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Secondary degenerative changes in the intervertebral disc due to zygapophyseal (para-vertebral) facet joint lesions.

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3.0 MATERIALS AND METHODS

3.1 Introduction

Five embalmed cadavers were randomly selected for the study. The cadavers had been used by students for dissection, but the spines had been left intact.

The specimens were numbered alphabetically A-B-C-D-E. The AD number represents the cadaver register number in the School of Anatomical Sciences.

Table 3.1: Summary of specimen used in the study

SPECIMEN	AGE	GENDER	WEIGHT	HEIGHT	CAUSE OF DEATH
A-AD8121	29	M	46kg	168cm	Bronchial Pneumonia
B-AD8140	42	M	60kg	165cm	Hepatic Encephalopathy
C-AD8095	51	F	48kg	158cm	Pneumonia
D-AD8059	45	F	46kg	152cm	Cardio Vascular Accident
E-AD8072	59	M	50kg	171cm	Pleural Effusion

3.2 Removal of specimens from the cadavers

The cadavers were placed in the prone position. An incision was made bilaterally; down to the level of the lamina and posterior aspect of the sacrum, immediately adjacent to the spinous processes from T11 to mid sacrum level S2. Figure 3.1

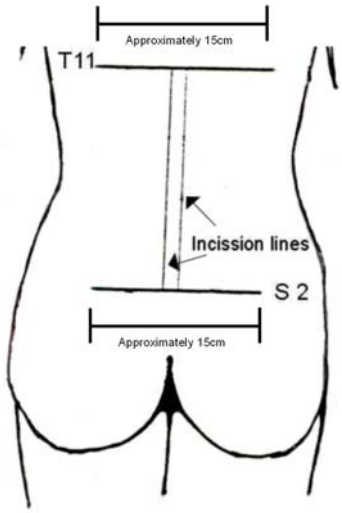


Figure 3.1: Position of incision on the lateral aspect of the spinous processes down to the lamina of the lumbar vertebra from T11 – S2

A further incision was made transversely across T11, about 15cm on each side, and similarly at the mid sacrum to just beyond the sacroiliac articulation.

Using a sharpened chisel tool, the posterior musculature was stripped laterally from the underlying ribs, transverse processes and sacrum, exposing the vertebral column. Figure 3.2

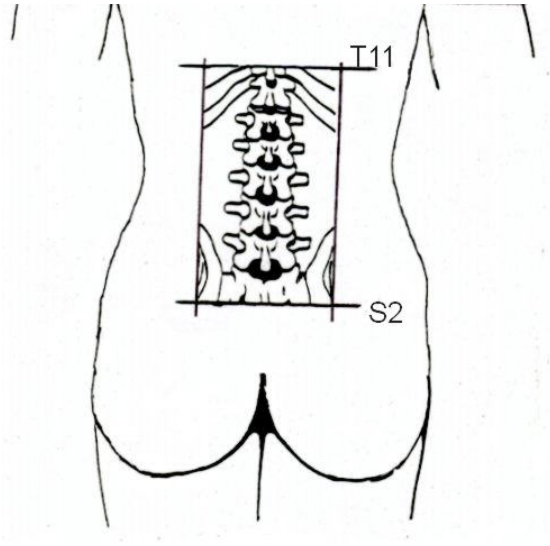


Figure 3.2: Transverse incision across T11, 15cm lateral on to the bony ribs of T11 and T12, and similarly laterally across S2 beyond the sacroiliac articulation and onto the posterior aspect of the ilium, to expose the vertebral column

The transverse processes and spinous processes were not part of the study, so they were severed from the bodies of the vertebrae. Figure 3.3

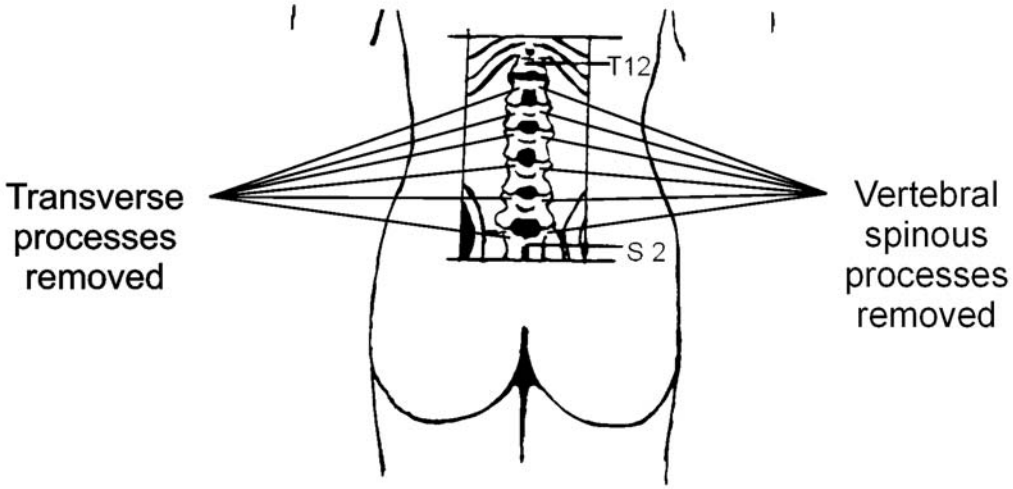


Figure 3.3: Removal of spinous processes and transverse processes

In order to remove the lumbar column, the T12 body was identified dy identifying the 12th rib attachment and using a bone saw a transverse incision was made through the middle of the body of T12 and the pedicle supporting the articulating processes at T12 and L1. A further transverse separation was done at S1-S2, lateral to the sacroiliac joint and superior to the joint was prised open freeing the 1st sacral segment from the ilium. Figure 3.4

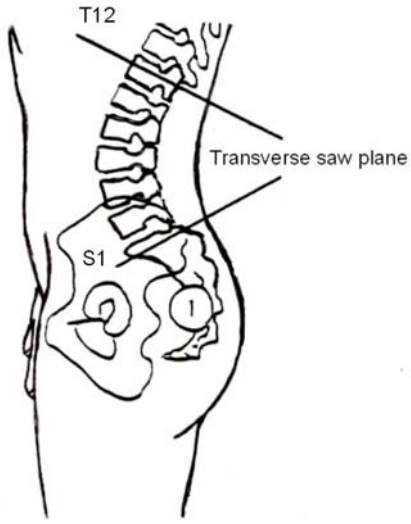


Figure 3.4: Removal of lumbar spine by mid transverse sectioning of body of T12 and S1

This procedure left the lumbar spine free for removal. Surrounding facias, blood vessels and nerve structures were severed and stripped away rendering the lumbar column free for removal from the cadaver. Figure 3.5

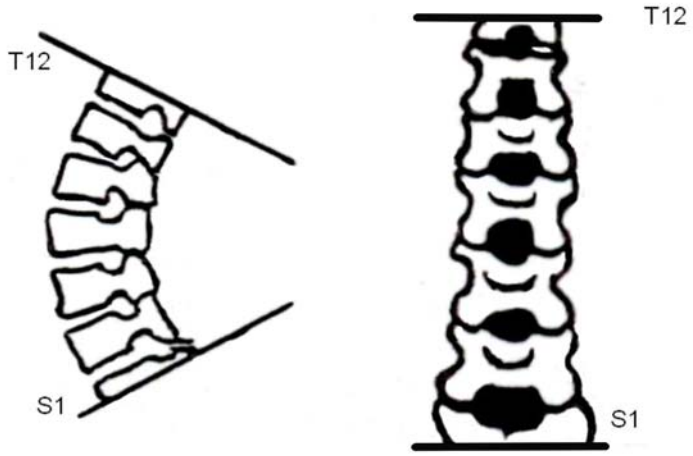


Figure 3.5: Lateral and posterior diagrams of the removed specimens

The structures that had been laterally displaced were repositioned on the cadaver and the body covered.

The vertebral bodies, devoid of the spinous processes and transverse processes were x-rayed in the A-P and lateral planes for reference purposes. Figure 4.1, 4.7, 4.13, 4.19, 4.25

The posterior elements consisting of the pedicles, articular processes and lamina were left attached to the lumbar vertebral specimen for the A-P and Lateral x-ray studies.

Once this had been completed, the posterior elements were removed at the pedicle, vertebral body junction and the articulating processes further removed from the pedicle and lamina. Figure 3.6 & 3.7.

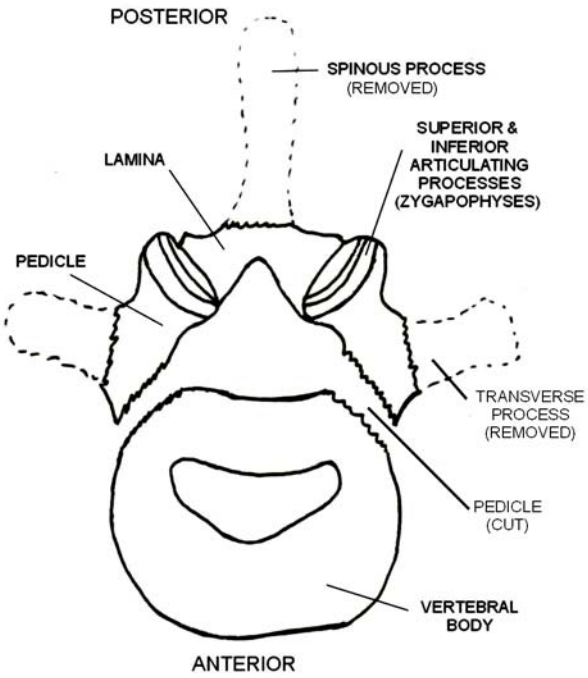


Figure 3.6: Schematic diagram of the removal of the facet joints

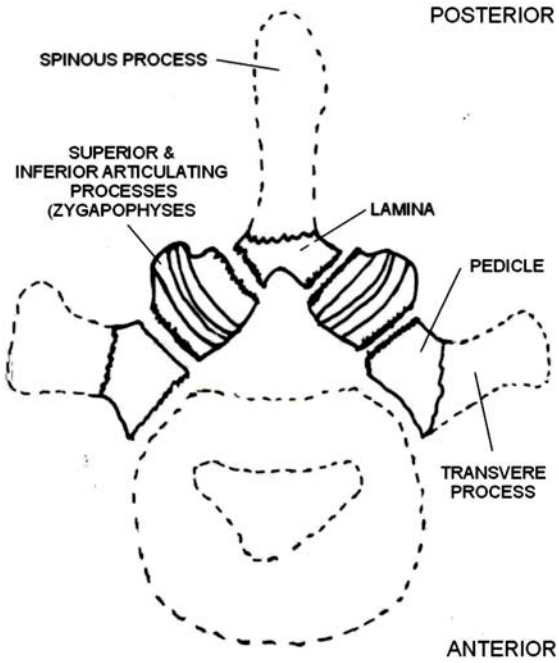


Figure 3.7: Schematic diagram of the removal of the facet joints, in the next step

The specimen that had been dissected out, containing the articulating processes and related discs was then further stripped of loose tissue, placed in a sealable container, containing 5% formalin. The container was marked, identifying the cadaver from

which it had been removed and the process was repeated in the remaining four cadavers.

3.3 Articulating facet joint dissection

3.3.1 Macroscopic dissection of the facet joints

In the protocol, it was planned to examine the state and constituency of the hyaline cartilage on the facet surface for signs and degrees of degenerative change. This would then be correlated with the studies on the anulus fibrosus failure.

The superior and inferior facet joints of each side were removed from the vertebral bodies (see A in figure 3.8)and placed in individually marked containers, with a preservative of 5% formalin.(figure 3.8 A – Shows the intact facet joints, whereas B shows the two separated surfaces after the capsule has been removed and the articulations separated)

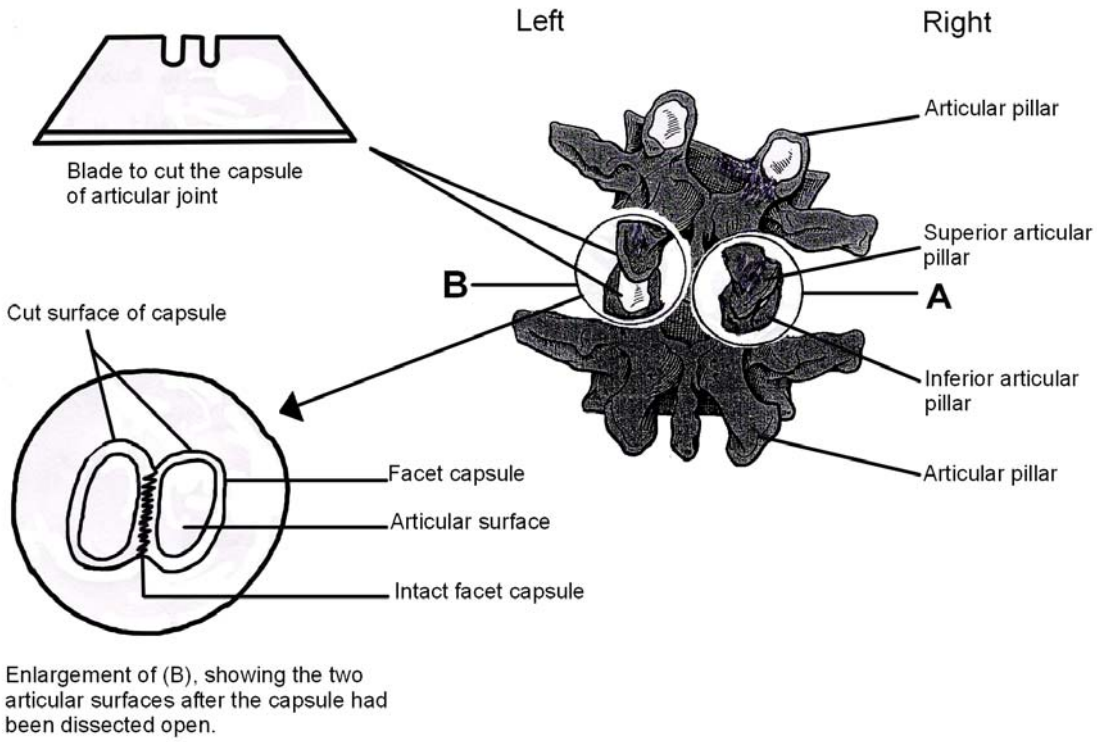


Figure 3.8: Diagram showing the removal of the intact articular process from the posterior vertebral body

A sharp scalpel blade was used to severe the capsule around the peripheral articulating border, Figure 3.8, and the joint was plied open. The incision was made to just inside the capsule to not damage the articular surface. The joint was opened and the hyaline cartilage surface exposed for examination.

All the facet surfaces were photographed using a stereomicroscope and ASA 100 colour print film. A record was kept of the exposure number and the corresponding facet. When the film was developed, the chronological number was matched with the exposure record, the facets and the photographs were filed accordingly.

Note:
In the protocol submissions, it was thought to microscopically examine the state of the hyaline cartilage on the joint surface, because of the irregular and undulating surfaces, it could not be done. It was then decided to use 5X enlargement, which resulted in loss of definition.

3.4 Intervertebral disc dissection

3.4.1 Gross dissection of the intervertebral disc

The lumbar spine was cut in the mid transverse plane of the vertebral bodies leaving the disc intact. Figure 3.9.

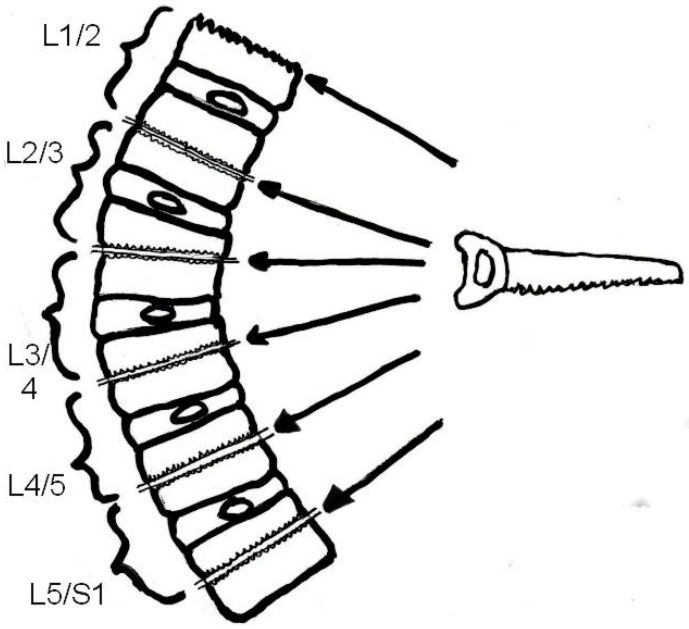


Figure 3.9: Cutting of lumbar vertebral column transversely through the bodies of the adjacent vertebrae

The units formed were L1/2, L2/3, L3/4, L4/5, and L5/S1. On the body of the superior vertebra, for example L2 on L3, a notch was made on the anterior superior aspect for identification. Figure 3.10.

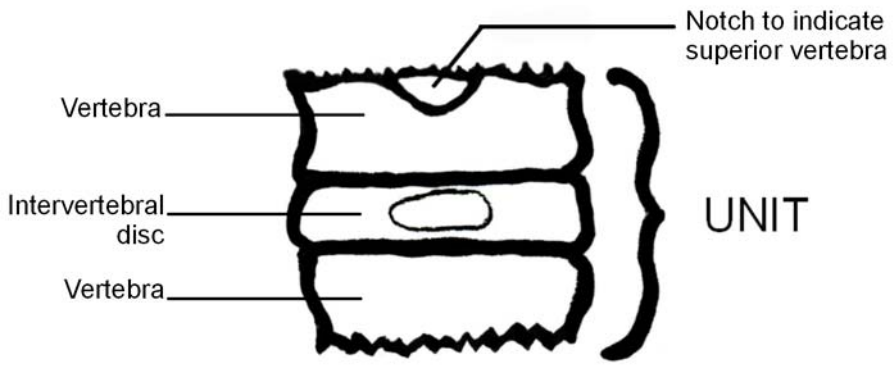


Figure 3.10: Vertebral unit

3.4.2 X-Ray procedure of the intervertebral disc

An integral part of the study was to observe the migration of dye in the nucleus pulposus, under pressure, hoping to identify early fissuring in the anulus fibrosus. To accomplish this, the nucleus pulposus would have to be infiltrated with a radio opaque dye and then x-rayed in the lateral and superior planes, which meant that each level would have to be done separately. Figure 3.11

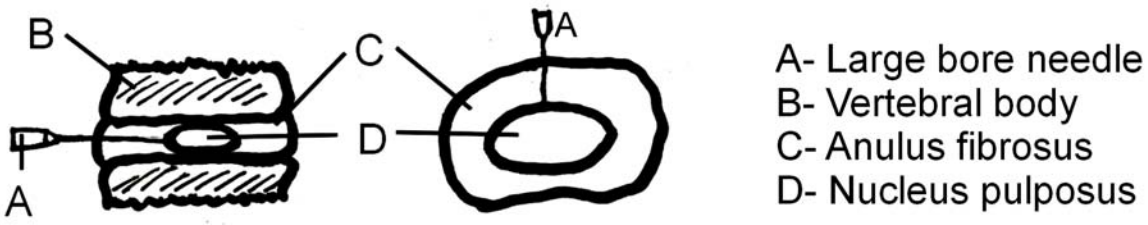


Figure 3.11: Positioning of needle into the nucleus pulposus

In order to observe the dispersion of radio opaque dye in the nucleus pulposus, a cage apparatus was constructed whereby the dye UROGRAFIN® could be injected into the nucleus pulposus under constant pressure.

The pressure apparatus was made of a 5mm x 30mm flat bar. It measured 20cmx10cm with a 7mm hole in the centre of one of the 10cm uprights. Figure 3.12

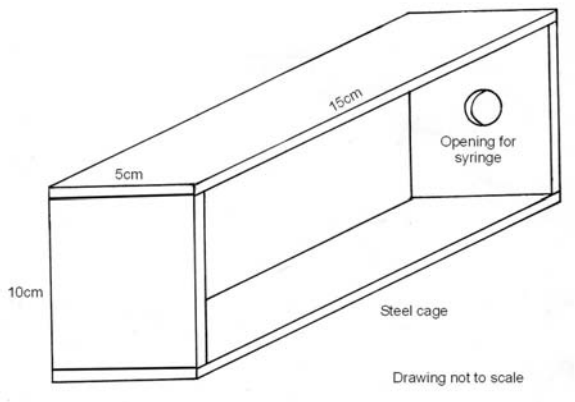


Figure 3.12: Diagram of cage apparatus

Five cubic centimetres of UROGRAFIN® was drawn up in the 20ml syringe, the excess air was expelled and the syringe was placed in front of the BP cuff. Figure 3.13

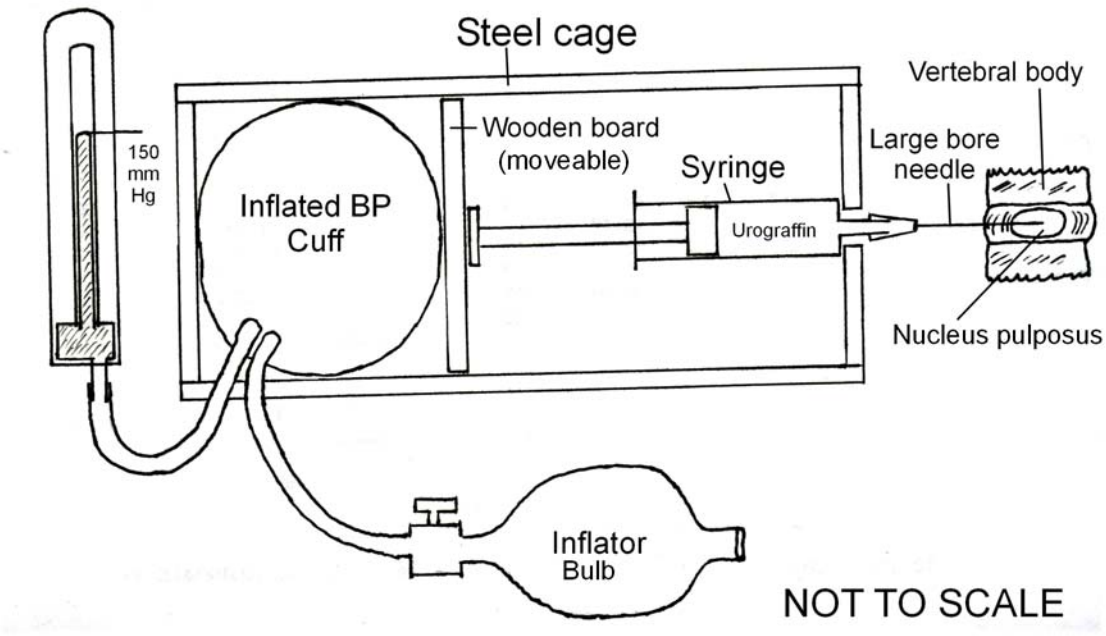


Figure 3.13: Placement of the syringe in cage and injection of dye

A fine bore rod/wire was inserted into the back of the large bore needle with the tip just penetrating from the front of the needle. In trial specimen, it was found that when the needle was inserted through the anulus, the anulus material blocked the opening of the needle and the dye did not reach the nucleus pulposus. Care was taken not to damage the posterior attachment of the anulus. The wire was removed from t he needle. The syringe, with the specimen attached, was then fitted to the needle holder of the syringe in the pressure apparatus.

An assistant held the needle in place with a pair of pliers. The BP cuff was inflated to 150 mm Hg and where possible maintained for 10 seconds. The BP cuff was then deflated. This process was repeated on all the specimens. (Figure 3.13)

In general, the pressure was well maintained and on average2.5 ml of dye was expelled from the syringe.

Some specimen showed posterior and lateral leakage during pressure resulting in all the dye being expelled from the syringe.

Note:
In the later stage of the experiment, the dispersed dye is observed on X Ray, and the possible reason for this phenomenon is taken up in the discussion chapter.

The specimens were then x-rayed in lateral and axial views. Great care was taken to accurately mark the superior and inferior bodies, as well as showing left and right. (Figure 3.10)

On the lateral aspect, a plastic ring was used to secure the specimen for X ray. This is visible as a white ring on the lateral x-ray.

Note:
This implication was not considered at the time and the specific area of leakage was not recorded. In the end, the required effect was obtained, but the added notes would have been of interest.

3.4.3 Macroscopic evaluation of the intervertebral disc

With the x-rays completed, the individual units were placed on the posterior aspect and with a very sharp “butchers knife” the discs were divided into two halves by separated slicing through the middle of the disc from anterior to posterior. It was attempted not to slice through the posterior longitudinal ligament. (Figure 3.14)

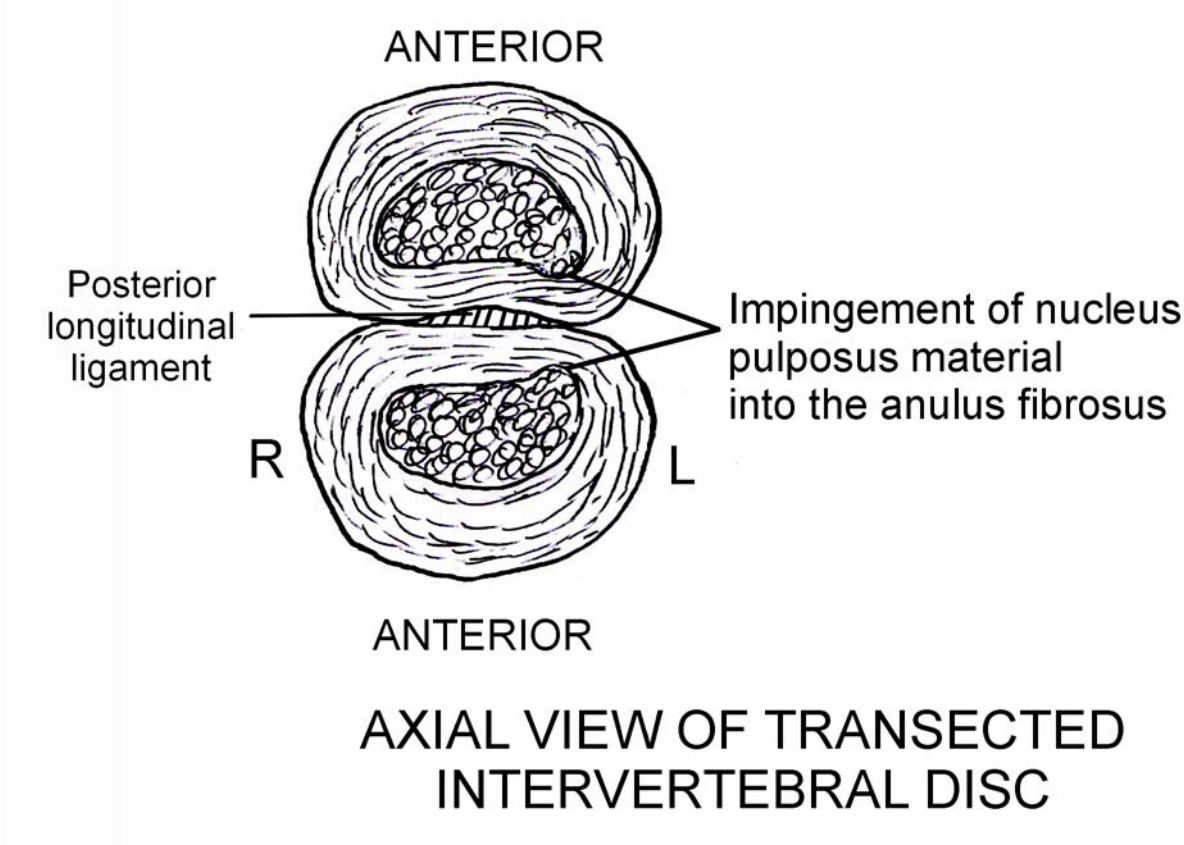


Figure 3.14: Transverse section through the intervertebral disc

Left and right markers were placed alongside the dissected units to identify the sides.

The two halves were plied open, and then photographed, using a digital camera. Again, the units were recorded in a register.

Digital technique was selected because the picture could be enlarged on the computer screen using an Adobe programme.

3.4.4 Microscopic dissection of the intervertebral disc

The vertebral bodies were then separated through the posterior longitudinal ligament. (Figure 3.15)

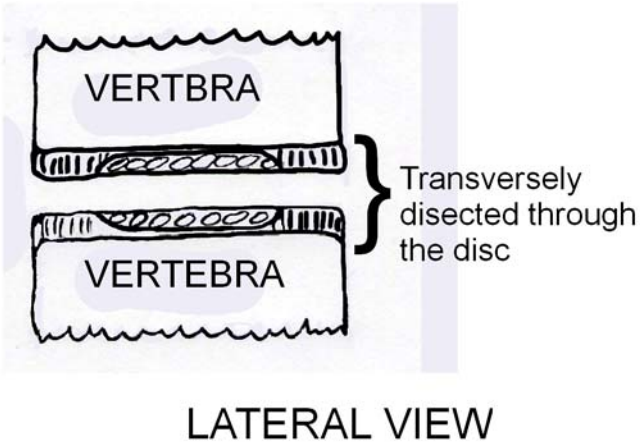


Figure 3.15: Sectioning through the intervertebral disc

A transverse section was made through the centre of the disc with a fine toothed fret saw. (Figure 3.15)

A coronal section was made through the intervertebral disc, separating the posterior aspect of the intervertebral disc. (Figure 3.16, 17)

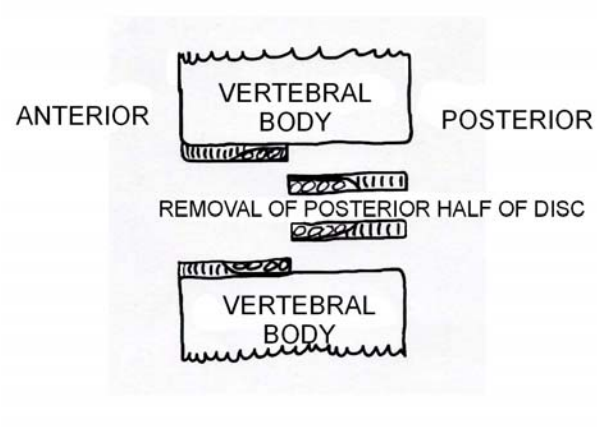


Figure 3.16: Separation of the posterior parts of the intervertebral disc into the superior and inferior sections

The posterior aspect of the disc was then divided into a left and right section through the sagittal plane. (Figure 3.17)

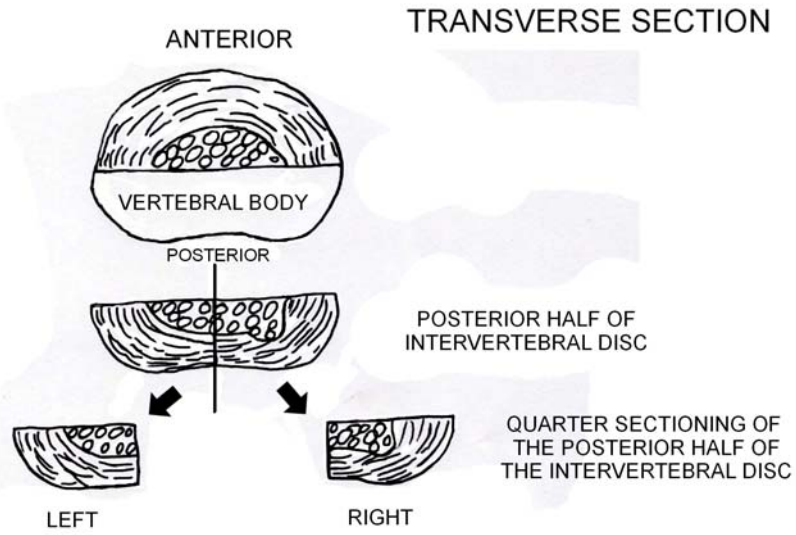


Figure 3.17: Removal of the posterior part of the intervertebral disc

Each half of the units that showed possible correspondence with the x-ray, facet and disc findings, was divided into posterior quarters and prepared for microscopic fixing and study.

These separated units were then placed into individual containers marked L – left and R – right and the identity of the articulation from whence it had been removed. The marking also identified whether it was the superior or inferior unit.

The quarters dissected off the vertebral body at the end plate, were labelled L and R, and S from the body above and I from the body below. A unit would thus be labelled L 4/5 S – A, where A denoted the origin of the specimen. The lower section was labelled L 4/5 I – A.

The units were then prepared for microscopy study

3.4.4.1 Staining and Mounting

The haematoxylin and eosin staining technique was used to prepare and block the different units. The solutions used were as follows:

Acid Haemalum (Modified Mayer's)

- Haematoxylin	2g
- Sodium Iodate	0.2g
- Potassium Alum	50g
- Citric Acid	1g
- Chloral Hydrate	50g
- Distilled water	1 litre

Allow haematoxylin, alum and sodium iodate to dissolve overnight. Add chloral hydrate and citric acid and bring to the boil. Continue boiling for 5 minutes after which the solution is cooled and ready for use.

Stock Eosin:

Eosin	8g
Erythrosin	2g
Distilled water	1 litre

Eosin working solution:

Stock eosin	250ml
Distilled water	750ml
Calcium chloride	20g

Method:

1 – De wax and hydrate sections.

2 – Stain in haematoxylin for 6-20minutes depending on the strength of the stain and the fixative used.

3 – Wash well in running tap water. The section may be examined microscopically at this stage to confirm a sufficient degree of staining. If insufficient, return to the stain.

4 – Remove the excess stain by differentiating in 1% hydrochloric acid in 70% alcohol (usually a few quick dips). The blue staining of the haematoxylin is changed to red by the action of the acid.
5 – Regain the blue colour by washing in alkaline running tap water. The stain should again be checked microscopically until the correct degree of staining is obtained.
6 – Stain in eosin for 1-2 minutes.
7 – Differentiate in tap water.
8 – Examine microscopically.
9 – Dehydrate in alcohol and clear in xylene.
10 – Mount in D.P.X.

Note:
The sections were stained with haematoxylin regressively, that is, with a stain of sufficient power and for long enough to ensure over staining of nuclei, the superfluous colouration of structures being removed by treatment with acid alcohol

Results:	Nuclei	Blue
	Erythrocytes	Orange
	Other tissue structure	Shade of pink

The above were photographed on a WILD Photomakroskop M400. Prints were made for illustration.

The hypothesis in this work suggests that a correlation exists between facet joint degeneration and ipsilateral fissuring or fragmentation in the anular rings of the anulus fibrosus.

In order to study this possibility, the following method was employed:

1. X Ray examination of the dye infiltrated discs.
2. Microscopic studies of the anulus fibrosus.
3. Macroscopic findings of the facet joint surfaces.
4. Macroscopic findings of the intervertebral discs.

A selective process was followed based on macroscopic examination of the discal dissection described. The selection was based on comparing the findings reported in the summary chart of the facet degeneration and laterisation or impinging of the nucleus pulposus into the anulus fibrosus, also observed in the axial x ray study with dye injection.

A control study was done where the ipsilateral correlation was not observed and the findings were recorded in the summary analysis Figure 5.1

3.5 X Ray examination of the dye infiltrated discs

As explained in the material and methods in 3.4.2, radio opaque dye was injected into the nucleus pulposus under controlled conditions.

Specimen A, B, C, D and E were X rayed in the anterior-posterior and axial planes prior to the segments/ units being dissected out separately. A unit, as described in figure 3.12, is made up of the dissected out structure of the inferior half of the vertebra above, the intact IVD and the superior half of the vertebra below. The purpose was to show a visual appearance of the intact specimen and also to rule out possible gross pathology in the columns. Note also the removal of the spinous processes and transverse processes as previously discussed in the material and methods section 3.4.

As the specimens were of embalmed cadaver material, it was difficult to determine the exact X ray exposure factors. After some experimentation, the following exposure factors were found to be the most satisfactory- 100 mA, 45-47 kV at 1/10 second. Variation in the exposure is the reason for some specimen’s vertebral bodies appearing more radio lucent, whilst others are more radio opaque.

No obvious pathological changes were observed in the vertebral bodies.

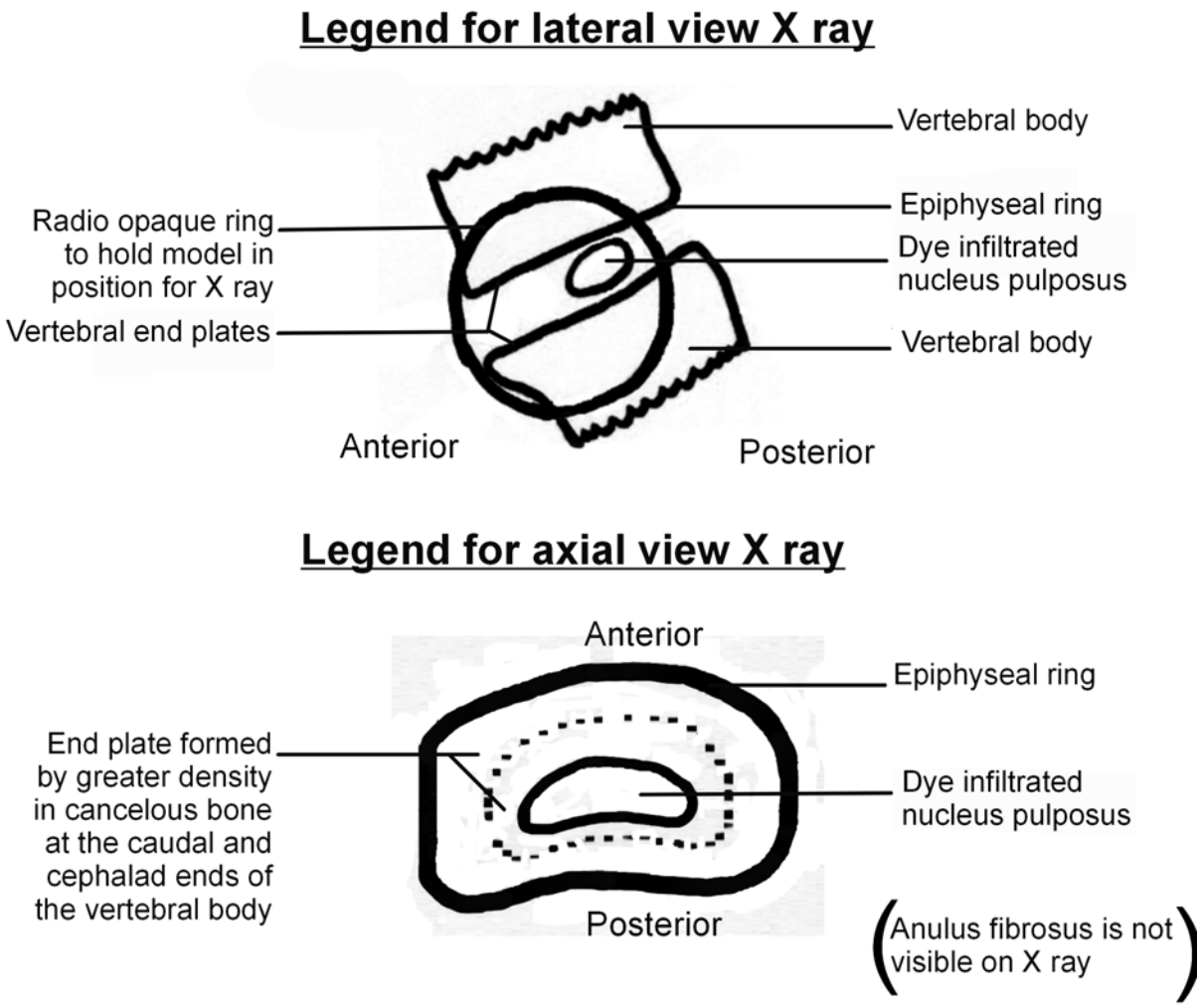


Figure 3.18: Schematic diagram of the lateral and axial view of the vertebral body structures to be x rayed

In order to report on the migration of dye within the injected nucleus pulposus, these symbols were used:

"X"	Refers to an observed migration of radio opaque dye.
"P" – Posterior.	Migration of the radio opaque dye either due to
"L" – Lateral.	early fissuring of the anulus fibrosus or degenerative changes in the transitional zone, which is the zone between the nucleus pulposus and the anulus fibrosus.

Note:

In the lateral view of the dissected out specimen, a white ring is visible. This ring is a support ring of PVC pipe that was used to secure the specimen for X-raying.