central region of the end-plates (Holm, Maroudas, Urban et al 1981).

This experimental study supports our earlier finding that probably all cases of discitis following intradiscal injections are due to bacteria introduced by the needle tips (Fraser, Osti and Vernon-Roberts 1985). On this basis the following recommendations are made to reduce the incidence of discitis after intradiscal injection: The procedure should only be carried out in a clean room with full aseptic technique. The skin should be prepared over a wide area to allow for movement of the drapes. The patient should be sedated to minimize unnecessary movement. Care should be taken to avoid contamination of the drapes by x-ray equipment. Only stilletted needles should be used, and a two needle technique for each level is recommended. The operator should avoid handling the shaft of the needle, particularly near the needle tip, and separate needles should be used for each skin puncture. The prophylactic potential of antibiotic injected at the time of chemonucleolysis is being assessed at the present time.

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