Assessment of spinal cord blood flow and function in sheep following antero-lateral cervical interbody fusion in animals with and without spinal cord injuries

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SIGNED STATEMENT

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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1. SUMMARY OF FINDINGS

The early management of cervical fractures and fracture-dislocations with or without neurological deficit has been the subject of considerable controversy for many years. Some clinicians advocate closed reduction and early surgical stabilisation, while others believe closed reduction followed by skeletal traction and postural nursing gives the patient the best chance of recovery.

Not only is there controversy related to the indications for operative intervention in these injuries, but also controversy in relation to the operative approach that should be used.

This study set out to evaluate the effect of an anterior surgical approach and antero-lateral inter-body fusion of the cervical spine on spinal cord blood flow and electrical function, using a sheep model.

The anatomy of the cervical spine and spinal cord of the sheep was studied by macro and micro-dissection, and vascular casting with both latex and methyl-methacrylate. The findings of this study were then compared with the anatomy of the spinal circulation in man. This comparison indicated the sheep was suitable to be used as a model of spinal cord injury.

The effect of an anterior approach and antero-lateral cervical fusion was evaluated using three groups of sheep. The first group was used to study the effect of surgery on the intact spinal cord. Spinal cord blood flow was monitored before, immediately after, and one hour after the surgery. The electrical function of the spinal cord was

monitored continuously using motor and sensory evoked potentials and recorded every fifteen minutes.

There was no difference in either the spinal cord blood flow or electrical function immediately after, or one hour after completion of the surgery when compared to the base line measurements. This result did not support the supposition that anterior exposure of the vertebral column, and antero-lateral cervical fusion adversely affected spinal cord blood flow or function.

The presence of a spinal cord injury may alter the response or sensitivity of the cord to fluctuations in its local environment. In the second and third groups of sheep the effect of an antero-lateral cervical fusion was evaluated in the presence of an incomplete spinal cord injury. Both groups were given a spinal cord injury and the third group went on to have an antero-lateral cervical fusion.

Twelve sheep were used in this part of the study and the incomplete spinal cord injury was inflicted by the instantaneous inflation of a Fogarty balloon catheter in the extradural space at the level of the C4/5 inter-vertebral disc. Half of these sheep went on to have an antero-lateral cervical fusion. The electrical function of the spinal cord was monitored every fifteen minutes by both motor and sensory evoked potentials, as for the first group of sheep. The spinal cord blood flow measurements were made four times; once the sheep were physiologically stable as a base-line. Immediately following inflation of the balloon in the extradural space. Fifteen minutes after deflation of the balloon in group given a cord injury and not proceeding to cervical fusion or immediately after completion of the surgery in the remaining sheep (the surgery was usually completed

within 15 minutes of deflating the balloon). Final measurements were made one hour after the third blood flow measurement in both groups.

The spinal cord injury had a marked, and significant effect on both the spinal cord blood flow and electrical function in all twelve sheep. The spinal cord blood flow at the level of the injury decreased by more than 50% as did the amplitude of the evoked potential. The latencies of the evoked potentials also increased significantly (by more than 10%) in all sheep. Release of the balloon resulted in a reactive hyperaemia which was evident in all segments of the cervical spinal cord studied. This hyperaemia was most marked at the level of the spinal cord injury, and had nearly returned to the baseline levels by the time of the final blood flow measurement.

Similarly the sensory evoked potential returned to near pre-injury levels following deflation of the balloon. The motor evoked potential response which became unrecordable at the instant the injury was produced did not recover prior to completion of the study in any of the twelve animals.

Analysis of the results failed to demonstrate a significant difference between the two groups in the degree of recovery of spinal cord blood flow or electrical function that could be related to the antero-lateral cervical fusion (ANOVAR).

It is accepted that the shortcomings of an experimental spinal cord injury model are numerous. An experimental spinal cord injury will not have the mechanical instability and associated patho-physiological changes that are a feature of these injuries clinically. However every effort was made to establish that the model was

anatomically valid to assess the hypothesis. The inability in this study to observe the neurological function and recovery of the animals, and the limited time and facilities available to observe the anaesthetised sheep may have reduced the opportunity to demonstrate a difference between the groups. It is unlikely however that the function of either group would have been modified by extending the period of observation.

The hypothesis that anterior stabilisation of the cervical spine adversely affects spinal cord blood flow and function could not be supported by the results of this study.

2. INTRODUCTION AND LITERATURE REVIEW

There has been considerable discussion and debate regarding the management of spinal injuries associated with neurological loss. Injuries to the cervical spine associated with an incomplete tetraplegia have not escaped this controversy. There is however little disagreement that the initial management of cervical injuries associated with complete neurological loss should be non operative.

2.1 MANAGEMENT OF INJURIES TO THE CERVICAL SPINE ASSOCIATED WITH INCOMPLETE TETRAPLEGIA.

Review of the more recent literature fails to give a clear indication of the course of management that is best for these patients.

The dilemma relates to the question; Which method of treatment will give the patient the greatest chance of neurological recovery and subsequent independence?

Cloward (1958, 1961 & 1973) reported the approach for anterior cervical fusion of the cervical spine for removal of herniated disc material and later applied this technique to the management of cervical injuries. This technique however was not favoured by many clinicians as it resulted in the loss of the structural integrity of the anterior longitudinal ligament. When this approach was used in the presence of posterior ligamentous disruption, stable fixation was not achieved resulting in a significant number of complications due to displacement of the graft and persisting instability (Stauffer et al, 1977; Capen et al, 1985).

The technique of antero-lateral disc clearance and dowel fusion was developed by J.R. Barbour in response to the clinical problem of post operative instability following anterior cervical fusion in some patients disruption of posterior ligamentous injuries. The technique was subsequently reported by Cornish (1965 & 1968). There was a major advantage of this procedure compared with the Cloward type of anterior inter-body fusion in the treatment of cervical trauma with the posterior osseo-ligamentous disruption. The antero-lateral fusion technique where the dowel graft is inserted transversely across the disc space enabled preservation of the anterior longitudinal ligament and greatly reduced the incidence of post operative instability (Cornish, 1965 & 1968; Verbiest, 1969).

Bohlman (1979) supports the use of either closed or open reduction, if necessary followed by surgical stabilisation. Anterior or posterior depending on the configuration and mechanism of the injury. He reports this operative, yet flexible approach offered the best chance of recovery of neural function and restoration of stability. Beatson (1963), Cheshire (1969) and Aebi et al (1986) support the use of operative stabilisation of anterior subluxations or dislocations and other injuries identified as having a high incidence of late instability, and the closed management of other cervical injuries.

Crutchfield (1933, 1936 & 1938) described the technique and recommended reduction by continuous traction in skull callipers. Rogers (1957) and Durbin (1957) advised the use of skull traction but advocated operative intervention in all cases in which displacement was not reduced by this method. Munro (1961) from a series of 176 patients treated conservatively reported lower mortality and morbidity than in the 158 cases treated operatively by Rogers

(1957) and Forsyth et al (1959). Geisler et al (1966) in a series of 645 patients found no statistically significant difference in results achieved by operative or non operative methods.

The non operative management of injuries to the cervical spine is supported by the reports of several prominent physicians in the field. (Guttmann, 1969; Frankel et al, 1969; Burke & Beryman, 1971; Bedbrook, 1979 & 1983; Kleyn 1984). Guttmann (1969) pointed out the need for gentleness in the handling of patients with spinal cord injuries and the avoidance of hasty immediate operative procedures. Bedbrook (1983) concluded that operative interference in the early, and even the late stages following injury may be difficult and risk further damage to the already injured spinal cord. He also indicated early surgery aimed at producing spinal fusion is contra-indicated when it is known that spontaneous fusion can be expected in a high percentage of cases.

Osti et al (1989) reported the findings from a retrospective study of 167 consecutive patients with cervical dislocations or subluxation treated between 1976 and 1986 at two major spinal injury units. Eighty five were managed at the Royal Adelaide Hospital, Adelaide, South Australia, by closed reduction and early surgical stabilisation. The operative technique used was a single level antero-lateral dowel fusion as devised by Barbour and reported by Cornish (1965 & 1968). The other eighty two cases were managed at the Royal Perth (Rehabilitation) Hospital, Perth, Western Australia, by closed reduction and maintenance of the reduction with skeletal traction and postural nursing.

The study by Osti et al (1989) reported a significant difference in the degree of neurological recovery between the two treatment groups.

Seventy percent of the patients treated by closed reduction and postural nursing with a modified Frankel grade of B, C, or D (Frankel et al, 1969) improved one or more Frankel grades from the time of their admission to the time of their discharge, while only 50% of the patients treated by closed reduction and early surgical stabilisation improved to the same extent ($p < 0.05$). This trend was seen in all patient groups and suggested early surgical stabilisation utilising a coronal dowel impaired neurological recovery.

The authors suggested that intra-operative interference with, or irritation of the spinal cord vasculature was responsible for the observed difference in the neurological outcome of the operative and non operative groups of patients. This seemed to be a reasonable assumption in light of the reports of Schneider (1951), and Morgan et al (1971) that posterior operations in the early stages after injury can interfere with the blood supply to an already damaged spinal cord.

The Perth and Adelaide series were similar in many respects but the retrospective nature of the study, deficiencies in the recorded data and uncertainty about the accuracy of the examination findings of the initial neurological assessment raised questions as to the validity of the reported findings and conclusions.

Despite these doubts the reported difference in the extent of neurological recovery could be of great clinical significance. If early surgical stabilisation of the cervical spine in patients with incomplete spinal cord injuries impaired neurological recovery and the chance of subsequent independence, this surgery should not be contemplated.

Conversely should the findings reported by Osti et al (1989) be due to factors not related to the surgery itself then this would also be of considerable importance as there are substantial benefits associated with the early surgical stabilisation, mobilisation and rehabilitation of patients with these injuries. Both the bed and hospital stay is reduced, decreasing the financial cost to the community and the incidence of the complications of prolonged bed rest, such as deep venous thrombosis, pulmonary embolism and pulmonary infection. Importantly early surgical stabilisation also corrects instability which persists in a number of patients treated conservatively who then require surgical stabilisation at a later date.

In the absence of any detrimental effect early surgical stabilisation could be offered to patients with suitable injuries without fear that the surgery in itself may adversely affect the patients' neurological recovery.

Because of the findings reported by Osti et al (1989), and the absence of a consensus relating to the management of injuries to the cervical spine associated with incomplete tetraplegia, further evaluation of the effects of antero-lateral cervical fusion on spinal cord blood flow and function was required.

This study therefore set out to evaluate the effect of surgical stabilisation of the cervical spine on spinal cord blood flow and function. The working hypothesis being that antero-lateral inter-body fusion of the cervical spine in the presence of an incomplete spinal cord injury hinders neurological recovery through adverse affects on spinal cord blood flow.

It was not ethical or technically possible to study the effect of this type of surgical stabilisation of the cervical spine, in the presence of an incomplete spinal cord injury, on spinal cord blood flow and electrical function in man. The use of evoked potential monitoring has enabled both the intra-operative, and post-operative monitoring of spinal cord function, but measurement of local spinal cord blood flow is not technically possible without risk of further spinal cord injury. A reproducible and valid animal model was required.

2.2 ANIMAL MODELS TO ASSESS THE PATHO-PHYSIOLOGY OF SPINAL CORD TRAUMA.

Review of the literature revealed an extensive number of animal models that had been used to evaluate the effect of ischaemia, injury and pharmacological agents on spinal cord blood flow and function. Unfortunately no one model has been universally accepted, and again no consensus has been reached as to the effect of different agents and insults on the spinal cord. The differences in study methodology, the animal model used, and variation in the degree and type of spinal cord injury produced may all have been important factors contributing to the conflicting results reported.

The requirement for the model to be anatomically and physiologically similar to man seems obvious. The disappointing fact however is that most studies are governed more by economy of space, time and money than the evaluation of the problem at hand, and utilise animal models that have not been properly validated.

Most researchers agree that the anatomy of a primate is likely to resemble that in man more closely than many other laboratory animals. This however may not be true of some of the smaller

primates used in laboratory research, and has not been established by a detailed anatomic comparison. Korbine et al (1975 & 1979) used rhesus monkeys to evaluate the effect of a spinal cord injury produced by the weight drop technique (Allen, 1911), on spinal cord blood flow. Sandler & Tator [1,2,3] (1976), Ducker & Assenmacher (1969), Assenmacher & Ducker (1971), Deecke & Tator (1973) and Bingham et al (1975) also studied the effect of spinal cord injury on spinal cord blood flow and patho-physiology using a primate model without comparing the anatomy and physiology of the animals used to that in man.

Small quadruped animals have been the main stay of research into the patho-physiology of spinal cord trauma. The relevance of these models to the situation in man is questionable. Cats were used by Croft et al (1972), Martin & Bloedel (1973), Smith et al (1978), Senter et al (1979), Dohrmann et al (1971, 1972 & 1973), Young et al (1980), Bennett (1983), Levy et al (1986), and Nacimiento et al (1986). Dogs have been used by Konrad et al (1987), Fairholm & Turnbull (1971), Ducker & Assenmacher (1969) and Griffiths (1975). Rats have been used extensively in the studies done by Black et al (1986 & 1988), Fehlings et al (1988 & 1989), Guha et al (1989) and Finkelstein et al (1990). Rabbits were utilised by Cheng et al (1984) and Fairholm & Turnbull (1971) and ferrets were used by Eidelberg et al [1,2] (1976). Finally sheep were used in the research by Kaplan et al (1987) to evaluate the effect of aortic occlusion on spinal cord blood flow and function.

None of the studies mentioned above have reported details of an anatomical evaluation of the spinal cord circulation in their chosen model, nor do they report the basis for accepting their model as valid

to assess the effect of various insults on the spinal cord and extrapolate these findings to the situation in man. The questions that need to be answered by all researchers, before proceeding is; "Is the model valid for evaluation of the stated hypothesis and can the results be extrapolated to the situation in man?"

For this reason it is not possible to draw definite conclusions from these studies until the animal models used are shown to be valid.

The initial stage of this study was therefore directed towards the selection, and anatomical validation of the animal model.

The animal used needed to have comparable anatomy (cervical spine and neck), and in particular, vascular anatomy of the cervical spine and spinal cord to that in man. The animal should also be large enough to use the same techniques and instrumentation for the cervical fusion that are used in man, and be amenable to the production of a spinal cord injury, comparable histologically and functionally with the cervical injuries associated with incomplete tetraplegia in man.

Sheep had been used locally in previous studies of spinal pathology and fulfilled many of the above criteria, they are also cheap, readily available and easy to handle. The bony anatomy of the sheep cervical spine has been documented (May, 1977) but not in sufficient detail for the purpose of this study. Despite a search of scientific and veterinary publications insufficient information and detail was available relating to the vascular anatomy of the cervical spine of sheep for comparison with that in man. It was therefore necessary to undertake a vascular and skeletal study to assess the comparative anatomy of the sheep's cervical spine.

2.3 EXPERIMENTAL SPINAL CORD INJURY MODELS.

The preferred method reported in the literature for producing a spinal cord injury seems to be the weight drop method (Allen, 1911), or a modification of this technique (Ducker & Assenmacher, 1969; Assenmacher & Ducker, 1971; Fairholm & Turnbull, 1971; Dohrmann et al, 1971, 1972 & 1973; Bingham et al, 1975; Griffiths, 1975; Korbine et al, 1975; Smith et al, 1978; Senter & Venes, 1979; Young et al, 1980; Levy et al, 1986; Black et al, 1988; Finkelstein et al, 1990). One of the main deficiencies of this technique is the requirement to expose the spinal cord through a laminectomy prior to inflicting the injury. The exposure itself is likely to interfere with local spinal cord blood flow (Schneider, 1951; Morgan et al, 1971) and possibly the electrical function of the spinal cord such that the validity of results obtained must be questioned.

Sustained or graded spinal cord compression has also been used by a number of researchers (Gelfan & Tarlov, 1956; Croft et al, 1972; Eidelberg et al [1], 1976; Nacimiento et al, 1986; and Black et al, 1986). Black et al (1986) found this model did not produce the type of injury required and went on in his subsequent research to use the weight drop method (Black et al, 1988). Other methods used to produce a spinal cord injury include a clip compression technique (Rivlin & Tator, 1978; Fehlings et al, 1989; and Guha et al, 1989) and circumferential compression using an inflatable cuff (Tator, 1973; Deecke & Tator, 1973; and Sandler & Tator [3], 1976). Again both techniques require exposure of the spinal cord through a laminectomy, and the circumferential compression cuff technique requires circumferential mobilisation of the dural sac which could

also have considerable effects on local blood flow and spinal cord function.

The techniques outlined above inflict the injury through a laminectomy with exposure of the spinal cord which may allow dissipation of part of any force applied to the spinal cord through deformation and expansion of the spinal cord and dural sac cephalad and caudal to the injury, where the spinal cord is no longer contained within its bony canal. This could alter the dynamics of the injury from that occurring when the spinal cord is completely contained within its bony canal.

Martin & Bloedel (1973) and Bennett (1983) produced experimental spinal cord injuries of varying severity by the rapid inflation of a balloon catheter in the extradural space. The catheter was inserted through a small fenestration, several spinal segments caudal to the level of the injury. This technique did not require exposure of the spinal cord at the level of the injury so the bony spinal canal remained intact. Inflation of the balloon anterior to the dural sac results in compression of the spinal cord anteriorly, by the balloon itself and posteriorly by the posterior arch of the spinal canal, as the balloon displaced the spinal cord dorsally. This mechanism of cord compression is similar to that seen in dislocations and fracture dislocations of the cervical spine, where the spinal cord is displaced and compressed by disruption of the vertebral column.

The balloon catheter method of producing an experimental spinal cord injury was chosen for use in this study.

2.4 USE OF EVOKED POTENTIAL MONITORING TO EVALUATE SPINAL CORD FUNCTION.

There is little disagreement as to the value of evoked potentials to monitor the function of the spinal cord. There is however some discussion as to the relative benefits of measuring somato-sensory potentials, spinal cord responses and motor evoked potentials (Raudzens, 1982 & 1984; Hahn & Latchaw, 1983; York et al, 1983; Ginsburg et al, 1985; Levy et al, 1986; Fehlings et al, 1988).

In this study the electrical function of the spinal cord was monitored with both motor and sensory evoked responses.

2.5 MEASUREMENT OF SPINAL CORD BLOOD FLOW IN SPINAL CORD INJURY MODELS.

The literature reports several different methods used for the measurement of spinal cord blood flow. The hydrogen clearance technique was reported by Aukland et al (1964) and has been used in spinal cord injury research (Korbine et al,1975 & 1979; Griffiths, 1975; Smith et al, 1978; Senter & Venes, 1979; Fehlings & Tator, 1989; Guha et al, 1989). This technique enables repeated measurement of local spinal cord blood flow but only measures the perfusion in a very small volume of tissue, immediately adjacent to the platinum electrode which is used in this technique. Placement of the electrodes into the spinal cord requires exposure of the spinal cord through either a laminectomy or multiple fenestrations. It is possible that the exposure of the spinal cord may affect the local blood flow and placement of the electrodes may alter the accuracy and reliability of the evoked potential measurements.

The C^{14}-antipyrine auto-radiographic technique described by Reivich et al (1969), and used by Sandler & Tator (1976) and Bingham et al (1975) allows differentiation of white and grey matter blood flow but permits only a single measurement. Xe ^{133} clearance used by Ducker & Assenmacher (1971) required the injection of Xenon into the spinal cord, which may result in local spinal cord trauma and also allows only a single measurements of spinal cord blood flow.

The use of radio nuclide-labelled microspheres to measure regional blood flow began with Grim & Lindseth (1958). Since that time the technique has been refined and the uniformity and circulatory dynamics of the technique have been improved. Buckberg et al (1971) reported "Some of the sources of error in measuring regional blood flow with radioactive microspheres", and a comprehensive summary of the technique was published by Heymann et al (1977). This technique enables repeated measurement of blood flow in any number of tissue samples or organs. The tissue to be studied does not need to be exposed in order to obtain the blood flow measurement. The number of measurements however is limited theoretically by the risk of producing tissue ischaemia as the result of multiple micro infarcts caused by the microspheres occluding vessels in the capillary bed. Kaplan et al (1987) demonstrated that the injection of three million microspheres into the left atrium of sheep, on five separate occasions resulted in no difference in the blood flow measured from the first injection when compared with the fifth injection. Indicating that tissue ischaemia had not occurred at the time of the fifth blood flow measurement due to the preceding injections of microspheres.

Of the techniques reported the use of radio-labelled microspheres was favoured due to the fact that the spinal cord did not need to be exposed and although continual blood flow measurement was not possible, it was felt several appropriately timed measurements would provide the necessary information to evaluate the hypothesis.

3. THE ANATOMY OF THE CERVICAL SPINE IN SHEEP

3.1 AIMS

To evaluate the vascular anatomy of the cervical spine and spinal cord in sheep, and assess the suitability of the sheep as a model to evaluate the patho-physiology of spinal cord injuries.

3.2 MATERIALS AND METHODS

3.2.1 MACROSCOPIC ANATOMY

Seven two year old merino wethers were used to study the bony and vascular anatomy of the sheep cervical spine. Two sheep were obtained from the Waite Agricultural Institute specifically for this study and five sheep were obtained from fellow researchers who had used the sheep in other research projects. Four of the sheep were dissected while anaesthetised and then used for vascular casting immediately after sacrifice, the other three sheep had been sacrificed prior to their use. None of the sheep had undergone operative procedures in the region of the cervical spine or cannulation of cervical vessels, and all sheep were neurologically intact at the time they were sacrificed.

The cervical spine of one of the dead sheep was removed in order to make a skeletal model. Each vertebra was cleared of its soft tissue attachments and macerated in a 4% solution of sodium hydroxide at $80^{o}C$ for 20 minutes. The vertebrae were then washed with tap water, cleared of any remaining soft tissue and bleached in a solution of hydrogen peroxide for twenty minutes. The dimensions of

the vertebrae were recorded and they were then reassembled over a plastic coated rod (in the spinal canal). Drawings were made and photographs taken of the cervical vertebral column in anterior, posterior and lateral elevations. The typical cervical vertebrae were evaluated in greater detail and drawings of the superior and inferior elevations of the fourth cervical vertebrae (C4) also made.

The bony and vascular anatomy of the cervical spine of the remaining six sheep were studied using techniques of macro and micro dissection in conjunction with vascular casting. Casts were made of the vertebral arteries and their tributaries in five sheep, and in the remaining sheep, latex was injected into the brachio-cephalic trunk of a sacrificed animal prior to dissection of the neck to assist in the identification of smaller vessels in and around the spinal canal. In this case the sternum was split and the chest opened anteriorly, the brachio-cephalic trunk was dissected from the aorta and cannulated. One hundred millilitres (ml) of latex was infused at a constant pressure of 200 mmHg via a pressurised reservoir (Figure 1). Any peripheral vessels leaking latex were ligated and the head and neck removed by dividing the inter-vertebral disc between C7 and T1. The cervical spine and head was then refrigerated for seven days to allow the latex to set before the specimen was dissected. At that time the head was disarticulated from the spine, latex was evident in the arteries at the base of the skull and over the surface of the brain. The vertebral vessels were then dissected free, in front of the lateral mass of C7 and followed cranially taking care to preserve its fine branches in the region of the inter-vertebral space.

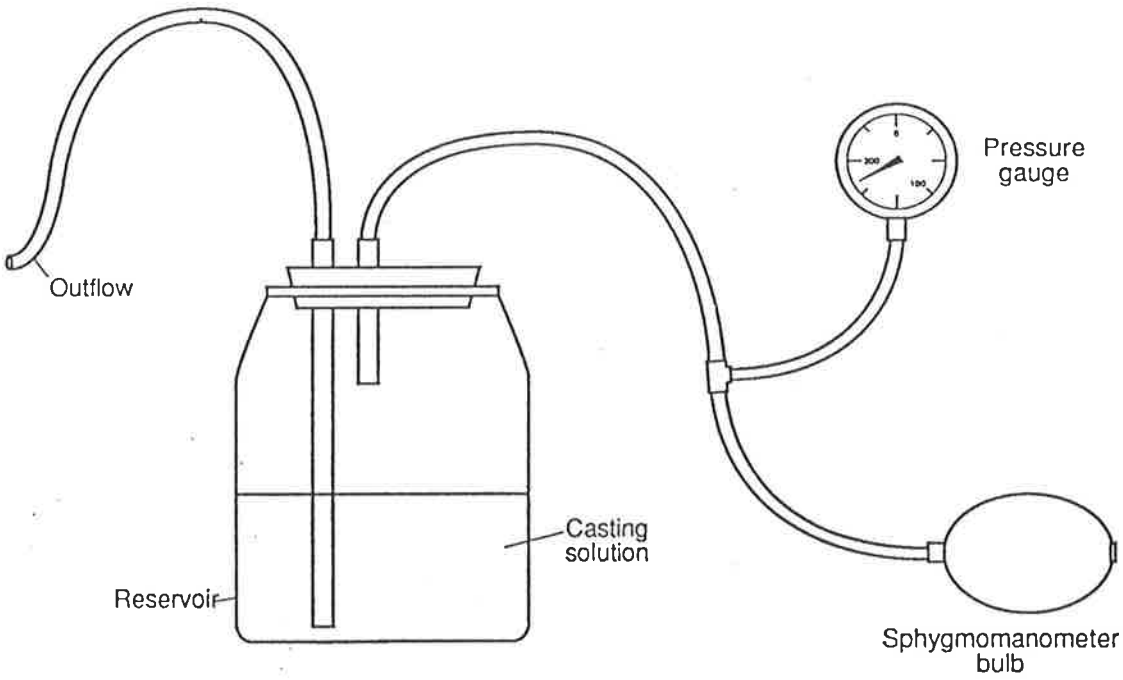


FIGURE 1: Diagram of the perfusion apparatus used to make both methyl-methacrylate and latex vascular casts. The apparatus consists of a reservoir, a sphygmomanometer bulb, a pressure gauge and outflow tubing.

Methyl-methacrylate casts were made of the cervical vasculature of three of the sheep and latex casts were made of the other two. All sheep used for casting were heparinised prior to sacrifice and all casting completed within 60 minutes of each animal's death.

The techniques used in the preparation for, and execution of vascular casting were based on those reported by Nakai et al, (1981). This technique has subsequently been used by the same group to evaluate the blood supply of the feline spinal cord (Naka et al, 1987) A comprehensive review of this technique has subsequently be reported by Lametschwandtner et al, (1990) indicating its use to study the vascular supply of organs using either whole body, single organs in situ or isolated organ preparations. The only requirement being the isolation of inflow and outflow vessels and the injection of adequate volumes of casting medium to fill the vascular tree.

3.2.2 METHYL-METHACRYLATE CASTING TECHNIQUE

3.2.2.1 PREPARATION OF METHYL-METHACRYLATE CASTING SOLUTION

One hundred millilitres of methyl-methacrylate monomer was mixed with 2g of 2,4-dichlorobenzoyl peroxide in 50% dibutyl pthalate. This was then polymerised under ultra violet light for 80 minutes, inverting the mixture every five minutes. After polymerisation was complete 0.25g of benzoyl peroxide and 0.01g of red waxyline dye were added.

Concurrently, in a separate container, the initiator solution was prepared. This consisted of 7ml of 2-hydroxy-propyl methacrylate and 0.4ml of N.N-dimethyl aniline. The initiator solution was then

combined with the polymerised methyl-methacrylate just prior to casting.

3.2.2.2 PREPARATION OF THE VERTEBRAL SPECIMEN

All the sheep used for vascular casting were heparinised with a bolus of 500 units/Kg of heparin sodium at least fifteen minutes, and not longer than 30 minutes before the casting was performed.

The cervical spine from C2 to C7 was removed en-block by dividing the intervertebral ligaments between C1 and C2 and the C7/T1 intervertebral disc. The adjacent para-spinal muscles, oesophagus, trachea and major vessels were left intact in the specimen. The left vertebral artery was identified and dissected free for cannulation proximal to its entry into the vertebral foramen of the sixth cervical vertebra. The left vertebral artery was then perfused with 600ml of a priming solution consisting of 60g of polyvinyl pyrrolidone 40 (PVP), 10,000 units of heparin sodium and 0.1ml papaverine in normal saline. The priming solution was perfused at a constant pressure of 120 mmHg. This cleared the vascular tree of blood and lined the vessels with PVP and aided curing of the methyl-methacrylate. Priming the specimen in this way also enabled identification and ligation of draining and branching vessels that were outside the field of interest. One main outflow vessel was identified and allowed to drain freely at the caudal end of the specimen to allow flow through and complete flushing of the cervical vascular tree.

The polymerised methyl-methacrylate and initiator solutions were then combined and perfused through the specimen at a constant pressure of 200 mm Hg, via the perfusion pump. Once methyl-methacrylate was seen flowing from the draining vessel, free of

priming solution and air bubbles, the draining vessel was clamped and the pressure maintained until the methyl-methacrylate set. This usually occurred within 10-15 minutes. The specimen was then refrigerated for at least 24 hours before the tissues were disturbed. Prior to the dissection and maceration of the specimens the vertebrae were stabilised using two stout axial Kirschner wires. This prevented movement of the vertebrae and damage to the fine vascular cast as the soft tissues were removed. The oesophagus, trachea and para-vertebral muscles which were outside the area of interest were removed using sharp dissection. A cuff of tissue was left around the vertebral arteries and exiting nerve roots in the intervertebral space so as not to damage the finer vessels present in these regions.

Specimens were sodium hydroxide at 80^{o}C for 20 minutes and then washed with a fine jet of hot water while immersed in a water bath. Sharp dissection under magnification was used to remove the remaining soft tissue. This was followed by another 5 minutes in the sodium hydroxide solution and repetition of the washing procedure.

In all cases a limited laminectomy was performed to assist the circulation of the sodium hydroxide around the spinal cord. The spinal cord itself was quite resistant to maceration using this technique which resulted in poor quality casts of the fine vessels on the surface of the spinal cord which were damaged during processing. The vertebral specimens and casts were then dried, examined and photographed.

This technique provided detailed fine casts of both the arterial and venous vascular network as the low viscosity methyl-methacrylate

solution crossed the capillary bed. The casts obtained identified an extensive anastomotic network of vessels around the spinal cord but their fragility and the combination of both venous and arterial casts made interpretation difficult and limited their value.

It was decided a more viscous moulding solution, one unable to cross the capillary bed would improve the quality of the arterial cast. Moulding Latex (Beta Chemicals No. 1302) was used to make casts in the remaining two specimens with excellent results.

3.2.3 LATEX CASTING TECHNIQUE

The sheep were heparinised in the same way as the sheep used for methyl-methacrylate casting, and the cervical vertebrae from C2 to C7 were removed en-block with the adjacent para-spinal muscles, oesophagus and carotid sheaths as outlined above. The left and right vertebral arteries were identified cephalad and caudally. The right vertebral artery was ligated caudally and the left vertebral artery cephalad. This was to encourage filling of both right and left arterial trees through the arterial anastomosis around the spinal cord. The specimens were then firmly wrapped, first in plastic and then in a crepe bandage, leaving both ends of the specimen open to the environment. This occluded both the veins and the arteries outside the area of interest and prevented external loss of the casting solution. In this way the filling of the vasculature was improved in and around the intervertebral foramen, in the spinal canal and around the spinal cord.

The left vertebral artery, which had been cannulated prior to wrapping the specimen which was then primed with heparinised saline. All draining vessels were ligated proximally except the right

vertebral artery at the cephalad end of the vertebral segment. The latex solution was perfused at a constant pressure of 200 mmHg. Once latex drained from the right vertebral artery, free of saline or bubbles the right vertebral (outflow) and then the left vertebral (inflow) vessels were ligated. The specimens were refrigerated for at least seven days to allow the latex to set sufficiently to prevent dissolution of the casts during dissection and maceration.

The para-vertebral musculature and other soft tissue structures were then removed leaving a cuff of tissue around the inter-vertebral foramen and exiting nerve roots. Finally the specimens were macerated in a 4% solution of sodium hydroxide as described in Section 3.2.2.2.

Partial laminectomies were again required to assist digestion of the neural tissue. The quality of the casts obtained in both macerated and wet specimens are indicated in Figures 7,8, &10c. The latex casts proved to be far more durable than the methyl-methacrylate and provided more useful information, despite the casts having less vascular detail.

3.3 RESULTS

3.3.1 BONY ANATOMY

The bony anatomy of the sheep cervical spine is illustrated in Figure 2, and a comparison of the fourth cervical vertebrae of the sheep and man is shown in Figure 3. Sheep have seven cervical vertebrae, each vertebra having a body, a neural arch, laminae, pedicles, lateral masses from which the transverse processes emanate, a spinous process and superior and inferior articular processes and facets. Nerve roots exit through the intervertebral foramen which are bounded anteriorly by the posterior annulus of the intervertebral disc and the adjacent vertebral bodies, superior by the inferior lip of the pedicle of the vertebra above, posteriorly by the anterior border of the superior articular facet of the vertebra below and inferiorly by the superior lip of the pedicle of the vertebra below.

In the lateral masses of the upper six cervical vertebrae, just anterior to the origin of the pedicle is a bony canal. The vertebral arteries travel cranially in this canal and the relationship of the exiting nerve roots to the vertebral artery is shown in Figure 4.

In the sheep as in man, the first, second and seventh vertebrae are "atypical", the remaining four vertebrae are of similar size and shape and are referred to as the "typical" cervical vertebrae. The first cervical vertebra (C1), or Atlas vertebra of the sheep has no body as such, but has an elongated shield-like anterior and posterior arch joined by two rather solid lateral masses. It has two cup-like superior articular facets for articulation with the base of the skull which allows flexion and extension but little or no rotation.

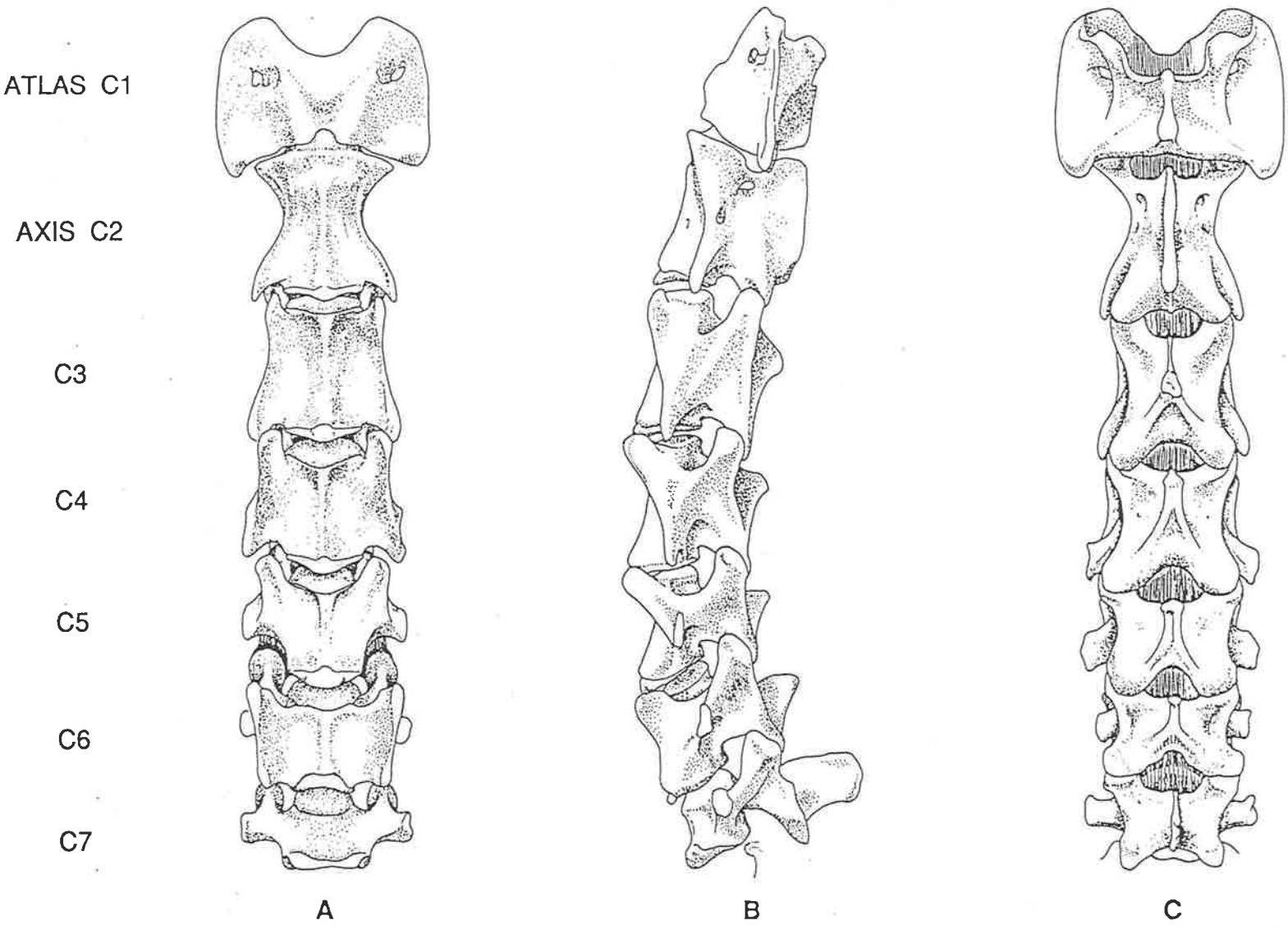


FIGURE 2: The cervical spine of the sheep; (A) Anterior elevation, (B) Lateral elevation, (C) Posterior elevation, showing the appearance and relationship of the seven cervical vertebrae.

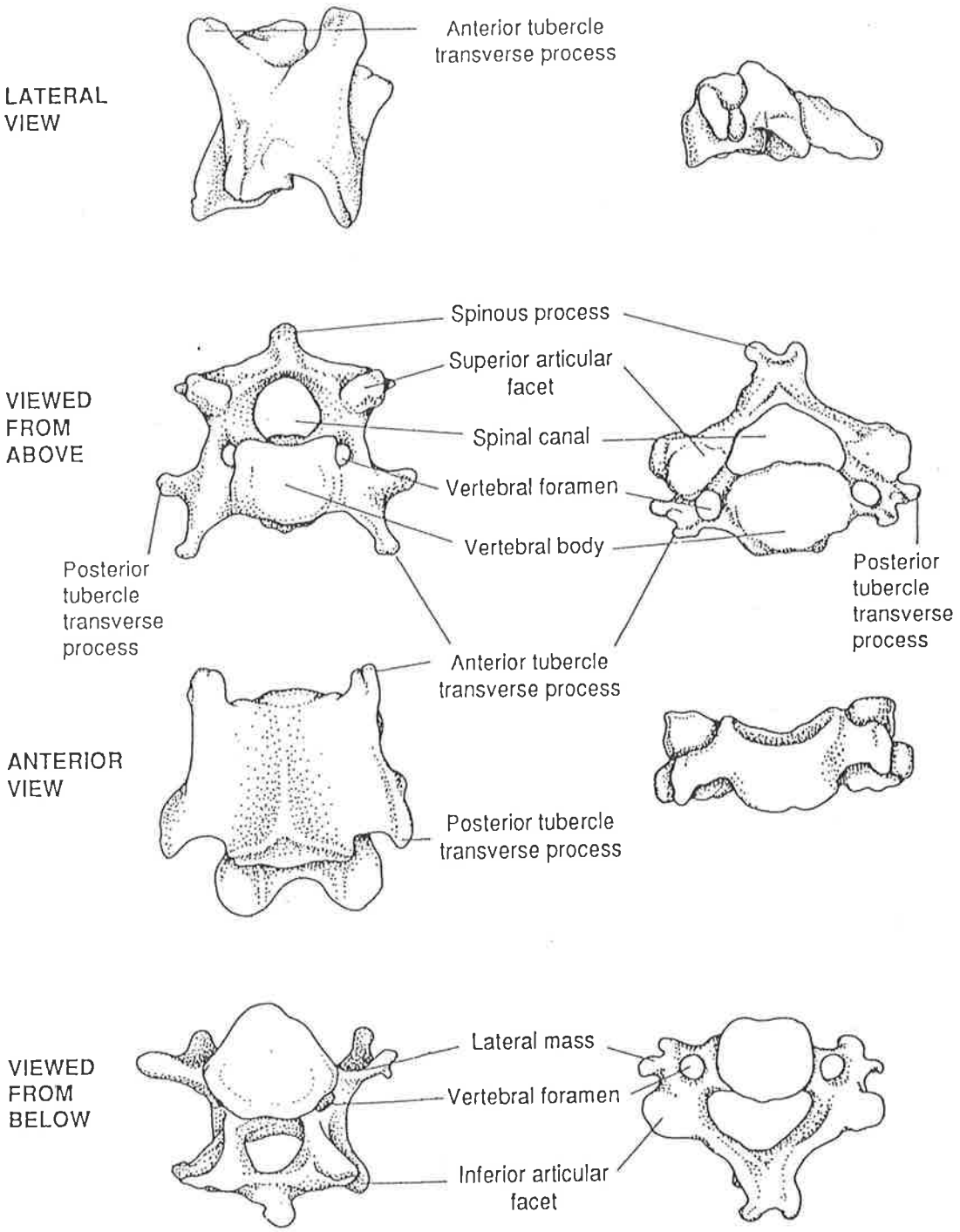


FIGURE 3: Scale drawings of the fourth cervical vertebra of the sheep (Left), and man (Right).

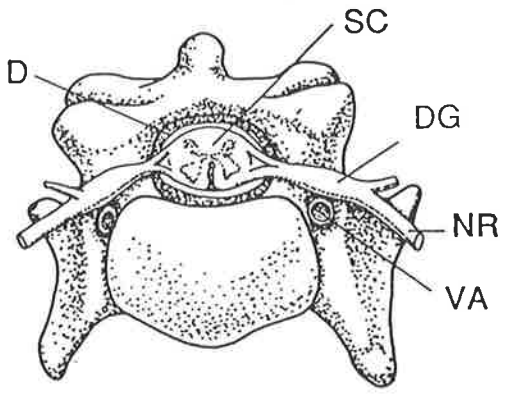


FIGURE 4: Transverse section of the sheep cervical spine at the level of the C4/5 intervertebral disc. The fifth cervical vertebra is viewed from above and the relationship of the vertebral artery (VA) to the exiting nerve root (NR) is identified. The position of the spinal cord (SC), dura (D), and dorsal root ganglion (DG) are also indicated.

On the inner and inferior surfaces of the Atlas are two smooth crescentic articular facets which are lined by articular cartilage for articulation with the articular process of the second cervical or Axis vertebra (C2).

The Axis vertebra has a semi circular articular process and a crescentic superior articular facet which are both lined with articular cartilage and articulate with the inferior articular facets of C1. These two vertebrae are different in appearance to the Atlas and Axis vertebrae in man but similar in function as the atlanto-axial articulation in sheep allows free rotation but no flexion or extension.

The seventh cervical vertebra (C7) does not have a vertebral foramen. It is shorter than the vertebrae above it, has a more prominent spinous process and has two large rounded transverse processes. The vertebral arteries pass in front of these transverse processes on their way to enter the vertebral foramen of C6. The seventh cervical vertebra is therefore a transitional vertebra between those of the cervical and those of the thoracic spine.

3.3.2 VASCULAR ANATOMY

The sheep has a single brachio-cephalic trunk which branches from the aorta near its origin. This brachio-cephalic trunk is really a continuation of the ascending aorta as the aorta proper arches to the left and descends to the abdomen. The first branches of the brachio-cephalic trunk are the fore-limb or brachial arteries (analogous to the subclavian arteries in man) the left arising slightly lower than the right behind the first rib at the levels of the third (T3) and second (T2) thoracic vertebral bodies respectively. The brachio-cephalic trunk continues on to divide into the right and left

carotid arteries in front of first thoracic vertebrae (T1). It is from the brachial arteries that the vertebral arteries originate.

The right vertebral artery arises immediately after the brachial artery branches form the brachio-cephalic trunk. The left takes its origin between two and three centimetres distal to the origin of the left brachial artery. The anatomy of the vertebral arteries and major vessels in the mediastinum of the sheep is illustrated in Figure 5.

The vertebral arteries pass cranially and laterally in contact with the antero-lateral aspect of the trachea on the right, and the oesophagus on the left, then over the para-spinal muscles in close proximity to the origin of the first rib. The vertebral arteries then pass anterior to the transverse process of C7 in a groove between transverse process and body. It then continues on to enter the vertebral foramen in the lateral mass of the sixth cervical vertebra, Figure 6. Branches are given off in the inter-vertebral space to supply the para-spinal muscles, nerve roots, spinal cord and the vertebral bodies (Figure 7).

The spinal cord itself is supplied via radicular vessels of varying calibre which join the dorsal and ventral spinal roots in the intervertebral space. These radicular vessels branch medially to supply the spinal cord and laterally to supply the nerve roots and brachial plexus. Radicular vessels travelling with the ventral roots anastomose on the anterior surface of the spinal cord to form a single anterior longitudinal arterial trunk (Figures 8 & 9). This trunk can be divided over short distances as shown in Figure 8.

Radicular branches travelling medially with the dorsal roots anastomose on the dorsal surface of the spinal cord. The

arrangement of these dorsal vessels was however quite variable. In several of the specimens studied there appeared to be definite dorsal longitudinal vascular channels over some segments of the spinal cord as illustrated in Figure 10A. The arrangement was however more random in the majority of cases (Figure 10B & 10C).

A fine subdural plexus of vessels provides anastomotic communication between and around the main longitudinal arterial trunks on both the ventral and dorsal surface of the spinal cord.

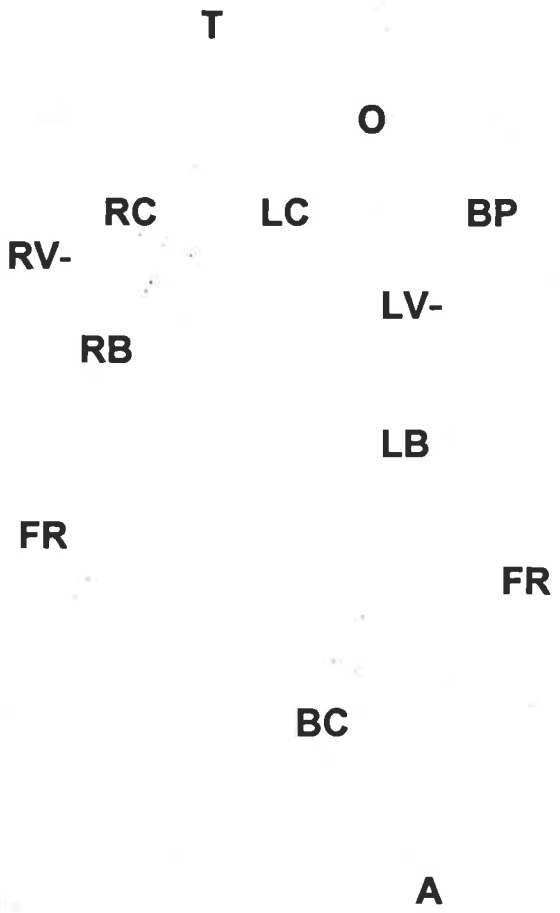
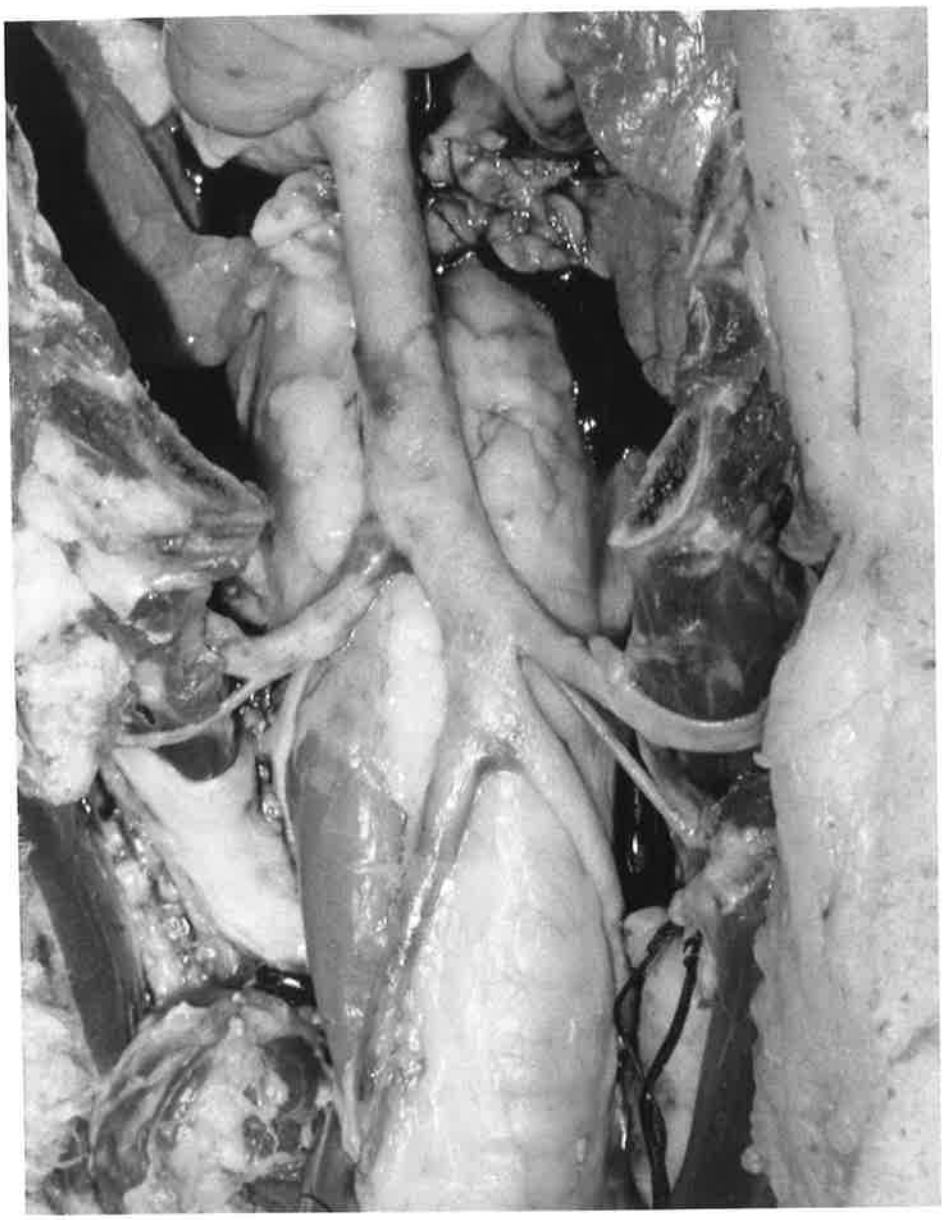


FIGURE 5: Macroscopic dissection of the sheep mediastinum showing the relationship of the major vessels to the oesophagus, trachea and first rib; aorta (A), brachio-cephalic trunk (BC), right and left brachial arteries (RB&LB), right and left carotid arteries (RC&LC), right and left vertebral arteries (RV&LV), first rib (FR), oesophagus (O), trachea (T) & brachial plexus (BP).



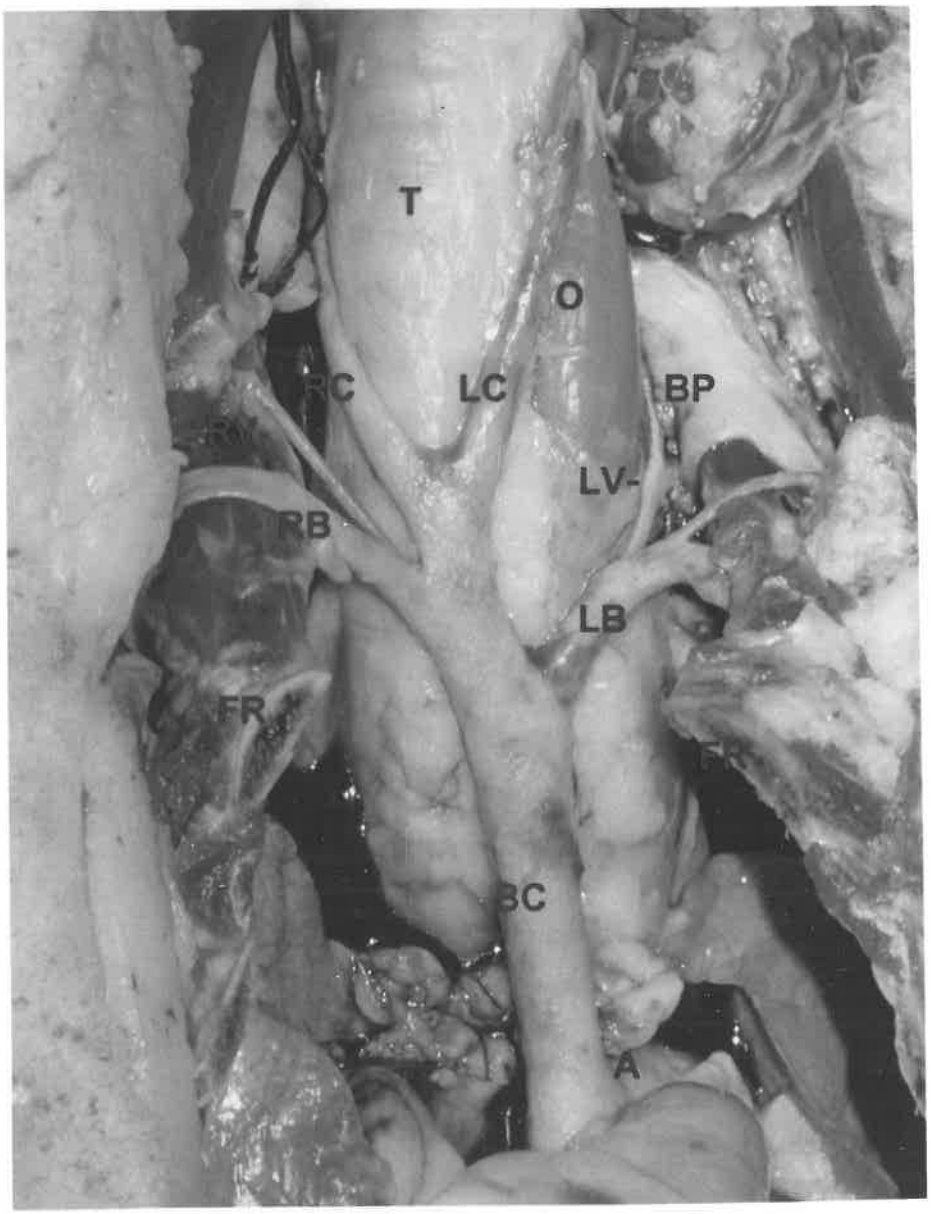


FIGURE 5: Macroscopic dissection of the sheep mediastinum showing the relationship of the major vessels to the oesophagus, trachea and first rib; aorta (A), brachio-cephalic trunk (BC), right and left brachial arteries (RB&LB), right and left carotid arteries (RC&LC), right and left vertebral arteries (RV&LV), first rib (FR), oesophagus (O), trachea (T) & brachial plexus (BP).

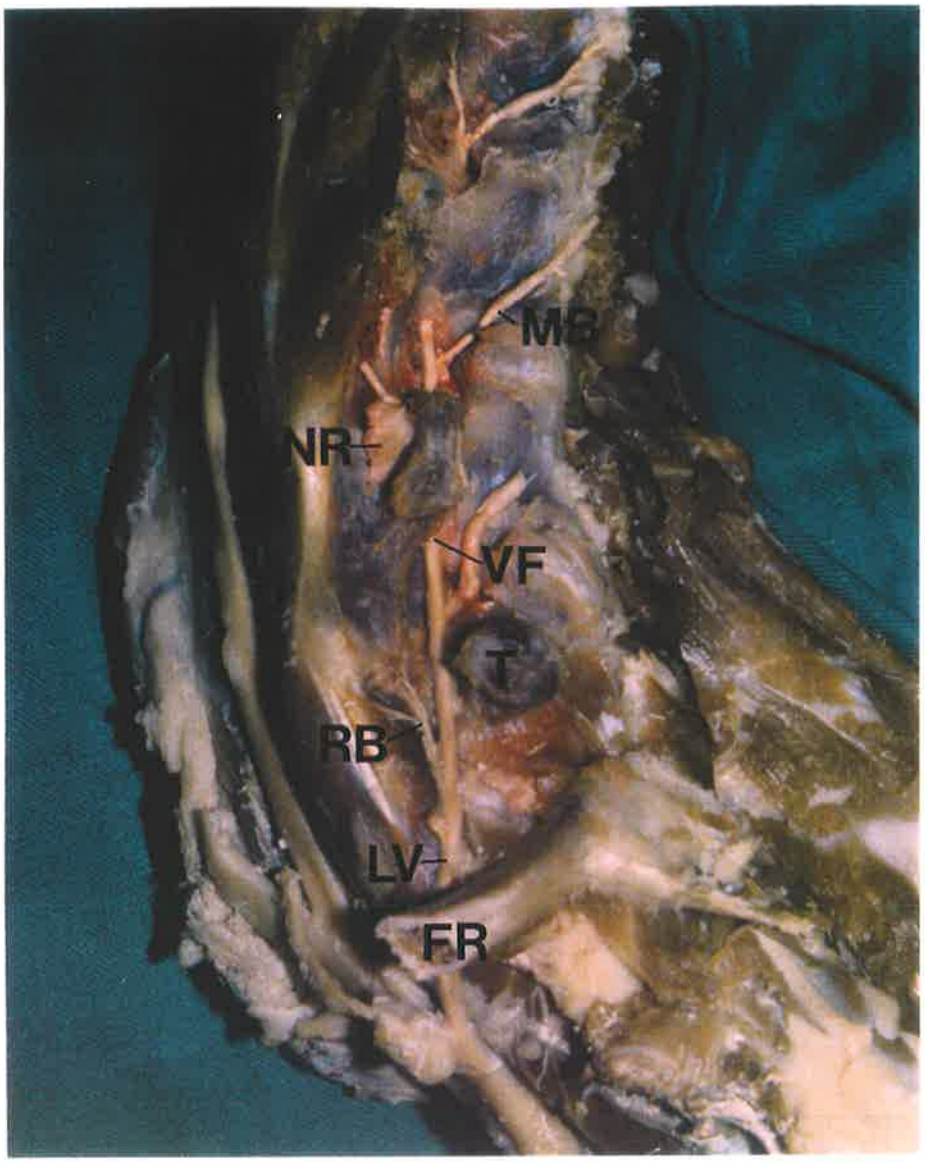


FIGURE 6: Macroscopic dissection of the left vertebral artery following injection with latex and fixation in formalin. The path of the left vertebral artery (LV) can be followed from its origin, beneath the first rib (FR), anterior to the tubercle of C7 (T) to enter the vertebral foramen of C6 (VF). The sixth cervical nerve root (NR) can be seen exiting the C5/6 intervertebral foramen above the lateral mass of C6. Radicular branches (RB) to the partially removed C7 nerve root are seen as are branches to the para-spinal muscles (MB).

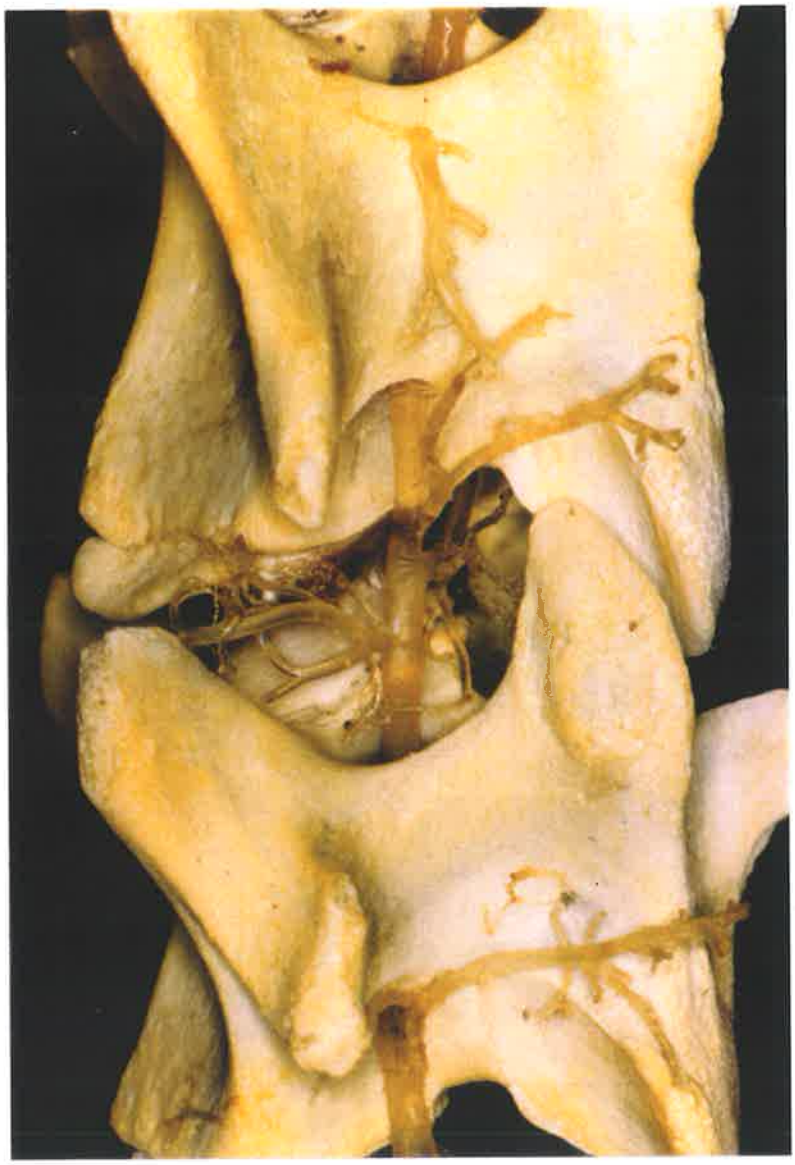
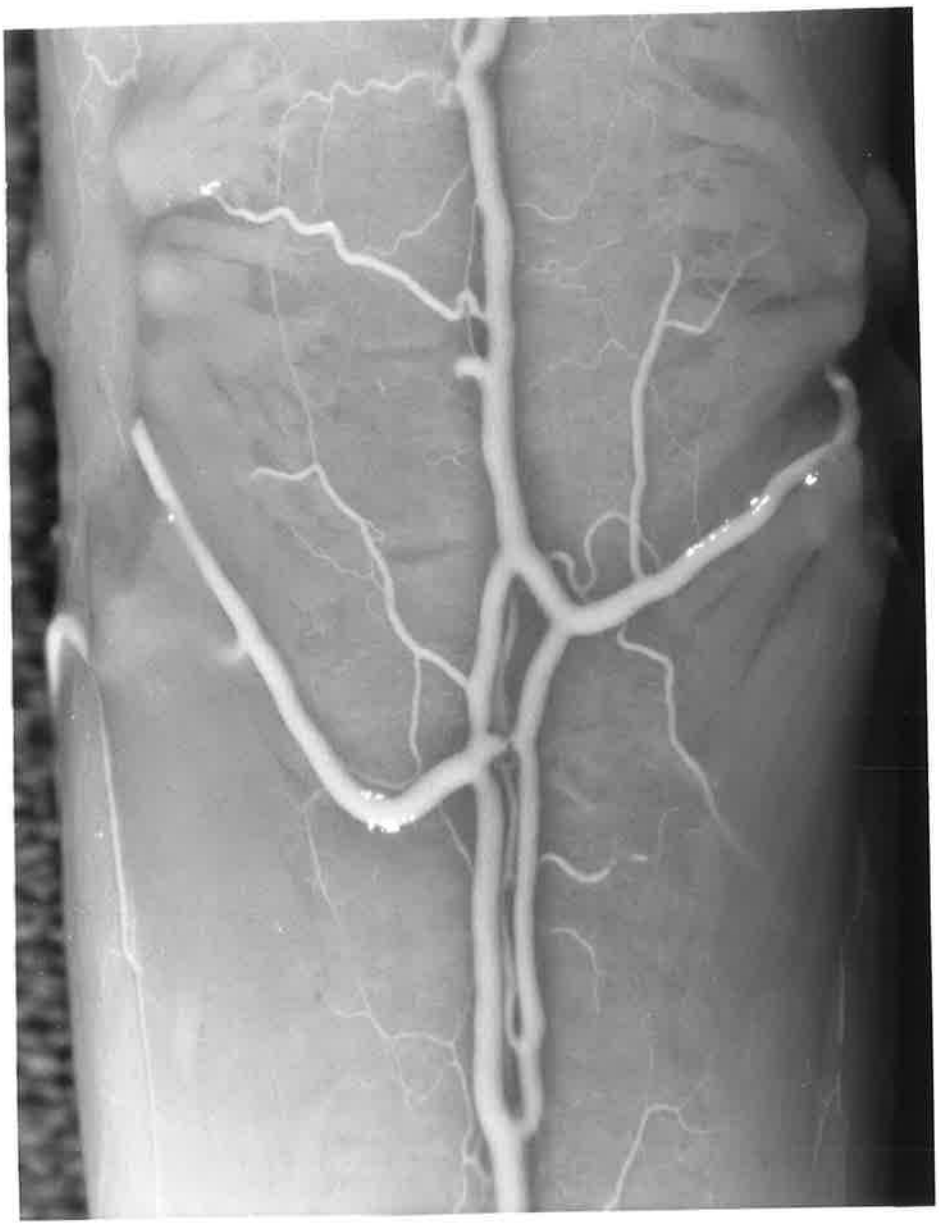


FIGURE 7: Latex cast of the C4/5 intervertebral space showing muscular branches (M), extradural vertebral branches (E) and radicular branches (RB) of the left vertebral artery (V). The radicular branches travel back along the nerve roots, enter the dura and supply the spinal cord.

FIGURE 8: Anterior view of a partially macerated segment of the cervical spinal cord following latex casting. The ventral roots (VR) can be seen exiting the spinal cord. Anterior radicular branches of the vertebral artery (RB) anastomose to form the anterior longitudinal arterial trunk, divided over a short distance in this specimen. Central arterial branches (CB) are also evident in this specimen.



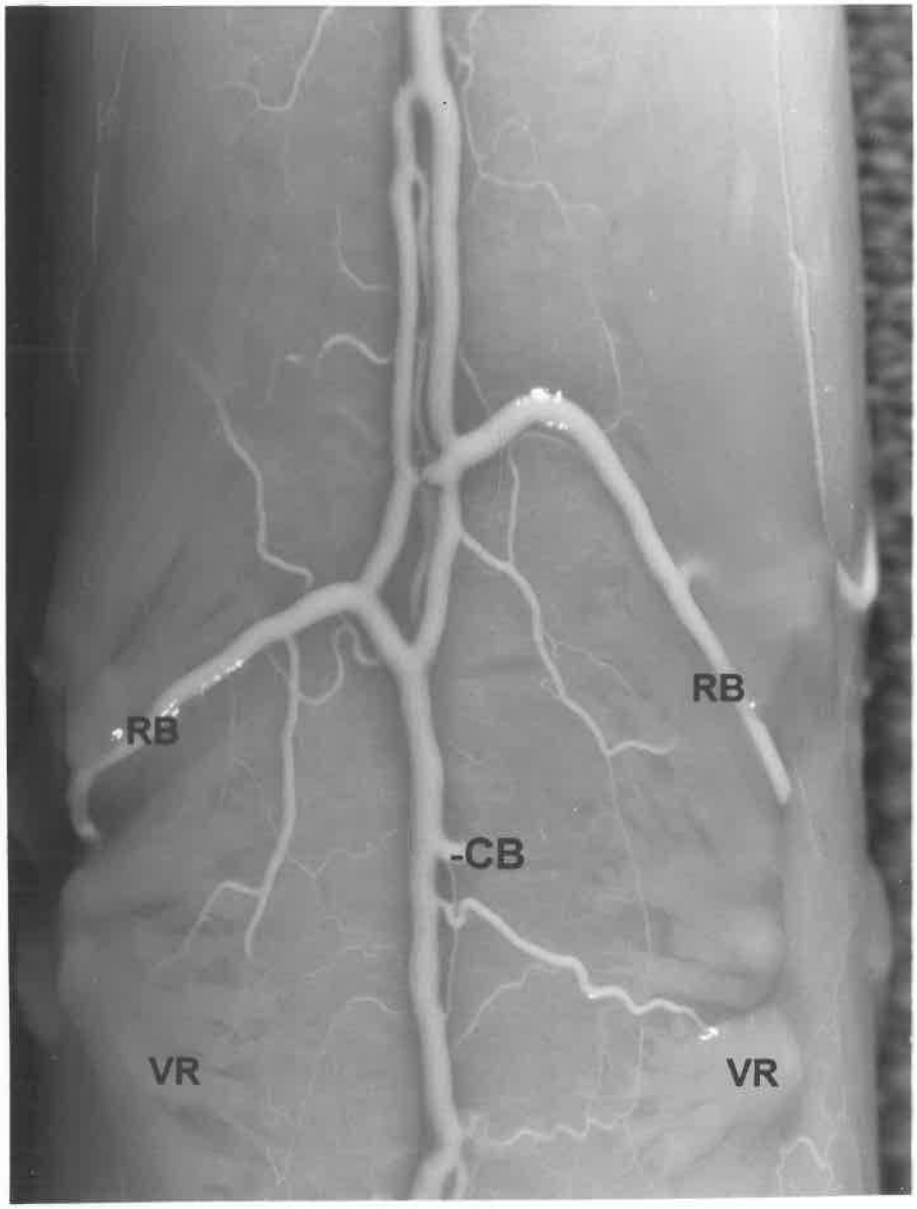


FIGURE 8: Anterior view of a partially macerated segment of the cervical spinal cord following latex casting. The ventral roots (VR) can be seen exiting the spinal cord. Anterior radicular branches of the vertebral artery (RB) anastomose to form the anterior longitudinal arterial trunk, divided over a short distance in this specimen. Central arterial branches (CB) are also evident in this specimen.

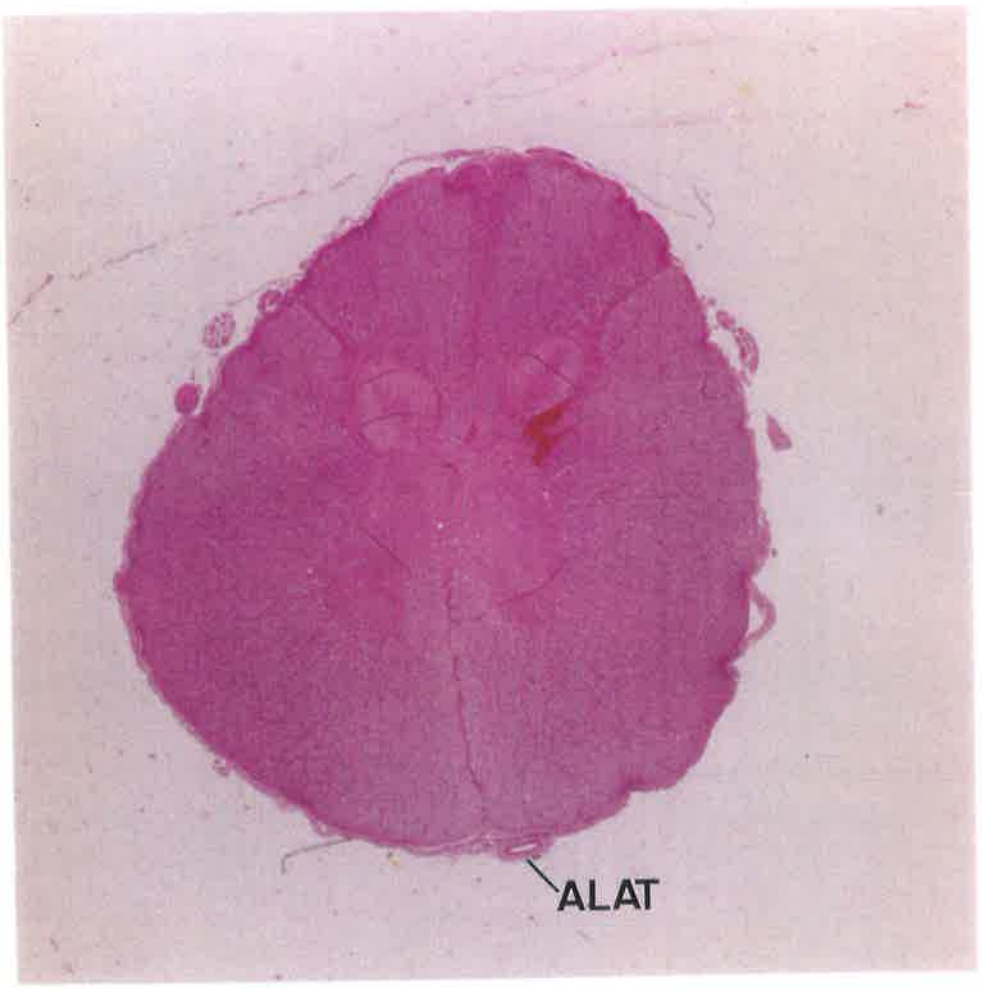


FIGURE 9: Histological section of the spinal cord at the level of the C4/5 intervertebral disc from one of the sheep given an incomplete spinal cord injury. The specimen indicates the location and size of the anterior longitudinal arterial trunk (ALAT). The smaller irregular dorsal vessels are less well seen. (H&E)

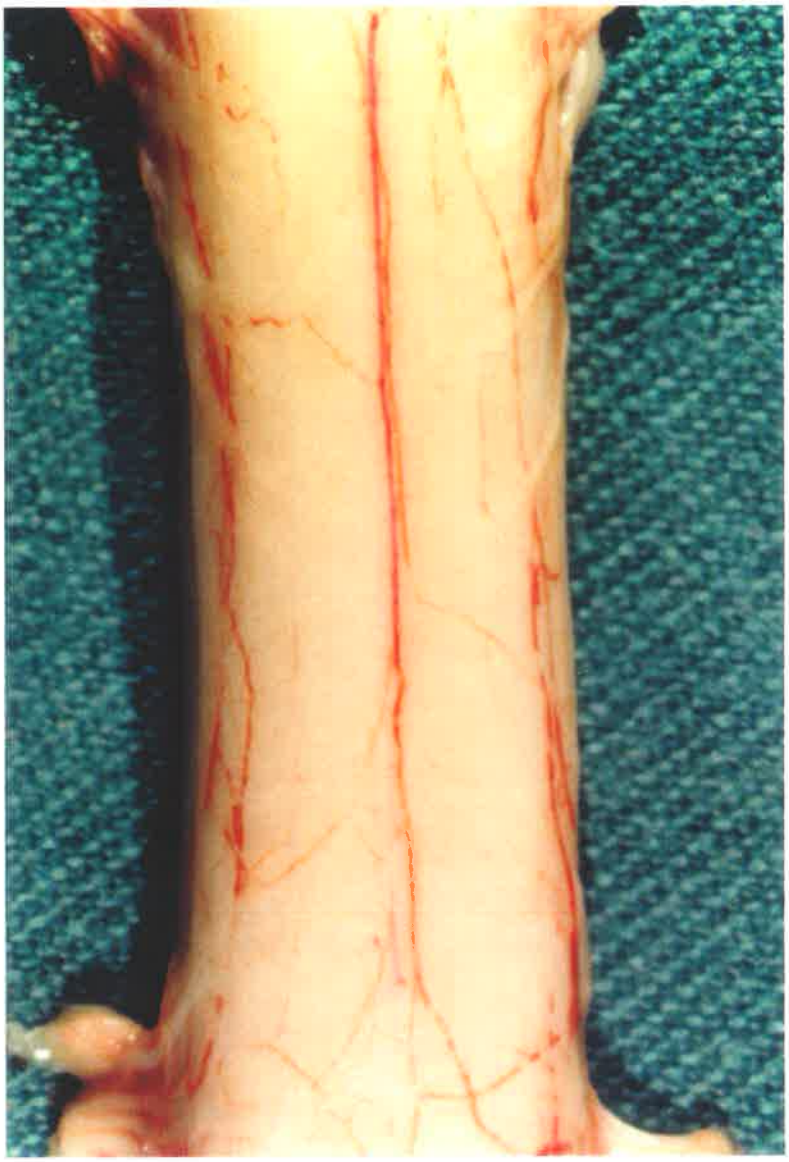


FIGURE 10A: Dorsal view of a fresh specimen demonstrating a segment of the spinal cord where dorsal longitudinal vascular channels are present.

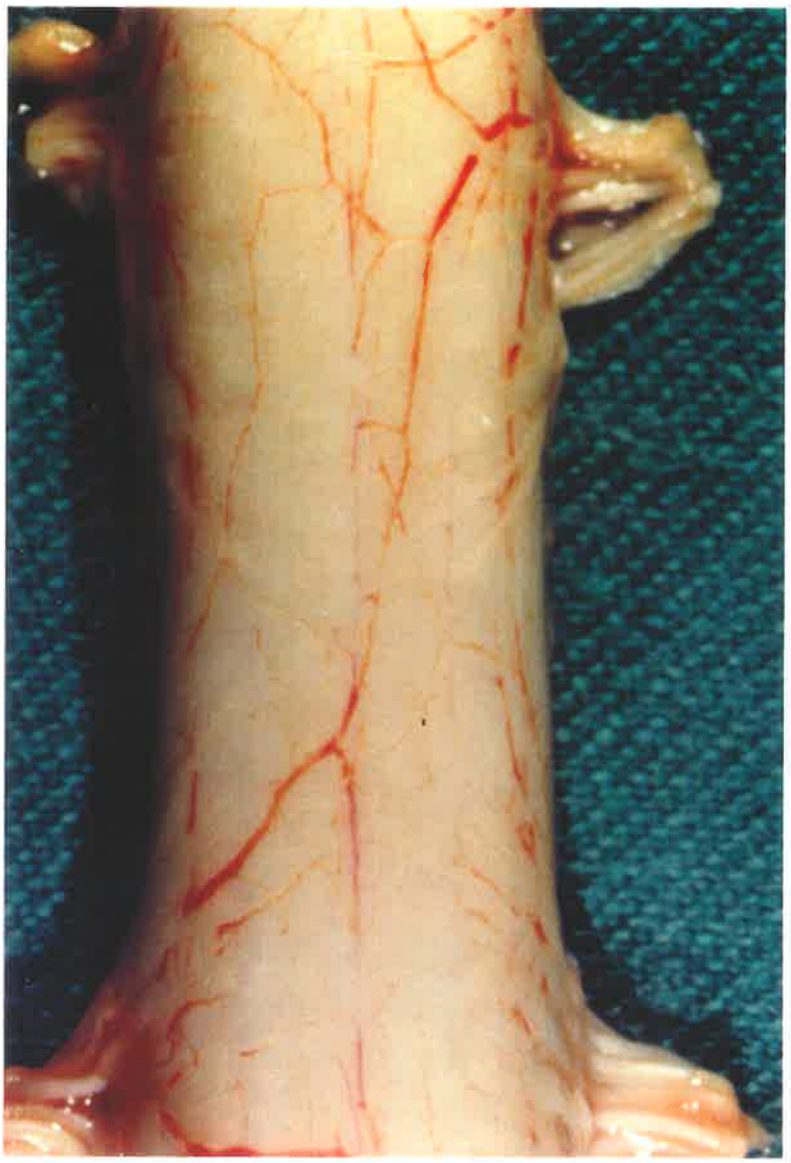


FIGURE 10B: Dorsal view of a fresh specimen of cervical spinal cord where the arrangement of vessels is random. Radicular branches are also evident as they contribute to the dorsal anastomosis of vessels.

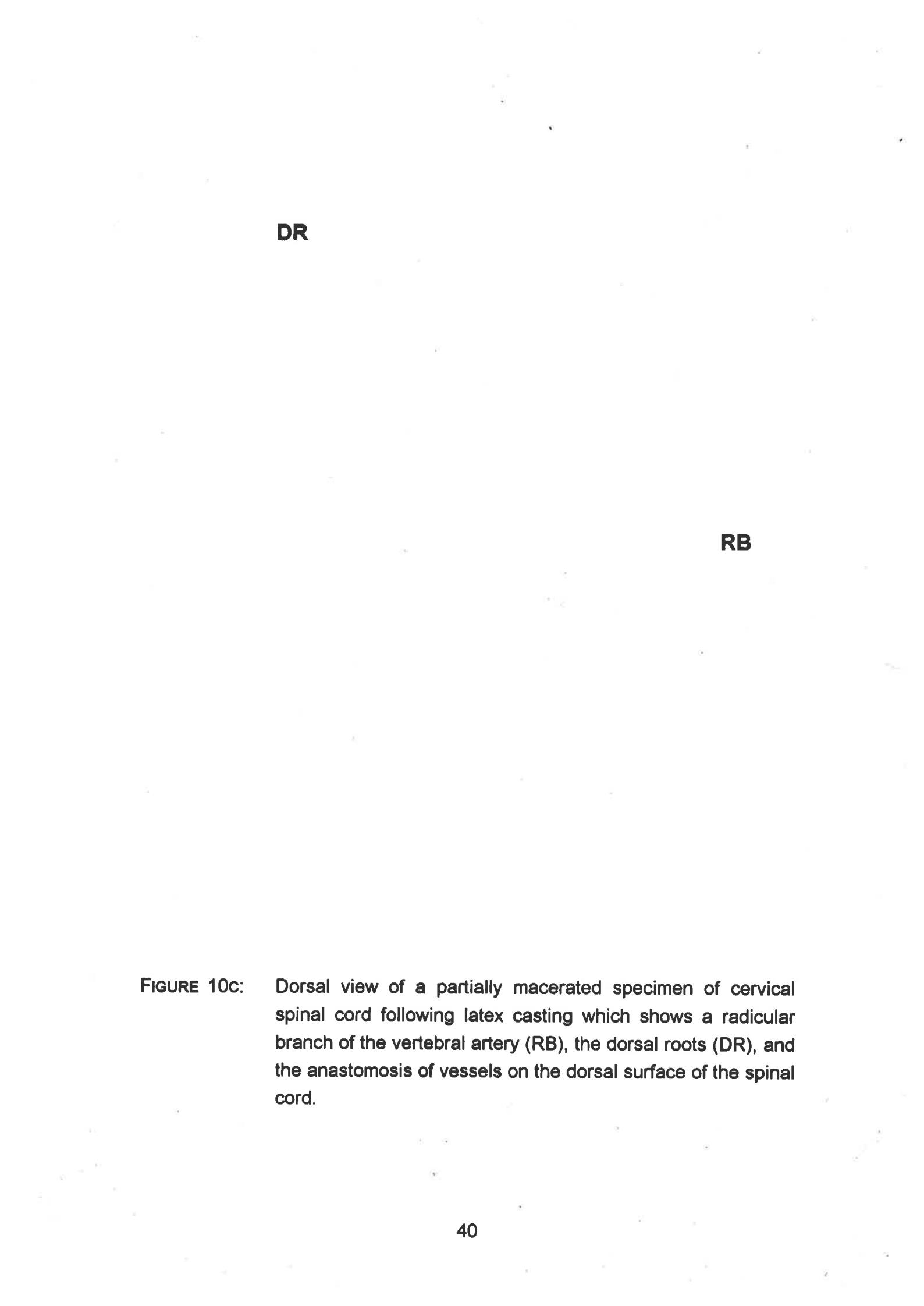
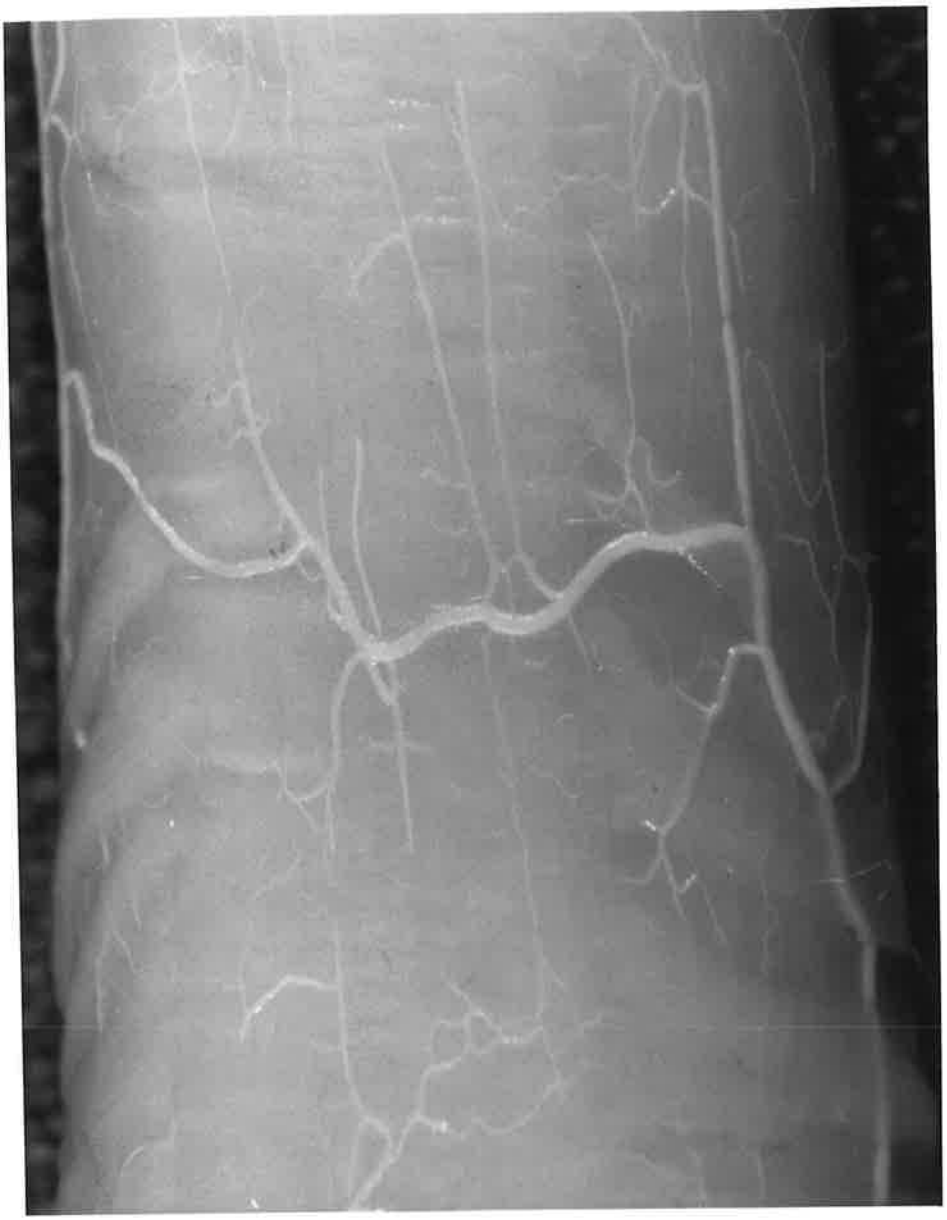


FIGURE 10c: Dorsal view of a partially macerated specimen of cervical spinal cord following latex casting which shows a radicular branch of the vertebral artery (RB), the dorsal roots (DR), and the anastomosis of vessels on the dorsal surface of the spinal cord.



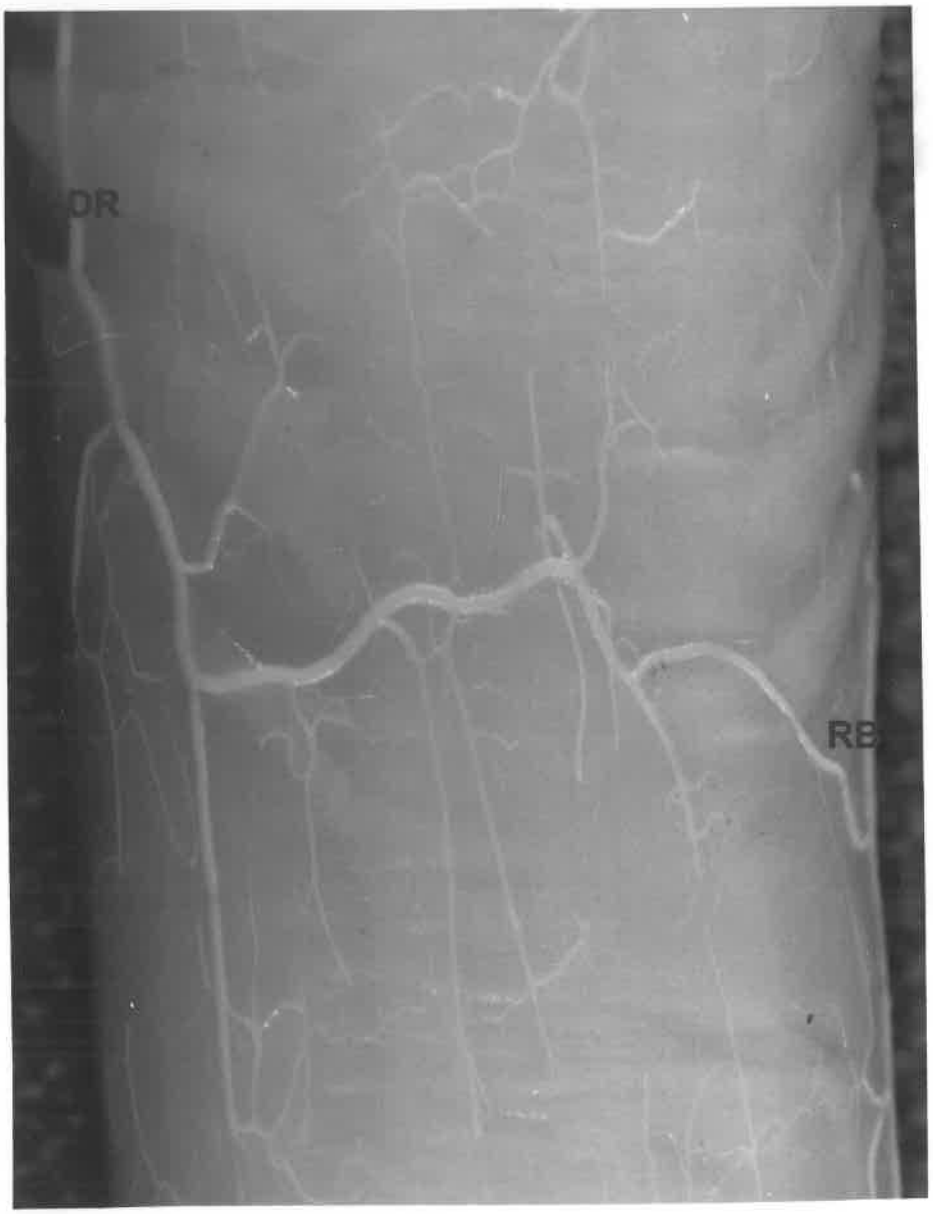


FIGURE 10C: Dorsal view of a partially macerated specimen of cervical spinal cord following latex casting which shows a radicular branch of the vertebral artery (RB), the dorsal roots (DR), and the anastomosis of vessels on the dorsal surface of the spinal cord.

3.4 DISCUSSION

There are both similarities and differences in the cervical vertebrae of sheep and man. The sheep cervical vertebrae are taller and the typical cervical vertebrae are cube like in their overall external shape. The human cervical vertebrae are shorter and have a triangular outline, base anterior, when viewed from above. The cross-sectional configuration of the spinal canal in sheep is almost circular, while it is kidney shaped in man. Thus the anterior-posterior dimensions of the spinal canal are similar but the transverse dimensions of the human spinal canal are almost twice that of the sheep (Figure 3). Human cervical vertebrae have unco-vertebral joints at the lateral margins of the vertebral endplates for articulation with the vertebrae above and below. These are absent in the sheep.

In both species the vertebral artery passes cranially through a bony canal or foramen in the lateral mass of each of the upper six cervical vertebrae. In man this foramen is called the vertebral foramen or foramen transversarium. In this text the canal for the vertebral artery in the sheep will be referred to as the vertebral foramen. The vertebral foramen in man is circular and has a maximal transverse diameter of 4-7mm (measurements made of the upper six cervical vertebral of three different dried human skeletons). The sheep vertebral foramen is a little smaller with a maximal transverse diameter of 3-6mm (measured form the typical cervical vertebrae of 4 of the sheep used in this anatomical study). The left vertebral foramen was an average of 1mm larger than its counterpart on the right, in all four of the sheep studied. The smaller size of the vertebral artery in sheep no doubt reflects the lower perfusion

demands of the less complex sheep central nervous system than that in man.

The relationship of the vertebral artery to the exiting cervical nerve roots in the intervertebral space is similar to that in man. The vertebral artery passing just anterior to the dorsal root ganglion (Figure 4).

The vascular anatomy of the human spinal cord has been studied and reported in some detail (Adamkiewicz, 1882; Kadyi, 1889; Turnbull et al, 1966; Dommisse, 1975 and Crock & Yoshizawa, 1977). The pioneering work reported by Adamkiewicz and Kadyi remained unchallenged for many years. More recently however as a result of improved casting and imaging techniques visualisation of greater detail of the vascular anatomy has been possible. Subsequently the segmental nature of the vascular supply of the spinal cord has been emphasised. Crock & Yoshizawa (1977) provided an extensive and very detailed atlas of the vascular anatomy of the vertebral column and spinal cord, so that today our knowledge and understanding of the vascular anatomy of the human spinal cord is almost complete.

In man the vertebral arteries provide the majority of the blood supply to the cervical spinal cord. This is by way of radicular vessels which are given off in the intervertebral space and reach the spinal cord by branching medially along the exiting nerve root. Branches also travel laterally to supply the nerve root itself, and the brachial plexus.

Turnbull et al (1966) reported that radicular arteries reached the cord by travelling with some, but not all of the anterior and posterior cervical nerve roots. Crock & Yoshizawa (1977) reported from his

more recent and detailed study that segmental radicular vessels accompany all nerve roots and pass medially towards the spinal cord. He also pointed out that the calibre of these vessels is variable and that the finer vessels are easily missed in cadaver studies where vessels may become occluded or clotted prior to the investigation. Some of the earlier techniques employed by Adamkiewicz (1882) were unable to identify these finer vessels.

In the 25 sheep used throughout this study (7 in the anatomical study reported in this Chapter and 18 animals used in the subsequent research reported in Chapters 4 & 5), segmental radicular vessels were found to accompany all of the cervical roots examined. The size and configuration of these vessels varied considerably, consistent with Crock & Yoshizawa's (1977) findings in man. These branches from the vertebral arteries passed medially to anastomose on the anterior and posterior surface of the spinal cord, (Figures 7, 8, 10A, B & C) to form the anterior longitudinal arterial trunk and the posterior vascular anastomosis. A pial plexus of very fine vessels was evident from the methyl-methacrylate vascular casts but these casts were extremely fragile and usually damaged during the maceration process. It was not possible to differentiate between the venous and arterial channels in this network but it was evident that this plexus of vessels formed anastomotic communications between the radicular vessels, the anterior longitudinal arterial trunk and the posterior anastomosis of vessels. Perforating arteriolar branches passed centrally, primarily from the anterior longitudinal arterial trunk, but also from the vessels of the posterior arterial anastomosis to supply the central region of the spinal cord (Figure 8).

Despite the different macroscopic appearance of the sheep's cervical vertebrae, and the different gravitational forces acting on the sheep's cervical spine, the anatomy of this region in the sheep is remarkably similar to that in man. The cross-sectional area of the vertebral bodies, the size of the spinal canal and intervertebral discs are similar to that in man. The vertebrae of the sheep are also large enough that the instruments and techniques used in an antero-lateral cervical fusion in man can be applied directly to the sheep.

The neural and vascular relationships of the operative site, particularly in the intervertebral region are comparable in the sheep to that in man. The proximity of the vertebral artery to the vertebral body, the location and relations of the exiting nerve root and radicular branches of the vertebral artery are all very much like that seen in man. In particular the arrangement of the vessels on the anterior surface of the spinal cord with a single anterior longitudinal arterial trunk is identical in the two species. The anatomy of the vessels on the dorsum of the spinal cord is however a little more variable in sheep than in man. It was felt this variation did not reduce the validity of the model as the relative size, and the overall distribution of vessels on the ventral and dorsal aspects of the cord are similar. The location of the sympathetic trunk, which may regulate or influence local spinal cord blood flow, was not evident during the dissection of these animals. Its role in the auto-regulation of spinal cord blood supply could therefore not be evaluated further.

On the basis of this anatomical study the sheep was considered a suitable model for the study of the effects of antero-lateral cervical fusion on spinal cord blood flow and function.

4. ASSESSMENT OF SPINAL CORD BLOOD FLOW AND FUNCTION IN SHEEP FOLLOWING ANTERO-LATERAL CERVICAL INTER-BODY FUSION

4.1 AIMS

To examine the effect of an anterior exposure and an antero-lateral cervical fusion on spinal cord blood flow and electrical function in a neurologically intact animal.

4.2 INTRODUCTION

The hypothesis for this study was that an antero-lateral inter-body cervical fusion has an adverse effect on spinal cord blood flow and neurological function in the presence of an incomplete spinal cord injury.

The effect of an antero-lateral cervical fusion was studied in neurologically intact sheep as if it could be shown that the this surgery adversely affected spinal cord blood flow and function in animals without a spinal cord injury the hypothesis would be supported and no further research required. Negating the additional costs of developing a spinal cord injury model to study the effect of this surgery in the presence of an incomplete spinal cord injury.

If the hypothesis was not be supported by the results obtained the sheep used in this section would act as controls for further evaluation of the hypothesis. Normal variations in spinal cord blood flow, motor and sensory evoked potentials and in physiological variables such as the $PaCO_2$ would become evident. The minimum

number of sheep required to identifying a significant difference in the outcome of sheep with incomplete spinal cord injuries having an antero-lateral cervical fusion, compared to those that did not could be calculated. Results from the study of neurologically intact animals would also assist in the differentiation of the changes in the spinal cord blood flow and evoked potentials responses due to the surgery from those due to a spinal cord injury.

4.3 MATERIALS AND METHODS

Six two year old Merino wethers were used in this part of the study. The number of sheep was determined after evaluation of the variation in results obtained from the first four animals studied.

4.3.1 ANAESTHETIC PROCEDURE

Anaesthesia was induced by injection, directly into the right external jugular vein, of 15-20 ml 2.5% thiopentone sodium and 4 mg pancuronium bromide. This was administered with the sheep in a holding pen close to the operating theatre. The sheep was then taken into the operating theatre and held supine on the operating table during intubation. The sheep was then ventilated using a BIRD ventilator with the rate and depth of ventilation adjusted every fifteen minutes in accordance with the results obtained from arterial blood gas analysis until the animals were physiologically stable. The aim was to maintain physiological variables within their normal range (Table 1). Anaesthesia was maintained with 1.5% to 2.0% halothane in a nitrous oxide and oxygen mixture.

After securing the animal on the operating table, intravenous access was obtained, usually through a vein in the right cubital fossa, and an infusion of normal saline commenced.

Once the animals were physiologically stable arterial blood gas analysis was performed just prior to, and following each blood flow measurement. This was done so that corrections could be made in the blood flow readings for variations in the pH and $PaCO_2$.

PHYSIOLOGICAL VARIABLE	UNITS	NORMAL VALUES
Respiratory rate	RPM	12-30
Tidal Volume	ml	280-330
Minute Volume	L/min	5-6
Heart rate	BPM	70-80
Arterial blood pressure	mmHg	110/70
Arterial pH	pH	7.38-7.46
$PaCO_2$	mmHg	34-42
PaO_2	mmHg	100
Haemoglobin	gm/100ml	10-12
Blood Volume	ml/kg	60
Expected terminal exsanguination	ml/kg	25
Safe max single blood sample	ml/kg	6
Body weight	kg	45

TABLE 1: Normal values for physiological variables and respiratory volumes for the anaesthetised sheep.
From Veterinary Anaesthesia, 8th edition; Hall & Clarke, Bailliere Tindall, London; and Laboratory Animal Handbooks No.8: Animal Anaesthesia; Green, CJ; Published by Laboratory Animals Ltd. London;(1982)

The variation in the spinal cord blood flow of the lumbar spinal cord segments, obtained as remote controls reflected changes in blood flow due to the anaesthetic agents used or changes in physiological parameters, particularly the $PaCO_2$ and pH.

The corrected spinal cord blood flows were calculated as outlined below.

$$100 - \frac{(CT \times 100)}{BLC} = \% \text{ Change in BF}$$

$$\text{Corrected BF} = \text{BFT} - (\% \text{ Change in BF} \times \text{BLBF})$$

CT	=	Control (lumbar) blood flow at Time X
BLC	=	Base-line control (lumbar) blood flow
BF	=	Blood flow (cervical cord)
BFT	=	Blood flow (cervical cord) at Time X
BLBF	=	Base-line blood flow (cervical cord)

The arterial PaO_2 was maintained above 100 mmHg throughout the study, and the $PaCO_2$ between 34 mmHg and 42 mmHg. Arterial blood pressure was monitored continuously through a cannula positioned in the right carotid artery, on a Nihon Kohden "Lifescope 6" monitor (supplied by Med Tel Pty. Ltd.). The mean arterial pressure was recorded along with the blood gas analysis results performed just prior to, and immediately after each blood flow measurement.

4.3.2 CANNULATIONS

4.3.2.1 FEMORAL ARTERY

Once the sheep were physiologically stable the right femoral artery was exposed and cannulated in the groin. This was done through a longitudinal incision over the femoral artery which was then exposed over a distance of approximately 3 cm using blunt dissection (Figure 11A). Black silk ties were placed around the vessel in the cephalad and caudal limits of the wound. The caudal ligature was tied to occlude the artery. The tie at the cephalad end was used to control flow in the isolated segment of artery during cannulation. A 5 mm longitudinal incision was made in the artery using a No. 11 scalpel, and a 12 gauge silastic cannula introduced. The cannula had been prepared with a small silastic collar positioned 12 cm from its tip. The cephalad ligature was tied around the artery containing the cannula, on the proximal side of the silastic collar. The ligature which had been tied around the femoral artery in the caudal extent of the wound was then tied around the cannula itself distal to the collar (Figure 11B). This prevented accidental removal of the cannula. The cannula tip was advanced 12 cm into the femoral artery so that it was situated at the level of the aortic bifurcation. The cannula was then connected through a 3 way tap to one of two 50 ml glass syringes mounted on a Harvard Double Barrel Infusion / Withdrawal pump (Harvard Apparatus Co., Inc., Millis, Massachusetts). It was from this arterial line that blood samples were collected for blood gas analysis.

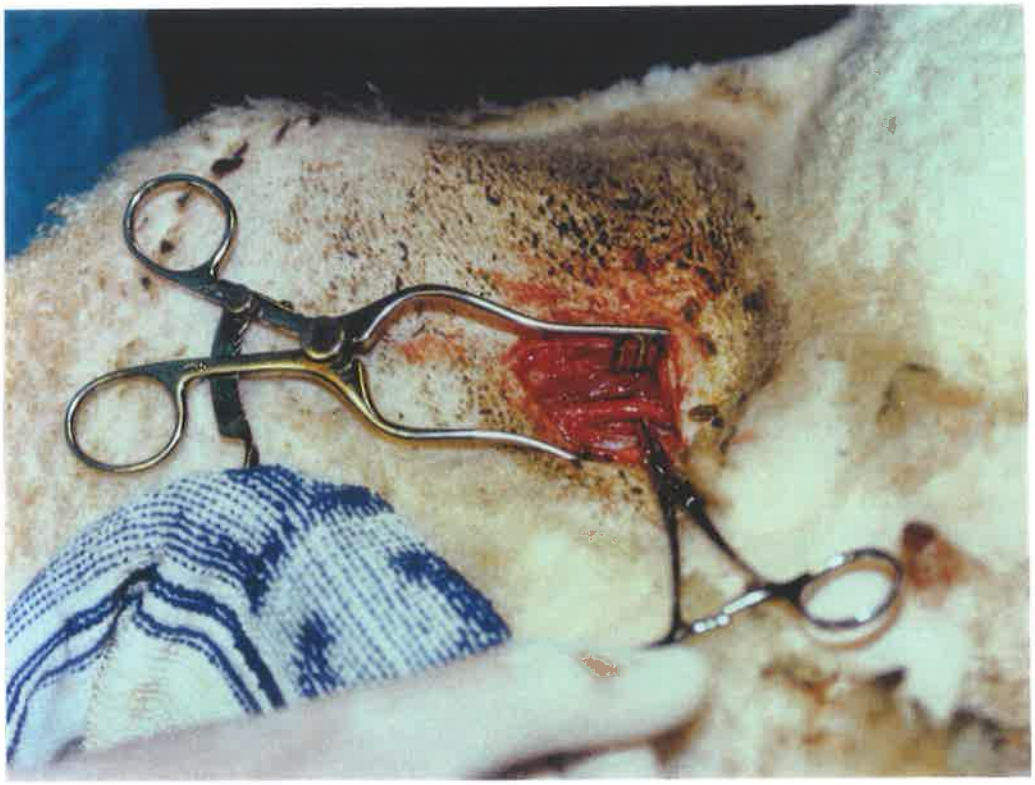


FIGURE 11A: Operative photograph of the right groin of one of the sheep studied with the femoral artery exposed prior to cannulation.

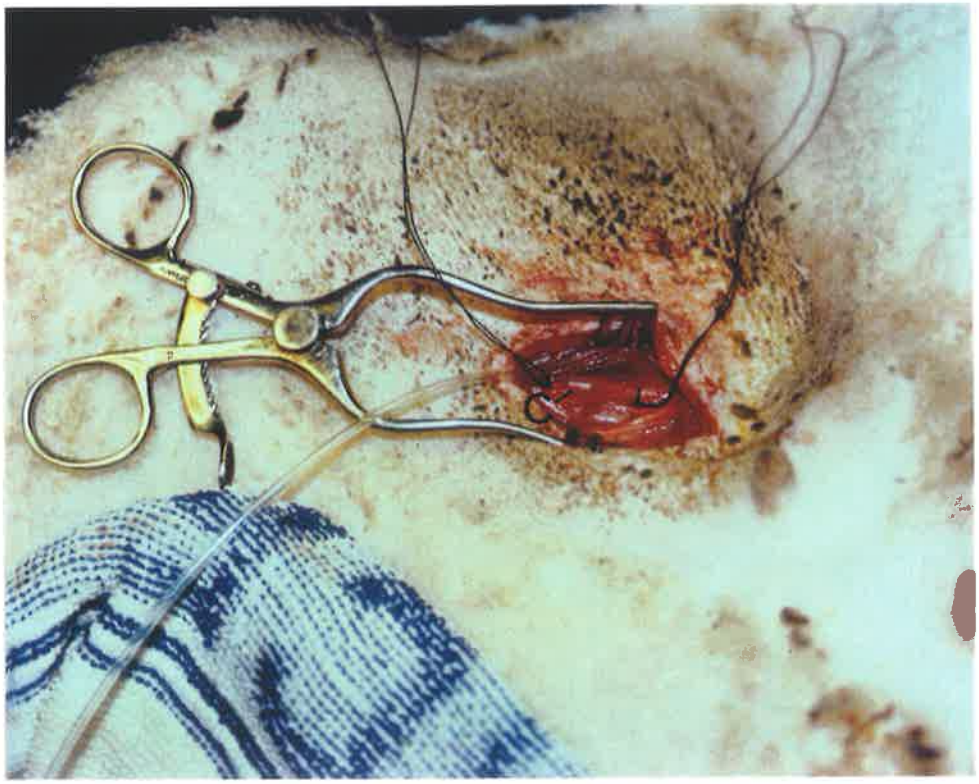


FIGURE 11B: The femoral artery is shown following insertion and fixation of the cannula. The collar (C) is indicated which prevented accidental extraction of the cannula.

4.3.2.2 CAROTID ARTERY

The right carotid artery was exposed in the mid cervical region through a longitudinal para-median incision just lateral to, and in line with the trachea. Blunt dissection was used to expose the plane between the trachea and the anterior strap muscles of the neck. The superficial jugular vein was retracted laterally with the superficial cervical musculature. The carotid artery was readily identified by palpation and exposed using blunt dissection over a distance of approximately 4 cm. Silk ties were then placed around the artery at the cranial and caudal extent of the wound. The cranial ligature was then tied to occlude the vessel. This had no detrimental effects on the cerebral circulation as there is a free anastomosis of right and left carotid systems at the base of the skull. The tie at the caudal end of the exposed artery was again used to control flow in the isolated segment during cannulation. This was performed as described for the femoral artery cannulation. The 12 gauge silastic cannula was introduced and advanced towards the heart approximately 8 cm so that the tip of the cannula was just below the level of the bifurcation of the carotid arteries. The cannula was prepared, and secured in the same way as the femoral cannula and then connected through a 3 way tap to the pressure transducer and the second 50 ml glass syringe mounted on the Harvard pump. The 3 way tap remained open to the blood pressure monitor at all times following its calibration except when the Harvard pump was in operation during blood flow measurements.

The sheep were then repositioned on their right side and were not moved again during the remainder of the study.

4.3.2.3 LEFT ATRIAL CATHETER

A left atrial catheter was inserted for infusion of the radio-labelled microspheres. The surgical approach was through the bed of the fourth rib which was identified by palpation from above. An incision was made over the rib from the costo-chondral junction to the mid axillary line, approximately 15cm in length. Muscle was divided and haemostasis maintained along the way with electro cautery. The fourth rib was exposed sub-periosteally and a 10 cm section over the left atrium excised.

Having notified the anaesthetic assistant that the ventilation pressures were about to be disturbed the periosteum and parietal pleura were divided to expose the underlying lung and pericardium. Appropriate corrections were then made in ventilation depth and rate, after a short period of manual ventilation. The lung and pericardium over the left atrial appendage was thus exposed, the lung retracted to expose the pericardium over the left atrium which was incised to expose the atrium itself (Figure 12A). A vascular clamp was used to isolate the tip of the left atrial appendage and a 00 black silk purse string suture placed around its tip (Figure 12B). A small hole was then made in the appendage for the insertion of a 12 gauge silastic cannula with a collar positioned 2.5 cm from its tip. A silk tie was secured to the cannula just distal to the collar, prior to its insertion. The cannula was introduced into the left atrium as the vascular clamp was released and purse string suture tightened. The tie around the cannula was then tied to the purse string suture to prevent movement in or out which may have caused cardiac arrhythmias and a reduction in cardiac output (Figure 12C).

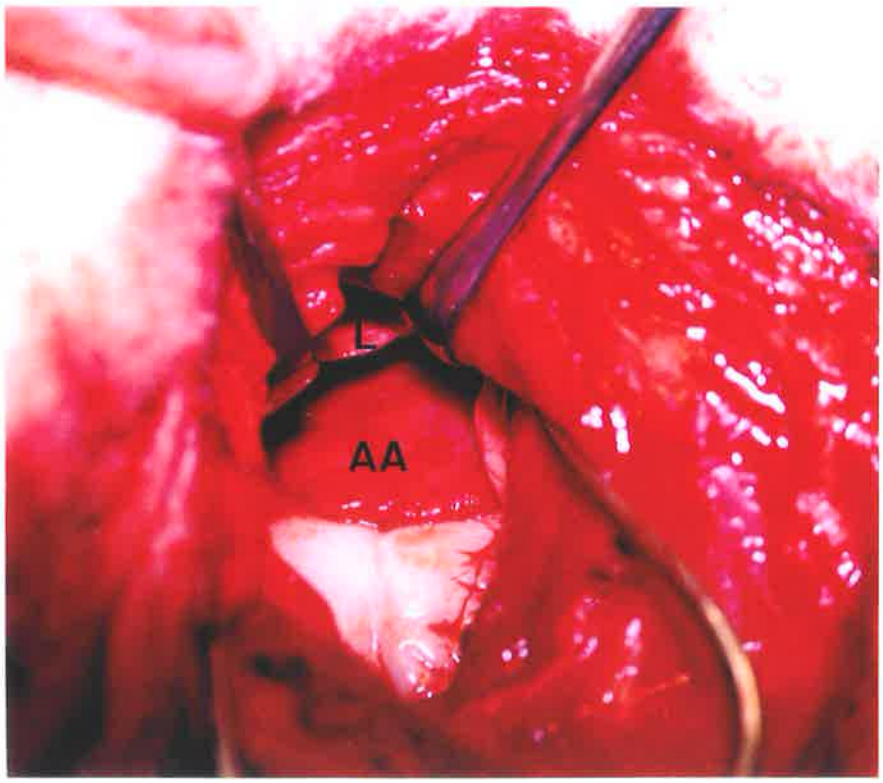


FIGURE 12A: Operative photograph of the left atrial appendage (AA) through the bed of the fourth rib after division and reflection of the pericardium. The lung (L) has been retracted superiorly.

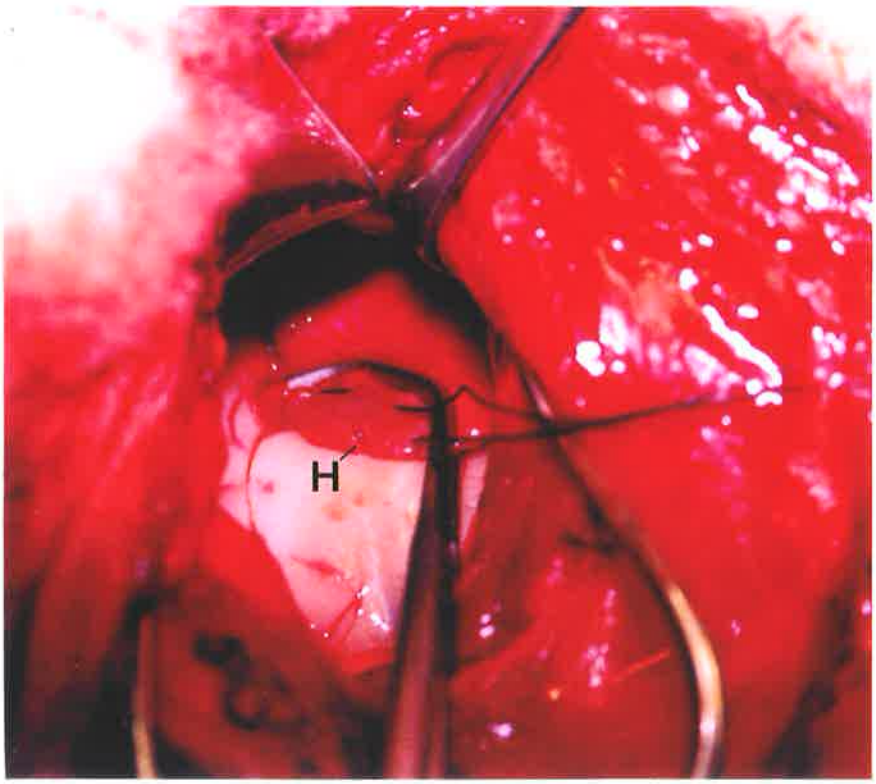


FIGURE 12B: A vascular clamp has been used to isolate the tip of the atrial appendage, a purse sting suture has been positioned and a small hole (H) made for the insertion of the cannula.

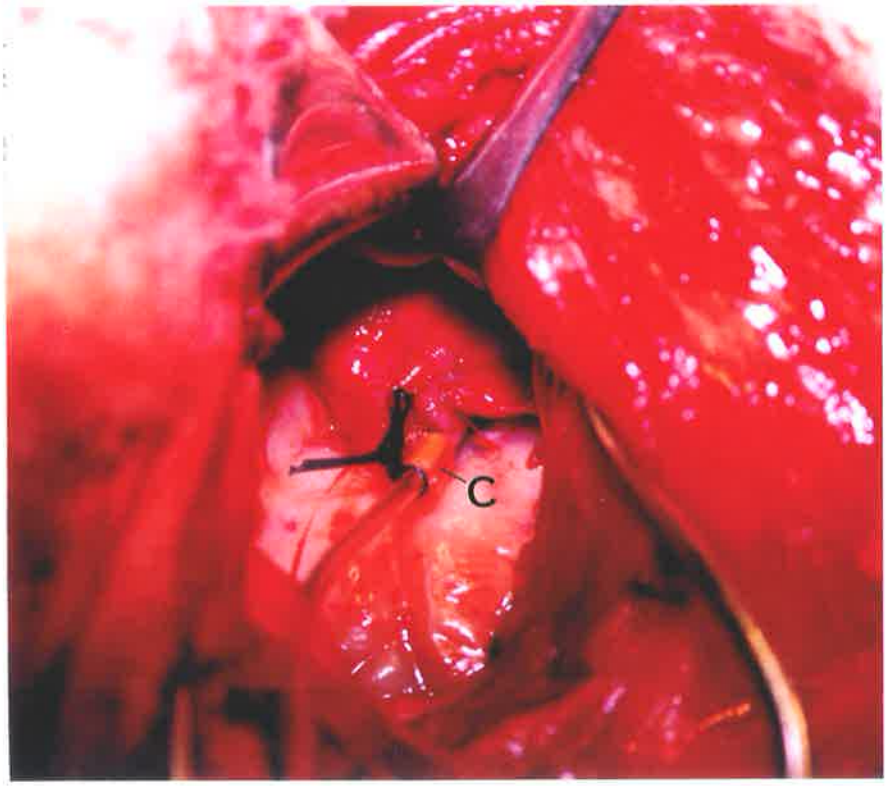


FIGURE 12C: The cannula has been introduced and the purse string secured and tied to the ligature around the cannula distal to the collar (C)

The cannula was flushed with heparinised saline to ensure patency before closure of the thoracotomy. Manual ventilation was used during closure of the chest to ensure full inflation of the lung and elimination of pleural air. After closure of the thoracotomy, blood gas analysis was performed and adjustments made to the depth and rate of ventilation if required.

All cannulas were flushed regularly with heparinised saline to prevent occlusion, and the dead space in the carotid and femoral lines primed with arterial blood prior to each blood flow measurement or collection of blood for blood gas analysis.

4.3.3 EVOKED POTENTIAL RESPONSES

It was not possible for the sheep to be assessed neurologically post-operatively as the Animal Ethics Committee considered survival would result in excessive suffering. Therefore motor (MEP) and sensory (SEP) evoked potentials were used to monitor the function of the spinal cord throughout the study.

Trials were performed with the equipment (donated for use during the study by Neuromed Aust Pty. Ltd.) and technique on the live sheep used in the anatomical study reported in Chapter 3. This indicated the use of surface scalp, and peripheral electrodes were unreliable for stimulation and recording of responses in the sheep. Direct spinal cord and cortical stimulation and monitoring, via an epidural bipolar cardiac pacing electrode and two cortical bolt electrodes was found to be much more reliable and subsequently used in all study animals.

A bipolar cardiac pacing electrode was chosen as the epidural electrode because of its small size, ease of insertion and suitability for both the motor and sensory studies.

4.3.3.1 INSERTION OF CORTICAL BOLT ELECTRODES

The cortical bolt electrodes were positioned, in contact with the dura, over the motor and sensory cerebral cortices. A scalp flap centred on the vertex of the sheep skull was raised and two burr holes (6.5 mm diameter) made for the placement of one active and one reference bolt electrode. The active electrode was positioned just to the left of the sagittal suture between the horn stumps. The reference electrode was placed along the left coronal suture 2 cm from the mid-line. The bolt electrodes were prepared in the Department of Biomedical Engineering, Royal Adelaide Hospital, and were made of stainless steel. They had an external thread diameter of 8 mm, an internal thread diameter of 6.5 mm. A 2 mm hole had been drilled beneath the head to accept a wire plug for connection to the evoked potential monitor, (Cadwell 7200). The thread of the bolts were made self tapping and the ends of the bolts rounded to prevent dural and cortical damage during their insertion, (Figure 13). The thickness of the skull was measured through each burr hole using an orthopaedic, AO type depth gauge.

The bolt electrode was then inserted and advanced just enough for the rounded tip to protrude from the inner surface of the skull and be in contact with the dura. Once positioned, the bolt electrodes were electrically insulated from surface moisture and surrounding tissue with methyl-methacrylate dental cement.

Scale (cm) 0 0.5

FIGURE 13: Photograph of the cortical bolt electrodes used for stimulation of the motor cortices and recording the sensory evoked potential response. The electrodes had a rounded tip to protect the dura, self tapping flutes (F) to aid insertion and a 2mm hole in the shaft to allow connection to the evoked potential monitor.

4.3.3.2 INSERTION OF EPIDURAL ELECTRODE

The bipolar electrode was introduced into the extradural space of the upper thoracic spine through a left inter-laminal fenestration between C7 and T1. It was not practical to perform the fenestration lower as the ribs made access to the epidural space difficult increasing the risk of damage to the spinal cord.

With the sheep positioned on their right side the left fore-limb was retracted caudally which pulled the scapula down out of the way. An incision was made on the left side over the transverse processes of the lower cervical and upper thoracic vertebrae from the transverse process of C6 to the supero-medial corner of the scapula. Superficial muscles were split in the line of their fibres and the scapula retracted to expose the erector spinae group of muscles. These muscles were again split in the line of their fibres between the transverse processes of C7 and T1. Muscular attachments to the transverse processes were divided to expose the spinous processes and laminae of the corresponding vertebrae.

The level of the dissection was confirmed by palpation as C6 has a short triangular spinous process angled cranially, C7 has a longer and wider spinous process with a rounded tip angled caudally, and T1 has an long narrow spinous process which is again angled caudally, (Figure 2). The ligamentum flavum was separated from the inferior border of the lamina of C7 and the fenestration performed using a small 45^{0} up-cutting punch and fine pituitary rongeurs.

The tip of the extradural electrode was introduced and passed caudally in the spinal canal approximately 10 cm to lie behind the body of the fourth thoracic vertebra. Both the extradural and cortical

electrodes were then connected to the evoked potential monitor and test recordings obtained of both the motor and sensory evoked potential responses. If the position of the extradural electrode required adjustment this was done and once adequate responses were obtained the epidural electrode was secured to the capsule of the adjacent facet joint with a 00 silk suture.

The motor evoked potentials were obtained using a square pulse DC stimulus with an amplitude of 128 mA, a pulse duration of 1.0 millisecond and a stimulation rate of one pulse per second. The sensory evoked potentials were obtained using a similar square pulse DC stimulus with the amplitude set at 12.2 mA, a pulse duration of 0.1 millisecond and stimulation rate of one pulse per second. Both the motor and sensory evoked potentials were averaged over 32 impulses. Tracings were made of the baseline evoked potentials and any response varying significantly from the baseline. The latency of the origin, latency of the peak and the amplitude of the responses were recorded every fifteen minutes.

4.3.4 BLOOD FLOW MEASUREMENTS

Blood flow measurements were performed using radio-nuclide labelled microspheres as described by Heymann et al (1977). Labelled microspheres were chosen in preference to other methods of blood flow measurement, particularly the hydrogen clearance technique (Young et al, 1980) so that surgical exposure of the spinal cord could be avoided.

A licence to use and handle radioactive substances pursuant to Section 28 of the Radiation Protection and Control Act, 1982 was required, and obtained from the Radiation Control section of the South Australian Health Commission. Following completion of the study of each of the sheep, all contaminated tissue, disposable products and residual nuclides were disposed of in specially marked plastic lined bags, as stipulated by the South Australian Health Commission. The Royal Adelaide Hospital radiation safety officer supervised both the handling of the nuclides and disposal of the sheep.

Fifteen micron diameter NEN-TRAC^{TM} microspheres, (Dupont, Medical Products, Biotechnology Division, Wilmington, DE. 19898) labelled with cerium 141 (Ce ^{141}), chromium 51 (Cr ^{51}) and niobium 91 (Ni ^{91}) were chosen for this part of the study as their specific activities were suitably spaced for differential activity counting and their half lives long enough to prevent significant loss of activity during the course of the study. The half lives were however short enough not to pose either a medical or environmental hazard if handled and disposed of correctly. The microspheres were transported, and used as a suspension in 0.9% saline and 0.01% Tween 80. Upon receiving each consignment of microspheres the

energy spectrum of each nuclide was analysed in order to set accurate window settings for differential activity counting in a LKB Wallac Universal Gamma counter (Compugamma Model No. 1282). This gamma counter was used for both the energy spectrum analysis and the subsequent tissue activity counts. The gamma counter had the software capability to count the activities of up to five nuclides with different energy spectrums concurrently and make spill-over corrections automatically. The combined energy spectrum of the nuclides used is shown in Figure 12.

The accuracy of the technique is dependant on the injection of enough microspheres to give adequate tissue concentrations, with random distribution of microspheres. The number of microspheres injected is calculated based on the lowest expected blood flow of the tissues to be studied (Hof, 1982).

The results of this technique become unreliable when insufficient numbers of microspheres are injected, when measuring the blood flow in small volumes of tissue and where microsphere distribution is non random due to incorrect preparation or injection techniques or disturbances of cardiac output at the time of injection. Reliability is also reduced when the microsphere shelf life has expired so that despite adequate tissue concentrations of the microspheres the nuclide has insufficient activity to give reliable activity counts. In this study all microspheres were used within one half life of being received.

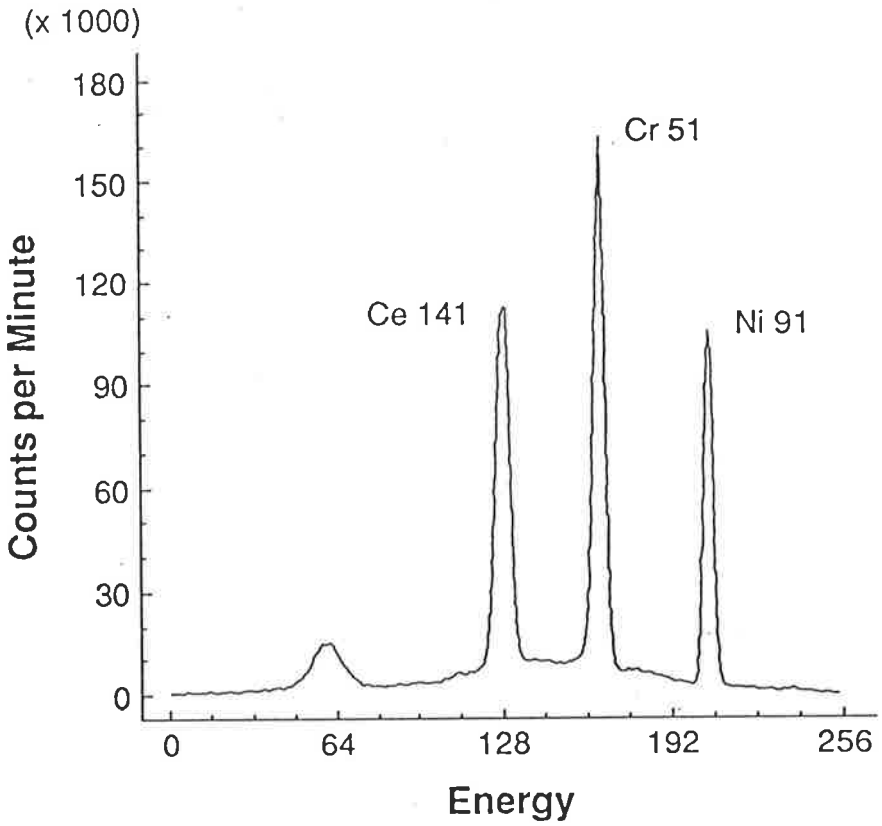


FIGURE 14: The combined energy spectrum plot of Cerium 141 (Ce ^{141}), Chromium 51 (Cr ^{51}) and Niobium 91 (Ni ^{91}).

The volume of the suspension injected was calculated from the product information (Annex A) accompanying each batch of microspheres. This product information detailed the number of microspheres per milligram and the number of milligrams of microspheres per 20ml vial. Previous studies have indicated that a concentration of around 400 microspheres per gram of tissue is required to produce sufficient tissue activity and ensure accurate and reproducible tissue counts and blood flow calculations (Heymann et al, 1977). Kaplan et al (1987) in their study of the effects of aortic occlusion on regional spinal cord blood flow in sheep injected three million microspheres through a left atrial catheter. They found injection of this number of microspheres resulted in enough tissue activity for accurate and reproducible blood flow measurements to be made from cervical, thoracic and lumbar spinal cord segments.

The formula used to calculate the volume of the nuclide suspensions to be injected is shown below with the aim of injecting a total of three million microspheres.

$$V = 3 \times 10^6 / \frac{(mg/v \times M/mg)}{20}$$

V	=	Volume of suspension for injection
mg/v	=	Milligrams of microspheres per vial
M/mg	=	Microspheres per milligram

The microsphere suspensions were prepared for injection in the Department of Nuclear Medicine, Royal Adelaide Hospital under the supervision of the Radiation Safety Officer. The microsphere suspensions were aspirated directly from the storage vial into the syringes used for injection into the sheep. The microspheres in the storage vial were mixed on a vortex mixer for five minutes while

contained in a lead pot behind a lead glass screen. Once the pot containing the storage vial was removed from the vortex mixer it was agitated by hand while the required volume of the microsphere suspension was aspirated into a 10 ml syringe. The syringes were then capped and placed in lead transportation pots, where they stayed until just prior to their injection. The storage vials were replaced in lead storage bins and the syringe along with needles and other contaminated material disposed of in appropriate disposal bins.

The local radiation safety officer checked the activity levels of the nuclide suspensions at the time of their preparation, while contained in the lead transportation pots and during their injection. The activity of tissue samples obtained from the sheep for analysis of blood flow was assessed as was that of sheep carcasses prior to disposal. All radiation readings were found to be well within recommended safety limits.

Once the preparative surgery and cannulations had been completed a base line blood flow measurement was performed. All blood flow measurements were done following the same procedure as outlined in the following text.

Just prior to each blood flow measurement the syringe containing the appropriate microsphere suspension was removed from its transportation pot and mixed in the syringe with 1000 units of heparin sodium. The volume in the syringe was then made up to 10 ml with fresh arterial blood, obtained from the femoral arterial cannula. The syringe was then agitated on a vortex mixer for five minutes. While the microsphere suspension was being mixed an

assistant flushed the femoral, carotid and left atrial lines with heparinised saline and then primed the femoral and carotid lines with arterial blood.

Blood was taken at this time from the femoral cannula for blood gas analysis and the femoral and carotid lines connected to the Harvard double barrel withdrawal pump. The Harvard pump was tested for a brief period to ensure free flow of blood into the two 50 ml glass syringes mounted on the pump. The Harvard pump was set at a constant rate of withdrawal and the 50 ml glass syringes became the "reference organ" for the calculation of tissue blood flow. The nuclide activity in this blood was measured and as the rate of blood flow into the syringes was set at a constant rate, the blood flow in other tissue could be calculated from the nuclide activity and weight of the tissue sample.

After adequate mixing, the microsphere suspension was taken to the sheep and the syringe connected to the left atrial catheter. The Harvard withdrawal pump was turned on and the injection of the microspheres commenced using a two syringe technique.

A 10 ml syringe containing heparinised saline and the syringe containing the microsphere suspension were connected via a three way tap to the left atrial catheter (Figure 15). The tap was initially opened to the syringe containing the microspheres and 4 ml of the microsphere suspension injected in a controlled fashion. The tap was then closed to the left atrium and 3 ml of heparinised saline flushed back into the "microsphere" syringe. The tap was then reopened to the left atrial catheter and a further 5 ml of microsphere suspension injected. The tap was again closed to the left atrial

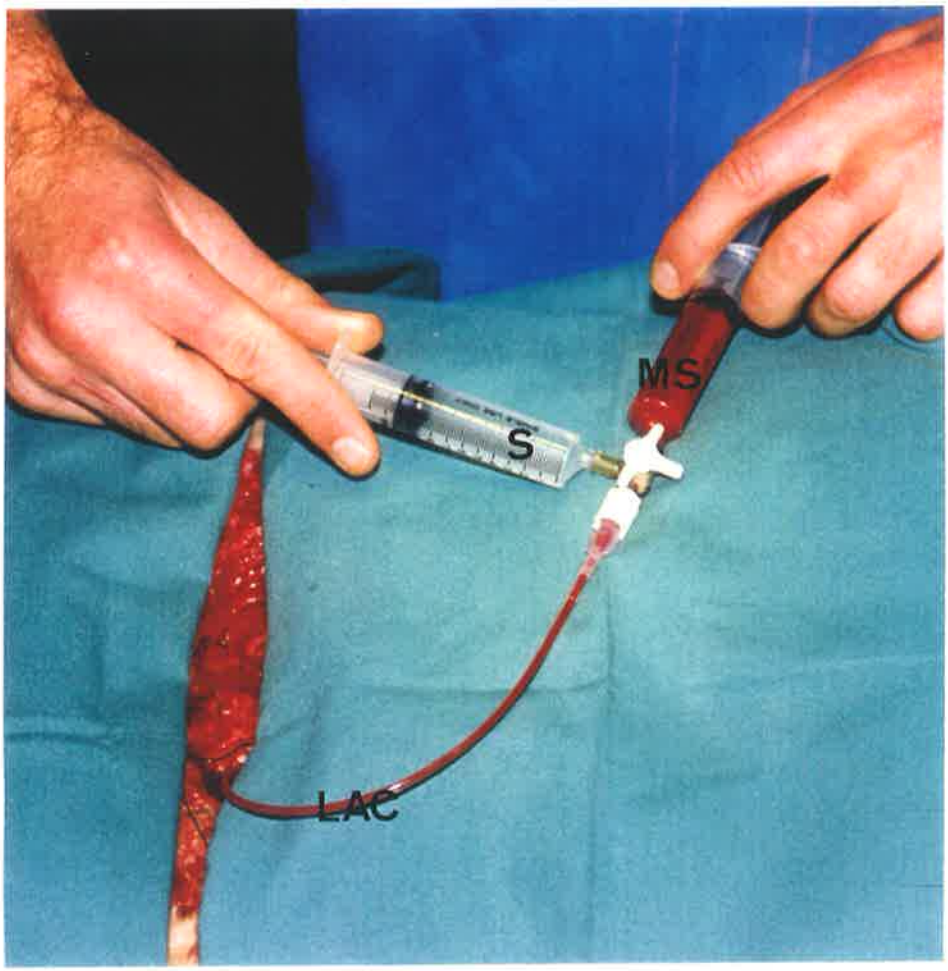


FIGURE 15: Photograph of the set up used for the injection of microspheres into the left atrium via the left atrial catheter (LAC). The syringe containing the suspension of microspheres (MS) and saline (S) are also indicated.

catheter and 3 ml of heparinised saline flushed back into the "microsphere" syringe. The remaining 7 ml of the now diluted microsphere solution was then injected into the left atrium and the 4ml of saline left in the second syringe used to finally flush the left atrial line and clear it of any microspheres. The entire injection procedure took no more than 20 seconds to complete in any of the animals studied and the total volume injected was 20ml.

Buckberg et al (1971) reported that no microspheres were found in the tubing leading to the withdrawal pump if the pump was left running 60 seconds after completing the injection of microspheres. The Harvard withdrawal pump in this study was left running for 90 seconds after completing the injection of microspheres and the blood left in the arterial lines aspirated into the collecting syringes after turning off the pump.

A second arterial blood sample was then taken from the femoral arterial line for blood gas analysis. The heart rate, mean arterial blood pressure and motor and sensory evoked potentials were also recorded immediately before and after each blood flow measurement. This enabled correction of blood flow measurements for changes in physiological parameters ($PaCO_2$, PaO_2 & pH) and with the functional state of the spinal cord.

Approximately 21ml of blood was collected in each of the 50ml glass syringes during the 110 seconds they were running, (20sec injection time and an additional 90sec withdrawal time). All of this "reference organ" blood was then transferred into sixteen previously labelled gamma counting tubes, eight tubes for the carotid and eight for the femoral blood. The syringes were then rinsed with 10ml of distilled

water, which was added in equal amounts to the blood in the tubes resulting in a volume of approximately 3.8ml in each of the tubes (2.6ml blood and 1.2 ml distilled water). The distilled water haemolysed the blood and allowed the microspheres to settle to the bottom of the tubes so that loss of activity to the surroundings from the top of the counting well of the gamma counter would be kept to a minimum.

The Harvard withdrawal pump was set at the same rate of 11.3ml/min for all the sheep studied and the nuclide activity of the blood measured directly. Calculation of tissue blood flow was then possible by measurement of the nuclide activity and weight of the tissue contained in each gamma counting tube by application of the equation shown below.

$$BF = TA \,/\, \frac{(TC + TF)}{2} \times 11.3 \times \frac{100}{W}$$

BF	=	Blood flow (ml/100gm/min)
TA	=	Tissue sample activity count
TC	=	Total carotid blood count
TF	=	Total femoral blood count
W	=	Weight of tissue sample (gm)
11.3	=	Blood flow in "reference organ" (ml/min)

Three blood flow measurements were performed. The first was the base-line measurement (Time 1), performed once the sheep was anaesthetised, all cannulations and preparatory surgery completed and the animal considered to be physiologically stable. The second blood flow measurement was made following completion of the

antero-lateral cervical fusion (Time 2), and the final blood flow measurement performed one hour after completion of the surgery (Time 3). Each of the three nuclides (Ce^{141}, Cr^{51} & Ni^{91}) were used at different times in each of the sheep studied. In other words Ce^{141} may have been used for the baseline blood flow measurement in the first sheep, but for either the first, second or third blood flow measurement in the subsequent sheep (Table 2). This was done to reduce the chance of a "bad batch" of microspheres, poor preparation of one of the microspheres suspensions, deterioration of nuclide activity or operator error rendering blood flow measurements at any one point in time invalid or unreliable.

Decay of nuclide activity did not prove to be a problem during the study as the activity of each of the nuclide suspensions was checked and adjustments made, if necessary to the volume of microspheres injected prior to the study of each animal.

Once the final blood flow measurement and evoked potential recordings had been performed and all necessary physiological variables recorded, the sheep were killed. This was done by injecting 20ml of Lethobarb into the left atrial catheter. The tissues required for blood flow calculation were then removed (cervical spinal cord, lumbar spinal cord, right and left kidneys) and the remainder of the carcass disposed of in the manner stipulated by the South Australian Health Commission.

	Sheep Number	Blood Flow Measurement		
		Time 1	Time 2	Time 3
Operation Only	1	Ce^{141}	Cr^{51}	Ni^{91}
	2	Cr^{51}	Ni^{91}	Ce^{141}
	3	Ce^{141}	Ni^{91}	Cr^{51}
	4	Ni^{91}	Ce^{141}	Cr^{51}
	5	Ce^{141}	Cr^{51}	Ni^{91}
	6	Cr^{51}	Ni^{91}	Ce^{141}

Table 2: Radio-labelled microspheres used for each blood flow measurement in each of the sheep studied.

The third to sixth cervical vertebrae and the third and fourth lumbar vertebrae were removed en-block and debrided of their dorsal muscular attachments. The laminae of each vertebrae was then excised to allow removal of the intact cervical and lumbar spinal cord segments.

The cervical spinal cord was then divided into four vertebral segments between the exiting nerve roots and each vertebral segment was further subdivided into three segments of equal size. This was done so that the blood flow in the spinal cord could be evaluated over short segments and the intact vertebral segment of spinal cord was too large to be counted in a single gamma counting tube. The lumbar spinal cord was subdivided in a similar fashion (Figure 16). All 18 spinal cord segments were placed into labelled gamma counting tubes for analysis of nuclide activity and calculation of blood flow.

Blood flow in the upper and lower two segments of the cervical spinal cord studied, did not alter significantly throughout the study and were not included in the subsequent analysis of the results. Blood flows from the segments labelled as "Cont C1" and "Cont C2" (Figure 16) were used as local controls and the blood flows from the lumbar spinal cord segments labelled "Cont L", used as a remote control to differentiate the effect of the surgery from the effect of the anaesthetic agents used and fluctuations in physiological parameters.

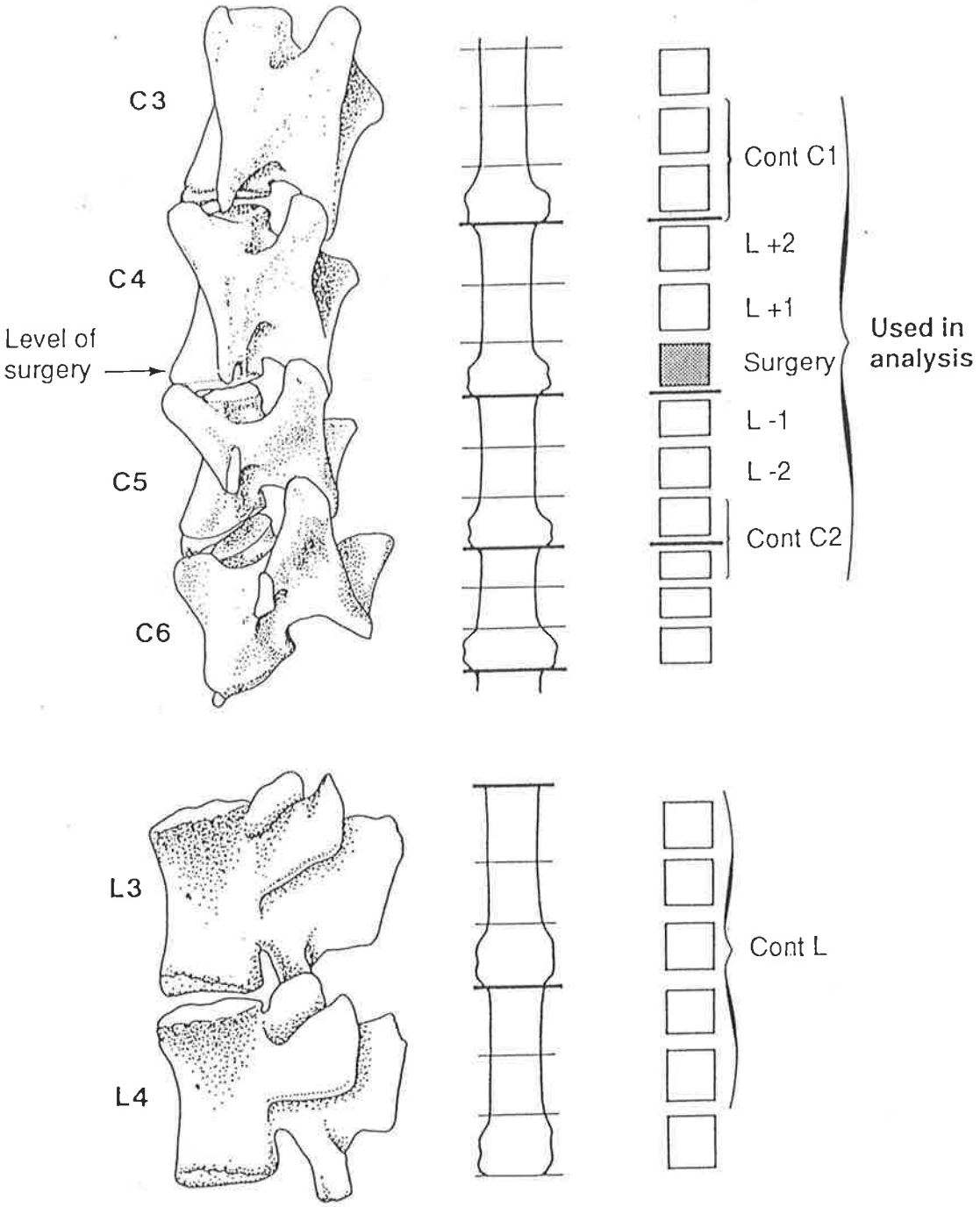


FIGURE 16: Diagrammatic representation of the cervical and lumbar vertebral segments removed from each sheep showing how the spinal cord was subdivided and labelled. The level of the surgery is also indicated.

A control to check the adequacy of microsphere mixing and distribution was required as clumping or poor injection technique could result in non-random distribution and unreliable blood flow calculations. The kidneys being paired organs with a high blood flow (relatively large percentage of the cardiac output) were ideal for this function. The right and left kidneys should have similar calculated blood flows (within 5%) if the blood flow method and technique was accurate and reliable.

The right and left kidneys were minced, keeping tissue from each kidney separate. Approximately three gram aliquots of kidney were then placed into each of 30 labelled gamma counting tubes, 15 for the left kidney and 15 for the right.

The gamma counting tubes containing spinal cord and renal tissue were then weighed and the net weight (weight of tissue and tube minus the weight of the empty tube) recorded. All 96 counting tubes (48 containing blood, 18 containing spinal cord and 30 containing kidney) were then placed in the gamma counter, along with standard nuclide samples for activity counting.

The Compugamma gamma counter produced a print out indicating the specimen number, total activity count and the spill-over corrected counts for each nuclide. The spill-over corrected counts for each individual tissue sample, along with their net weights were then entered into a Lotus 123 spreadsheet (version 2.2). The total activity of the "reference organ" blood was determined, and the tissue blood flows calculated using the formula shown above.

It is accepted that both cerebral and spinal cord blood flow increases with hypercarbia and decreases with hypocarbia, (Harper et al, 1961 & 1965; Smith et al, 1969). Despite every attempt to maintain a stable physiological environment, there were fluctuations in the $PaCO_2$ during the study. In order to evaluate the effect of the surgery on spinal cord blood flow and function it was necessary to make corrections in the blood flows for changes in the $PaCO_2$ and pH as outlined above.

Griffiths (1973) reported the findings of a dog study where he found the relationship between spinal cord blood flow and $PaCO_2$ to be linear. He also reported that there was no significant change in spinal cord blood flow due to variations in the PaO_2 above 60 mm Hg. Analysis of the relationship between the spinal cord blood flow and changes in the $PaCO_2$ following the completion of this study confirmed that relationship. Spinal cord blood flow increased and decreased in a direct relationship with variations in the $PaCO_2$ (Figure 17 & 18).

Adjustments were therefore made to the blood flows in the cervical spinal cord, in proportion to the changes that occurred in the lumbar control spinal cord segments primarily due to changes in the $PaCO_2$. The calculation used for this adjustment is outlined in the text above, and was programmed into the Lotus 123 spreadsheet and calculated automatically. It was this corrected spinal cord blood flow that was used in the subsequent analysis of results.

In all sheep studied the PaO_2 was maintained above 90 mmHg. Variations in the PaO_2 above this level were found not to influence spinal cord blood flow.

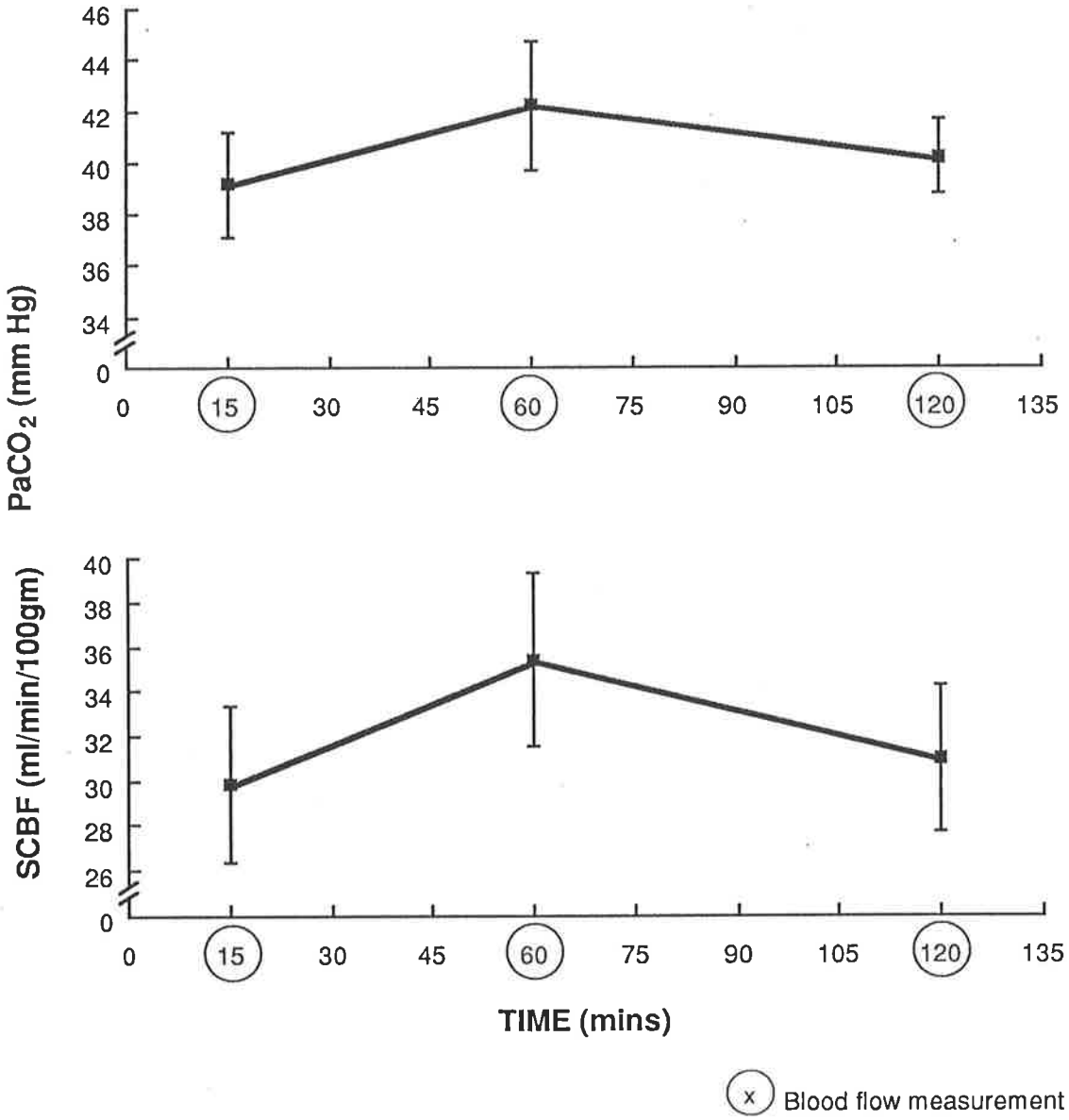


FIGURE 17: Change in spinal cord blood flow associated with changes in the $PaCO_2$ in the 18 sheep studied. The spinal cord blood flow was that recorded in the lumbar control spinal cord segments (Cont L).

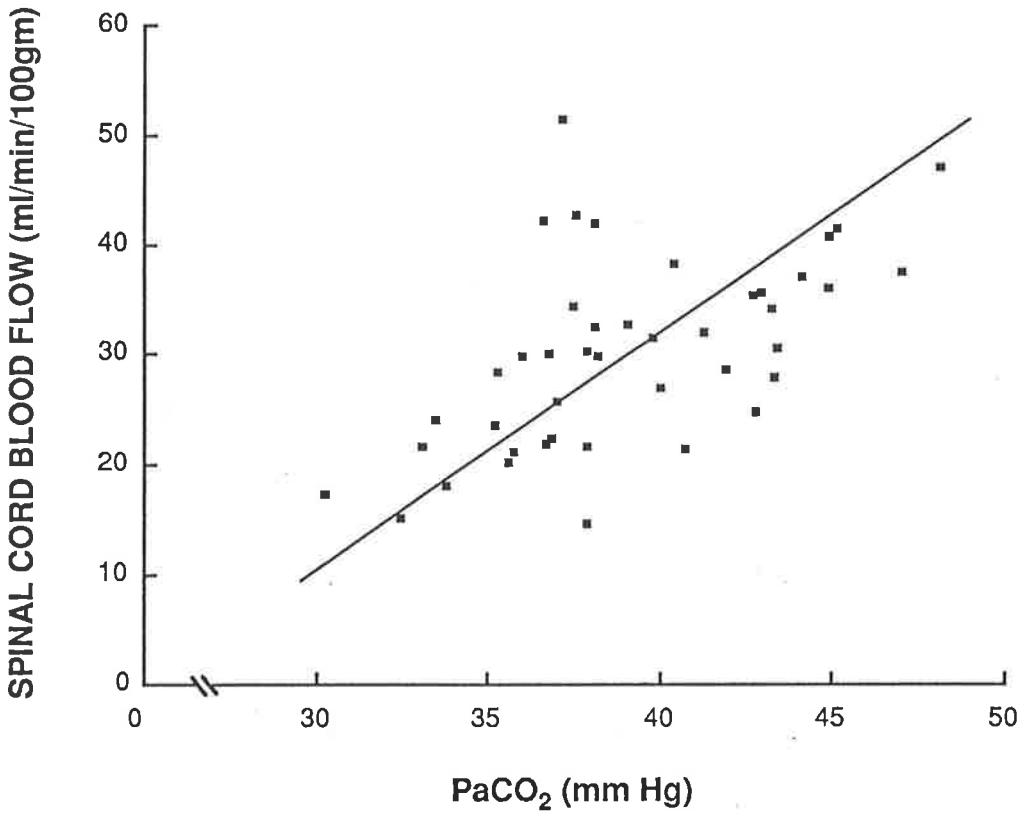


FIGURE 18: Relationship between $PaCO_2$ and spinal cord blood flow from the sheep used in this study.

4.3.5 ANTERO-LATERAL CERVICAL FUSION

With the sheep positioned on its right side, (left side up), a sand bag was placed transversely under the neck to prevent lateral sagging and provide support for the cervical spine during the fusion procedure. A longitudinal para-median incision was then made which was centred at the level of the anterior tubercle of the transverse process of the fifth cervical vertebra.

The deep fascia was divided and blunt dissection used to identify the intermuscular plane behind the left sternomastoid muscle, retracting the sternomastoid, trachea, oesophagus, carotid sheath and jugular vein medially. This exposed the anterior spinal musculature and the anterior tubercles of the transverse processes of the cervical vertebrae. The vertebral level was then checked again by palpation and subsequently confirmed radiographically prior to proceeding with the cervical fusion. All the typical cervical vertebrae (C3, C4, C5 and C6) have a prominent anterior tubercle of the transverse process. The seventh has no such anterior tubercle but instead just a rounded lateral mass (Figure 2). By slipping a finger down over the pre-vertebral musculature the anterior tubercle of the transverse process of C6 was easily identified. The fourth and fifth cervical vertebra were then identified by counting up from C6.

The anterior tubercle of C5 was exposed by dividing its ligamentous attachments, and stripping its muscular attachments. It was necessary to remove the anterior tubercle in order to gain access to the antero-lateral margin of the C4/5 intervertebral disc. A 23 gauge needle was introduced into the centre of the disc to identify the inclination of the end-plates and it was at this stage that a radiograph was taken to confirm the operative level.

The antero-lateral margin of the annulus of the C4/5 intervertebral disc was then incised at its attachments to the end plates and a 10mm tube saw centred over the disc. The tube saw was stabilised with a central trocar and guide pin and directed towards the opposite postero-lateral corner of the disc, parallel with the inclination of the end plates. Once the tube saw had engaged both vertebral bodies the central trocar was removed and the orientation of the saw checked and adjusted if necessary. The tube saw was then advanced to a depth of 15mm. In none of the seven vertebral specimens used in the anatomical study reported in Chapter 3 did the vertebral bodies have a diameter less then 20mm in this direction. The core of intervertebral disc and adjacent vertebrae were then removed using fine nibblers, pituitary rongeurs and a small curette. A bi-cortical bone dowel with an external diameter of 11mm was harvested from the left iliac crest, measured, cleared of soft tissue and inserted into the hole prepared in the C4/5 interspace. Longitudinal traction was applied to the neck during insertion of the dowel and once inserted, the dowel was checked for stability. A radiograph was taken at this time to ensure the dowel had been positioned correctly and that the tube saw had not penetrated the spinal canal.

The extent of bony resection and the orientation of the tube saw and dowel are illustrated in Figure 19. Motor and sensory evoked potentials were monitored throughout the procedure. Figure 20 displays diagrammatically the experimental set-up used throughout the study. The position of the carotid and femoral arterial cannulas, the cortical bolt electrodes and bipolar cardiac pacing electrode and the left atrial catheter are indicated.

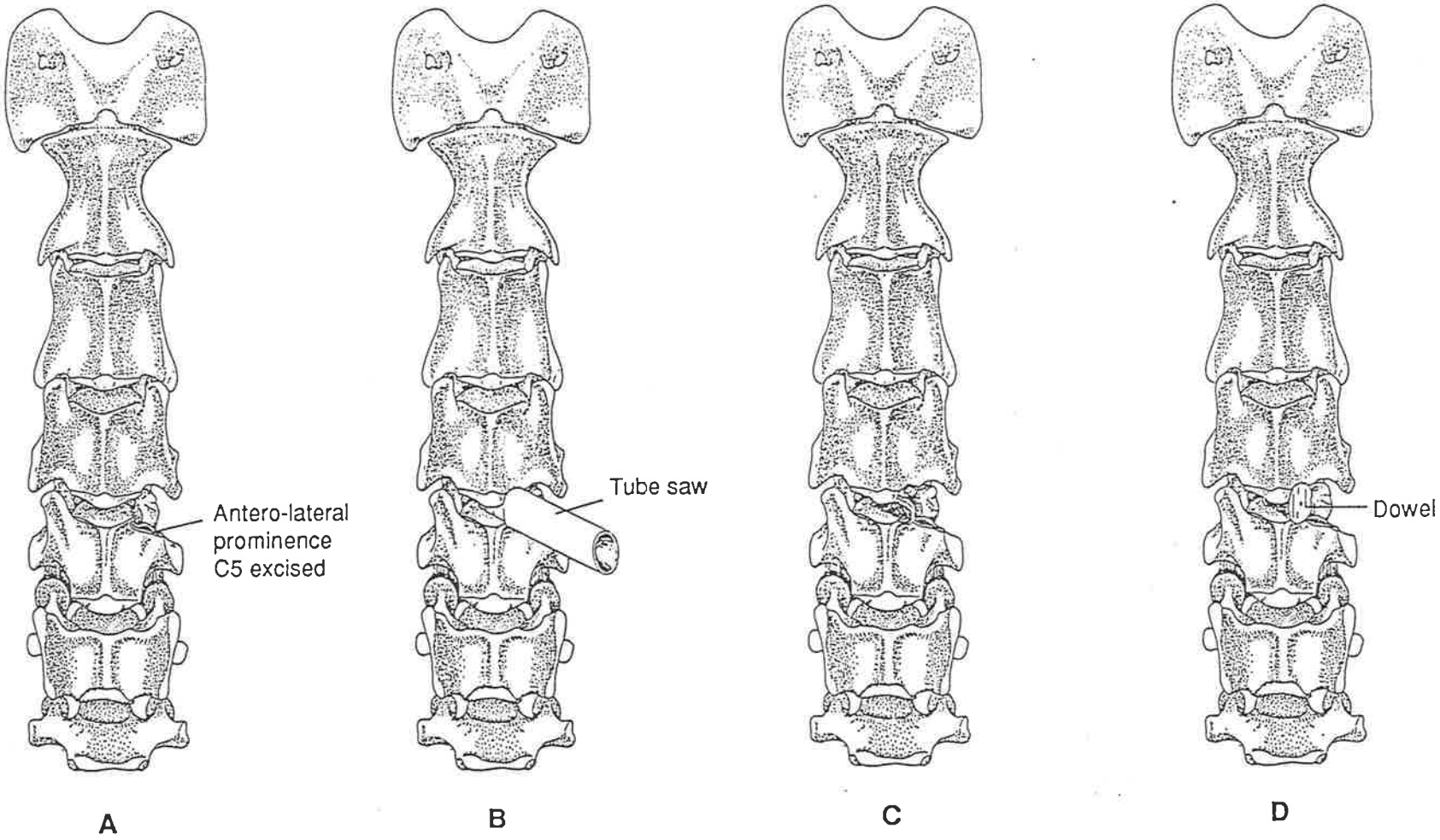


FIGURE 19: Illustrations showing the extent of bony resection and the orientation of the tube saw and dowel graft. (A) exposure and excision of the tip of the antero-lateral prominence of C5. (B) Placement of the tube saw over the intervertebral disc, (C) clearance of the dowel hole and (D) Insertion of the dowel.

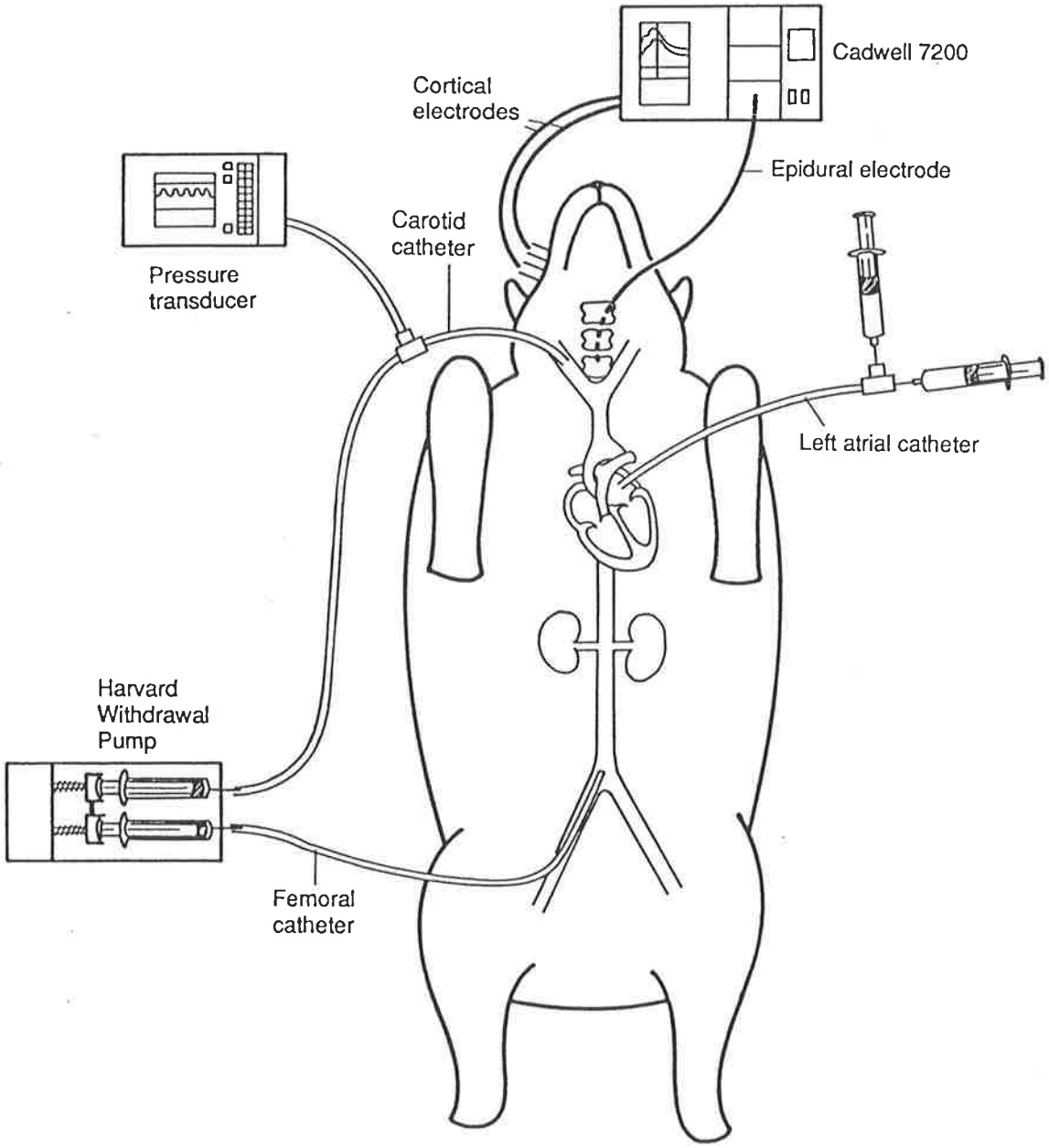


FIGURE 20: Experimental set up used throughout the study to assess the effect of surgical stabilisation of the cervical spine on spinal cord blood flow and function. Showing the left atrial catheter for injection of microspheres, the carotid and femoral cannulas connected to the pressure transducer and Harvard pump, and the cortical bolt and epidural electrodes connected to the evoked potential monitor.

Three assistants were required at the time of each blood flow measurement for a period of ten to fifteen minutes to ensure all tasks were performed and data recorded without interruption. During the surgical preparation of the animal, cannulations and placement of epidural and cortical evoked potential electrodes, one assistant was required to maintain anaesthesia and keep the sheep physiologically stable. Despite this assistance, the time required to complete the study of each individual sheep was 7 hours.

4.4 RESULTS

The changes in the spinal cord blood flow and evoked potential responses that were considered significant were set after consideration of the local clinical experience and published literature (Martin & Bloedel, 1973; Korbine et al, 1979; Bennett, 1983; Cheng et al, 1984; Mizrahi et al, 1984; Kaplan et al, 1987; Konrad et al, 1987; Fehlings et al, 1988 & 1989; Guha et al, 1989). Studies investigating the effect of hypotension, ischaemia and aortic occlusion on spinal cord blood flow and function (Kaplan et al, 1987), have established that a relatively large reduction in spinal cord blood flow, at least 50% is required to produce permanent ischaemic damage to the spinal cord.

Significant ischaemic or traumatic damage to the spinal cord is usually associated with a reduction in the amplitude of the evoked potential response of at least 50% and an increase in the latency of the motor and/or sensory evoked potential of at least 10% (Korbine et al, 1979; Senter & Venes, 1979 and Kaplan et al, 1987).

The spinal cord blood flow and the motor and sensory evoked potentials recorded immediately after the cervical fusion and one hour after completion of the surgery were compared with the base line measurements.

4.4.1 SPINAL CORD BLOOD FLOW

It can be seen from the results shown in Tables 3 & 4 that there was a slight reduction, 7.7% overall (range 0.9% - 16.2%), in the mean cervical spinal cord blood flow (SCBF) as the study progressed (base line SCBF compared with SCBF 1 hour post fusion).

LEVEL	BASE-LINE SCBF	SCBF IMMEDIATELY POST OPERATION	SCBF 1 HOUR POST OPERATION
Cont C1	23.45 +/- 3.88	22.05 +/- 3.27	22.49 +/- 2.41
L + 2	31.88 +/- 6.13	30.42 +/- 5.12	30.31 +/- 5.38
L + 1	28.61 +/- 4.15	27.83 +/- 3.04	27.25 +/- 4.21
Surgery	25.16 +/- 5.00	24.11 +/- 2.74	23.97 +/- 3.26
L - 1	32.78 +/- 5.29	29.95 +/- 3.90	30.26 +/- 4.16
L - 2	38.98 +/- 6.80	35.01 +/- 5.30	35.21 +/- 5.32
Cont C2	44.16 +/- 8.34	38.57 +/- 5.49	38.19 +/- 6.16
Cont L	27.70 +/- 4.59	28.02 +/- 4.56	26.82 +/- 4.55

TABLE 3: Mean Spinal Cord Blood Flow (ml/min/100gm), (+/- SD) for the cervical and lumbar spinal cord segments studied (refer Figure 16).

LEVEL	SCBF 1 HOUR POST OPERATION	PERCENTAGE OF BASE-LINE SCBF
Cont C1	22.49 +/- 2.41	95.91 +/- 8.9
L + 2	30.31 +/- 5.38	95.08 +/- 17.4
L + 1	27.25 +/- 4.21	95.25 +/- 15.0
Surgery	23.97 +/- 3.26	95.27 +/- 12.9
L - 1	30.26 +/- 4.16	92.31 +/- 12.7
L - 2	35.21 +/- 5.32	90.33 +/- 13.6
Cont C2	38.19 +/- 6.16	87.48 +/- 11.7
Cont L	26.82 +/- 4.55	96.82 +/- 16.4

TABLE 4: Mean spinal cord blood flow, (ml/min/100gm) 1 hour post operation (+/- SD) expressed as a percentage of the base-line (Time 1), spinal cord blood flow.

The reduction in blood flow was most marked in the lower cervical segments studied. There was also a reduction in blood flow in the control lumbar spinal cord segments which averaged 3.1%, (range 1.5% to 4.7%). There was also a reduction in the renal blood of both right and left kidneys of 8.0% (Figure 21). None of the changes that occurred in tissue blood flow were significance.

It is unlikely that the changes in the lumbar spinal cord and kidneys are related to the surgery performed on the cervical spine at the level of the C4/5 intervertebral disc. Nor can it be explained by a reduction in circulating blood volume and fluid depletion as haemostasis was maintained throughout the study, and intravenous fluid replacement was based on the estimated fluid loss and the mean arterial blood pressure. Also both the mean arterial blood pressure and the heart rate remained stable throughout.

4.4.2 EVOKED POTENTIAL RESPONSES

Typical motor and sensory evoked potential responses are illustrated in Figure 22. The parameters measured during the study to evaluate the electrical function of the spinal cord and used in the subsequent analysis of results are also indicated.

There was considerable inter-animal variation in the amplitudes of both the motor and sensory evoked potentials but there was no significant intra-animal variation during the study of each individual animal. There was a slight increase in the amplitudes of both the motor and sensory evoked potentials during the study from the time of the base-line measurement to that made 1 hour post operation (Time 1 to Time 3), the magnitude of which was 1.4% for the motor response, and 6.1% for the sensory response. At the same time

there was a prolongation of both the motor and sensory evoked potential latencies ranging from 1.0% to 2.2%, (Table 5 and Figures 23 & 24). Again none of these variations reached significance, based on the criteria outlined above.

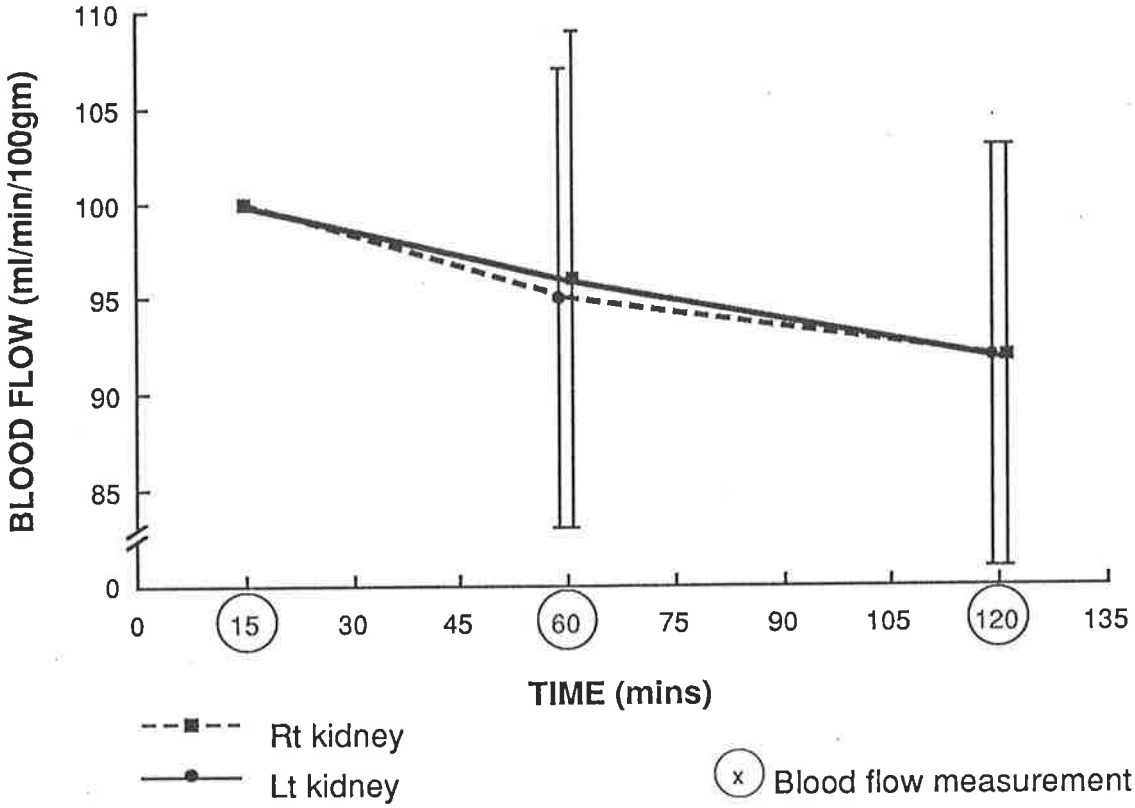


FIGURE 21: Changes in the mean renal blood flow with time expressed as a percentage of the base line for all eighteen sheep studied. There was an 8% reduction in mean renal blood flow of both kidneys during the study period.

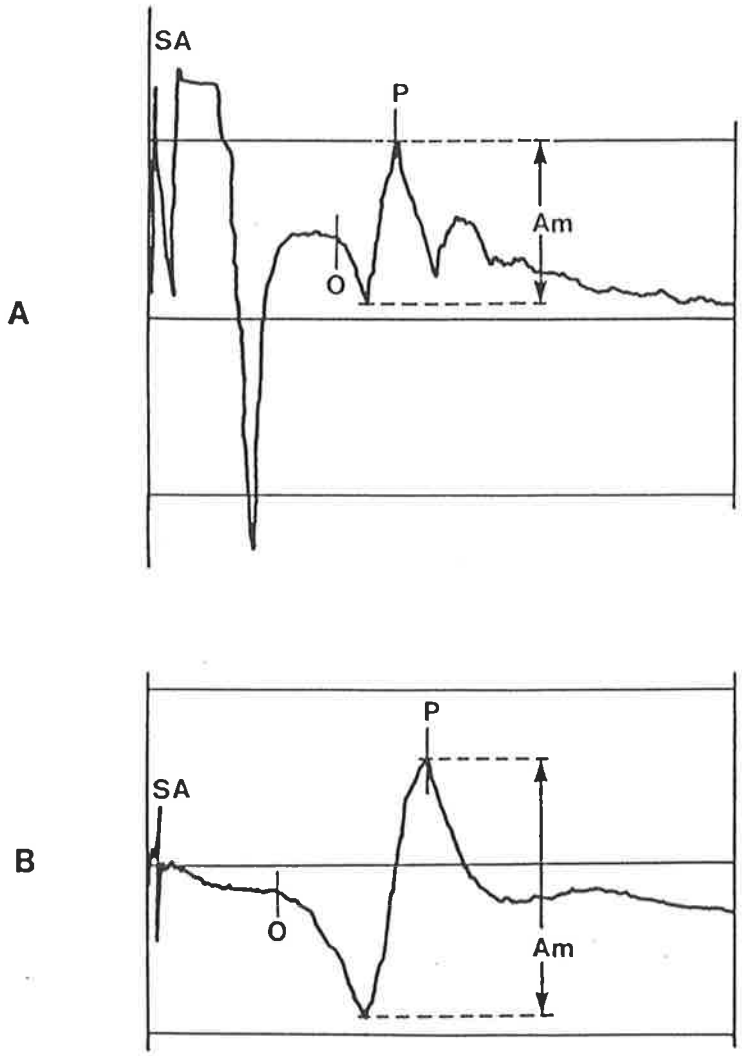


FIGURE 22: Tracings of typical base-line [A] motor and [B] sensory evoked potential responses. The parameters recorded are also indicated; stimulus artefact (SA), origin latency (O), peak latency (P), amplitude (Am).

	ELECTRICAL FUNCTION TIME 1	ELECTRICAL FUNCTION TIME 2	ELECTRICAL FUNCTION TIME 3
MEP			
Amplitude	17.50 +/- 11.31	17.21 +/- 10.60	17.74 +/- 12.33
Latency "O"	2.72 +/- 0.09	2.76 +/- 0.07	2.78 +/- 0.09
Latency "P"	3.03 +/- 0.11	3.04 +/- 0.11	3.06 +/- 0.11
SEP			
Amplitude	29.14 +/- 10.78	30.45 +/- 9.49	30.92 +/- 9.88
Latency "O"	15.03 +/- 0.50	15.44 +/- 0.70	15.32 +/- 0.43
Latency "P"	18.82 +/- 0.89	19.38 +/- 1.58	19.09 +/- 1.35

Time 1 = Time of base-line blood flow measurement
Time 2 = Following completion of the cervical fusion
Time 3 = One hour after completion of the cervical fusion
Latency "O" = Latency of origin
Latency "P" = Latency of peak

TABLE 5: Mean amplitude and latencies of the take off and peak for the motor and sensory evoked potentials (+/- SD) at the time of each blood flow measurement.

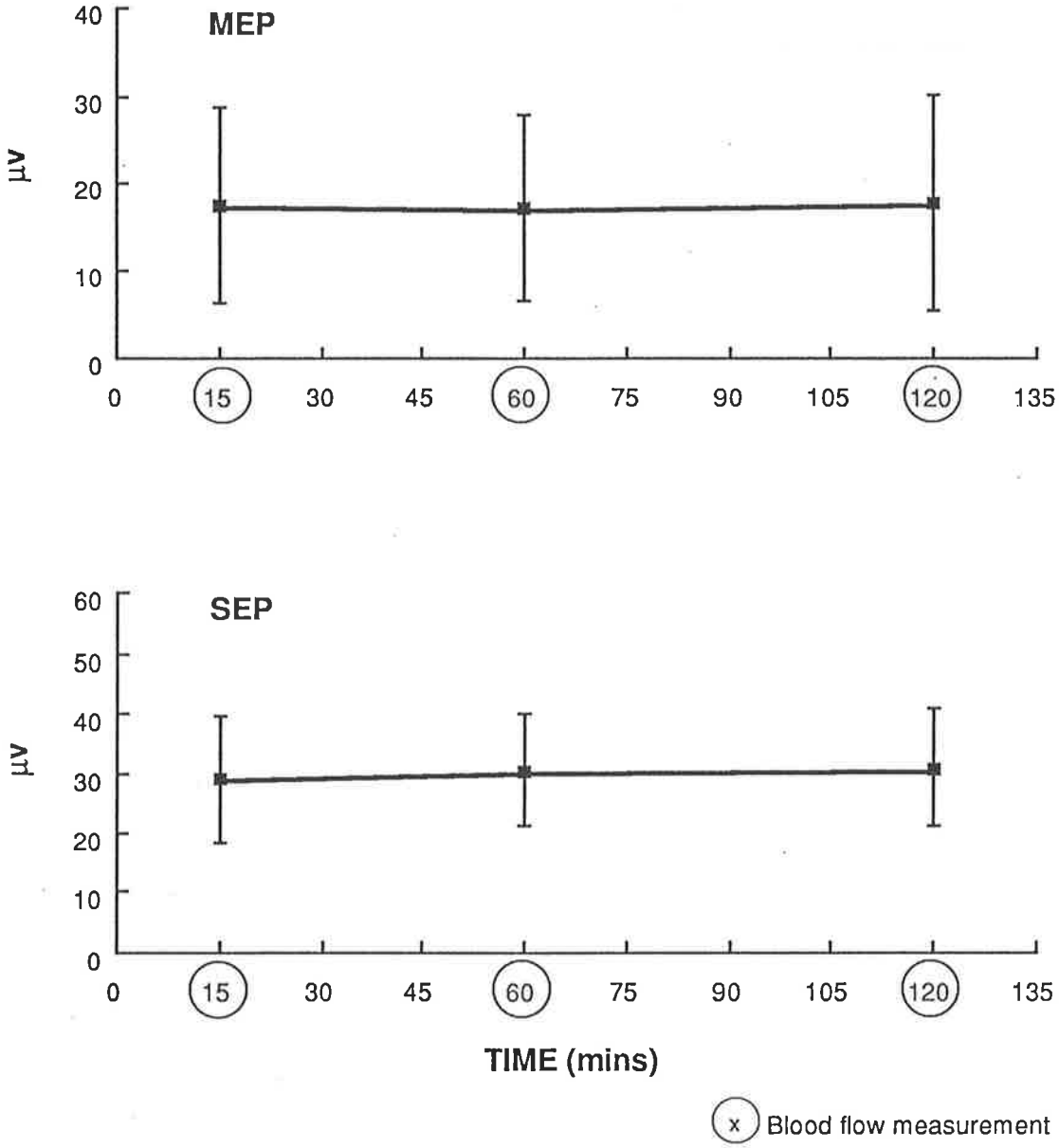


FIGURE 23: Graphs showing the variation in the mean amplitude of the Motor (MEP) and Sensory (SEP) evoked potentials, recorded at the time of each blood flow measurement from the "Operation Only" sheep.

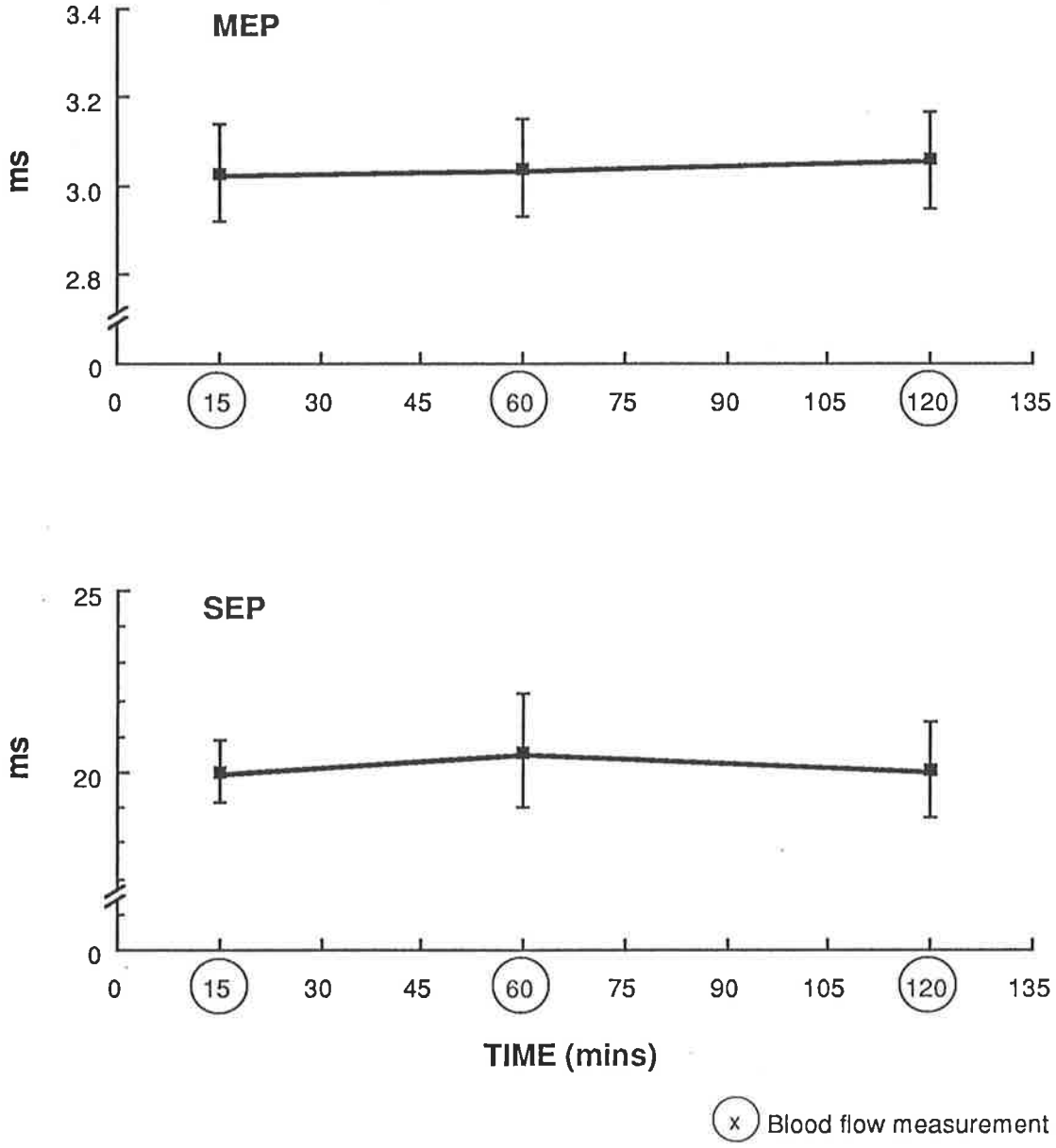


FIGURE 24: Graphs showing the variation in the mean peak latency of the motor (MEP) and sensory (SEP) evoked potentials, recorded at the time of each blood flow measurement from the "Operation Only" sheep.

4.5 DISCUSSION

The aim of this study was to evaluate the effect of an anterior approach and antero-lateral cervical fusion on spinal cord blood flow and function, in the presence of an incomplete spinal cord injury. It could therefore be argued that there was no need to evaluate the effect of the surgery in the absence of such a spinal cord injury. There are however, several good reasons for studying the effect of the surgery in neurologically intact sheep. Firstly, if the antero-lateral cervical fusion did have an adverse affect on spinal cord blood flow and electrical function in the absence of a spinal cord injury, the hypothesis would have been supported. There would then be no need to proceed and develop a spinal cord injury model to evaluate the effect of the surgery in the presence of a spinal cord injury. Secondly, the results obtained would indicate the normal variance of spinal cord blood flow and evoked potential measurements with time in individual animals and between different animals. The results obtained would also be controls for the subsequent study of the effect of the surgery in sheep given an incomplete spinal cord injury and assist in differentiating changes in spinal cord blood flow and electrical function due to the surgery from those due to the injury. Thirdly, the methodology of the study could be tested and evaluated. This allowed adjustments to be made to the technique, to improve the accuracy of the results and streamline the experimental procedures where possible. Few such adjustments were required, and it was reassuring that reliable results were obtained from the first sheep studied.

4.5.1 SPINAL CORD BLOOD FLOW

Regional blood flow can be measured by a variety of techniques as discussed in Chapter 2 (Introduction & Literature Review). The radio-labelled microspheres technique was chosen because exposure of the spinal cord was not required and blood flow could be studied in any body organ or in different parts of the same organ simultaneously without further operative intervention. This enabled assessment of blood flow at salient points throughout the experimental period. The timings of the blood flow measurements, immediately after, and one hour after completion of the surgery should have identified significant alterations in blood flow as a result of the surgery. It is unlikely that transient fluctuations in blood flow that may have been missed by this method of blood flow measurement would have any effect on the final outcome of the study. This is especially true in the absence of any alteration in the evoked potential responses which were monitored continually throughout the study.

The adequacy of microsphere distribution was evaluated by calculation of the blood flow in each kidney. There was no more than 5% difference in the blood flow of each kidney in all but one of the sheep studied. This sheep had hydro-nephrosis of the right kidney which only became evident at the time of obtaining tissue specimens. In this animal the blood flow in the right kidney was less than half of that of the normal left kidney. It was felt that as the difference in blood flow could be explained by the pathology in that kidney, and because the blood flow measurements in the control lumbar spinal cord segments were consistent with those of the other

sheep studied, the results obtained from this sheep were included in the final analysis of results.

The radio-labelled microsphere technique used to measure spinal cord blood flow was found to be reliable and reproducible as there was little variation in the calculated segmental spinal cord blood flows between the baseline, immediately post surgery or delayed post surgery measurements of all six sheep studied.

The reduction in spinal cord blood flow observed may have been due to a depressant effect of the anaesthetic agents used. The same anaesthetic agents were used in all animals studied with maintenance of physiological parameters within the normal range (Table 1). Any depressant effect due to the agents used should have been the same in all animals and it was felt they were not related to the surgery.

The fact that the reduction was greatest in the lower cervical segments is possibly the result of the preparatory surgery for the insertion of the extradural electrode. Exposure of spinous process of C7 and T1, and performing the inter-vertebral fenestration at this level may have resulted in a slight increase in local blood flow. This local increase in blood flow, greatest in the lower cervical and upper thoracic spinal segments would have been present at the time of the base line measurement of spinal cord blood flow. The final blood flow measurement was made some 120 minutes later, by which time this reactive increase in blood flow should have settled.

If the base line blood flow measurements in the lower cervical spinal cord segments were artificially elevated due to this surgery, any reduction in blood flow due to factors such as the anaesthetic agents

used would appear greater if occurring in conjunction with the normalisation of this hyperaemia.

The reduction in blood flow at the level of the surgery was no greater than that in the adjacent spinal cord segments and was also consistent with the trend, mentioned above, of increased reduction in blood flow in the lower cervical segments.

4.5.2 EVOKED POTENTIALS

Motor and sensory evoked potentials were monitored to assess the functional state of the spinal cord both during and after the performance of the antero-lateral cervical fusion. The amplitude of the response was found to vary with alterations in the position of the extradural electrode, as did their latencies in accordance with the distance the impulse had to travel. For this reason the extradural electrode was secured to the capsule of the adjacent facet joint to prevent any inadvertent movement and alteration in its position.

During the study there was a slight increase in the amplitude of the evoked potential responses which was associated with a minor increase in the latencies of the motor and sensory evoked potential responses. These changes do not reflect compromise of the spinal cord which is typically associated with an increase in the latencies and a decrease in the amplitude of the response. The observed change may however represent an alteration in the normal neural activity as a result of the type and duration of anaesthetic agents used. The slight increase in the amplitude of both the motor and sensory evoked potentials may have been due to an exaggerated neural response following a period of reduced neural activity. The

associated increase in the latency of the responses may be due to slower propagation of an impulse due to alterations in ion transport.

Neither the spinal cord blood flow nor the evoked potential responses varied significantly from the base line measurements, made at Time 1 to the final measurements made at Time 3. Also the changes that were evident, namely the reduction in blood flow in the cervical and lumbar spinal cord segments, the reduction in renal blood flow and the increase in the amplitude and latencies of the evoked potentials could not be related to the surgery performed on the cervical spine at the level of the C4/5 intervertebral disc.

It was therefore necessary to go on to evaluate the effect of the antero-lateral inter-body fusion in the presence of an incomplete spinal cord injury.

5. THE EFFECTS OF ANTERO-LATERAL INTER-BODY FUSION ON SPINAL CORD BLOOD FLOW AND FUNCTION IN THE PRESENCE OF AN INCOMPLETE SPINAL CORD INJURY

5.1 AIMS

To develop a closed model for the study of spinal cord injury in the sheep, and to evaluate the effect of an anterior exposure and an antero-lateral cervical fusion on spinal cord blood flow and function in the presence of an incomplete spinal cord injury.

5.2 INTRODUCTION

As outlined in Chapter 4, an antero-lateral cervical fusion resulted in no significant effect on either spinal cord blood flow or electrical function in the neurologically intact animals. The presence of a spinal cord injury, with its associated haemorrhage and oedema may however significantly alter the effect this type of surgery on the spinal cord. Ischaemia may result from compression of the cord from extradural haemorrhage following vertebral fractures and disruption of epidural vessels. Haemorrhage and oedema within the cord due to rupture of vessels within the cord or venous congestion may further hinder spinal cord perfusion.

Intra-neural haemorrhage often occurs during the reactive hyperaemia that follows tissue ischaemia where ischaemic vessel walls are unable to maintain their integrity following restoration of perfusion. Inflammatory exudate and oedema develop from the time of the injury in perfused but damaged tissue, and in ischaemic tissue once circulation has been re-established. Venous congestion will be

present as long as the extradural vessels are compressed and may persist following decompression of the spinal canal due to venous thrombosis. These pathological changes may interfere with the spinal cord circulation sufficiently that the addition of an antero-lateral cervical fusion may reduce spinal cord blood flow enough to compromise its function and potential for recovery.

In order to evaluate the effects of the antero-lateral inter-body fusion of the cervical spine in the presence of an incomplete spinal cord injury it was necessary to develop a spinal cord injury model and to evaluate the effect of the injury itself on local blood flow and spinal cord function. Study of the effect of the injury itself was necessary to distinguish changes in spinal cord blood flow or function due to the surgery from those due to the spinal cord injury.

The study hypothesis that the antero-lateral cervical fusion has an adverse effect on spinal cord blood flow and electrical function, in the presence of an incomplete spinal cord injury was evaluated using two groups of sheep. The "Spinal Cord Injury Only" group (SCI Only) would represent spinally injured patients managed conservatively, and the "Spinal Cord Injury and Operation" group (SCI & Operation) would represent these patients managed by antero-lateral cervical fusion.

The number of sheep required was estimated from (a) the variance in blood flow and evoked potential results obtained from "Operation Only" sheep, (Chapter 4) and (b) the variations in spinal cord blood flow and the evoked potentials considered to be significant. A nomogram for a two sample comparison was used to make this estimation (Altman, 1980) to increase the chance that sufficient

animals would be studied to identify a significant difference ($p < 0.05$) between the two treatment groups if in fact one existed.

This calculation was performed with the assistance of an epidemiologist employed in the Department of Orthopaedic Surgery and Trauma, Royal Adelaide Hospital and indicated twelve sheep, six in each group would be sufficient.

All of the sheep studied were given an incomplete spinal cord injury at the level of the C4/5 intervertebral disc. In addition sheep in the "SCI and Operation" group went on to have an antero-lateral inter-body fusion of the cervical spine at the level of the spinal cord injury. Statistical analysis, using a paired t test, was performed on the results to identify significant variations in spinal cord blood flow or evoked potential responses with time. Analysis of variance was then used to evaluate any difference between the treatment groups in relation to the final spinal cord blood flow and evoked potential responses.

5.3 MATERIALS AND METHODS

All twelve sheep were prepared for surgery and anaesthetised as described in Chapter 4 Section 3.1. The cannulations were performed as outlined in Chapter 4 Section 3.2, and the evoked potential electrodes positioned as described in Chapter 4 Section 3.3. The method used to produce the spinal cord injury is detailed below and the surgical technique for those sheep going on to have an antero-lateral inter-body fusion is described in Chapter 4 Section 3.5.

5.3.1 SPINAL CORD INJURY

As discussed in Chapter 2 several different techniques have been used to produce both complete and incomplete spinal cord injuries in a variety of animal models. To my knowledge there have been no studies reported in which a reproducible incomplete spinal cord injury has been produced in sheep.

Martin and Bloedel (1973) evaluated experimental spinal cord injuries produced by the sudden inflation of a Fogarty balloon catheter in the epidural space cephalad to a laminectomy in cats. By inflating the balloon with different volumes of fluid, neurological lesions of varying severity were produced. They studied the predictive value of the evoked potential changes at the time of the injury by observing the animals' neurological deficit and subsequent recovery. Martin and Bloedel (1973) emphasised however that in their study the inflation of the balloon was not used to produce lesions of quantitatively reproducible graded severity.

The requirement of the present study was however to produce a quantitatively reproducible incomplete spinal cord injury and to then

monitor the effect of a secondary insult, the antero-lateral cervical fusion, on spinal cord blood flow and function.

Use of a dropped weight or controlled pressure clamp were considered unsuitable for this study as discussed in Chapter 2. Inflation of a Fogarty balloon catheter in the extradural space, anterior to the spinal cord, inserted through a small fenestration several segments caudal to the level of interest required neither exposure of the spinal cord at the level of the injury or disruption of the posterior elements. The severity of the injury could also be varied by alteration of the volume of fluid used to inflate the balloon.

The spinal cord in the sheep is larger than that of the rat, cat and monkey and closer in size to that of man. The average diameter of the cervical spinal cord in the sheep studied was 9.5mm. The shape of the sheep spinal canal at the level of the C4/5 intervertebral disc is almost circular, (Figure 4). With the average diameter of the spinal canal at this level in the 13 sheep studied (7 used in the anatomical evaluation & 6 "Operation Only" sheep) being 13mm (range 11.5mm - 14mm). Other soft tissue contents of the spinal canal reduced its effective diameter of the canal to 12mm. The spinal cord in the mid cervical region is also nearly circular in cross-section, (Figure 9). The cervical spinal cord therefore occupies approximately 63% of the spinal canal, calculated using the equation for the area of a circle ($A = \pi r^2$), the radius of 6mm for the spinal canal and 4.75mm for the spinal cord. Selection of the appropriate size of Fogarty balloon catheter and the volume of fluid to be injected was determined on the basis of the dimensions of the spinal canal outlined above and experimentation with the technique in animals used in the anatomical study.

Patients presenting with incomplete spinal cord injuries due to burst fractures of the thoracic and lumbar spine often have restriction of the spinal canal volume in the order of 50%. The degree of neurological loss however varies with the mechanism of the injury, and the severity of spinal canal compromise evident during investigation at hospital may not reflect the canal compromise that occurred at the time of the injury. It is therefore not possible to accurately quantitate the severity of a spinal cord injury seen in man with the extent of canal compromise.

It was felt that inflation of a balloon in the extradural space resulting in a 60-70% reduction in spinal canal volume would produce a suitable incomplete spinal cord injury. This was based on the results reported by Martin & Bloedel (1973) and Bennett (1983), who used the extradural balloon compression technique to produce injuries of varying severity by inflating an extradural balloon with different volumes of fluid. A balloon catheter with an inflated diameter of 9.4mm - 10mm was required to produce this degree of compression.

Fogarty balloon catheters come in a range of sizes, none however with a fully inflated diameter within the specified range. The number 4 French Fogarty catheter having an inflated diameter of 9mm and the number 5 French Fogarty catheter 11mm. As none of the catheters were an ideal size the number 5 French Fogarty catheter (balloon capacity 1.5ml; order number 12-080-5F) was tried with sub maximal inflation in two of the live sheep used in the anatomical study. In these animals the balloon was inflated with 1.0ml of fluid which resulted in an external diameter of the balloon of 9.6mm. In these two trial sheep a pressure transducer was connected to the catheter to record the pressure within the balloon when inflated in

the extradural space. The pressure in the balloon peaked at 740 mmHg during inflation and stabilised at 430 mmHg, where it remained until the balloon was deflated. The pressure transducer recorded the pressures within the balloon and not the spinal canal. However, as the balloon was sub-maximally inflated (two thirds capacity) these pressures are probably also an indication of the pressure produced within the spinal canal. Regardless of the actual pressure generated, inflation of the balloon resulted in the instantaneous loss of the motor evoked potential, while the sensory evoked potential remained unchanged. The balloon was left inflated for 30 minutes and the motor and sensory evoked responses were recorded every five minutes during this period. The motor evoked response did not recover during the period of observation, while the sensory evoked response persisted.

These evoked potential changes, (loss of the motor, but persistence of the sensory response) were consistent with an incomplete spinal cord injury. Therefore a number 5 French Fogarty balloon catheter inflated with 1.0ml of fluid was subsequently used to produce the spinal cord injury in all study animals.

The Fogarty catheter was introduced into the epidural space of the cervical spinal canal through the fenestration in the C7/T1 interspace made for the insertion of the epidural electrode. The surgical approach is described in Chapter 4, Section 3.3. A new catheter was used for each of the sheep studied and the balloon tested for patency prior to its use. The catheter was introduced into the epidural space over the wire stylet supplied with the catheter and the catheter tip was curved slightly prior to its insertion allowing it to follow the natural lordotic curve of the sheep cervical spine. The

catheter was advanced cranially approximately 10cm, usually without resistance or difficulty, so that the tip of the wire was situated at the level of the caudal endplate of C4. Motor evoked potentials were monitored during introduction of the catheter and in none of the animals did insertion affect the amplitude or latencies of the evoked potential response. The position of the catheter was then checked with a lateral cervical radiograph before removing the wire stylet (Figure 25) and if necessary the catheter was advanced or retracted.

Once positioned correctly the catheter was secured with a 00 silk suture to the capsule of the adjacent facet joint and the wire stylet removed. The Fogarty catheter was then connected to a three way tap via a short length of silastic tubing (Figure 26). This tubing was then connected to a 5ml glass syringe containing 1.0ml fluid, a mixture of 1 part water and 1 part Urografin, which was used to inflate the balloon. This enabled radiographic localisation of the inflated balloon for correlation with the level of the lesion and segmental spinal cord blood flow measurements. The three way tap and tubing were primed with 0.5ml of the water/Urografin mixture prior to connection to the 5ml syringe and Fogarty catheter. The tubing was clamped following balloon inflation to prevent back flow and decompression of the balloon.

Preparation of the animal then continued as outlined in Chapter 4, and the baseline blood flow measurement made after the completion of all other preparatory surgery and physiological stabilisation of the animal.

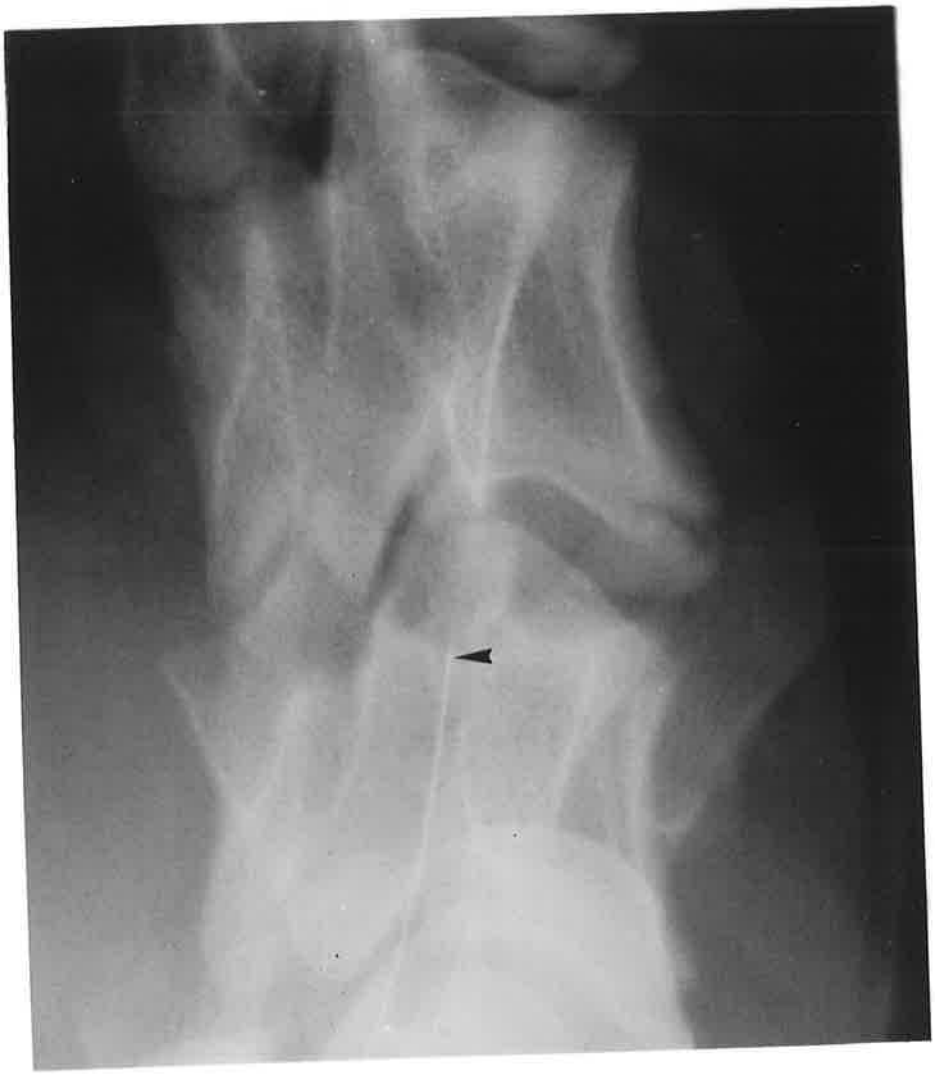


FIGURE 25: Intra-operative lateral radiograph of the sheep cervical spine showing the position of the tip of the wire in the Fogarty catheter (Arrow), indicating the position of the balloon in the spinal canal.

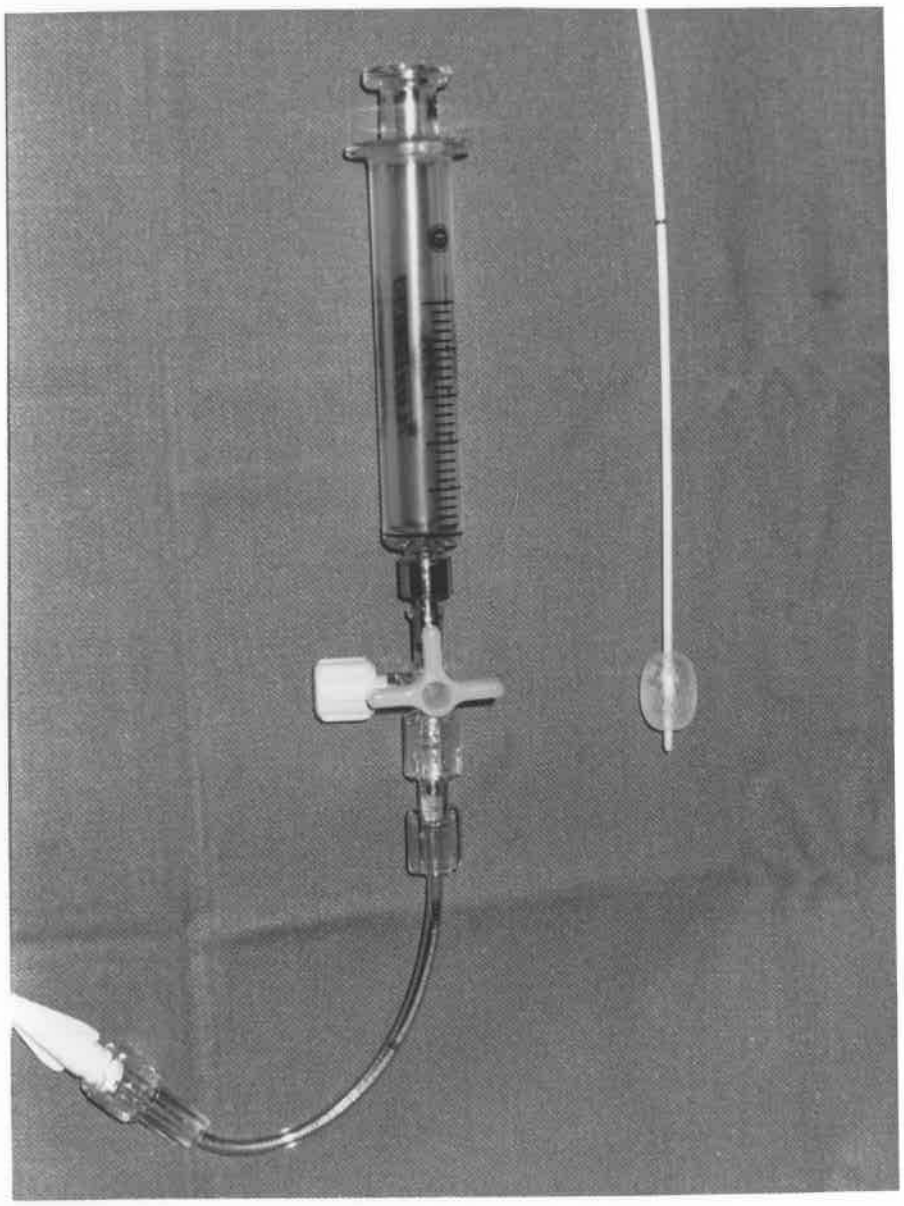


FIGURE 26: Photograph of the Fogarty catheter connected to a 5ml glass syringe via three way tap and a short length of silastic tubing.

Sheep used in this part of the study were arbitrarily allocated, to either of the two treatment groups (SCI Only or SCI & Operation) once the preparation of the animal had been completed.

The spinal cord injury was produced by the sudden inflation of the balloon within the spinal canal following the baseline blood flow measurement and recording the baseline motor and sensory evoked potentials, arterial blood gas results and mean arterial pressure. The balloon was left inflated for 30 minutes, after this time the clamp was released, the balloon deflated and the clamp re-applied. The catheter was left in place until the completion of the study to prevent any inadvertent neural damage or alteration in the position of the epidural electrode which may have distorted the evoked potential results obtained subsequently.

It was hoped the rapid inflation of the balloon would simulate the sudden forceful compression of the spinal cord that occurs at the time of a traumatic dislocation or subluxation, and the compression on the spinal cord by the inflated balloon would simulate the effect of a displaced vertebral body or vertebral fragments. Deflation of the balloon would then simulate decompression of the spinal cord at the time of closed or open reduction. While the balloon was inflated a lateral cervical radiograph was taken without moving the neck of the animal, by placing the X-Ray film cartridge beneath a sheet on which the sheep was lying (Figure 27). This was done to document the exact location of the inflated balloon for later correlation with the level of the spinal cord injury, spinal cord blood flow results and the histology.

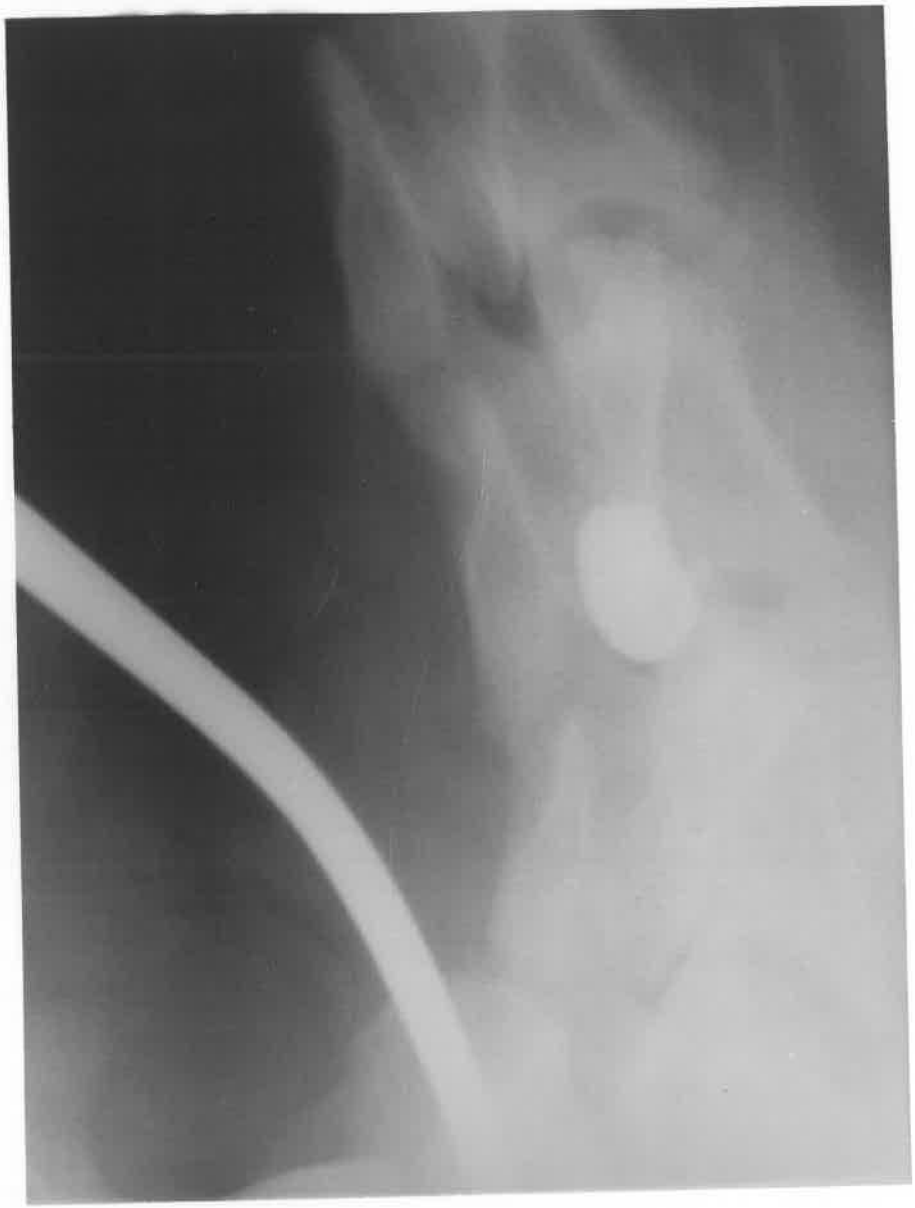


FIGURE 27: Intra-operative lateral radiograph of the sheep cervical spine showing the contrast filled balloon in the spinal canal at the level of the C4/5 intervertebral disc.

5.3.2 BLOOD FLOW MEASUREMENTS

Blood flow measurements were performed using the same methods described in Chapter 4, Section 3.4. Different nuclides were used as Dupont was unable to supply Niobium 95 labelled microspheres, and the use of a fourth blood flow measurement following inflicting the spinal cord injury necessitated the selection of a fourth nuclide with a suitable energy of emission. The four nuclides used were Cerium 141 (Ce ^{141}), Chromium 51(Cr ^{51}), Ruthenium 103 (Ru ^{103}) and Scandium 46 (Sc ^{46}).

The combined energy spectra of these nuclides are shown in Figure 28 and the nuclides used for each of the blood flow measurements is indicated in Table 6.

The base line measurement was performed following completion of the preparatory surgery and physiological stabilisation of the sheep as in the "Operation Only" sheep, (Time 1). The second blood flow measurement was made immediately following inflation of the balloon and production of the incomplete spinal cord injury, (Time 2). The third blood flow measurement was made approximately forty-five minutes later, fifteen minutes after deflation of the balloon in the "SCI Only" group and immediately after completion of the surgery in the "SCI & Operation" group (Time 3). The fifteen minute delay in the "SCI Only" group was introduced to ensure comparable timings of blood flow and evoked potential measurements in each group as the surgery was not usually completed until fifteen minutes after the balloon was deflated. The electrical function of the spinal cord was monitored throughout this period. The final blood flow measurement was made one hour after the third measurement in both groups (Time 4).

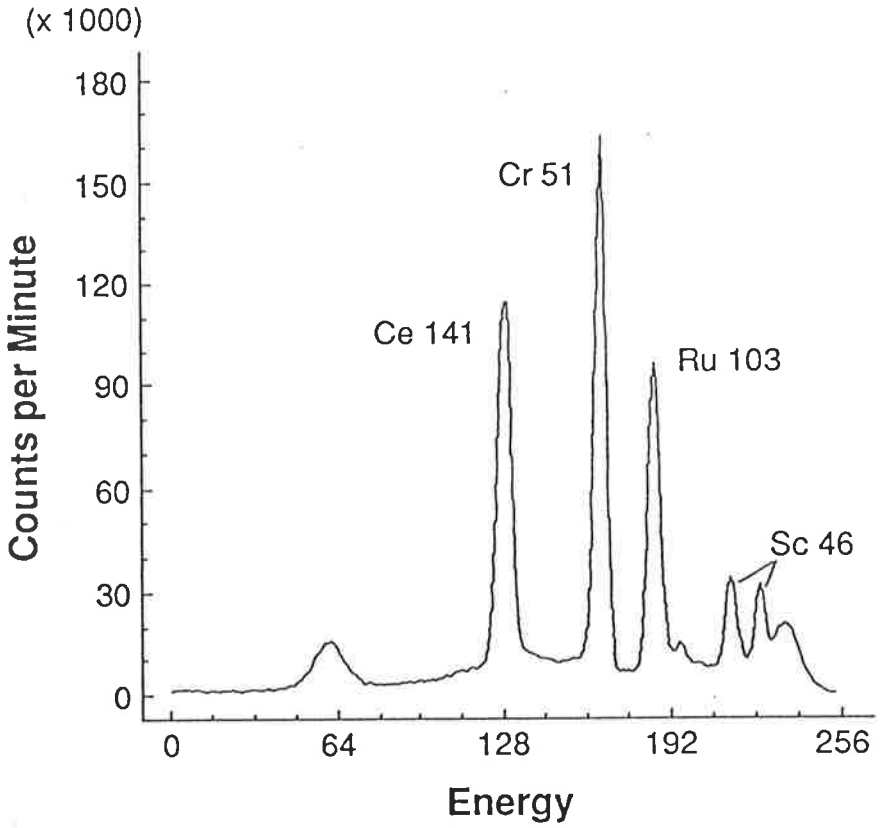


FIGURE 28: The combined energy spectrum plot of Cerium 141 (Ce^{141}), Chromium 51 (Cr^{51}), Ruthenium 103 (Ru^{103}) and Scandium 46 (Sc^{46}).

	Sheep Number	Blood Flow Measurement			
		Time 1	Time 2	Time 3	Time 4
SCI Only	1	Ce^{141}	Cr^{51}	Ru^{103}	Sc^{46}
	2	Sc^{46}	Ce^{141}	Ru^{103}	Cr^{51}
	3	Ru^{103}	Sc^{46}	Cr^{51}	Ce^{141}
	4	Sc^{46}	Cr^{51}	Ce^{141}	Ru^{103}
	5	Ce^{141}	Cr^{51}	Ru^{103}	Sc^{46}
	6	Ru^{103}	Ce^{141}	Sc^{46}	Cr^{51}
SCI & Operation	1	Sc^{46}	Ru^{103}	Cr^{51}	Ce^{141}
	2	Ce^{141}	Sc^{46}	Cr^{51}	Ru^{103}
	3	Cr^{51}	Ce^{141}	Ru^{103}	Sc^{46}
	4	Ru^{103}	Ce^{141}	Sc^{46}	Cr^{51}
	5	Cr^{51}	Sc^{46}	Ce^{141}	Ru^{103}
	6	Ru^{103}	Cr^{51}	Ce^{141}	Sc^{46}

TABLE 6: Radio-labelled microspheres used for the blood flow measurement of each of the sheep studied from "SCI Only" and "SCI & Operation" groups.

The blood flow measurements from the first group of sheep studied (Operation only) correspond to the timing of blood flow measurements in the "SCI Only" and "SCI & Operation" groups as follows;

Operation only		SCI +/- Operation
Time 1	=	Time 1
-	=	Time 2
Time 2	=	Time 3
Time 3	=	Time 4

The sheep were sacrificed, and tissue specimens obtained as outlined in Chapter 4, Section 3.4. The spinal cord was divided as shown in Figure 29 and a thin section of spinal cord from each vertebral segment was taken for histological examination.

5.3.3 ANTERO-LATERAL CERVICAL FUSION

The antero-lateral inter-body fusion was performed on the sheep in the "SCI & Operation" group using the technique described in Chapter 4, Section 3.5. The surgery commenced just prior to the deflation of the balloon catheter to simulate the effect of reduction and progression to early operative stabilisation.

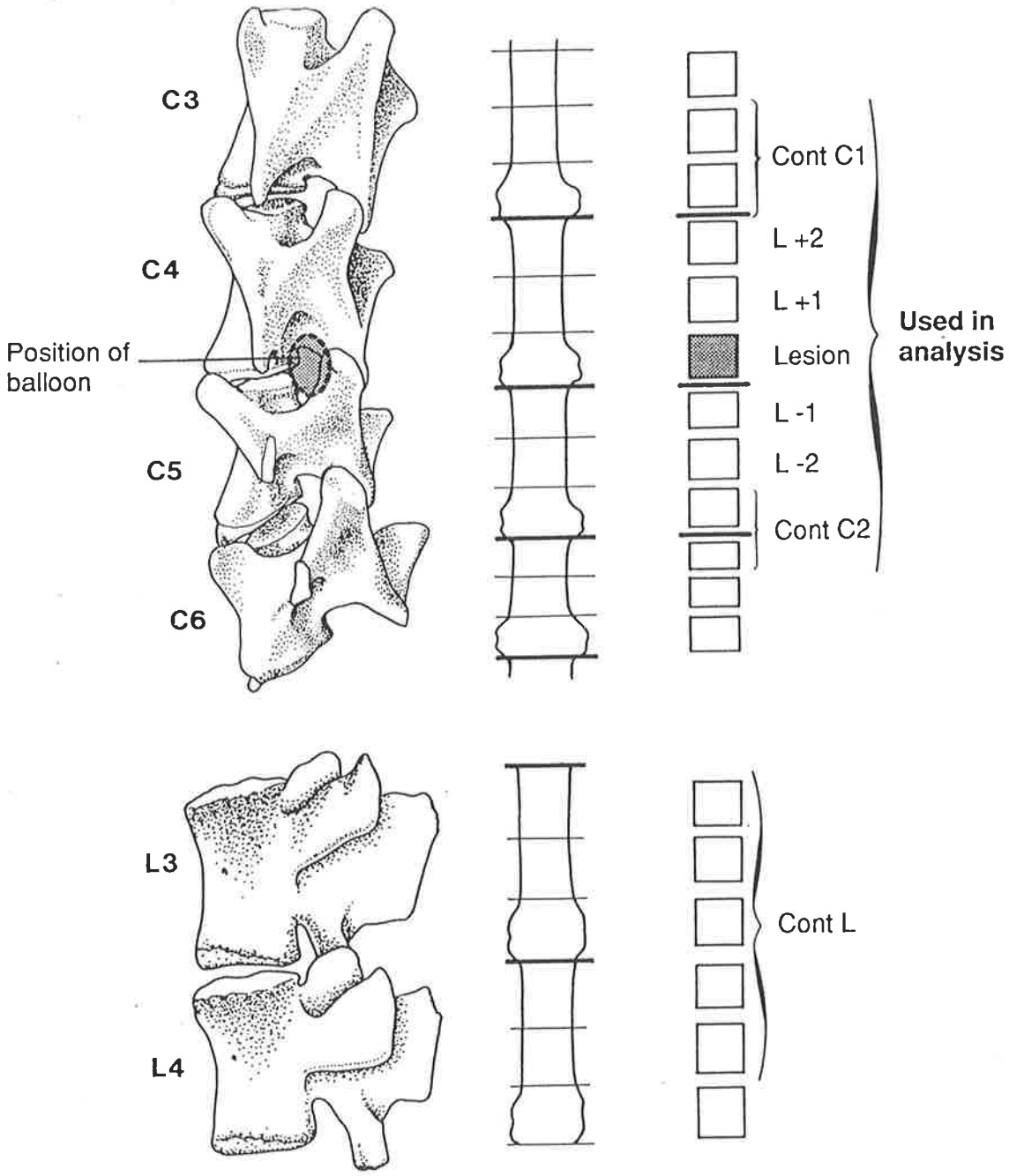


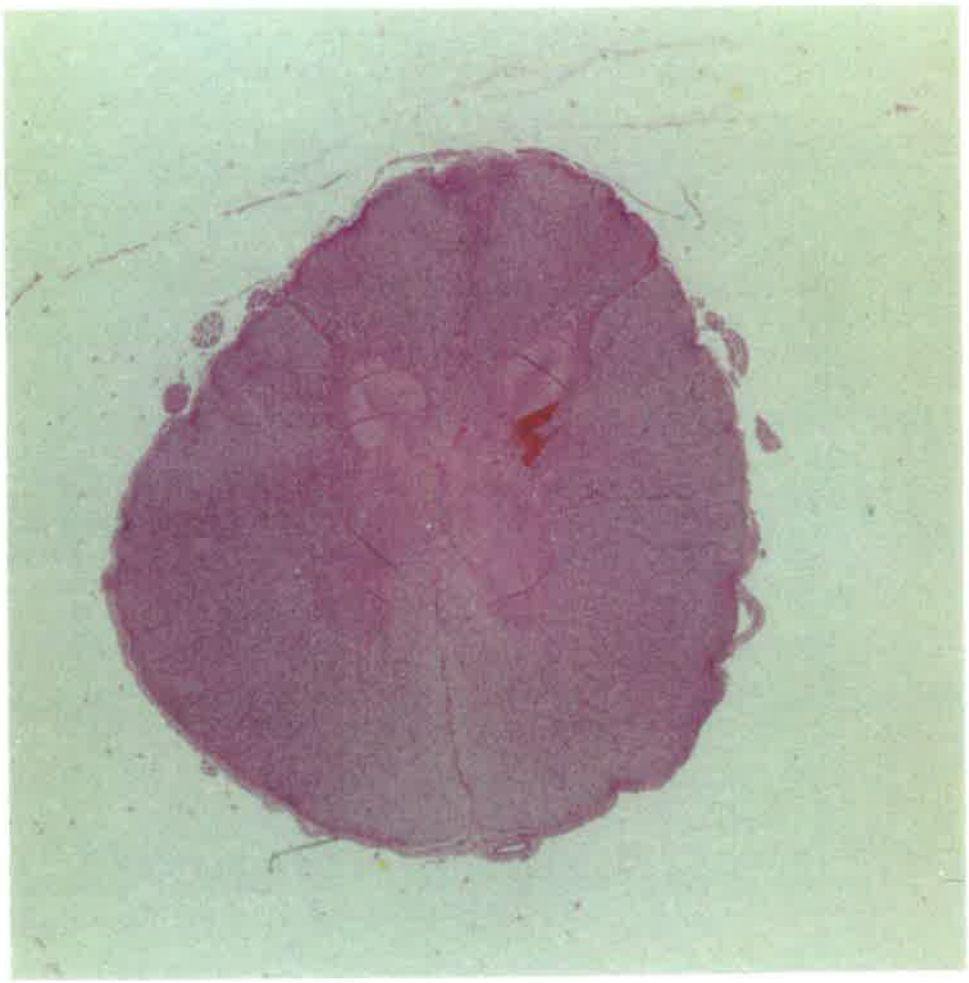
FIGURE 29: Diagrammatic representation of the cervical and lumbar vertebral segments removed from each sheep showing how the spinal cord was subdivided and labelled. The location of the balloon used to produce the spinal cord injury is also indicated.

5.4 RESULTS

5.4.1 HISTOLOGY

Histological specimens were examined and reported by Dr P. Blumbergs, Neuropathologist, Institute of Medical and Veterinary Science, Adelaide, South Australia. All of the spinal cord sections taken from the vertebral segments away from the level of the cord injury, (C4/5) were reported as normal. Sections taken through the spinal cord at the level of the injury showed evidence of acute haemorrhage and ischaemic cell damage. Figure 29 shows a transverse histological section of the spinal cord at the level of the C4/5 intervertebral disc, (Haematoxylin & Eosin (H&E)) demonstrating scattered haemorrhage in and around the central region of the spinal cord. Figure 30, from the same specimen (H&E x 250) demonstrates recent haemorrhage into the central white matter of the spinal cord and Figure 31 (H&E x 250) shows "acute ischaemic cell change" in anterior horn cells.

The earliest stage of ischaemic cell change is microvacuolation. Here the contour of the nerve cell is unaltered and the dimensions and staining properties of the nucleus are normal. Small apparently empty spherical bodies or microvacuoles (diameter 0.16 μm to 2.5 μm) are present in the cytoplasm. The stage of simple ischaemic cell change follows that of microvacuolation where the cell body is shrunken and the cytoplasm usually stains a vivid pink with eosin. These changes are evident from 30 minutes to 6 hours after a hypoxic episode. The process progresses to nuclear fragmentation and dissolution of the cell.



Scale (mm) 0——1

FIGURE 30: Transverse histological specimen of the cervical spinal cord from the level of the C4/5 intervertebral disc in one of the sheep given an incomplete spinal cord injury. A localised area of haemorrhage is evident in the central region of the spinal cord. (H&E)

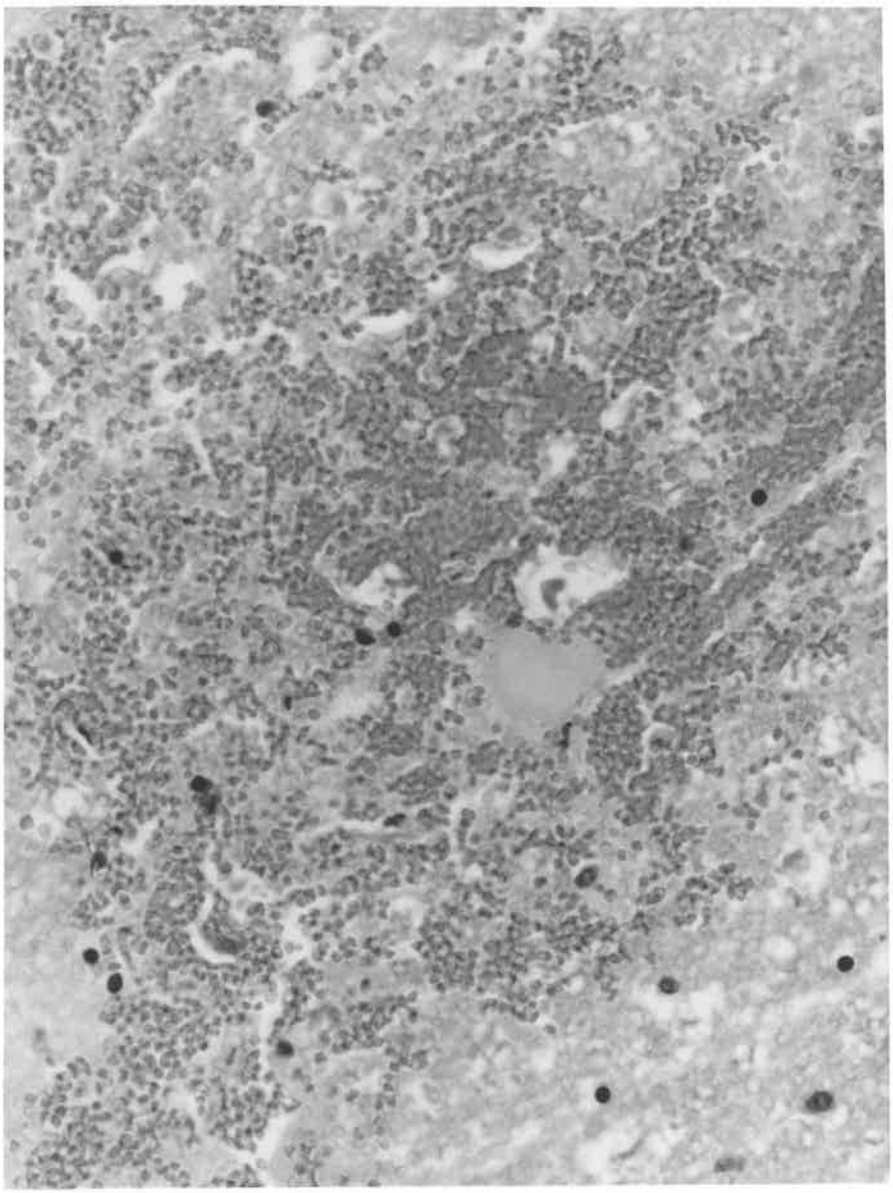


FIGURE 31: Histological specimen of the cervical spinal cord of a sheep given an incomplete spinal cord injury showing recent haemorrhage into the central white matter of the spinal cord. (H&E x 250)

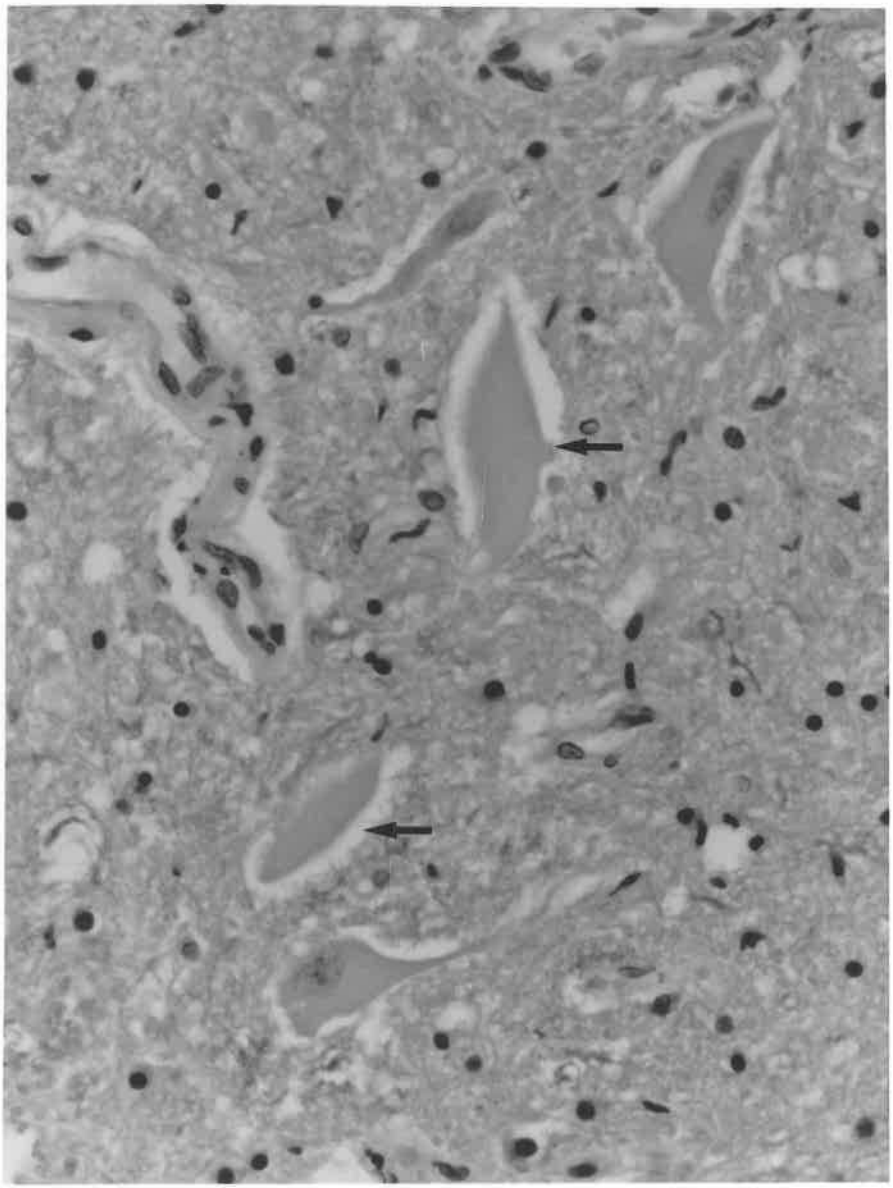


FIGURE 32: Histological specimen of the cervical spinal cord of a sheep given an incomplete spinal cord injury (H&E x 250). The anterior horn cells (Arrows) demonstrate the features of early acute ischaemic cell change (See page 116).

5.4.2 EVOKED POTENTIALS

Inflation of the Fogarty balloon catheter resulted in an instantaneous and complete loss of the motor evoked potential (Figure 32). This occurred in eleven of the twelve sheep given a spinal cord injury. One of the sheep from the "SCI Only" group had a persistent motor response. The responses in this animal were however significantly affected by inflation of the balloon, and the sensory response was more severely affected than in the other animals studied. In three of the sheep, two from the "SCI Only" group and one from the "SCI & Operation" group some motor function was evident at the time of the final blood flow measurement (Time 4).

There was a reduction in the amplitude of the SEP of 13.5% in the SCI Only group and 44.6% in the SCI & Operation group. A prolongation of the latencies of the sensory evoked potential while the balloon remained inflated in the extradural space was also observed (7.3% increase in the peak latency in the SCI Only group and 10.5% increase in the SCI & Operation group). The changes seen in the sensory evoked potential were not instantaneous but developed, and progressed slowly as long as the balloon remained inflated. The extent of the deterioration varied slightly with each animal but in no case did the sensory evoked potential become unrecordable. Following release of the balloon the sensory evoked potential began to recover, and in all animals returned to within 90% of the pre-injury state by the completion of the study, (Figure 33). The evoked potential responses of both groups of animals (SCI Only and SCI & Operation) at the time of each blood flow measurement are summarised in Table 7 and 8.

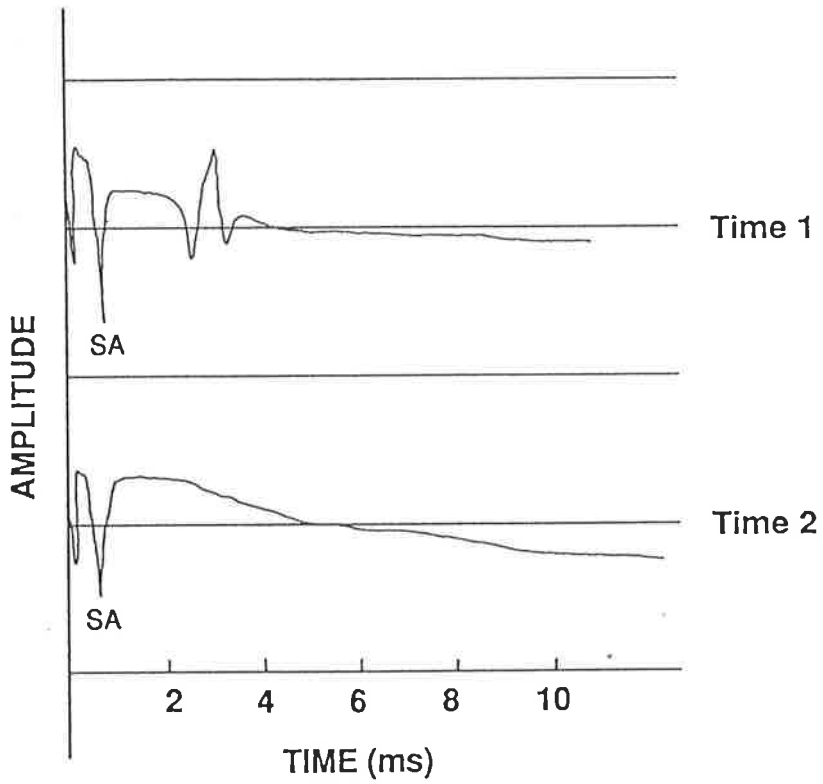


FIGURE 33: Tracing of the motor evoked potential at the base-line (Time 1) and immediately following inflation of the balloon in the extradural space (Time 2). Responses averaged over 32 impulses. Stimulus artefact (SA).

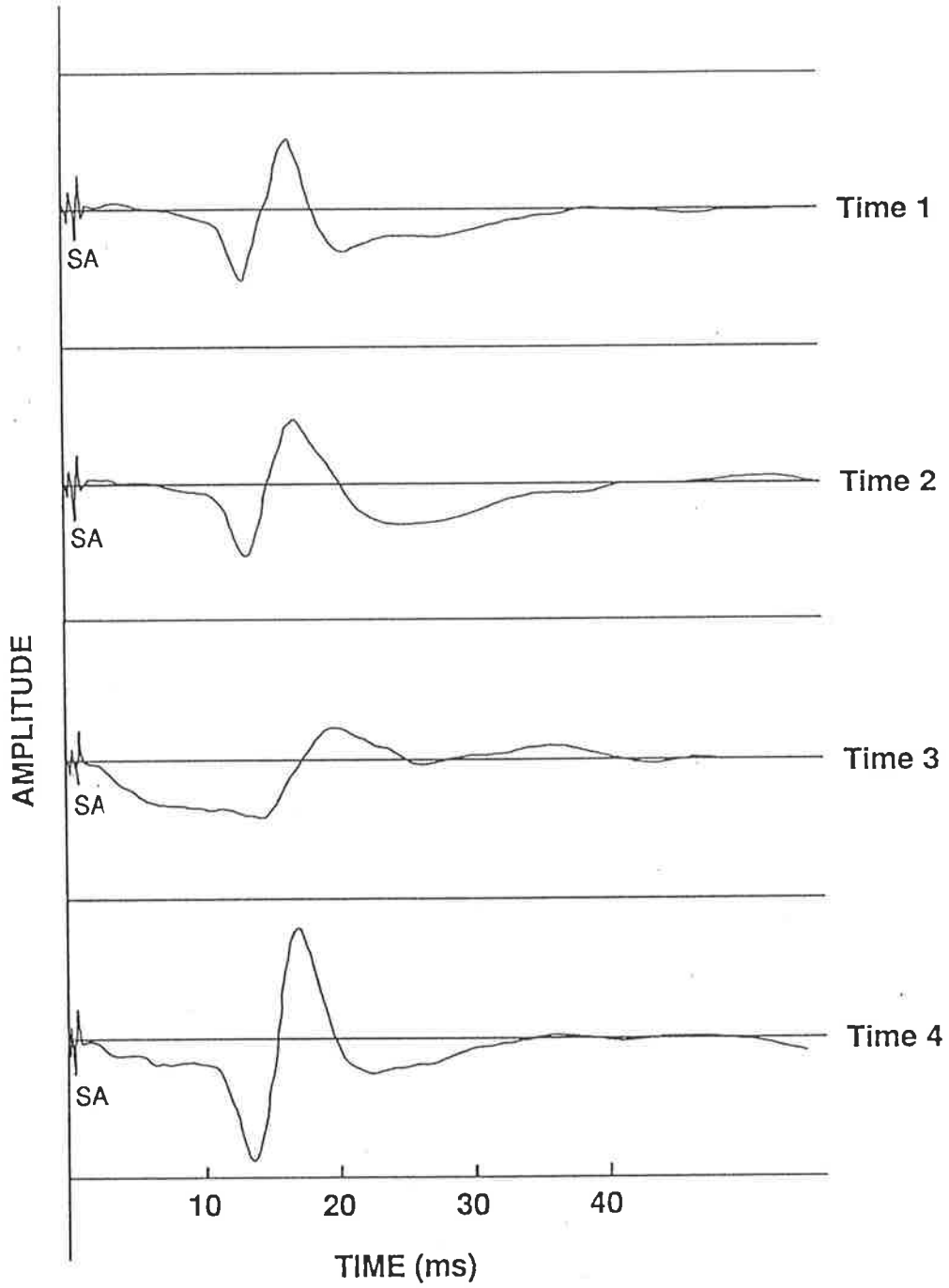


FIGURE 34: Tracing of the sensory evoked potential at the base-line (Time 1), immediately following inflation of the balloon in the extradural space (Time 2), following deflation of the balloon (Time 3) and at the time of the final evoked potential recording (Time 4). Responses averaged over 32 impulses. Stimulus artefact (SA).

		AMPLITUDE	ORIGIN LATENCY	PEAK LATENCY
Time 1	SCI Only	10.79 +/- 4.80	2.75 +/- 0.17	3.06 +/- 0.24
	SCI & Operation	9.80 +/- 5.71	2.99 +/- 0.46	3.27 +/- 0.48
Time 2	SCI Only	1.00 +/- 0.94	0.53 +/- 0.35	0.59 +/- 0.74
	SCI & Operation	0.00	0.00	0.00
Time 3	SCI Only	0.83 +/- 0.57	0.53 +/- 0.29	0.59 +/- 0.31
	SCI & Operation	0.00	0.00	0.00
Time 4	SCI Only	2.33 +/- 0.82	0.98 +/- 0.67	1.08 +/- 0.83
	SCI & Operation	1.30 +/- 0.61	1.17 +/- 0.94	1.29 +/- 1.08

Time 1 = Time of Base line Blood Flow Measurement
Time 2 = Immediately following inflation of the Balloon
Time 3 = Following deflation of the Balloon +/- Operation
Time 4 = One hour after deflation of the Balloon +/- Operation

TABLE 7: Mean motor evoked potentials (+/- SD) recorded at the time of each spinal cord blood flow measurement for both the SCI Only and SCI & Operation groups.

		AMPLITUDE	ORIGIN LATENCY	PEAK LATENCY
Time 1	SCI Only	21.25 +/- 7.61	15.36 +/- 1.59	20.80 +/- 1.78
	SCI & Operation	19.58 +/- 5.32	14.75 +/- 0.33	20.64 +/- 0.89
Time 2	SCI Only	18.60 +/- 8.21	16.16 +/- 1.62	22.33 +/- 1.78
	SCI & Operation	10.87 +/- 6.45	16.39 +/- 0.81	22.61 +/- 1.36
Time 3	SCI Only	18.96 +/- 9.55	16.12 +/- 1.81	22.24 +/- 1.08
	SCI & Operation	10.84 +/- 4.86	16.92 +/- 1.47	22.81 +/- 1.19
Time 4	SCI Only	19.67 +/- 10.64	15.98 +/- 1.71	22.04 +/- 1.09
	SCI & Operation	11.59 +/- 5.98	17.00 +/- 1.02	22.73 +/- 1.12

Time 1 = Time of Base line Blood Flow Measurement
Time 2 = Immediately following inflation of the Balloon
Time 3 = Following deflation of the Balloon +/- Operation
Time 4 = One hour after deflation of the Balloon +/- Operation

TABLE 8: Mean sensory evoked potentials (+/- SD) recorded at the time of each spinal cord blood flow measurement for both the SCI Only and SCI & Operation groups.

The incomplete nature of the injury enabled continual monitoring of the residual electrical function of the spinal cord while the balloon was inflated and its recovery once the balloon had been deflated.

A comparison was then made between the electrical function of the spinal cord in each group at the time of each blood flow measurement.

The sheep in the "SCI & Operation" group demonstrated a greater reduction in the amplitude of the sensory response and increase in the latencies when the balloon was inflated compared to those in the "SCI Only" group. This difference between the groups became evident before the surgery was performed, persisted throughout the study, failed to reach significance and was not altered further by the antero-lateral cervical fusion.

These results suggest the neurological injury caused by the inflation of the balloon was a little more severe in the "SCI & Operation" group, than it was in the "SCI Only" group. The reason for this difference is not clear as sheep were selected for use by people not involved directly with the study and allocated arbitrarily to either group. The method of producing the injury was identical in all sheep studied. There was however a slight difference in the mean weight of the sheep in each group with the "SCI only" group being a little larger. It may be that associated with this difference in size, the larger animals had a little more room in their spinal canals, and as a result the severity of their neurological injuries when the balloon was inflated was a little less than the smaller sheep.

The change in the peak latency of the sensory evoked potential with time is shown in Figure 34. The results from the operation only group are included as a control for comparison.

It was the effect of the surgical stabilisation of the cervical spine in the presence on an incomplete spinal cord injury, and not the effect of the injury itself that was under evaluation. The effect of the injury therefore needed to be eliminated or at least considered in the evaluation of the outcome of each group. This was done by expressing the blood flows and evoked potentials at Times 3 and 4 as a percentage of those obtained at the time the spinal cord injury was produced, (Time 2). The differences between the two groups due to the surgery then became evident (Figure 35). The difference in blood flows and evoked potentials from Time 2 to Time 3 and from Time 2 to Time 4 in each group were then evaluated, and analysis of variance used to assess any difference between the two groups. This analysis should have identified any detrimental effect of the surgery on the spinal cord.

The difference in the amplitude of the responses from the "SCI & Operation" and the "SCI Only" group at Time 3 were not significant and no difference was evident at Time 4. The electrical function of the spinal cord was monitored continuously in most animals and recorded at least every fifteen minutes in all the sheep studied. Comparison of these evoked potential responses gives an indication of the physiological, and hence the blood flow changes that occurred in the spinal cord throughout the study. The SEP amplitude, latency of the origin and peak from three individual sheep, one from each of the three groups, (Operation Only, SCI Only and SCI & Operation) are shown in Figure 36.

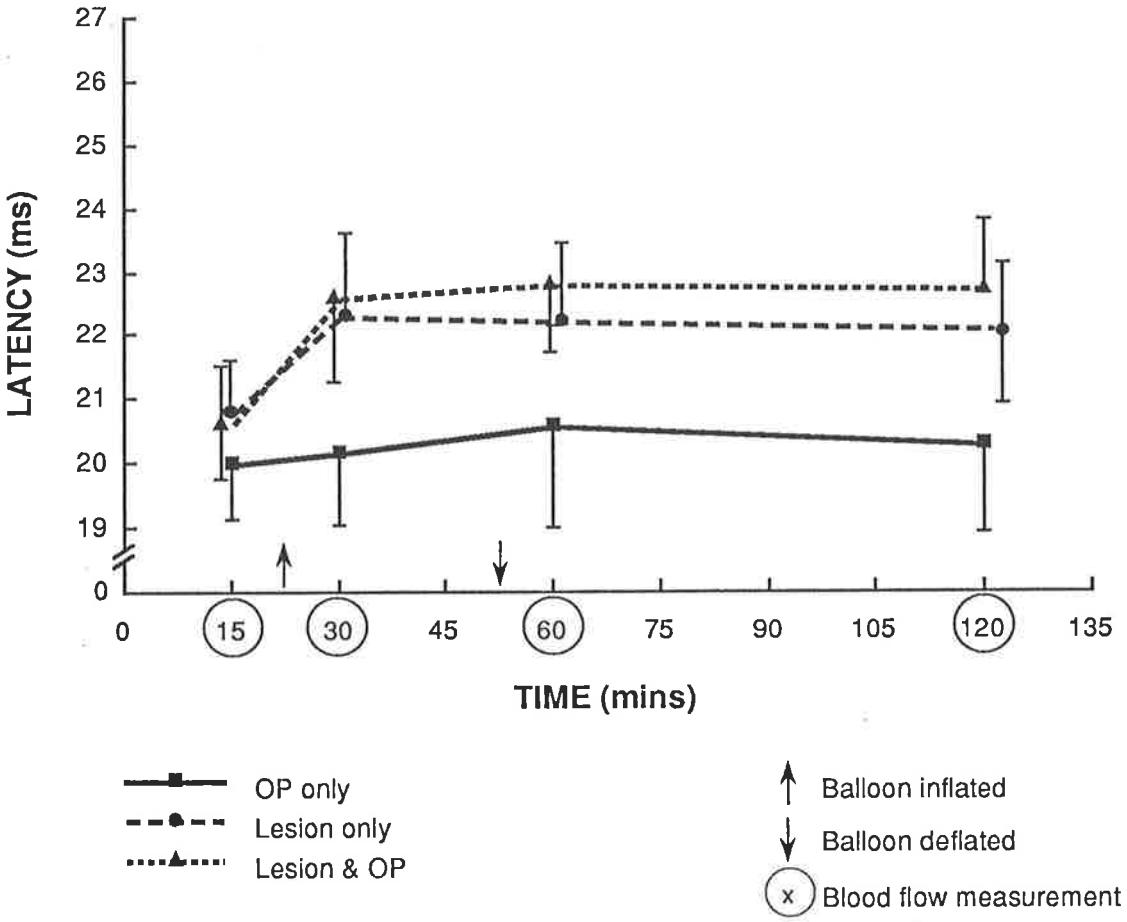


FIGURE 35: Graph showing the change in the mean peak latency of the sensory evoked potential with time for all three groups of sheep.

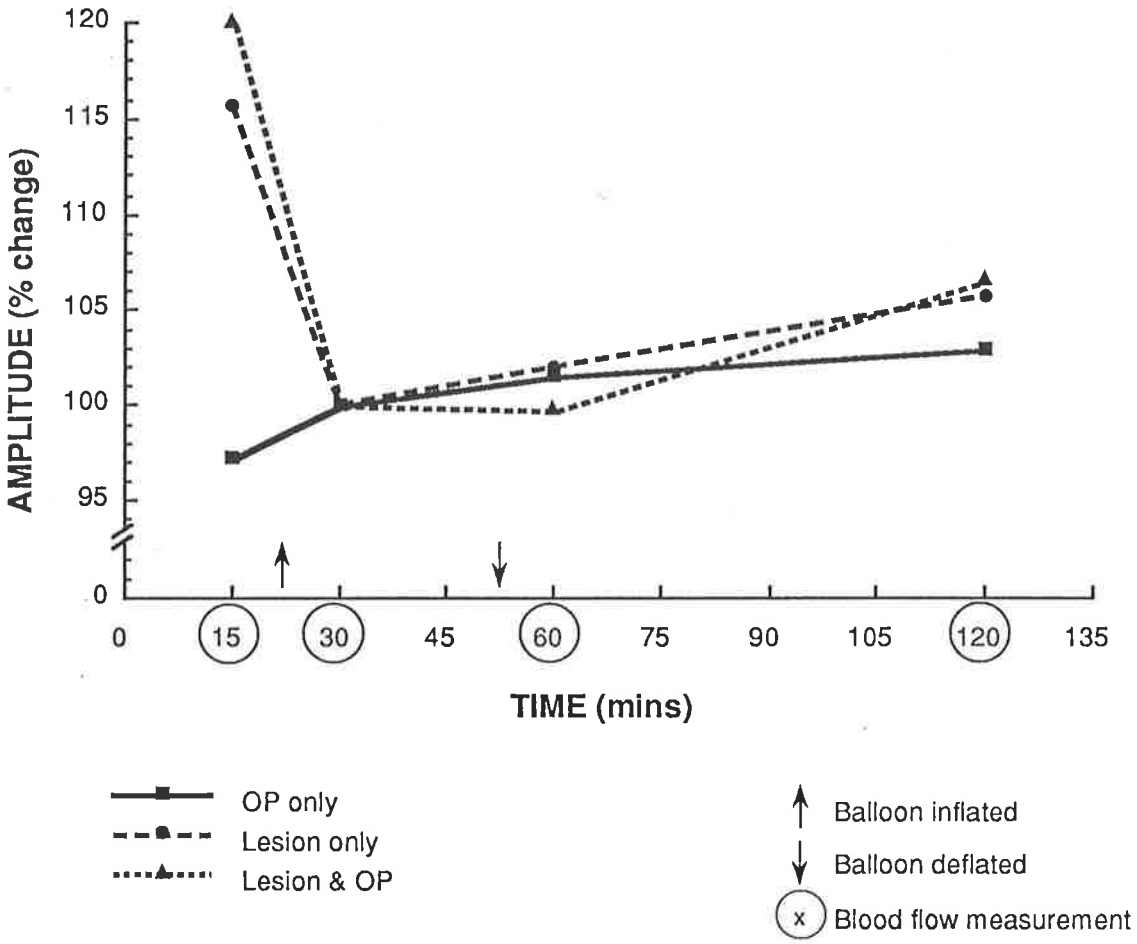


FIGURE 36: Graph showing the change in the amplitude of the sensory evoked potential with time expressed as a percentage of the amplitude immediately after production of the spinal cord injury (Time 2).

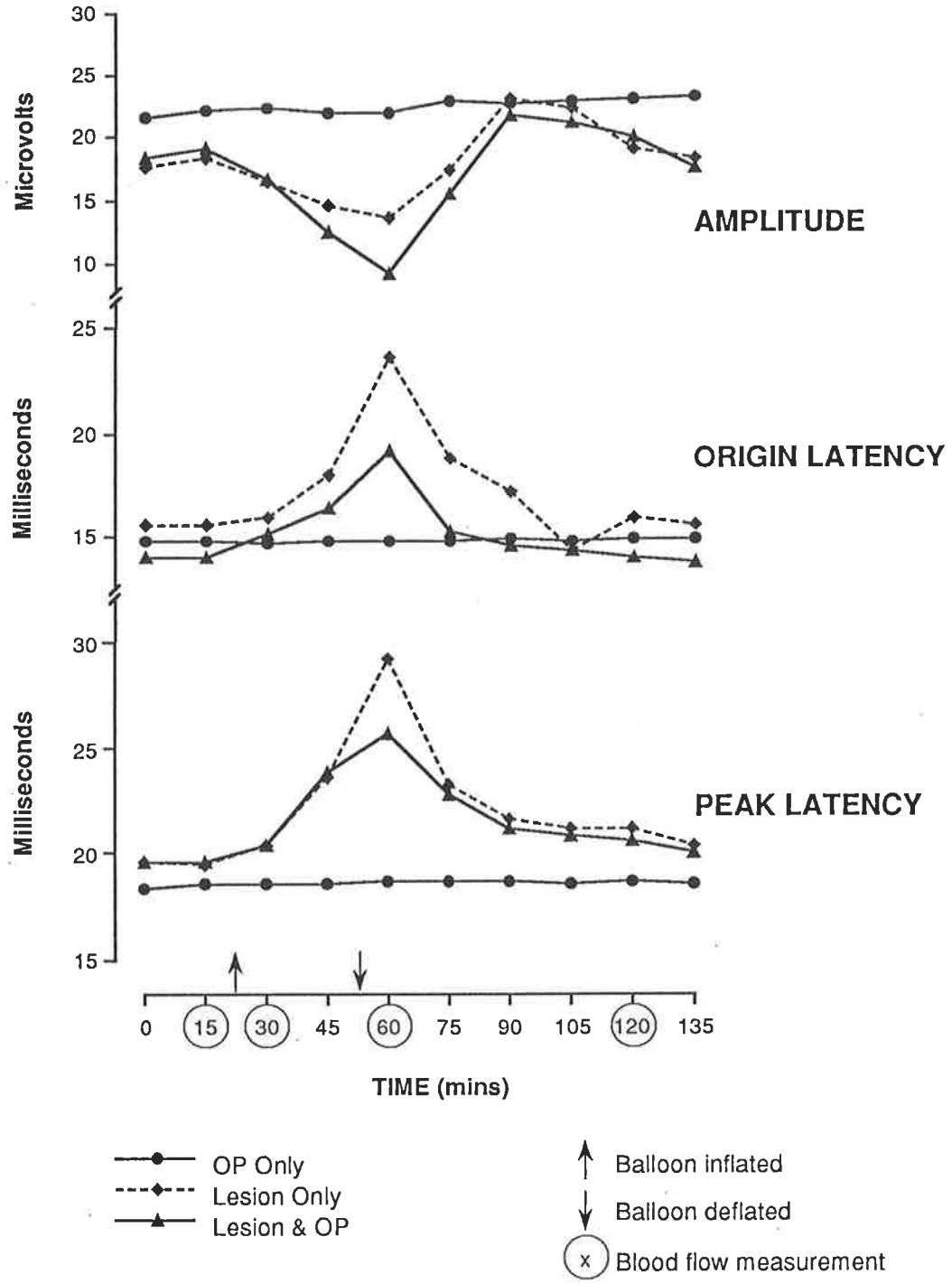


FIGURE 37: Comparison of the sensory evoked potential responses from one sheep from each of the three groups. Responses were recorded every fifteen minutes. The time of each blood flow measurement as well as the time the balloon was inflated and deflated is indicated along the horizontal axis.

5.4.3 SPINAL CORD BLOOD FLOW

Inflation of the balloon resulted in a clinically, (greater than 50%) and statistically significant ($p < 0.005$) reduction in the spinal cord blood flow at the level of the spinal cord injury. Deflation of the balloon was followed by a reactive hyperaemia affecting all of the cervical spinal cord segments examined, but which was more pronounced at the level of the injury. This hyperaemia resolved and the spinal cord blood flow in all segments had almost returned to the base-line levels at the time of the final blood flow measurement (Tables 9 & 10). Table 11 indicates the change in blood flow, as a percentage, from the first to the last blood flow measurement, (Time 1 to Time 4) for each segment of spinal cord studied. The change in spinal cord blood flow at the level of the spinal cord injury is shown in Figure 37. The spinal cord blood flow from the "Operation Only" sheep is again included as a control for comparison.

5.4.4 STATISTICAL ANALYSIS

A paired t-test was used to test the hypothesis for each variable that there was no difference in spinal cord blood flow or evoked potential responses at Time 4 when compared to Time 2 in each group of sheep. Analysis of variance was then used to assess any differences between the groups for both the parameters of electrical function and spinal cord blood flow.

5.4.4.1 EVOKED POTENTIALS

None of the evoked potential variables (amplitude or latencies) showed differences that were statistically significant (Table 12).

	TIME 1	TIME 2	TIME 3	TIME 4
Cont C1	28.61 +/- 3.71	28.51 +/- 3.42	29.91 +/- 3.15	31.50 +/- 5.68
L + 2	28.69 +/- 3.61	25.64 +/- 2.81	36.79 +/- 3.79	34.57 +/- 5.65
L + 1	29.27 +/- 3.84	18.49 +/- 2.41	44.76 +/- 4.82	40.69 +/- 7.45
Lesion	32.37 +/- 3.70	6.36 +/- 2.87	55.64 +/- 5.92	42.07 +/- 9.37
L - 1	30.77 +/- 4.12	19.27 +/- 3.46	46.91 +/- 4.71	40.58 +/- 8.11
L - 2	35.26 +/- 6.34	29.95 +/- 5.29	42.79 +/- 5.13	40.71 +/- 8.90
Cont C2	45.52 +/- 7.41	46.28 +/- 7.91	53.01 +/- 6.43	48.66 +/- 9.13
Cont L	30.87 +/- 7.49	30.87 +/- 7.24	30.84 +/- 6.37	30.83 +/- 7.40

TABLE 9: Mean Spinal Cord Blood Flow measurements (+/- SD) for the cervical and lumbar spinal cord of the six sheep in the spinal cord injury only group.

	TIME 1	TIME 2	TIME 3	TIME4
Cont C1	20.22 +/- 3.06	23.43 +/- 3.09	24.82 +/- 3.17	21.99 +/- 7.19
L + 2	22.49 +/- 4.19	22.56 +/- 3.14	25.95 +/- 3.82	25.54 +/- 9.18
L + 1	23.98 +/- 3.79	15.24 +/- 3.86	40.26 +/- 4.19	31.62 +/- 7.31
Lesion	22.00 +/- 6.22	7.76 +/- 3.55	52.93 +/- 5.94	38.16 +/- 8.20
L - 1	23.90 +/- 3.91	22.26 +/- 4.23	38.41 +/- 5.71	38.26 +/- 8.23
L - 2	28.23 +/- 3.74	31.96 +/- 6.91	31.18 +/- 6.89	30.62 +/- 8.48
Cont C2	34.68 +/- 5.83	40.59 +/- 6.59	38.68 +/- 7.13	36.35 +/-10.31
Cont L	22.09 +/- 6.19	21.92 +/- 6.87	22.00 +/- 7.49	22.04 +/-10.42

TABLE 10: Mean Spinal Cord Blood Flow measurements (+/- SD) for the cervical and lumbar spinal cord of the six sheep in the spinal cord injury and operation group.

	MEAN SCBF GROUP 2 TIME 1	MEAN SCBF GROUP 3 TIME 1	% CHANGE GROUP 2 TIME 1-TIME 4	% CHANGE GROUP 3 TIME 1 - TIME 4
Cont C1	28.61	20.22	+ 10.1	+ 8.8
L + 2	28.69	22.49	+ 20.5	+ 13.6
L + 1	29.27	23.98	+ 39.0	+ 31.9
Lesion	32.37	22.00	+ 30.0	+ 73.5
L - 1	30.77	23.90	+ 31.9	+ 60.1
L - 2	35.26	28.23	+ 15.5	+ 8.5
Cont C2	45.52	34.68	+ 6.9	+ 4.8
Cont L	30.87	22.09	- 0.1	- 0.2

TABLE 11: Mean spinal cord blood flow at the time of the base line measurement and the percentage change in blood flow evident at the time of the final blood flow measurement for both the spinal cord injury only and spinal cord injury and operation groups.

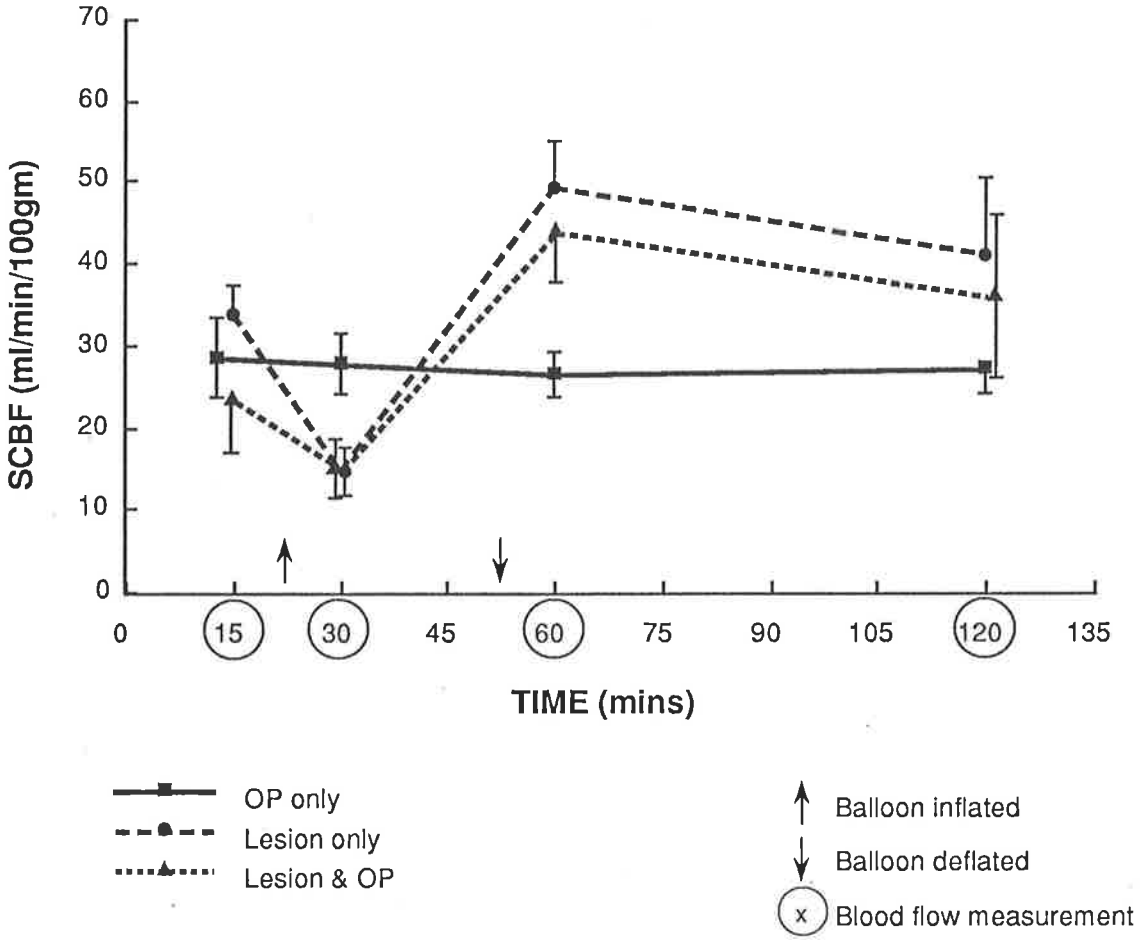


FIGURE 38: Graph showing the changes that occurred in the mean spinal cord blood flow at the level of the C4/5 intervertebral disc (L+1, Lesion & L-1) for all three groups of sheep. The spinal cord blood flow was reduced to near zero immediately following inflation of the balloon and a period of reactive hyperaemia followed deflation of the balloon. The blood flow normalised towards the end of the study period.

GROUP	VARIABLE	MEAN DIFFERENCE (+/- SE)	t	p
SCI Only	SEP (A)	4.7 +/- 4.33	1.09	0.328
	SEP (O)	-0.13 +/- 0.37	-0.36	0.732
	SEP (P)	-0.20 +/- 0.54	-0.37	0.728
SCI & Operation	SEP (A)	0.82 +/- 3.58	0.23	0.829
	SEP (O)	0.67 +/- 0.57	1.17	0.296
	SEP (P)	0.03 +/- 1.36	0.02	0.982

TABLE 12: Differences in the sensory evoked potential results from Time 2 to Time 4 and the results of a "paired t test". The deterioration in electrical function, as indicated by the sensory evoked potential did not reach statistical significance but was clinically significant.

The recovery of electrical function of the "SCI & Operation" group was then compared with that in the "SCI Only" group using analysis of variance (Table 13). No significant difference between the two groups was identified. That is to say that after the spinal cord injury was produced there was no further deterioration in the electrical function of the spinal cord that could be related to the cervical fusion. Also the neurological function recovered to the same extent in both treatment groups during the period of post operative observation.

5.4.4.2 SPINAL CORD BLOOD FLOW

Application of the paired t-test to the segmental spinal cord blood flow results did however demonstrate a significant difference in the blood flows at Time 4 when compared to those at Time 2.

This difference reflected the increase in spinal cord blood flow that occurred following release of the balloon and decompression of the spinal cord (Table 14). The three spinal cord segments were at the level of the spinal cord injury (Lesion), and immediately above (L + 1)and below (L - 1) the spinal cord injury (refer Figure 16).

Having identified this difference, the possibility that the recovery of spinal cord blood flow at Time 4 was greater in one group (SCI Only) than in the other (SCI & Operation), that is the mean difference was higher in one group than the other, needed to be addressed. Analysis of variance was again used to evaluate this possibility.

The results shown in Table 13 indicate that there is no statistically significant difference in the recovery of spinal cord blood flow between the "SCI Only" and "SCI & Operation" groups.

Antero-lateral fusion of the cervical spine utilising a coronal dowel in the "SCI & Operation" group resulted in no significant difference in either the spinal cord blood flow or electrical function when compared with the same parameters in "SCI Only" group.

VARIABLE	MEAN	F	p
L +1	25.79	< 0.00	0.998
Lesion	47.79	0.05	0.500
L -1	17.66	0.26	0.619
SEP (A)	2.77	0.48	0.505
SEP (O)	0.27	1.38	0.267
SEP (P)	-0.83	0.03	0.877

TABLE 13: Analysis of variance of the mean difference of variables in the "SCI Only" group with that in the "SCI & Operation" group.

GROUP	VARIABLE	MEAN DIFFERENCE (+/- SE)	t	p
SCI Only	L + 1	25.78 +/-11.54	2.23	0.075 *
	Lesion	40.43 +/- 8.45	4.78	0.005
	L - 1	19.75 +/- 5.96	3.32	0.021
SCI & Operation	L + 1	25.81 +/- 9.04	2.86	0.036
	Lesion	55.16 +/- 9.10	2.89	0.034
	L - 1	15.56 +/- 5.58	2.79	0.039

TABLE 14: Differences in the spinal cord blood flow results from Time 2 to Time 4 for the levels demonstrating a statistically significant differences from a "paired t test". All animals given a spinal cord injury had clinically and statistically significant reductions in spinal cord blood flow.

* Not a statistically significant result, but included for comparison with the result calculated from the SCI & Operation group.

5.5 DISCUSSION

There are many variables to be considered in the evaluation of the results of a study of this type. From the findings of the initial anatomical study outlined in Chapter 3 the sheep model was established as being suitable for the study of the effect of surgery on spinal cord blood. It is also likely, from the results of this study, that the physiological response of the spinal cord to ischaemia and trauma in the sheep, is similar to that in a variety of other animals used in spinal research and to that in man (Griffiths, 1975; Konrad et al, 1987; Korbine et al, 1975 &1979; Guha et al, 1989; Sandler & Tator, 1976; Senter & Venes, 1979; Finkelstein et al, 1990).

By far the majority of the information relating to the pathology of acute spinal cord injuries has come from animal studies as outlined in Chapter 1. The outcome of Individuals who sustain traumatic cervical injuries with incomplete tetraplegia either survive or die as a result of associated injuries. The majority die in the first hour after sustaining their injuries or survive for several hours or days. Very few however die in the period from two to three hours after sustaining their injuries and despite the frequency of cervical injuries in road crash fatalities (25%, Cain et al, 1989) few are studied histologically due to limitations of time and money. For these reasons, direct comparisons of the histological changes seen in the sheep, two hours after the injury, cannot be made with those seen in man. Certainly the histological changes reported in this study are consistent with those reported from other experimental studies (Finkelstein et al, 1990; Young et al, 1980). The neuropathologist who reviewed the spinal cord histology in this study felt the changes seen were consistent with an incomplete spinal cord injury of two to

three hours duration. Also the ischaemic cell change and central haemorrhages were consistent with the type of changes seen in the spinal cord of spinal trauma patients who fail to survive as a result of injuries to other body regions or, where the cervical injury was recognised prior to death.

The spinal cord was compromised to a greater extent by the spinal cord injury in the "SCI & Operation" group than it was in the "SCI Only" group. There was a greater decrease in the amplitude of the sensory response (44.5% compared to 13.5%) and a larger increase in the latencies (10.1% compared to 6.9%). None of these variations reached statistical significance ($p > 0.3$; Table 13). This difference resulted from the spinal cord injury and not the antero-lateral cervical fusion, as the cervical fusion was performed after the changes were established. The antero-lateral cervical fusion itself did not result in any further impairment of either the spinal cord blood flow or electrical function of the spinal cord.

The main advantage of using the radio-labelled microsphere technique to measure spinal cord blood flow was that the spinal cord did not need to be exposed. Disadvantages of the technique include the lack of continuous blood flow measurements and a limitation in the number of blood flow measurements that can be made. The half lives of the nuclides and their radiation decay also limits the time interval over which blood flow measurements can be performed accurately. It would have been of interest to measure blood flow more frequently. It is however unlikely that this would have affected the results obtained or their significance as the same trends were evident in all animals studied. Also there were no concurrent fluctuations in the evoked potential responses, which were measured

every fifteen minutes and should have indicated any significant reduction in spinal cord blood flow.

Osti et al (1989) postulated in their study that the observed difference in neurological recovery, favouring the non-operative management of patients with an incomplete tetraplegia was due to an intra or peri-operative interference with the blood supply to the cervical spinal cord. This was thought to be due to either irritation or occlusion of the vertebral artery or it's branches. The distribution of patients between the two centres reported by Osti et al (1989), and the patients admission and discharge neurological status has been reproduced in Table 15. As already indicated the study of Osti et al (1989) was retrospective, and the results depended on the details recorded about the neurological status of each patient at the time of admission and discharge. They accept this data was incomplete in a number of cases. The conclusions made about the management of injuries to the cervical spine in the presence of an incomplete spinal cord injury were based on the outcome of 72 patients (34 managed in Perth and 38 in Adelaide), and the number of patients in each Frankel grade grouping was therefore quite small. When the extent of neurological recovery for patients with injuries of Frankel grade B,C or D were combined, a significant difference in the neurological recovery was claimed. Statistical evaluation of the results reported by Osti et al (1989) was performed by the Statistics Department of the University of Adelaide. The statistician who performed the analysis is no longer with the university, and it was not possible to ascertain the actual method of analysis that was used. The results contained in the paper by Osti et al (1989) were re-evaluated and this analysis failed to identify a significant difference between their

SERIES AND NUMBER		NEUROLOGICAL STATUS ON ADMISSION	NEUROLOGICAL STATUS AT FOLLOW UP					
			A	B	C	D1	D2	E
Perth*	14*	A	7	5	-	-	-	-
Adelaide#	16#		5	4	-	-	-	-
Perth+	7+	B	-	-	4	2	-	-
Adelaide	4		-	2	1	1	-	-
Perth	2	C	-	-	-	2	-	-
Adelaide	4		-	-	1	3	-	-
Perth	7	D1	-	-	-	2	-	5
Adelaide	7		-	-	-	1	2	4
Perth	19	D2	-	-	-	-	5	14
Adelaide	23		-	-	-	-	11	12
Perth	33	E	-	-	-	-	-	33
Adelaide	31		-	-	-	-	-	31

*two deaths; #seven deaths; +one death

Table 15: Neurological status of patients on admission and at follow-up (modified Frankel grading).
Osti, O.L., Fraser, R.D., & Griffiths, E.R. Reduction and stabilisation of cervical dislocations: An analysis of 167 cases. *J Bone Joint Surg* [B] 1989;71B:275

two treatment groups (Table 16). Due to the small numbers in each group, the accepted deficiencies in some of the recorded data, and the doubt about the statistical significance of their results, their conclusions must be questioned.

The controversy relating to the management of spinally injured patients has been discussed in Chapter 2. The publication of the paper by Osti et al (1989) resulted in the re-evaluation of the surgical management of this group of patients. Their conclusions having been accepted by those clinicians favouring the non-operative management of these injuries, and dismissed by those who favour surgical stabilisation.

Despite the deficiencies of the paper by Osti et al (1989), the reported findings were of considerable clinical importance and stimulated the re-evaluation of treatment protocols for these types of injuries. As there is no definite answer as to the correct management of these injuries in the literature, it was necessary to assess the effect, on the spinal cord, of surgical stabilisation of the cervical spine, particularly the use of an anterior approach and antero-lateral cervical fusion in greater detail.

From the results of this study the base-line spinal cord blood flow measurements and evoked potential responses obtained were not significantly different in the three groups studied (Figures 34 & 37). Neither did these parameters alter significantly throughout the study period in the "Operation Only" group of sheep (Figures 23 & 24, and Tables 2 & 3).

FREQUENCY EXPECTED	NOT IMPROVED	IMPROVED	TOTAL
PERTH	7 10.4	27 23.6	34
ADELAIDE	15 11.6	23 26.4	38
TOTAL	22 30.6%	50 69.4%	72 100.0

CHI Square	=	3.02 with DF = 1	p=0.083
CHI Square (Yates correction)	=	2.19 with DF = 1	p=0.139
Fisher Exact Test (one tail)			p=0.069

TABLE 16: 2-Way contingency table evaluating the significance of the different rate of neurological recovery in Perth and Adelaide (Osti et al, 1989)

The spinal cord injury resulted in a significant change in all the measured parameters. This change in the case of the motor evoked potential remained complete for the duration of the study. Sensory evoked potentials became attenuated and decreased in amplitude. Associated with this deterioration in spinal cord function was a reduction in spinal cord blood flow of greater than 50%. This was followed, after deflation of the balloon, by a period of reactive hyperaemia which was most marked at the level of the spinal cord injury. Both the spinal cord blood flow and sensory evoked potentials had returned to near base-line levels by the end of the study period.

As it was the surgery performed on the injured spine, and not the injury itself under evaluation, the changes that resulted from the spinal cord injury were not relevant to the study hypothesis. It was therefore necessary to separate those changes due to the injury from those due to the operation. This was done by calculating the difference in the spinal cord blood flow and evoked potential measurements for each group from the time the spinal cord injury was produced to the end of the study (Time 2 - Time 4). Analysis of variance was then used to determine if the difference in the spinal cord blood flow and evoked potential results between Time 2 and Time 4 for the "SCI Only" group were significantly different from those of the "SCI & Operation" group.

The results shown in Figures 34 & 37 suggest that there was a difference between the two treatment groups at the completion of the study. However eliminating the effect of the spinal cord injury by considering Time 2 as the base-line and expressing the results obtained at Time 3 and Time 4 as a percentage of those at Time 2,

there was no difference in the final sensory evoked potential responses of the two treatment groups (Figure 35).

The number of sheep studied (six in each group) is relatively small. The estimate of the number of sheep required to test the study hypothesis was based on the results obtained from the Operation Only group of sheep (Chapter 4). However greater variation occurred in the spinal cord blood flows and evoked potential responses in the animals given an incomplete spinal cord injury than those just undergoing the cervical fusion. This was particularly true in relation to the reactive hyperaemia that followed release of the balloon. A sample size calculation performed on the data obtained from sheep studied in SCI Only and SCI & Operation groups suggested as many as 30 sheep in each group would be required to confirm the findings of this study with a statistical power of 0.8. It was considered that the expense of a study of this magnitude was not justified in light of the results obtained. None of the animals studied demonstrated deterioration of spinal cord function or blood flow as a result of the surgical exposure or stabilisation of the cervical spine, either in the presence, or absence of an incomplete spinal cord injury.

The results of this study, have not demonstrated any significant difference in the outcome of sheep with and without operative intervention following a compression spinal cord injury as assessed by evoked potential responses or spinal cord blood flow. Yet this study was still unable to provide a definitive answer to the question posed.

The validity of the spinal cord injury model must be examined and will always be questioned. Both the macroscopic and microscopic appearance of the spinal cord injury produced in the study animals, and the resultant evoked potential changes are consistent with an incomplete injury.

The supporting structures of the vertebral column were however not disturbed. The effect of vertebral instability and damage to adjacent soft tissue structures which are associated with ligamentous and capsular disruption remains unanswered.

None of the animal models used in the evaluation of spinal cord injuries and their subsequent management are ideal in terms of the reproduction of all of the components of injuries of this type seen in clinical practice. It is not possible at this time to reproducibly inflict injuries to the spine and spinal cord of experimental animals which have the same soft tissue and bony deficits seen clinically in man.

It may be that bony and ligamentous instability is of considerable significance in terms of their outcome, and "recoverability" of spinal cord injuries. Instability and persistent or repeated displacement of the bony elements onto the spinal cord, resulting in ischaemia and inflammation contribute to the secondary spinal cord injury. The primary injury being that which occurs at the instant the spinal column is disrupted and the spinal cord compromised, compressed or divided. The primary injury may well be fixed from the outset. It is the secondary injury to the cord which can best be limited by improved treatment methods and which may be least well evaluated in the study of animal models.

The hypothesis that anterior exposure of the cervical spine and the antero-lateral cervical fusion in the presence of an incomplete spinal cord injury adversely affects spinal cord blood flow and function could not be supported by the results of this study.

Of clinical interest and significance was the fact that the descending motor tracts, and the motor evoked potentials were affected earlier and more extensively than the ascending sensory tracts. The motor response was lost immediately the spinal cord injury was produced where the sensory evoked potential did not change significantly for between 10 and 20 minutes. This supports the findings of other researchers and clinicians that the use of the sensory evoked potential alone as an indicator of spinal cord function may not indicate immediately when the spinal cord has been compromised (Levy et al, 1986; Katayama et al, 1986; Fehlings et al, 1989). Fehlings et al, (1989) reported that the motor evoked potential was extremely sensitive to mild cord injury, but did not distinguish between mild and moderate injuries as readily as the sensory evoked potential. He concluded that the combination of an abnormal motor evoked potential with a normal sensory evoked potential suggested the presence of a mild spinal cord injury, whereas an abnormality of both the motor and sensory evoked potential indicated a moderate to severe lesion. The motor and sensory evoked potentials are therefore complementary and indicates the advantage of monitoring both the sensory and motor evoked potentials in spinal surgery where there is a risk of spinal cord compromise.

6. CONCLUSION AND SUMMARY

Patients with spinal injuries produce a considerable long term financial burden to the health system and loss of individual productivity. This results in considerable medical and community cost for their subsequent support. Many such patients remain dependent on family members or community and government institutions for all activities of daily living, while for others this dependence is limited in duration and extent. Anything that may improve the chances of neurological recovery and subsequent independence should be done. A relatively small functional improvement, may result in a considerable improvement in independence and productivity.

The lack of a consensus relating to the acute treatment of these injuries, the number of studies reported which have been undertaken in an attempt to evaluate their patho-physiology, treatment and the comparative retrospective study of Osti et al (1989) prompted the initiation of this study.

The research outlined in the preceding chapters has established the anatomical validity of the sheep model for the study of spinal cord patho-physiology and blood flow. This study also documented the vascular anatomy of the neck and cervical spine of the sheep for future reference. The sheep model is suitable to evaluate surgical procedures, to evaluate the effect of alterations in the physiological environment, posture and spinal cord compression as well as the effect of drugs, such as steroids and vasodilators, on spinal cord blood flow and function.

The effect of anterior surgical exposure and antero-lateral cervical fusion of the cervical spine has been evaluated in both the presence and absence of an incomplete spinal cord injury. This type of surgery did not adversely affect either the spinal cord blood flow or electrical function of the spinal cord. The pathology and effect of the incomplete spinal cord injury was also evaluated as a control for the animals given an incomplete spinal cord injury and proceeding to antero-lateral cervical fusion. Statistical analysis and comparison of the results obtained from the sheep given a spinal cord injury alone (SCI Only) with those going on to have surgery (SCI & Operation) failed to demonstrate a difference in the outcome of the two groups.

The spinal cord injury produced an appropriate pathological lesion in the cord but did not render the cervical spine unstable which may be a significant component of this type of injury in man. The absence of haemorrhage, oedema and perhaps bony fragments in the para-vertebral region around an unstable injury may have lessened any detrimental effect that the surgery may have on the spinal cord. However it is unlikely that such an effect would result in significant compromise of the spinal cord or its potential for recovery.

There is increasing evidence to support the case for early surgical stabilisation of the vertebral column in certain vertebral fractures and dislocations associated with spinal cord injuries (Beatson, 1963; Cheshire, 1969; Bohlman, 1979; Aebi et al, 1986). This enables early mobilisation, a reduction in bed and hospital stay and more rapid rehabilitation with enormous benefits to both the patients and the community. The findings of this study should give comfort to those clinicians concerned that early surgery in the patient with an incomplete tetraplegia may adversely affect the patient's recovery.

Where anterior surgery is indicated the use of anterior internal fixation such as the AO cervical locking plate which provides rigid and stable fixation negates the need to preserve the anterior longitudinal ligament. For this reason the antero-lateral cervical fusion devised by Barbour ad reported by Cornish (1965 & 1968) which preserves the anterior longitudinal ligament ad outer fibres of the anterior annulus has fewer indications today. The technique has proved to be effective in stabilising the injured segment without internal fixation or rigid external support (Osti et al, 1989) in contrast with anterior fusion by the Cloward method (Stauffer & Kelly, 1977; Capen et al, 1985; Aebi et al, 1986; Maiman et al, 1986;)

The results of this study, although performed on a spinal column that was not mechanically unstable could not support the hypothesis that anterior stabilisation of the cervical spine utilising a coronal dowel adversely affects spinal cord blood flow and function. This study indicates anterior exposure of the cervical vertebral column combined with disc clearance and inter-body fusion should not jeopardise spinal cord function, or the potential for recovery from a spinal cord injury in the absence of operative mishap.

For the future the optimal treatment of injuries to the cervical spine and spinal cord may not depend so much on technological advances in the methods or techniques of stabilisation, but on factors that permit the rapid assessment and reduction of these injuries and minimising the secondary injury and insult to the spinal cord, with appropriate progression to internal fixation when the primary reduction is inadequate or unstable.

7. DIRECTIONS FOR FUTURE RESEARCH

This study now sets the scene for further research. The sheep and spinal cord injury model reported can be used to assess many as yet poorly understood facets of spinal cord injuries and their treatment. The effect of the time from injury to treatment could be assessed and perhaps the "point of no return" identified at which any neurological deficit becomes fixed. The effect of steroids and other drugs such as vasodilators and anaesthetic agents on the pathological and electro-physiological changes that take place in the spinal cord as a result of these injuries and their treatment could be evaluated.

There is also a requirement for the evaluation of the effect of experimental surgical exposure of the spinal cord, for the placement of electrodes or the application of external cord compression devices. Until this is done the validity of the results of many previously reported studies should be questioned. There is also a need for prospective, controlled, randomised clinical trials to evaluate the results of both the operative and non-operative treatment of these injuries.

The ultimate goal of research into, and the treatment of these injuries being greater independence for spinally injured patients through maximising their potential for recovery. The effects of time, temperature and drugs on the outcome of spinal cord injuries needs to be evaluated, directing attention to the reversible component of spinal cord trauma that is the damage caused by ischaemia secondary to compression, oedema and instability. This will probably be more important than improved surgical techniques.

Annex A:

NEN®

NEN-TRAC™ MICROSPHERES

RESEARCH PRODUCTS — TECHNICAL DATA

NEM-032C Chromium-51 NENC# 876489/0

Lot Number : CR15187

Calibration Date : 08-Sep-1989

Total Activity : 18.5 MBq 0.50 mCi

Specific Activity : 115.8 MBq/g 3.13 mCi/g
23 DPM/Microsphere

Volume : 20.0 mls.

Suspending Medium : 0.9% Saline with
0.01% Tween 80

Radioactive Contaminants : None Detected

Microspheres per mg : 3.04×10^5

Milligrams per Vial : 159.7 mg

Mean Size : 16.5 u ± 0.1 u

Leach data *

in Saline : 0.10 %

in Plasma : 0.10 %

<u>Nuclide Physical Characteristics</u>

Nuclide : Chromium-51

Half Life : 27.7 Days

Principal Photon : 0.320 MeV

Radiation Unshielded : 1.6 uSv/hr/MBq
6 mR/hr/mCi

HANDLING GUIDE

A. In order to assure a homogeneous suspension:
- Vortex or physically shake the vial until the beads are mixed in the solution. (Beads will not sonicate properly if they are floating or settled out on the bottom of the vial.)
- Immediately prior to use, sonicate in warm water (about 40°C.) for 15 to 30 minutes. (Warm water helps to suspend the beads in more viscous solutions such as dextran.)
- We recommend the use of a surfactant or wetting agent such as TWEEN-80. When requested, NEN-TRAC microspheres are shipped with 0.01% TWEEN-80, or when ordered dry, with a vial of 1% TWEEN-80. Use this complimentary TWEEN-80 to adjust the suspending medium to 0.01%.

B. The viscuous nature of dextran makes it more difficult to obtain a homogeneous suspension. If your experiments allow the use of saline to suspend the microspheres, it may be advantageous.

C. If for any reason microscopic analysis of the product is performed, prepare the slides immediately before examination. A small drop, containing 5 to 10µg of the sonicated microspheres, should be used for a slide. Slides that are not prepared fresh or contain too many microspheres may show some aggregation not representative of the product.

D. For extended shelf life (beyond 60 days), we recommend storage of NEN-TRAC microspheres at 2-8°C.

E. Venting with a 22 gauge needle is recommended prior to breaking seal.

* These microspheres meet DuPont's stringent leach criteria of <2% in vitro testing at 37 degrees centigrade for 48 hours in plasma, and <1% after 4 days in saline. Long term (i.e. greater than 24 hours) in vivo use may result in different leach characteristics.

ANNEX B:

PROCEDURAL OUTLINE:

1. Sheep anaesthetised and stabilised;
   a) Intubation and ventilation (supine).
   b) Insertion of carotid and femoral cannula, monitor BP, perform base-line arterial blood gas.
   c) Turn sheep onto right side and insert left arterial line.

2. Insert cortical bolt electrodes for evoked potential monitoring.

3. Perform fenestration of C7/T1 intervertebral space for insertion of epidural electrode (and Fogarty balloon catheter if applicable followed by a lateral cervical X-Ray).

4. Perform base-line motor and sensory evoked potentials and repeat every 15 mins.

5. Perform base-line blood flow measurement;
   a) Mix microsphere suspension with arterial blood and 1000 units Na Heparin on vortex mixer for 5 minutes.
   b) Ensure free withdrawal of Harvard pump.
   c) Inject microsphere/blood suspension into left atrial cannula over 20 seconds.
   d) Flush left arterial cannula with heparinised saline.
   e) Turn Harvard pump off 90 seconds after completing injection of microspheres.
   f) Flush lines with heparinised saline.
   g) Transfer blood to Gamma counting tubes.

6. Group I (Operation Only)
   Perform antero-lateral inter-body dowel fusion at the level of the C4/5 intervertebral disc.

   Group II (Spinal Cord Injury Only)
   Inflate Fogarty balloon in the epidural space at the level of the C4/5 intervertebral disc, followed by a lateral cervical X-Ray.

Group III (Spinal Cord Injury & Operation)

Inflate Fogarty balloon in the epidural space at the level of the C4/5 intervertebral disc, followed by a lateral cervical X-Ray.

7. Perform second blood flow measurement and repeat motor and sensory evoked potentials and arterial blood gas analysis.

8. Group II (Spinal Cord Injury Only)

Deflate the balloon 30 minutes after its inflation.

Group III (Spinal Cord Injury & Operation)

Deflate the balloon 30 minutes after its inflation and proceed to perform the antero-lateral dowel fusion at the level of the C4/5 intervertebral disc.

9. Perform third blood flow measurement and repeat motor and sensory evoked potentials and arterial blood gas analysis.

10. Perform the final blood flow measurement 60 minutes after the third and repeat motor and sensory evoked potentials and arterial blood gas analysis.

11. Sacrifice the sheep by the intra-arterial injection of 20ml Lethobarb and obtain tissue specimens.
    a) Cervical spinal cord from C3-C6
    b) Lumbar spinal cord from L3-L4
    c) Right and left kidneys

12. Divide tissue specimens and place in marked Gamma counting tubes.

13. Dispose of animal.

14. Weigh tissue specimens and place in Gamma counter.

15. Calculate tissue blood flows.

ANNEX C:

SURGICAL INSTRUMENT LIST:

Item	No.
Scalpel & 15 blade	1
Scalpel & 20 blade	1
Dissecting scissors	1
Mayo scissors	1
Gillies forceps	1
Heavy toothed forceps	1
Fine toothed forceps	1
Artery clips	5
Mosquito clips	5
Suture forceps (long handle)	1
Tube saw set	1
Single action bone nibblers (fine)	1
Double action bone nibblers (heavy)	1
Pituitary rongeurs (straight)	1
Rib cutter	1
45^{o} up-cutting punch	1
Periosteal elevator	1
7/16" drill bit and sleeve	1
Hand drill	1
Spanner	1
Self retaining retractors	2
2" right-angle retractors	2

ANNEX D:

EQUIPMENT AND DISPOSABLES LIST:

Item	No.
Pressure transducer and cable	1
Blood pressure monitor	1
Double barrel extraction pump	1
50 ml glass syringe	2
Vortex mixer	1
Evoked potential monitor	1
Earthing electrode	1
Bipolar cardiac pacing electrode	1
Cortical bolt electrode	2
Intravenous cannula	2
Manometer tubing (60cm)	2
Manometer tubing (150cm)	2
Three way tap	4
No. 5 Fogarty balloon catheter	1
Silastic intravenous cannula	3
Gamma counter tubes	100
Prepared microsphere suspensions	4
Heparinised saline	200ml
Sodium heparin (1000 units/ml)	10ampoules
Methyl-methacrylate dental cement	10ml
00 black silk sutures	2
00 black silk ties	3
10ml syringes	10
2ml syringes (blood gas)	20

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