



Pathophysiology of Syringomyelia

by

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ABSTRACT

The normal physiology of cerebrospinal fluid (CSF) in the spinal subarachnoid space and spinal cord and the pathophysiology of non-communicating syringomyelia are poorly understood. The hypothesis examined in this thesis is that CSF is driven from the subarachnoid space into perivascular spaces and the central canal by arterial pulsations and that this is the driving force for the development of non-communicating syringomyelia. Horseradish peroxidase (HRP) was used as a CSF tracer in rats and sheep. In normal rats and in normal sheep CSF flowed rapidly from the subarachnoid space, through perivascular spaces and into the central canal. Flow into the central canal was not via the fourth ventricle or the caudal opening of the central canal. The effect of arterial pulsations on this flow was examined by ligating the brachiocephalic artery in sheep before injecting HRP into the subarachnoid space. There was no flow into the central canal in sheep with damped arterial pulsations. Reducing the spinal subarachnoid pressure did not appear to alter flow into the central canal. CSF flow was also studied in the rat intraparenchymal kaolin model of non-communicating syringomyelia. Rapid flow into the central canal occurred at 1 day, 3 days, 1 week and 6 weeks after kaolin injection. There was rapid flow into isolated, enlarged segments of central canal even when there was evidence that the enlarged segments were causing pressure effects on surrounding tissue. These results support the hypothesis that arterial pulsation-driven CSF flow from perivascular spaces into the central canal is the driving force for the development of non-communicating syringomyelia. An additional finding from this work was that rapid perivascular CSF flow occurs in the cerebellum as it does elsewhere in the nervous system. A technique for studying the three-dimensional morphology of the human central canal was also developed.

DECLARATION

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

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

Marcus A. Stoodley

August, 1996

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ABBREVIATIONS

3-D	Three-dimensional
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computerised tomography
HLA	Human leucocyte antigen
Ig	Immunoglobulin
IMVS	Institute of Medical and Veterinary Science
MR	Magnetic resonance
MRI	Magnetic resonance imaging
H&E	Haematoxylin and eosin
HRP	Horseradish peroxidase
SCO	Subcommissural organ
TMB	3,3',5,5' Tetramethylbenzidine
U of A	University of Adelaide

STYLE CONVENTIONS

The abbreviations, punctuation and reference style used in this thesis conform with the guidelines of the *AMA Manual of Style*²³⁰ and the *Style Manual*.³ The spelling is Australian English and conforms with *The Australian Concise Oxford Dictionary*.²

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INTRODUCTION

The literature on syringomyelia and the theories of its pathogenesis will be reviewed, followed by a discussion of certain anatomical and physiological aspects of central nervous system (CNS) extracellular fluid and its spaces.



1 Syringomyelia

1.1 History

Knowledge of spinal cord cysts and cavitation has existed for several centuries. Descriptions of cystic cord cavitation were made by Estienne in 1546,²⁶⁸ Brunner in 1688,⁴⁰⁶ and Morgagni in 1740.¹⁵² In 1824, Ollivier d'Angers described pathological dilatation of the central canal (believing it to be a developmental anomaly).¹⁸⁰ He applied the term 'syringomyelia' to a condition he called 'cavité existant dans l'intérieur de la moelle,' or 'dilated central canal.' The first clinical correlation was made by Portal,¹⁸⁰ who in 1804 recognised that limb paralysis was associated with cystic spinal cord cavitation; further observations were made by Gull in 1862 and Clarke in 1865.¹⁵⁹ Schultze⁴⁰⁸ described the classical clinical syndrome in 1882, and in particular noted the reduction in pain and temperature sensation. The presentation of the disease was well recognised by Gowers in his *Manual of Diseases of the Nervous System* in 1886.¹⁸¹ Since the first operative decompression of a spinal cord cyst by Abbe and Coley⁵ in 1892, the treatment of syringomyelia has essentially been surgical, with the first shunting of a syrinx being performed by Puusepp in 1926.¹¹⁵

The first association between syringomyelia and other abnormalities of the nervous system was made by Langhans, who described a case associated with cerebellar deformity.¹⁵⁹ Chiari described three types of cerebellar malformation in 1891; types I and II were associated with 'hydromyelia.'¹⁵⁹ Syringomyelia is now known to be associated with many pathologies

of the nervous system but despite being the subject of extensive speculation, its pathogenesis remains unknown.

1.2 Definitions

Ollivier d'Angers introduced the term 'syringomyelia' in 1827, using the Greek *syrinx*,* meaning a tube or pipe, and *myelos*, meaning marrow.³⁴³ Stilling⁴²⁷ used the term 'hydromyelia' to describe a dilated central canal. Hallopeau¹⁸⁰ in 1870, and Simon³³³ in 1875, made a distinction between 'hydromyelia' (a dilatation of the central canal) and 'syringomyelia' (a cyst independent of the canal). Leyden,²⁶⁹ in 1876, argued that there was no difference between the two conditions. Chiari, in 1888, found that most cavities had connections with the central canal and contended that syringomyelia and hydromyelia were identical, perhaps with minor pathophysiological differences.³³³ Other authors had also held this belief: Virchow in 1863 and Kahler and Pick in 1879.^{152,180} In an attempt to avoid confusion over the two terms, Ballantine et al²¹ coined 'syringohydromyelia' to describe a variety of spinal cord cystic cavities. Many authors have since used this term, or the variation 'hydrosyringomyelia.' 'Pseudosyringomyelia' has been used to describe cysts occurring in association with tumours, haemorrhage or necrosis, in the belief that these cysts are not formed as a result of altered cerebrospinal fluid (CSF) dynamics.¹³⁸ Cavities extending into, or occurring in, the brainstem, are referred to as 'syringobulbia.'^{190,333} In this thesis, the term 'syringomyelia' will be used to describe all abnormal fluid-filled spinal cord cavities except small non-enlarging cavities secondary to trauma or necrosis. Qualifying terms, such as 'posttraumatic,' will be used where necessary to differentiate various types of syringomyelia. In the description of other authors' work, the term 'hydromyelia' will be used to describe a

* The Greek *συρίγξ* means "a shepherd's pipe." In Greek lore, the pipe was named for the nymph Syrinx, who when being chased by Pan prayed to be turned into a clump of reeds. When Pan tried to embrace her, he found he was clutching a handful of reeds. Letting out a sigh, he elicited a pleasant sound from the hollow reeds.²⁰⁴

dilated central canal where it is necessary to understand their hypotheses. The terms ‘cyst,’ ‘syrinx’ and ‘cavity’ will be used to describe the fluid-containing cavity of syringomyelia.

1.3 Epidemiology

Syringomyelia has historically been regarded as a rare condition.⁵³ Two factors probably responsible for this belief were the difficulty in diagnosing the condition before modern neuroimaging techniques became available, and the course of the disease which is slow and infrequently fatal. Since the introduction of computerised tomography (CT) and magnetic resonance imaging (MRI), the diagnosis of syringomyelia is being made more frequently and is even being detected in asymptomatic patients.^{38,166,167,302} It affects mainly children and young adults.^{157,333}

There is a paucity of epidemiological studies of syringomyelia. The prevalence of syringomyelia in England has been estimated at from 5.6 to 8.4 per 100,000 population.^{320,333} There may be a geographical variation with higher rates being reported in parts of Germany and Russia.³³³ Patients with syringomyelia have accounted for 0.4% to 1% of cases admitted to neurosurgical clinics.³³³

1.4 Associated conditions

Syringomyelia occurs in association with a wide array of congenital and acquired conditions (Table 1). Most cases are associated with an abnormality at the craniocervical junction^{16,158} or occur following spinal trauma. Other associations include occult spinal dysraphism, intramedullary and extramedullary tumours and arachnoiditis.^{311,335,346} Very few cases occur without a known associated pathology.

Table 1. *Conditions associated with syringomyelia.*

1.	Malformations at the craniocervical junction
a)	Congenital malformations
i)	Chiari malformation types I and II
ii)	Dandy-Walker malformation ²⁵
iii)	Apert's syndrome with tonsillar herniation ^{90,470}
iv)	Crouzon's syndrome with tonsillar herniation ⁹⁰
v)	Noonan's syndrome ²⁰
vi)	Achondroplasia with tonsillar herniation ⁴⁵⁷
b)	Acquired malformations
i)	Tonsillar herniation secondary to lumbar CSF shunting ⁴⁸³
ii)	Tonsillar herniation secondary to intracranial mass lesions ³¹⁸
iii)	Lhermitte-Duclos disease of the cerebellar vermis ⁴¹⁴
iv)	Basilar impression ³¹¹
v)	Pannus deformity of the odontoid ³¹¹
2.	Spinal trauma
3.	Congenital spinal malformations
i)	Myelomeningocele
ii)	Tethered spinal cord ⁴⁵²
iii)	Diastatomyelia ^{226,404}
iv)	Lipomyelomeningocele ^{63,80}
v)	Spinal dermoid ²²⁶
vi)	Neurenteric cyst ^{102,180,364}
vii)	Sacral agenesis ³³⁵
4.	Spinal intramedullary tumours
i)	Virtually any primary intramedullary tumour
ii)	Metastatic intramedullary tumours ¹⁵¹
5.	Acquired conditions causing chronic spinal cord compression
a)	Neoplastic
i)	Meningioma ^{210,476}
ii)	Schwannoma ²³³
iii)	Lymphoma ²¹⁷
iv)	Myeloma ^{365,376}
b)	Non-neoplastic
i)	Intervertebral disc protrusion ^{8,302}
ii)	Cervical spondylosis ³⁰²
iii)	Lipoma ³⁶⁵
6.	Arachnoiditis
i)	Spinal or posterior cranial fossa arachnoiditis of any cause ^{31,32,57,69,81}

1.4.1 Malformations at the craniocervical junction

In 1891, Chiari described hindbrain abnormalities seen in association with hydrocephalus and divided them into three types:^{40,84}

- I Displacement of the cerebellar tonsils into the cervical spinal canal, without caudal displacement of the medulla,
- II Displacement of the inferior vermis into the cervical canal and caudal displacement of the lower pons and medulla, with elongation of the fourth ventricle, and
- III Herniation of the cerebellum in a meningoencephalocele combined with caudal displacement of the brainstem and medulla.

A fourth type was described later:²²

- IV Cerebellar hypoplasia. No longer used, this denotation may have corresponded to the Dandy-Walker malformation.

Types I and II are commonly seen in association with syringomyelia, although not all patients with a Chiari malformation have syringomyelia.^{38,73,213} Studies prior to the introduction of MRI found 20% to 75% of patients with a Chiari type I malformation have syringomyelia.^{40,67,358} Using MRI, Pillay et al³⁵⁸ recorded a rate of 57%. Stovner and Rinck⁴²⁹ reported that the prevalence of syringomyelia is significantly higher in patients with a cerebellar herniation measuring 9 to 14 mm than in patients with less or more herniation, but this has been disputed by Masur and Oberwittler.²⁷⁹ Syringomyelia is seen in up to 88% of Chiari type II deformities.⁴⁰

Besides displacement of neural tissue, there is often scarring around the cerebellar tonsils and the foramen of Magendie, sometimes with complete obstruction of the foramen.^{49,353} Often hydrocephalus and deformities of other parts of the neuraxis are seen.

Chiari type II is commonly, if not always, seen with myelomeningocele^{22,320,372} and may occur with other abnormalities such as the Klippel-Feil syndrome.^{22,372} Chiari malformations not associated with spina bifida or other lesions have been estimated to occur in 1 per 25,000 live births.³²⁰

Chiari believed the hindbrain malformations were secondary to hydrocephalus,²² a theory that has persisted through this century. However, the frequent presence of skeletal abnormalities suggests that not all the manifestations of the deformity are pressure effects.³⁷² An alternative theory was of traction on the hindbrain by tethering of the cord to a spina bifida defect,²² but this was later abandoned when cases were reported without an associated dysraphic defect and experimental tethering of the spinal cord in animals did not produce a Chiari malformation.⁴¹³ Current theories involve local overgrowth of the neural tube or dysplasia of the occipital bone.²² Against these theories is evidence that the Chiari malformation can occur postnatally.^{148,219}

Cystic dilatation of the fourth ventricle and dysgenesis of the vermis constitute the Dandy-Walker malformation.¹³³ Originally thought to result from failure of the apertures of the fourth ventricle to open,¹³³ embryological and clinical studies now cast doubt on this theory.¹³³ The Dandy-Walker syndrome is usually associated with hydrocephalus^{133,293} and is occasionally associated with syringomyelia.²⁵

Several authors have reported cerebellar descent forming after lumboperitoneal shunting.^{87,350,483} Payner et al³⁵⁰ reported 10 cases; none of these had syringomyelia. Two of the cases reported by Welch et al⁴⁸³ had cervical syringomyelia. The rate of tonsillar herniation following lumboperitoneal shunting was 70% in a paediatric population studied by Chumas et al.⁸⁷

Syrinxes may also be seen with chronic tonsillar herniation associated with other congenital abnormalities, or with herniation secondary to cerebellar tumours or supratentorial

lesions.^{318,483} Syringomyelia may also accompany other congenital or acquired chronic compressions at the craniocervical junction. Syrinxes associated with abnormalities at the craniocervical junction are caudal to the lesion, with or without an intervening segment of normal spinal cord.³¹¹

1.4.2 Spinal trauma

Until recently, posttraumatic syringomyelia was considered a rarity.^{27,103,164,207,334} Clinical examination and CT scanning reveal syringomyelia in 0.9% to 3.2% of patients with severe spinal cord injuries.^{12,187,333,385,386,477} More recently, MRI has been used to record syrinxes in up to 22% of patients with spinal cord injuries.⁴²³ It appears to be more common after tetraplegia than after paraplegia,^{34,385} or after a complete cord lesion than a partial lesion.^{125,333} An incidence of 8% in cases of complete tetraplegia has been reported by Rossier et al.³⁸⁵ Other authors have reported a higher incidence following thoracic or lumbar spinal injuries compared with cervical spinal injuries.^{12,285}

Symptoms may occur as early as two months or as long as 35 years after the injury.^{103,333,426,467} Hida et al²⁰⁷ found the mean interval from injury to presentation with a syrinx was nine years, while in the series of Rossier et al³⁸⁶ the mean interval was 13 years. Symptoms and signs usually begin unilaterally and slowly progress cephalad in almost all patients, although a caudal progression may also be seen.^{19,103,333} The cysts are usually juxtaposed to the injury site: rostral in 81%, caudal in 4% and in both directions in 15%.¹²⁵

1.4.3 Congenital spinal malformations

Syringomyelia has been reported in association with diastematomyelia,⁴⁰⁴ tethered spinal cord,⁴⁵² lipomyelomeningocele,^{63,80} spinal dermoid²²⁶ and neurenteric cyst.^{102,180,364} The most frequent association is with diastematomyelia—37% to 53% of cases have syringomyelia.²²⁶ Syrinxes associated with occult spinal dysraphism occur predominantly in

the lower one third of the spinal cord and may form rostral or caudal to the lesion, but are usually close and rostral.²²⁶

1.4.4 Intramedullary tumours

Some tumours are commonly associated with syringomyelia: two thirds of haemangioblastomas and half of spinal ependymomas have an associated syrinx,^{162,322,329,333,394} however syringomyelia may be seen with virtually any intramedullary tumour,³⁷ including lymphoma,²⁵⁶ paraganglioma⁴²⁵ and metastases.¹⁵¹ Poser's review of postmortem cases in 1956³⁶² established an incidence of associated intramedullary spinal cord tumours in patients with syringomyelia of 16.4%; the incidence of syringomyelia in patients with intramedullary tumours was 31%. Other authors have reported higher incidences in postmortem studies. Sloof et al⁴¹⁷ found a syrinx in 58% of 33 autopsied cases of intramedullary tumour, and Ferry et al¹⁴⁴ found neoplasms in 75% of 38 patients with clinically diagnosed syringomyelia. Fifty-three percent of the patients reported by Conway⁹³ had tumours. Of 100 patients with intramedullary tumours reported by Samii and Klekamp,³⁹⁴ 45 had an associated syrinx. Of these, 49% of cysts were rostral to the tumour, 11% were caudal and 40% were above and below the tumour. Syringes were seen in 50% of adult patients, but only 21% of paediatric patients.³⁹⁴

Most tumours associated with a syrinx are in the cervical or thoracic cord, while tumours of the conus or cauda equina are uncommon.³²² Samii and Klekamp noted a higher incidence of syringomyelia with tumours at higher spinal levels.³⁹⁴ Williams and Timperley⁵⁰⁸ reported three cases of syringomyelia secondary to midbrain gliomas.

1.4.5 Chronic spinal cord compression by extramedullary lesions

Syringomyelia in association with an extramedullary tumour is not common, but may be seen with meningioma,^{210,476} Schwannoma,²³³ enterogenous cyst,⁸³ lymphoma,²¹⁷ lipoma and myeloma.^{365,376} Rarely, removal of such a tumour is complicated by the development of

syringomyelia years later.^{76,101} Syringomyelia may also be associated with cord compression from cervical disc disease or spondylosis.^{114,296,302,306,329} The syrinx may be caudal or rostral to the lesion,⁴⁷⁶ but is usually caudal.³⁰⁶

In a series of 51 cases of syringomyelia reported by Milhorat et al,³⁰² 25 were associated with extramedullary lesions causing chronic cord compression, although he included 14 cases of Chiari malformation in this group. Cervical discs, tumours and craniospinal deformity accounted for the other cases. In each case, the syrinx arose immediately caudal to the lesion and did not extend rostrally. Longer lesions were found in younger patients.³⁰² In two of three cases of cervical disc protrusion, the syrinx resolved following discectomy.³⁰² Excision of other extramedullary masses resulted in resolution of the syrinxes.

1.4.6 Arachnoiditis

Spinal cord cavitation in association with arachnoiditis was first reported in 1869 and may be seen with spinal or posterior cranial fossa arachnoiditis.^{31,32,57,69,81} The arachnoiditis may be caused by bacterial meningitis, subarachnoid haemorrhage, tuberculosis, syphilitic pachymeningitis, trauma (including birth-related trauma), injected radiopaque dyes, surgery, or spinal anaesthetics.^{57,69,160,333,500} Some cases of arachnoiditis associated with syringomyelia have no known preceding cause.³³³ A common feature may be blockage of CSF flow at the level of the foramen magnum.¹⁶⁰ The syrinx usually forms caudal to the area of arachnoiditis.³³³

1.4.7 Other associations

Syringomyelia has been reported in association with Apert's syndrome (with severe invagination of the occipital bone obliterating the cisterna magna),⁴⁷⁰ Noonan's syndrome (a case with Chiari I),²⁰ multiple sclerosis (central lesions in the cord reported by Ransohoff et al³⁶⁹ which they did not believe was simple cavitation of plaques), sarcoidosis (in a patient

who had arachnoiditis),³⁷¹ craniofacial dysostosis (with hydrocephalus),⁴⁴ achondroplasia with Chiari malformation,⁴⁵⁷ sacral agenesis³³⁵ and Lhermitte-Duclos disease of the cerebellar vermis⁴¹⁴ (with herniation of the cerebellar tonsils). A case of syringomyelia associated with tentorial meningioma has been reported.⁴⁵⁸ There was tonsillar herniation and the syrinx resolved after the tumour was removed. Syringomyelia may be seen after treatment of hydrocephalus by shunting of the lumbar CSF.^{148,483} It has been suggested that this is due to the development of tonsillar herniation, or to arachnoiditis altering the CSF dynamics.¹⁴⁸ Post-operative or posttraumatic intradural arachnoid cysts have been associated with syrinxes.¹¹

There have been several reports of familial syringomyelia, and of syringomyelia occurring in siblings with healthy parents.^{42,77,82,92,178,320,333} Newman et al³³¹ found human leucocyte antigen (HLA) A9 to be significantly increased in patients with syringomyelia. A genetic link appears likely in the cases of syringomyelia associated with myotonic dystrophy reported by Kuhn.²⁵⁴ There is no clinical difference between sporadic and familial cases.^{153,333} The number of cases is too small for the nature of genetic transmission to be determined.⁹²

1.5 Classification

There have been several classifications of syringomyelia.^{29,39,333,500} The classification presented in Table 2 is adapted from those of Batzdorf³⁹ and Williams⁵⁰⁰ and is based on the associated pathology. Milhorat et al^{295,296,303,304} have classified syrinxes according to the MRI and pathological findings, regardless of the associated pathology, into: 1) communicating central canal syrinxes; 2) non-communicating central canal syrinxes; and 3) extracanalicular syrinxes (see *Pathology*, page 17).

Table 2. *Classification of syringomyelia. Percentages are from Williams.*⁵⁰⁰

1.	Syringomyelia related to abnormalities at the craniocervical junction. (81%)
a)	Adult forms - Chiari type I, membranous rhombic roof. (71%)
b)	Infantile forms - Chiari type II and type III, Dandy-Walker cyst. (4%)
c)	Tumours at the level of the craniocervical junction. (1-2%)
d)	Meningeal fibrosis
2.	Primarily spinal syringomyelia. (19%)
a)	Posttraumatic. (12%)
i)	Associated with spinal injuries
ii)	Postoperative
b)	Postinflammatory. (3%)
i)	Infectious
ii)	Chemical
3.	Spinal tumours. (5%)

1.6 Clinical features

The mean age at onset of symptoms is approximately 30 years, regardless of whether a Chiari malformation is present,^{278,405} but symptoms may occur from infancy to 70 years.^{153,333} The onset of symptoms in patients with syringomyelia is earlier than in patients who have a Chiari malformation but no syrinx.²⁴ The clinical features are dictated by the spinal cord damage caused by the syrinx.⁴⁹⁵ Although there is considerable variation there is usually an insidious onset of motor and sensory symptoms.^{268,405,440} The most common presenting symptoms are lower limb stiffness and pain and numbness of the hands.¹⁵⁵ Other presenting features include oscillopsia, diplopia, stridor, urinary incontinence, dysaesthesia, ataxia and respiratory paralysis.^{152,155,200,333} The initial symptoms may be suggestive of a lesion of the conus medullaris.^{79,250,286,326} There may be a rapid onset after mild cervical trauma;^{30,155,278} after coughing, straining or sneezing; or spontaneously.^{46,274,405,518}

Pain may be of two types:²⁷⁴ 1) restricted to the suboccipital and neck area, associated with coughing (probably due to cervicomedullary compression); and 2) a burning, aching pain on the side of the sensory loss.²⁴ If the dorsal root entry zone is affected, all sensation may be lost unilaterally.²⁷⁴ Sensory disturbance may extend to the face if the syrinx extends into the upper cervical cord. The classic dissociated sensory loss affects the ulnar border of the

forearm, extending into the arm and the chest in a cape or half-cape distribution, but this is in fact uncommon.⁴⁴⁰ The first motor signs are often seen in the upper limbs, with wasting and weakness of the hands.^{24,153,333} Hypotonia, areflexia and fasciculations may be seen.^{24,152,333} Spastic paraparesis and brisk reflexes may be due to cervicomedullary compression or from involvement of descending motor tracts.²⁷⁴ Upper limb girdle joints may be affected by arthropathy, spontaneous fractures and calcification of the soft tissues in the late stages of the disease.^{333,494,500,517}

The presenting features differ in adults and children. In adults, pain and numbness are the most common initial symptoms.^{10,53,152,268,287} The first manifestation in children may be scoliosis, while neurological deficits may not follow for many years.^{152,188,228,395,494,512} Scoliosis may be present in up to one half of the cases of hindbrain-related syringomyelia,^{53,67,500} and it may improve after treatment of the syrinx.¹⁸⁸

Syringobulbia usually occurs as an extension of syringomyelia, but it may occur separately.³³³ Nystagmus is the most common sign, which may be due to either a hindbrain malformation or the syrinx. Facial sensory loss in an onion-skin pattern, pharyngeal and laryngeal dysfunction and other bulbar signs may be seen.³³³ Other symptoms, such as bradycardia, may also be due to the Chiari malformation.⁴¹⁰ Damage to the chemosensitive regions and the nucleus tractus solitarius may cause CO₂ insensitivity and respiratory failure.^{201,333} Syringobulbia may be more common in non-hindbrain related cases; perhaps compression of the upper end of the cord prevents upward extension of a cyst.⁵⁰⁰ The syrinx may extend to the pons, midbrain, or centrum semiovale.⁴⁵ Bertrand⁴⁶ postulates that coughing or straining raises the pressure in a spinal cord syrinx; if the fluid is unable to pass into the fourth ventricle, it forces its way up into the soft grey matter of the medulla.

The severity of symptoms and signs is not proportional to the dimensions of the syrinx.¹⁸² Foster¹⁵² believed that syringomyelia becomes clinically apparent when the fluid

within the dilated central canal ruptures through the ependymal lining and dissects through the tracts.

1.7 Diagnostic tests

Prior to the introduction of modern neuroimaging techniques, the diagnosis of syringomyelia was essentially clinical, and was difficult to make.³²⁹ Almost all the 32 patients reported by Cahan and Bentson⁶⁷ had previously been misdiagnosed, as had most of the patients of Wisoff and Epstein⁵¹² and 43 of the 127 cases described by Levy et al.²⁶⁸

Several laboratory investigations such as electrophysiology have been used in an attempt to improve the diagnostic accuracy. Sensory nerve conduction is normal, because the process is proximal to the dorsal root ganglia, while motor conduction velocity is usually also normal.³³³ F-wave latencies in the upper limbs are prolonged if the syrinx affects C8-T1 segments.³³³ Failure of the F-wave latencies to improve following surgery is an indication of permanent motor cell loss.³⁸⁵ Chronic partial denervation is seen in electromyographic studies.³³³ The process is slowly progressive, so fibrillation potentials and positive sharp waves are rarely seen.³³³ Brain stem evoked potentials,²¹⁵ and somatosensory and spinal evoked potentials^{317,430,474,475} have been used in the assessment of syringomyelia patients with or without Chiari malformation, but their role in clinical assessment is unclear.³¹⁷

Several investigators have analysed the fluid from syringes: that associated with hindbrain anomalies is usually clear, with a normal protein level;^{152,175,333,405} that associated with tumours may have a high protein level and be xanthochromic.^{132,333} Posttraumatic syringomyelia fluid is clear or yellow, with a protein level ranging from 0.35 g/L to 3.9 g/L.³⁸⁵

1.7.1 Plain X-rays

Plain X-rays may reveal basilar impression or platybasia,^{10,24,49,67,161,494} the cervical spinal canal may be enlarged^{67,129,484,494} or there may be segmentation abnormalities of the cervical spine.^{13,49,67,161,333,500} Wells et al⁴⁸⁴ found that the vertebral canal was more

commonly enlarged in patients whose symptoms began prior to age 30. They concluded that the evidence favoured adaptation of the canal during growth as the main factor.⁴⁸⁴

1.7.2 Myelography

A syringomyelic cord may appear enlarged on myelography, but according to Nogués,³³³ the cord diameter may be normal. When air myelography was used, the 'collapsing cord' sign was taken as a certain indicator of syringomyelia.^{67,333} With the patient in a vertical posture, the upper part of the cord collapsed, while the dependent segment of cord dilated.¹²⁹ Conway⁹³ believed that it represented a syrinx in continuity with the fourth ventricle. Occasionally, a communication is demonstrated between the spinal subarachnoid space and a post-meningitic syrinx.⁴⁰³ Levy et al²⁶⁸ found syringes at surgery in 12 of 16 patients with normal myelograms. Nor is myelography a reliable method of demonstrating the Chiari malformation.⁴⁹

1.7.3 CT

Plain CT is not a reliable test for syringomyelia, primarily due to image degradation by surrounding bone.¹²⁹ CT combined with contrast myelography is capable of demonstrating Chiari malformations, but 20-50% of cavities may not be detected.¹⁵² Davis and Symon¹⁰³ claim that CT myelography is capable of detecting 90% of cysts.

Following myelography, the syrinx cavity may fill with contrast.^{67,176,222,333} In delayed CT scans taken three hours after contrast injection, there is loss of the sharp margins of the cord; at six to 24 hours, the cavity is filled with contrast medium and the subarachnoid space has been cleared of contrast.³³³ Contrast penetration into the cysts may be immediate,¹⁰³ particularly in complete traumatic cord lesions. Areas within the cord that take up contrast may be degenerative material rather than cysts in up to 10% of cases.^{103,426} The route of entry of contrast is controversial. Proposed routes of entry include a communication with the fourth ventricle, 'diffusion' through the perivascular spaces,¹⁷⁶ or through tears in the cord.⁴²⁶

Alternatively, the higher attenuation within the syrinx has been attributed to differential absorption of the fluid within and outside the cord.¹⁸⁰

1.7.4 MRI

MRI is currently the imaging modality of choice for the diagnosis and follow-up of syringomyelia.^{147,180,248,363,366,380,416,500} Syrinx fluid has a signal intensity the same as or slightly higher than CSF on T1-weighted images.¹²⁹ Wilberger et al⁴⁸⁸ showed that one third of syringes demonstrated on MRI were either not seen or not seen adequately by myelography. MRI has demonstrated that syringes are complex; they are often asymmetrical, loculated or multiple.^{103,358,380} MRI is not infallible: due to partial volume effects, it may not adequately demonstrate cavities in cases with small spinal cords, or in cases with scoliosis.^{333,396,500} Chiari malformations are clearly demonstrated.^{248,333} MRI is able to show septa within syringes,^{103,303,380} which may aid in operative planning.¹⁰³

MRI has been used to attempt quantification of the severity of syringomyelia. Iskandar et al²²⁶ measured the cross-sectional area of syringes and calculated the percentage of spinal cord area occupied by the cyst. The thinner the neural tissue around the cyst, the more severe the syrinx was thought to be. Cysts were classified as small if they occupied less than 50% of the cross sectional area of the cord and they were less than 2 cm in length.²²⁶

Dittmann and Hoffmann used electrocardiography-gated cine-MRI to examine CSF movements in patients with syringomyelia.¹¹² They demonstrated increased flow velocity in the cerebral aqueduct in a patient with syringomyelia, Chiari malformation and hydrocephalus. The flow velocity in a patient with syringomyelia and Chiari malformation but not hydrocephalus was normal.

1.7.5 Intraoperative imaging

Intraoperative ultrasound has been used to image cysts. It is claimed that surgical planning is improved, and the placement of shunts can be seen.^{113,333} Intraoperative contrast

imaging of syringes has been used;^{260,378} the authors claim it aids in determining the continuity of the cavity in a septated syrinx, which aids in the placement of shunt devices.

1.8 Natural history

The natural history of syringomyelia is not well established.²⁶⁸ Progress of the disease is extremely variable; in some cases symptoms may progress for a few years and then remain static, or there may be a slow and intermittent or continuous deterioration.³²⁹ Conway followed six patients who refused treatment; five worsened and one remained unchanged.⁹³ Boman and Iivanainen⁵³ followed 55 untreated cases for up to 45 years at the University of Helsinki from 1920 to 1965 (the firm diagnosis of syringomyelia in these cases must be in doubt since it was prior to CT and MRI). There was slow progression of symptoms and signs in most of the cases. Eleven patients died, but the cause of death was not specified. They concluded that the prognosis of syringomyelia was good, but of the 44 surviving patients, 22 were disabled. Anderson et al¹⁰ described 20 untreated patients with symptoms lasting from 5 years to 43 years. Seven patients did not deteriorate and five died (two unrelated to syringomyelia). Mariani et al²⁷⁸ followed 14 patients for a minimum of 3 years. Five had a clinically arrested course, six had a slow progression, and three rapidly progressed. Netsky³²⁹ described the usual course of syringomyelia as being 10 years to 15 years, with occasional cases lasting up to 50 years. According to Peerless and Durward,³⁵¹ up to 50% of patients may remain stable for 10 years following diagnosis. Tator and Agbi⁴⁴² believe that the natural course in most cases is a relentless progression of symptoms. Rapid deterioration may occur following myelography³⁸⁵ or after haemorrhage into the syrinx from vessels in the wall of the cavity.^{333,352} There may be spontaneous resolution in rare cases,^{329,342,351,397,398,431} which may be due to development of a communication between the syrinx and the subarachnoid space.^{93,397}

1.9 Pathology

Syrinxes most often involve the lower cervical and upper thoracic segments of the cord.^{26,333} The cord may be enlarged, normal or collapsed in the anteroposterior diameter.³³³ The predilection for the cervical enlargement has been attributed to mechanical forces arising from neck movements.³³³

The cavity often occupies the central grey matter and involves the crossing pain and temperature fibres. Cavities may extend into the anterior grey matter but more commonly they involve the posterolateral cord and the posterior grey commissure.^{333,375} This may be due to the relative lack of connective tissue in this part of the cord.¹² Cysts may be uni- or bilateral.³³³ In advanced cases, the cavity extends to the pial surface at the tips of the dorsal horns.³³³ Cysts may be uniform, convoluted, or multichambered and may be present at different levels within the same cord.^{175,180}

Fibrillary glial tissue (Rosenthal fibres) lines the cavities, but part of the cavity may be lined by ependyma.^{123,142,333,469} The walls of the cavity vary greatly from case to case, and also from segment to segment in the same patient.³³³ Around the cavity, vascular changes including hyalinised and thickened vessels, oedema and haemorrhages may be seen.³³³ Aberrant nerve fibres may be seen in the wall of the cavity. They may arise from spinal ganglia and enter through the dorsal horns.³³³ The Virchow-Robin spaces may be enlarged.¹²³

Brainstem cavities are usually unilateral and occupy one of three positions:³³³
1) ventrolateral to the fourth ventricle, external to the hypoglossal nucleus; 2) as an extension of the fourth ventricle along the median raphe; or 3) as a slit between the pyramid and inferior olive. Syrxinx cavities may extend rostrally to the diencephalon or even the centrum semiovale.^{45,340}

Syrinxes associated with tumours typically involve the posterior half of the cord,³³³ although some authors suggest that there is no pathological difference between syrxinxes

associated with tumours and other syrinxes.¹⁴² Most cysts extend rostrally from the tumour, although some extend both rostrally and caudally.³³³

Milhorat et al^{295,296,303,304} recently classified syrinxes according to the MRI and pathological findings into: 1) communicating central canal syrinxes; 2) non-communicating central canal syrinxes; and 3) extracanalicular syrinxes (Figure 1).

Dilatations of the central canal anatomically continuous with the fourth ventricle (communicating central canal syrinxes) are associated with hydrocephalus (such as secondary to meningitis or haemorrhage). There is enlargement of all four ventricles and the communicating segment of the central canal. In patients with Chiari II or Dandy-Walker malformation and an obstructed aqueduct of Sylvius, the fourth ventricle is isolated from the cerebral ventricles and its outlets are occluded. In these cases, the dilated upper central canal communicates with the isolated fourth ventricle. The cavities are lined wholly or in part by ependyma and are defined at their caudal end by central canal stenosis. Paracentral dissections are only occasionally present. This type of syrinx is asymptomatic or associated with non-specific clinical findings.²⁹⁶

Non-communicating central canal syrinxes are found at a variable distance caudal to the fourth ventricle. They are associated with conditions such as Chiari I malformation, cervical spinal stenosis, spinal arachnoiditis and basilar impression. Most cavities are closed at their rostral end by canal stenosis but some have an apparent communication with the fourth ventricle through a patent, although not enlarged, central canal. The cavities are complex, with extensive ependymal denuding, septations and paracentral dissection. Patients with this type of syrinx are more likely to have segmental neurological deficits.²⁹⁶

Extracanalicular syringes are associated with spinal trauma, infarction, haemorrhage or transverse myelitis. The cavities are usually in the central grey matter, dorsal and lateral to the central canal. All patients have segmental neurological deficits.

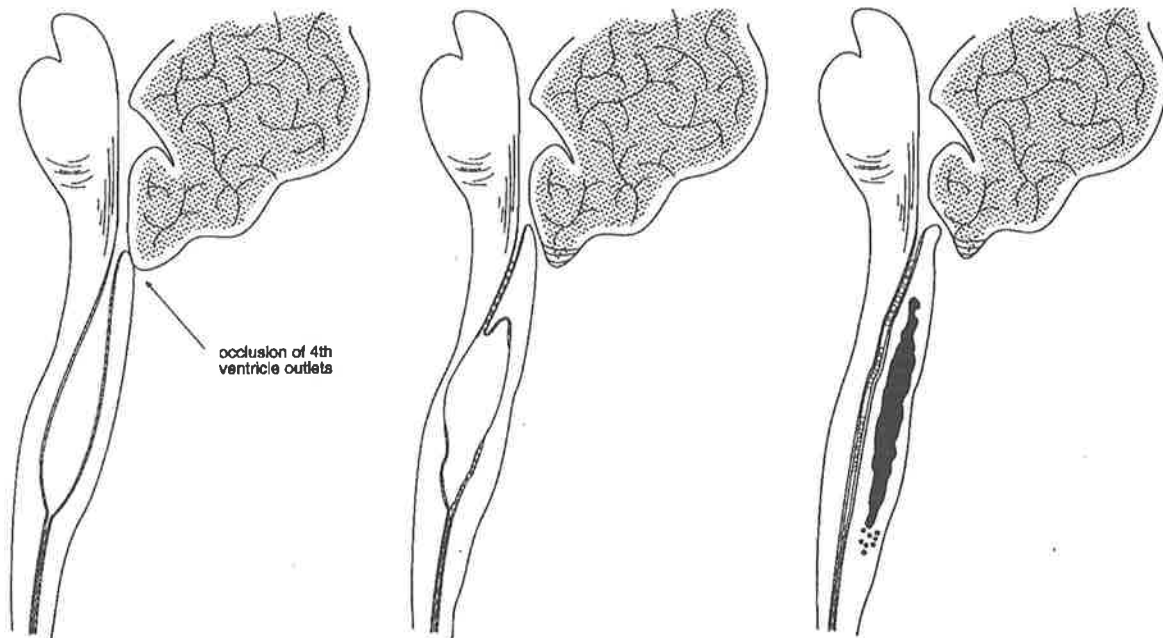


Figure 1. *Anatomical classification of syrinx cavities. The communicating central canal type (left) is associated with obstruction of the outlets of the fourth ventricle and there is often hydrocephalus. The syrinx communicates with the fourth ventricle via the central canal. The non-communicating central canal type (middle) is seen with chronic compressive lesions and appears to begin as a dilated central canal that may rupture into the spinal cord parenchyma. The extracanalicular type (right) forms by enlargement of a cavity that is separate from the central canal. (Adapted from Milhorat TH, Capocelli AL, Anzil AP et al: Pathological basis of spinal cord cavitation in syringomyelia: analysis of 105 autopsy cases. J Neurosurg 82:802-812, 1995)*

1.10 Treatment and outcome

There is no entirely satisfactory treatment for syringomyelia.⁴⁴² Treatment has essentially been surgical, although belief that syringomyelia resulted from secretion of fluid by neoplastic glial tissue led to treatment with radiotherapy earlier this century.^{53,93,329,351,462}

Surgery has included posterior fossa decompression, various shunting procedures, opening the cyst directly into the subarachnoid space and laminectomy (Table 3). There is

often short term improvement,²⁷⁸ but the variable and long course of the disease makes it difficult to determine the effectiveness of treatment based on small studies with short follow-up.^{167,268} The few large studies of long term outcome indicate that symptoms progress in 25% to 50% of cases regardless of the treatment method.^{70,141,268,351} In a review of the literature in 1983, Levy et al²⁶⁸ calculated that the overall results of surgical treatment were: improved 46%, unchanged 32% and worse 20%, but these included cases of Chiari malformation without syringomyelia. Surgical intervention appears to be most effective at stabilising or alleviating pain, sensory loss and weakness. Spasticity, headache, and sphincter dysfunction are less likely to be reversed.²⁸

Table 3. *Surgical treatments for syringomyelia.*

1.	Foramen magnum decompression
	a) Bony
	b) Dural
	c) Arachnoid
	d) Plugging the opening of the central canal*
2.	Syrinx shunting and fluid diversion
	a) Ventriculo-peritoneal
	b) Ventriculo-atrial
	c) Syringo-subarachnoid
	d) Syringo-pleural
	e) Syringo-peritoneal
	f) Syringo-atrial
3.	Opening of the syrinx into the subarachnoid space
	a) Syringotomy
	b) Cord transection
4.	Laminectomy ³⁶⁰

The appropriate timing of surgical treatment is controversial. Anderson et al,¹⁰ Foster,¹⁵² Tator and Agbi,⁴⁴² Lesoin et al,²⁶⁵ Bidzinski⁴⁸ and Peerless and Durward³⁵¹ advocated surgical treatment only in cases with active progression of signs and symptoms. However, it seems that results are better in patients with a short duration of symptoms.^{48,51,124,351,375,443} Williams⁴⁹³ advised surgical treatment as soon as the disease is

* The opening of the central canal commonly has been referred to as the 'obex.' Strictly speaking, the 'obex' is the band of neural tissue between the nuclei of the area postrema and forms the dorsal roof of the central canal opening.³¹⁰

diagnosed, citing the tendency for rapid advancement of deficits in some cases. According to Williams,⁵⁰⁰ neurological deficits are unlikely to be reversed by treatment; this is the justification for treatment of asymptomatic cases. Barbaro et al²⁸ cite the better results in patients with less severe deficits as an indication for early surgery.

1.10.1 Foramen magnum decompression

Foramen magnum decompression as a treatment for syringomyelia was first advocated by Gardner,¹⁷⁴ as a means of correcting the putative pathophysiological forces responsible for syrinx development. The place of this operation in the management of syringomyelia remains controversial. Some authors advocate foramen magnum decompression as the first step in treating cases associated with malformations at the craniocervical junction,^{115,274,333,351,357,449} followed by a shunting procedure if the syrinx persists.¹¹⁵ Transoral decompression has been performed in cases of syringomyelia associated with basilar impression, with good results.^{237,247} For other types of syrinx, a shunting procedure has been recommended as the initial procedure.^{163,333,351,460} Syrinxes resolve over several weeks after foramen magnum decompression.³⁸

The aims of decompression have been to: 1) relieve the craniospinal pressure differential; 2) restore the subarachnoid space; 3) eliminate the syrinx; and 4) relieve the brain stem compression.^{367,392} There have been many variations in the technique of decompression.³⁶⁷ Gardner¹⁷⁴ recommended plugging of the central canal opening to prevent CSF flowing into the central canal from the fourth ventricle. Williams,⁴⁹³ claiming that the smooth wall of the opening prevents secure plugging, advocated plugging the central canal opening with muscle secured with a suture passing through the dorsal medulla. Logue and Edwards,²⁷⁴ Levy et al²⁶⁸ and Vaquero et al⁴⁶⁴ found no advantage, and a higher rate of complications, after plugging the central canal opening. Others continue to advocate plugging the opening,^{51,214,348,351,358,450,463} but it seems that this is not essential for syrinx collapse.⁴⁶⁴ In

addition to decompression, Raftopoulos³⁶⁶ recommended subpial resection of the cerebellar tonsils. Batzdorf³⁸ recommended opening the arachnoid, dissecting it laterally and mobilising the tonsils to confirm flow of CSF from the fourth ventricle. Batzdorf³⁸ also suggested inserting a dural graft to prevent scarring of the musculature to the cerebellum. Williams preferred leaving the dura open, claiming that reconstituting it would reform the valvular mechanism which caused the syrinx.^{500,503} He also claimed that a dural graft could fall forward against the cerebellum and form adhesions, with constriction worse than before surgery.^{500,503}

The cerebellar hernia of a Chiari malformation may resolve after foramen magnum decompression.^{18,72} Movement of the hindbrain has been related to the extent of bone removal. Duddy and Williams¹²⁰ claimed that in most cases after decompression, the posterior fossa contents actually descended; it has been suggested that failure to form an artificial cisterna magna is the cause of this descent.³⁹² Conversely, it has been emphasised that the craniectomy should be relatively small to prevent hindbrain descent ('slump').³⁹² Sahuquillo et al³⁹² argued against a small craniectomy, claiming that formation of a new cisterna magna allows upward migration of the hindbrain and good clinical results. To achieve this they used an extra-arachnoidal technique with extensive bony decompression and tenting of a dural graft.

Significant risk has accompanied foramen magnum decompression. Williams reported a mortality rate of nearly 15%,⁴⁹³ citing postoperative respiratory difficulties (including sudden respiratory arrest) as a major factor. Other authors^{200,349,442} also have reported fatalities after decompressive surgery; the cause is probably damage to the respiratory centre during attempts to plug the central canal opening.^{280,442} Postoperative worsening or development of brain stem neurological deficits has also frequently been encountered.⁴⁹³ Hydrocephalus is an additional possibility.^{442,493}

Short term results after posterior fossa decompression have been encouraging, but it appears that early relief of pain and dysfunction is followed by deterioration months or years later in up to half the patients. Garcia-Uria et al¹⁶⁸ treated 31 adult patients with Chiari malformation and syringomyelia and followed them for 5-10 years. They used foramen magnum decompression with plugging of the central canal opening. Fifteen patients considered themselves improved by the operation, but only five showed objective improvement. Eight patients showed objective signs of deterioration, but only 5 of these considered themselves worse. None of the patients were asymptomatic after treatment. Peerless and Durward³⁵¹ did not achieve satisfactory long term results with decompression alone, nor with decompression and plugging of the central canal opening. They noted that early relief of pain and dysfunction often disappears months or years later.³⁵¹ Vengsarker et al⁴⁶⁶ claimed that only 30% of patients show some degree of improvement following decompression. Cahan and Bentson⁶⁷ found continued progression of symptoms in at least half of their patients. Matsumoto and Symon²⁸⁰ reported clinical progression in 40% of patients. Hadj-Djilani and Zander¹⁹⁰ reported improvement or stabilisation in the surviving 10 patients of 12 studied for 6-12 years after posterior fossa surgery. Bidzinski⁴⁸ followed 28 patients for longer than five years. He reported very good results in 29%, good results in 50% and poor in 21%. Mariani et al²⁷⁸ reported a short term improvement in 82% of their patients, but in 50% progression of the disease occurred in the longer term. Isu et al²²⁹ performed bony decompression with excision of the 'outer layer' of the dura; they reported good results in seven patients followed for two years.

Many series have compared the results of decompression in patients with Chiari malformation alone, and in patients with Chiari malformation and syringomyelia. Banerji and Millar²⁴ reported improvement in nine of 10 Chiari malformation patients without syringomyelia, but only three of 10 patients with syringomyelia improved. Piper et al³⁵⁹

reported favourable results for decompression of Chiari malformation, but not all patients had syringomyelia. Cahan and Bentson⁶⁷ reported improvement in 10 of 14 patients with Chiari malformation undergoing decompression, while only three of their 26 syringomyelia cases demonstrated sustained improvement.⁶⁷ Similarly, Pillay et al³⁵⁸ found improvement after decompression in 13 of 15 patients with Chiari malformation without syringomyelia, compared with improvement in only nine of 20 patients with syringomyelia. Saez et al³⁸⁹ performed foramen magnum decompression in 60 patients with Chiari malformation. They reported improvement in 65% of cases but found that central cord involvement was a detrimental prognostic factor, as did Paul et al.³⁴⁹ Carey et al⁷¹ reported relief of symptoms in 17 of 20 paediatric patients undergoing decompression for Chiari malformation, but not all these patients had syringomyelia. Appleby et al¹³ reported favourable short term results in a series of 15 cases of Chiari malformation, but only three of the cases had syringomyelia. According to Logue and Edwards,²⁷⁴ upper motor neurone weakness, joint position sense and central neck pain are the features most likely to improve, and this is most likely to result from relieving the medullary compression of the Chiari malformation rather than resolution of the syrinx. Cahan and Bentson⁶⁷ concluded that a large component of the benefit obtained by decompression is from the decompression of the Chiari malformation, rather than treatment of the syringomyelia.

The pathophysiological mechanisms for deterioration after decompression are unclear, as are the mechanisms of syrinx resolution in those patients who improve. Some authors have commented on the poor results obtained with decompression in the presence of arachnoiditis.^{389,493} Williams suggested that progression of a syrinx after posterior fossa surgery may be due to 'slosh' or ischaemic changes resulting from gliosis around the syrinx.⁴⁹³ He cautioned against re-operation on the posterior fossa in patients who do not improve, believing little or no benefit can be obtained.⁴⁹³

Williams⁵⁰⁰ believed foramen magnum decompression is the most effective treatment for hindbrain-related syringomyelia, but he acknowledged that the means by which this produces improvement is unclear. After observing that improvement may occur even where no change is demonstrated in craniospinal pressure dissociation,⁴⁹³ he postulated that improvement is due to alteration of the capacitance of the neuraxis and change in the pulsation characteristics. Heiss et al²⁰⁶ measured cervical and lumbar CSF pressure and craniospinal compliance before and after tonsillar decompression. Decompression improved compliance from 1.35 to 3.34 mL CSF/mm Hg and reduced cervical pressure from 16 to 12 mm Hg and lumbar pressure from 17 to 11 mm Hg. Syring size decreased in all patients.

1.10.2 Syring shunting and CSF diversion

Percutaneous aspiration of syringes has been used in an attempt to prevent neurological deterioration.⁴⁰⁵ Improvement has been only temporary at best³⁵¹ and if fluid drainage is used, most authors now recommend permanent shunting. Many drainage devices have been used, including rubber catheters³⁶⁰ and tantalum wire.²⁷⁶ Silastic catheters are the current preference.

Many authors advocate shunting of the syrinx or the spinal subarachnoid space as the primary treatment, regardless of the associated pathology.^{28,103,167,228,265,355,465} Robertson and Narayan³⁷⁸ claimed that shunting offers the best chance of reducing the pressure in a syrinx and of arresting or reversing neurological loss. Phillips and Kindt³⁵⁵ recommended shunting, claiming that shunts are capable of draining excess fluid regardless of its origin. Other authors have reserved shunting for specific indications. Williams and Page⁵⁰⁶ recommended syngo-pleural shunting for patients with spinal tumour, trauma or arachnoiditis, and for patients who deteriorate after foramen magnum decompression. Peerless and Durward³⁵¹ used drainage of the syrinx as the primary treatment for cases of non-communicating syringomyelia. Hida et al²⁰⁷ recommended syngo-subarachnoid shunting as the primary

procedure for posttraumatic syringomyelia. Matsumoto and Symon recommended shunting when foramen magnum decompression fails.²⁸⁰ Fujii et al¹⁶⁵ used shunting in addition to foramen magnum decompression for cases with large syrinxes and a Chiari I malformation. Shunting of the ventricles has been recommended as a treatment for syringomyelia both in the presence and absence of associated hydrocephalus.^{192,200,250,253,450,493,500} Milhorat et al³⁰⁶ used ventriculo-peritoneal shunting for communicating syrinxes occurring with hydrocephalus. Kruse et al²⁵³ believed that some cases of syringomyelia result from impaired CSF absorption and that ventricular shunting is indicated in these cases, whether hydrocephalus is present or not.

The fluid space to be drained and the destination of the drainage have varied. Shunting into the peritoneal space has been claimed to be superior to other shunting procedures because the peritoneal cavity can readily absorb fluid^{103,355} and tube patency can be assessed.⁴³³ It is claimed that syringo-subarachnoid shunts may not result in good absorption of fluid^{103,433} and CSF may reflux into the syrinx from the subarachnoid space.⁴³³ A syringo-pleural shunt is easier to perform but may not absorb fluid as well as a peritoneal shunt.^{103,487} Iskandar et al²²⁶ used shunting to the subarachnoid or pleural spaces to treat syrinxes in the caudal third of the cord associated with congenital spinal abnormalities. To bypass obstructions at or below the foramen magnum, Milhorat et al³⁰⁵ shunted syrinxes to the posterior fossa subarachnoid space. Nogués³³³ advocated shunting of the syrinx to the subarachnoid space when the spinal subarachnoid space communicates with the intracranial subarachnoid space.

Shunting procedures appear to have an approximately equivalent outcome to posterior fossa decompression.^{333,433,464} Dramatic short term improvement is common,¹⁵⁶ but shunting is often unsuccessful in the long term.³⁵³ Tator et al⁴⁴³ reported favourable results in 15 of 20 cases receiving syringo-subarachnoid shunts followed for an average of five years. Vaquero et

al⁴⁶⁴ found foramen magnum decompression and syringo-subarachnoid shunting equally successful. They also found collapse of septated syrinxes after syringo-subarachnoid shunting, claiming that communication between the septa exists.⁴⁶⁴ Padovani et al³⁴⁵ used syringo-subarachnoid shunting to treat 29 cases; symptoms stabilised in 17, improved in nine, and there was delayed deterioration in three. They proposed that the mechanism of late deterioration was damage to radicular vessels by stretching when the cord collapses.³⁴⁵ Sgouros and Williams⁴¹¹ reported their long term results with syringo-subarachnoid shunts. There was a 50% failure rate, and a 16% complication rate. They recommended opening CSF pathways as the primary treatment for syringomyelia and lowering the overall CSF pressure when this proves impossible.⁴¹¹ Philippon et al³⁵⁴ used syringo-peritoneal shunting and found that pain was the most likely symptom to improve. However, the overall outcome was unchanged. Park et al³⁴⁷ used lumbo-peritoneal shunting with myelotomy because they believed syrinxes form from raised pressure in the spinal subarachnoid space forcing CSF into the cord. They reported collapse of the syrinx in four of their seven patients.³⁴⁷ Vengsarker et al⁴⁶⁶ used lumbo-peritoneal shunting to dissipate the presumed abnormally high pressure generated by the Chiari malformation. They reported favourable results in their three patients.⁴⁶⁶ Vassilouthis et al⁴⁶⁵ used theco-peritoneal shunts in three patients; the syrinx reduced in size in each case. Milhorat et al³⁰⁵ shunted syrinxes to the posterior fossa subarachnoid space with collapse of the syrinx in all of their four patients. Hall et al¹⁹² reported encouraging results after shunting the ventricles of patients with syringomyelia complicating myelomeningocele. These cases were shown to have a communication between the syrinx and the fourth ventricle. Krayenbühl and Benini²⁵⁰ also reported good results after ventricular shunting in patients with demonstrated communication between the syrinx and the fourth ventricle. Milhorat et al³⁰⁶ used ventriculo-peritoneal shunting for communicating syrinxes occurring with hydrocephalus and all seven patients had an excellent result.

The mechanism of shunt failure in many cases is not known. Shunts block and they may cause arachnoiditis^{1,10} or scarring around the cyst end of the shunt,¹²⁵ although according to Tator et al,⁴⁴³ modern shunt materials reduce these problems. Edgar and Quail¹²⁵ quoted a rate of shunt blockage in experienced hands of 10% to 15%, and 50% in inexperienced hands. Piper et al³⁵⁹ report a failure rate of 50% for syring shunts. Krayenbühl and Benini²⁵⁰ believe catheter blockage was responsible for poor results seen with myelotomy and subarachnoid drainage. Williams cited 17 cases of recurrence in 19 shunted patients.⁵⁰² He stated that shunting should not be performed in the presence of a hindbrain hernia,⁵⁰⁰ or for posttraumatic syringomyelia.⁴⁹⁸ He claimed that an effectively draining shunt will fail because it will draw the wall of the syrinx around it.⁴⁹⁸ In addition, he claimed that shunts will increase the degree of fibrosis and make the situation worse.⁴⁹⁸

Other complications of shunting procedures include direct spinal cord damage, spinal instability (after extensive laminectomy), deafferentation pain, CSF fistula and infection (including pyosyrinx).⁴⁴² Ventricular shunting hazards include infection, epilepsy, over-shunting and shunt blockage.⁴⁹³

Inserting a drainage tube into a syrinx provides an opportunity for its study, but very few such studies have been reported. Intracyst pressures were measured by Davis and Symon.¹⁰³ They drained cysts at myelotomy, then inserted drainage tubes and measured the pressure after 10 minutes. In 15 of 17 cases, the developing intracyst pressure was 40-70 mm CSF. The possible explanations given were 1) the rate of fluid flow into the syrinx is much greater than had previously been thought, and 2) the cord is 'elastic' due to the longitudinal and circular fibre arrangement.¹⁰³

1.10.3 Direct opening of the syrinx into the subarachnoid space

Myelotomy and subarachnoid drainage of the cyst was the standard treatment prior to foramen magnum decompression.²⁵⁰ Various forms of myelotomy have been used, often

without drainage, in an attempt to collapse syrinxes. Williams suggested that the benefit sometimes seen after myelotomy could be the result of arachnoiditis interfering with the mechanism of 'slosh'.⁴⁹³

Midline or dorsal root entry zone opening of the cord into the syrinx may lead to transient improvement,^{333,505} but the syrinx tends to refill.⁴³³ Excision or transection of the spinal cord has been used,^{35,333} but drainage of the cyst is inhibited by postoperative adhesive arachnoiditis.⁴⁴³ Cord transection was used by Shannon et al⁴¹² to treat posttraumatic syringomyelia in patients with a complete cord lesion. Syringostomy was used in patients with incomplete lesions.⁴¹² In the patients whose main symptom was severe pain, complete relief of pain was observed after surgery.⁴¹² Wide opening of the cord over the length of the syrinx is a possible treatment that has been advocated as a last resort.¹⁰³

Terminal ventriculostomy (excision of the filum terminale and conus medullaris) has been used when foramen magnum decompression fails.^{48,173,505} Improvement is seen in some cases,^{48,351} but most patients continue to deteriorate.^{333,505} Even Gardner reported on the favourable results of terminal ventriculostomy long after he proposed foramen magnum decompression, without commenting on the reason for seeking an alternative treatment.^{173,493} Filizzolo et al¹⁴⁶ reported six patients who underwent terminal ventriculostomy; only one improved and the other five continued to deteriorate.

1.10.4 Other treatments

Samii and Klekamp³⁹⁴ used dural grafts in an attempt to decompress the subarachnoid space in posttraumatic syringomyelia. Infusion of heavy viscous fluid into the syrinx to dampen the effects of 'slosh' has been suggested by Williams.⁴⁹³ He also mooted the possibility of infusing a sclerosing agent.⁴⁹³

1.11 Theories of pathogenesis

Syringomyelia was originally thought to be a developmental abnormality, or a result of cavitation of an abnormal growth of glial tissue.^{93,117,142,489,500} Cameron⁶⁸ believed that the cavity occurred as part of the delayed closure of the caudal neuropore. Netsky³²⁹ and Schneider⁴⁰⁶ believed the cause was occlusion of vessels in a vascular anomaly of the spinal cord, resulting in cavitation, gliosis and connective tissue proliferation. Grund, in 1908, may have been the first to suggest a role of CSF hydrodynamics in the formation of syrinxes,⁹³ although a hydrodynamic theory had been introduced by Morgagni, who suggested that oversecretion of CSF distended the CNS, causing a dilated central canal and spina bifida.¹⁵²

Subsequent theories have centred on hydrodynamic or ischaemic factors. The details differ according to the associated pathology, and to whether the factors are held to initiate or propagate the syrinx.⁵⁰⁰ This review will discuss each of the theories according to the associated pathology. The issue of a communication existing between the fourth ventricle and the syrinx will be discussed separately at the end of this section.

1.11.1 *Malformations at the craniocervical junction*

With the realisation that most syrinxes were associated with abnormalities at the level of the craniocervical junction, several hydrodynamic theories have been proposed, notably by Gardner and Williams, but also by Ball and Dayan and others.

Gardner developed his hydrodynamic theory to explain the formation of myelomeningoceles; his theory was based on that of Morgagni 200 years earlier.¹⁷⁰ Gardner and his colleagues^{169-172,174,175} believe the basis for syringomyelia (and myelomeningocele and Dandy-Walker malformation) is obstruction of the outlets of the fourth ventricle (Figure 2). This obstruction may be of the foramina of the fourth ventricle or it may be a ball-valve effect of a cerebellar hernia. Failure of the fourth ventricle foramina to open during embryonal development is said to result in persistence of a 'physiological' hydrocephalus and

hydromyelia. If embryonal CSF forms too rapidly or escapes too slowly, the neural tube bulges and ruptures. In mild cases, the tube does not rupture; hydromyelia (and syringomyelia) is the consequence. The presence of hydromyelia and hindbrain hernia in conjunction with myelomeningocele and diastematomyelia is interpreted as an intermediate form of the disease spectrum.¹⁷⁰

The mechanism of central canal dilatation proposed by Gardner is the transmission of the arterial pressure wave originating in the choroid plexus down the central canal—the so-called ‘water hammer’ effect.¹⁷⁰ Gardner et al¹⁷⁵ believe this is the explanation for all syrinxes, and that patients who develop syringomyelia in association with other pathologies have a previously-existing congenital obstruction of the fourth ventricle outlets. The low protein content of syrinx fluid was said to be indicative of its origin from the choroid plexus.¹⁷⁰ Gardner’s explanation for enlargement of the central canal in preference to enlargement of the ventricles is that the central canal is lined by high water content grey matter and the ventricles are lined by white matter.¹⁷⁵ The explanation for initial enlargement of the lower cervical segments (rather than enlargement from the fourth ventricle downward) is that the central canal will tend to dilate where the grey matter is thickest—supposedly its weakest point.⁴⁸⁹ His explanation for septations is that overdistension of the central canal is lobulated at the somite stage due to the greater resistance of the dense bordering somites.¹⁷⁵

Gardner’s theory is based on failure of the ‘normal’ rupturing of the roof of the fourth ventricle. He described four stages of hydromyelia, resulting from inadequate compensation of the ‘physiological’ hydromyelia that exists at the time of neural tube closure:

1. Congenital hindbrain hernia as a result of lowering of the tentorium by the relatively greater effect of the choroid plexus in the lateral ventricles compared with the choroid plexus of the fourth ventricle. There are symptoms of syringomyelia in adulthood and

congenital abnormalities of the spine (widening of the canal); occult spina bifida or bulging myelomeningocele may be present.

2. More severe overdilation of the neural tube causes separation of the roof and floor plates of the spinal cord; subsequent closure of each half results in two hemicords. Scoliosis and widening of the spinal canal will be present. The spinal cord above and below the split will be hydromyelic.
3. Even more severe obstruction and overdilation results in rupture of the neural tube into the amniotic sac. The spinal cord above remains hydromyelic and there is a severe hindbrain hernia.
4. Severe overdilation may result in external rupture of the roof plate and internal rupture of the floor plate; in rare cases this heals to form a posterior enteric fistula.

Gardner¹⁷¹ argues that these four stages represent a morphological continuum resulting from a pathological degree of the physiological 'hydrocephalomyelia' which he claims is part of normal development. In the first stage the overdilation becomes compensated by communication with the subarachnoid space before significant damage occurs.

Crucial aspects of Gardner's theory are that the roof of the fourth ventricle normally ruptures under the pressure of CSF production, that in the disease state the foramina of the fourth ventricle remain occluded, and that syrinxes communicate with the fourth ventricle. It is now apparent that the foramina of the fourth ventricle do not form as a result of CSF pressure,¹³³ and that there is not always obstruction of the fourth ventricle outlets in patients with syringomyelia.^{67,117,489} Patency of the foramina of Luschka in such cases may be demonstrated by the passage of spinal subarachnoid radiopaque dye into the fourth ventricle.⁴⁸⁹ It is also now clear that not all syrinxes are in communication with the fourth ventricle³⁷⁷ (*vide infra*), although Pillay et al³⁵⁷ argue that extracellular spaces may act as a

pathway for fluid to be forced from the fourth ventricle into the syrinx. Other evidence against Gardner's theory has been cited by Ball and Dayan.¹⁹ They calculated that the pulse pressure wave transmitted through the central canal into a syrinx was of the order of 4×10^{-5} mm Hg and therefore most unlikely to cause a syrinx.¹⁹ In addition, they found no communication between different cavities in the same cord examined at autopsy.¹⁹ Williams⁴⁹² found no seasonal variation of births of patients who later developed syringomyelia; he believed this was evidence against the spinal dysraphism theory of Gardner.¹⁷¹

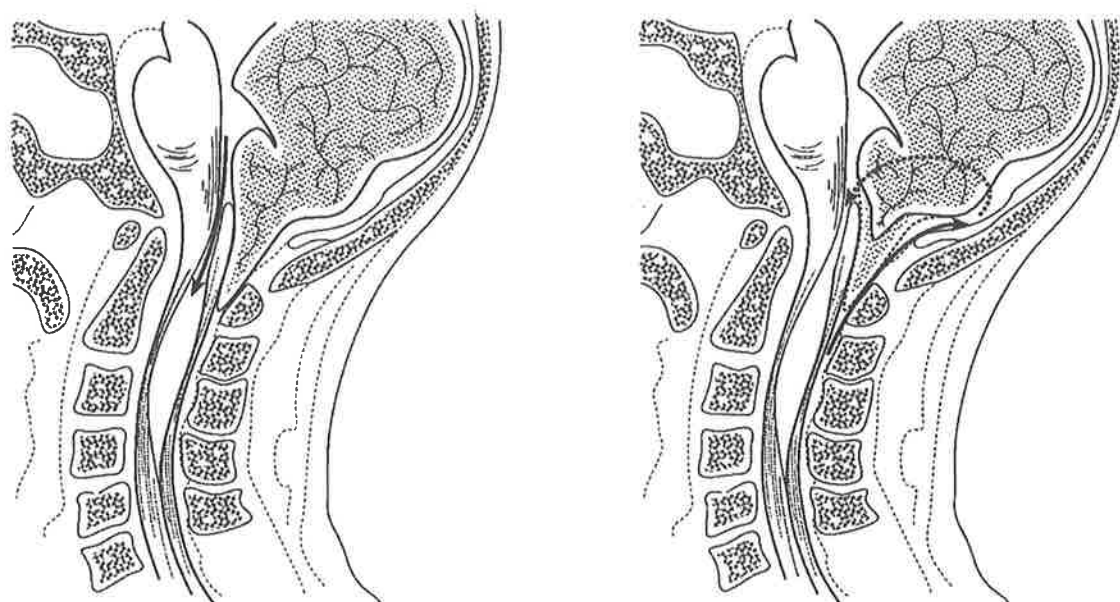


Figure 2. Theories of pathogenesis of syringomyelia. Occlusion of the fourth ventricle outlets is said to result in transmission of the arterial pulse wave from the ventricles into the central canal (left). An alternative explanation (right) is that the cerebellar tonsils act as a one-way valve, allowing spinal fluid to enter the cisterna magna during coughing and straining (solid arrow) then preventing fluid from returning to the spine, forcing it under pressure into the fourth ventricle and central canal (dotted arrow).

Williams and coworkers^{120,316,411,486,489-509} developed a similar theory proposing that syrinxes develop by a flow of CSF from the fourth ventricle into the central canal. Williams believed the pressure comes from the ball-valve mechanism of hindbrain malformations, rather than the 'water-hammer' effect. The hypothesis is that Valsalva manoeuvres force CSF

from the spine up past the foramen magnum, but that the hindbrain malformation then acts as a valve to prevent CSF from returning to the spinal canal. A pressure differential is thereby established between the posterior fossa and the spine. The only open communication then is into the central canal—so-called ‘suck’ forcing CSF into the central canal and dilating it (Figure 2). Williams claims that pressures developed by coughing and straining are much greater than the arterial pressure waves cited by Gardner. A surge of blood from the abdominal and thoracic cavities is thought to enlarge the epidural venous plexus, squeezing the dura. Williams believed the major causative factor in hindbrain-related syringomyelia is the lack of a cisterna magna.

Williams referred to a syrinx developing in this way as ‘communicating,’ meaning that there is a communication between the syrinx and the fourth ventricle. He claimed that some cases of syringomyelia start as ‘communicating,’ then lose the communication with the fourth ventricle (possibly due to the changes developing in the posterior fossa), and that different pathophysiological mechanisms maintain the syrinx cavities. According to Williams, ‘non-communicating’ syringomyelia begins with a cavity in the cord that is related to the associated pathology. Once a cavity has formed, it is said to enlarge by the process of ‘slosh:’ increased syrinx pressure associated with Valsalva manoeuvres dissecting cord tissue.⁴⁹³ Lorenzo, Maleci and Williams²⁷⁵ cite a case of neurological deterioration in a patient with syringomyelia undergoing lithotripsy to support this hypothesis. Fluid movement within syringes has been studied with MRI. Enzmann et al¹³⁰ found pulsations within syringes, but admitted that some cysts could enlarge without pulsations. Wisoff and Epstein⁵¹² argue that pressure external to a syrinx would tend to collapse it rather than enlarge it. Gardner¹⁷⁵ rejects the concept of non-communicating syringomyelia, claiming that all syringes communicate with the fourth ventricle.

Objective evidence for Williams's theory comes from measurements of pressure in the cranial and spinal subarachnoid spaces. Of 37 patients studied by Williams, 24 had pressure dissociation.⁴⁹⁵ Ellertsson and Greitz¹²⁸ also found a craniospinal pressure differential, but interpreted their results of increased cyst pressure and increased intracranial venous pressure as being evidence for communication of the cyst with the subarachnoid space.¹²⁸ In contrast, Park et al³⁴⁷ studied three patients and found that lumbar spinal pressure often equals, or exceeds, intracranial pressure in patients with syringomyelia.

The source of fluid within cavities not in communication with the subarachnoid spaces was an enigma to Williams. He proposed that fluid may be exuded from damaged cells and capillaries, and that fluid within a stable cavity follows Starling's laws for extracellular fluid.⁴⁹⁵ Williams did not accept the theory that fluid could initiate a syrinx by entering the cord through the perivascular spaces, because he believed that raised pressure within the subarachnoid space should act to compress the cord rather than expand it.⁴⁹⁵ He accepted that such a route may maintain a syrinx but that it could not initiate or expand it.⁴⁹⁵

Other routes of fluid entry into the cavities have been postulated. Nogués³³³ suggested the dorsal root entry zone, while Ball and Dayan¹⁹ believed the regular finding of dilated perivascular spaces is evidence for a route of CSF flow from the subarachnoid space into the syrinx. Ball and Dayan¹⁹ proposed that obstruction at the foramen magnum prevented the normal upward flow of fluid occurring with Valsalva manoeuvres. Increased pressure in the spinal subarachnoid space would therefore dissect the cord via the perivascular spaces and a syrinx would form by the coalescence of small pools of extracellular fluid. They did not believe that the central canal could be of primary importance, instead hypothesising that rupture of the cyst into the central canal was a secondary event.¹⁹ Olivero and Dinh³⁴² suggested that rather than increasing subarachnoid pressure and forcing fluid along perivascular spaces, compression at the cervicomedullary junction might prevent the outflow

of fluid which normally enters the cord. Based on their recent large autopsy series, Milhorat et al²⁹⁶ suggested that Chiari malformations cause a disturbance of CSF flow, forcing fluid into the central canal through the interstitial space.

Du Boulay et al^{116,117} suggested that cyst formation results from obstruction at the level of the foramen magnum; the CSF is diverted from the basal cistern with each systole and the tonsils 'milk' the fluid down the central canal to form a cyst below the level of the obstruction. Oldfield et al³⁴¹ proposed that the cerebellar tonsils in a Chiari malformation are forced down with systole, acting as a piston on the isolated spinal CSF. A pressure wave would be imparted on the surface of the spinal cord, causing progression of syringomyelia by compressing the cord and propelling the syrinx contents longitudinally. According to these authors, a syrinx may originate and be extended by the pressure wave forcing fluid along the perivascular and interstitial spaces. Atkinson and Lane¹⁵ argued that this theory cannot be applied to all types of syringomyelia and suggested that occlusion of the central canal by any mechanism may be the unifying aetiology.

The appearance of metrizamide in the cyst cavity after myelography (see *CT*, page 14) has been cited as supportive evidence for CSF entry through the dorsal roots or perivascular spaces.³³³ Ellertsson and Greitz¹²⁷ injected fluorescein into the lumbar space of patients with syringomyelia and were able to aspirate it from the cyst 2-3 hours later. They believed the fluorescein entered the cyst through a communication at the fourth ventricle, but considered a perivascular route possible.

A further theory is that of raised spinal venous pressure. Compression at the foramen magnum is claimed to interfere with venous drainage causing necrosis, a cavity and subsequently a syrinx.^{283,413,444,445} Williams⁴⁹³ regarded this theory as unlikely given the widespread venous anastomoses and alternative venous channels in this area, but there is no objective evidence to support or refute the theory.

Tachibana et al⁴³⁶ reported positive Queckenstedt tests in patients with Chiari malformation while their necks were flexed. The tests returned to normal after posterior fossa decompression and this was interpreted as evidence for a role of neck flexion in the pathogenesis of syringomyelia.⁴³⁶ These authors have also produced experimental evidence that intramedullary pressure increases with neck flexion.⁴³⁷ An increase in pressure was not seen in animals after spinal cord and nerve root transection, demonstrating that stretching of the cord was responsible for the pressure increase.⁴³⁷ They postulated that compression of the upper cervical cord by tonsillar herniation during neck flexion could convert a communicating syrinx to a non-communicating one, allowing different mechanisms of syrinx propagation to come into play.⁴³⁷

Several authors have studied the pulsatile movements of the CNS and CSF in patients with Chiari malformation. In normal patients undergoing positive contrast myelography, the cerebellar tonsils do not move, whereas in patients with Chiari malformation, the tonsils move downward with each systole.¹¹⁷ Terae et al⁴⁴⁶ used cine-MRI and found greater pulsatile movement of the hindbrain and spinal cord in patients with Chiari malformation, compared with a control group. They believe that normal downward movement of CSF in systole is impeded and the increased intracranial pressure forces the medulla and tonsils downward.⁴⁴⁶ They proposed that the pulsatile movements of the spinal cord act as a 'vacuum pump' that extends the size of a syrinx. Mechanical stress on the cord from pulsations is proposed to be a causative factor in syrinx formation.⁴⁴⁶ Armonda et al¹⁴ found decreased CSF velocity and shorter periods of caudal CSF flow in syringomyelia patients than in normal subjects.

Pillay et al³⁵⁷ propose a 'unified theory,' which essentially claims that any or all of the previous theories could act together or at different times in a given case.

1.11.2 Other congenital malformations

Syrinxes associated with congenital spinal malformations such as occult spinal dysraphism are virtually always close and rostral to the lesion.²²⁶ There is often no associated hindbrain abnormality or hydrocephalus. Muthukumar³¹⁹ and Li et al²⁷⁰ suggested that a single embryological insult results in both the syrinx and the occult dysraphic lesion. Pang³⁴⁶ proposed that premature termination of retrogressive differentiation leads to a low conus medullaris and thickened filum terminale, whereas complete lack of retrogressive differentiation leads to an extremely long conus with a persistent large terminal ventricle. Meltzer et al²⁸⁶ and Sigal et al⁴¹⁵ claimed that failure of the development of the connection between the terminal ventricle and the central canal could result in a pathological dilatation of the terminal ventricle. Iskandar and Oakes²²⁵ argued that these theories do not explain large high pressure syrinxes that cause neurological deficits. Cystic cavitation of the cord may follow ischaemia caused by tethering of the cord. Chapman and Frim⁸⁰ reported three cases of syringomyelia occurring after surgery to treat retethering of lipomyelomeningoceles. They hypothesised that the inflammatory response to surgery and interstitial debris may have obstructed the central canal, causing syringomyelia by a mechanism similar to the experimental model of Milhorat et al³¹³ (*vide infra*). Williams claimed that 'slosh' is the mechanism of cyst propagation in non-hindbrain congenital malformations.⁴⁹⁷

1.11.3 Spinal tumours

Early theories were that both the syrinx and an associated tumour arise from abnormal glial and mesodermal elements included in the cord as the result of faulty neurulation.^{93,144,362,417} Abnormal glial proliferation followed by degeneration and cavitation, or disintegration of congenitally unstable glia at the union of the embryonic basal and alar plates was said to be the cause of cysts in these cases.⁹³ Gardner¹⁷⁵ did not believe that cystic

cavities associated with spinal cord tumours should be classified as syringomyelia and other authors have suggested that the cysts are simply cavitations within the tumour.²⁷²

More recently, interference with vascular supply,³²² tumour haemorrhage,³⁶⁴ interference with tissue fluid drainage,²⁷¹ and secretion of fluid or an oedema-generating factor by the tumour^{142,419} have been the proposed aetiologies of cysts associated with intramedullary and extramedullary tumours.

Neoplastic growth may interfere with spinal cord blood supply, resulting in ischaemia, necrosis, and cavity formation.^{217,333,357,365} Pencil-shaped infarcts of the cord, with a shape and location similar to syringomyelic cysts, are seen in spinal trauma, arachnoiditis, intramedullary tumours, metastases and in compression myelopathy secondary to extradural tumour.^{202,211} In the series of Hashizume et al,²⁰² the infarct was in the ventral part of the dorsal column or the dorsal horn in all cases and there was minimal reactive change in the surrounding tissue. The area of softening communicated with a segment of complete or patchy transverse necrosis. These authors believed mechanisms other than vascular occlusion were involved, such as mechanical compression of the cord or penetration by necrotic tissue or oedema fluid.

It has been suggested that increased spinal venous pressure resulting from a spinal tumour may cause an influx of CSF along the perivascular spaces.³⁶⁴ Quencer et al³⁶⁵ proposed that compression of the spinal cord causes neural and connective tissue damage that leads to enlargement of the perivascular spaces. In conjunction with 'altered CSF dynamics,' this is said to cause an increase in CSF entering the cord. Nagahiro et al³²² proposed that the presence of a tumour may alter the CSF dynamics (subarachnoid block or cord tethering) and result in net inflow of CSF into the cord. An alternative explanation has been that tumours affect tissue fluid drainage through perivascular spaces, resulting in oedema, and cavity

formation.^{333,357} Once formed, secretion of fluid by neoplastic cells is the putative mechanism of cavity perpetuation.^{132,351}

Samii and Klekamp³⁹⁴ proposed that a combination of transudation and secretion from the tumour and disturbance of CSF and extracellular fluid flow is the underlying mechanism. According to Williams,⁴⁹⁵ syrinxes associated with tumours result from secretion by the tumour, and this is supported by the high protein content of such cysts.⁵⁰⁰ Cyst propagation is then by 'slosh,' although Enzmann et al¹³⁰ found no evidence for pulsations in cysts associated with spinal cord tumours.

Blaylock reported a case of a low thoracic meningioma with syringomyelia below it.⁵² He proposed several possible explanations, including one that the meningioma could have occluded the central canal and that CSF production into the central canal would cause a syrinx. This was the first suggestion that fluid normally crossed the ependyma into the central canal. Quencer et al³⁶⁵ argued that this theory would require all such cases to be located caudal to the tumour and this is not the case.

Syringomyelia often resolves after removal of the associated tumour; the pathophysiological explanation for this is not clear.³²² In contrast, syringomyelia may develop years after the tumour has been removed; the cases reported by Castillo et al⁷⁶ were probably related to arachnoiditis. Cusick and Bernadi¹⁰¹ cite several possible mechanisms for delayed syrinx development, including: transneuronal or perivascular space migration of CSF, tethering of the cord by adhesions exposing the cord to increased hydrodynamic stresses, and arachnoiditis causing ischaemia.

1.11.4 Posttraumatic

Gardner claimed that syringomyelia developed after spinal trauma because the injury caused a spinal block that amplified the effects of a pre-existing hindbrain hernia,¹⁷⁵ but in a series of 600 cases, Edgar and Quail¹²⁵ found no evidence of Chiari malformation. In

addition, Barnett³³ recorded an incidence of syringomyelia of 1.3% in spinal injury patients and said it was 'inconceivable' that this high figure could represent a random association. Van den Bergh⁴⁶¹ suggested that posttraumatic tethering of the cord may lower the tonsils, producing a pressure dissociation.

A compelling theory is that posttraumatic syrinxes form from cystic cavities at the level of cord injury. Necrosis, haemorrhage and microcysts may all develop early after spinal cord trauma.^{7,36,119,140,150,238} Rupture or coalescence of microcysts may then initiate a syrinx.^{12,333,426,515} Wagner et al⁴⁷¹ demonstrated that traumatic haemorrhagic lesions involve predominantly the central grey matter; resorption of the haematoma would therefore leave a central grey matter cyst. MRI evidence suggests such cavities can form within a few months of the injury.¹⁰³ Propagation of cysts could then be by 'slosh',^{493,498,502,507} or influx of fluid via perivascular spaces.^{150,333}

Arachnoiditis has been cited by many authors as a possible cause of posttraumatic syringomyelia. Williams^{498,507} suggested that the cord may be pulled open by fibrotic adhesions between the cord and the dura. Davis and Symon¹⁰³ claimed that in nearly all cases there remains a component of cord compression from bony or intervertebral disc fragments in the spinal canal, usually anteriorly. They suggested that the presence of continuing cord compression and arachnoiditis with central cord necrosis may result in a 'tension myelocyst.' McLean et al²⁸⁵ believe that arachnoiditis tethering the cord results in squeezing of cyst contents with neck movement or coughing, causing extension of the cyst. Pressure dissociation above and below the site of trauma and arachnoiditis may contribute to the caudal extension of the syrinx.²⁰⁷ Edgar and Quail¹²⁵ believed spinal cord tethering by arachnoiditis is an essential prerequisite to the development of posttraumatic syringomyelia.

Ball and Dayan suggested a mechanism similar to their theory for the formation of syrinxes associated with foramen magnum abnormalities.¹⁹ They claimed that a 'functional'

obstruction develops at the foramen magnum and that Valsalva manoeuvres result in influx of fluid into the cord along the perivascular spaces.¹⁹ Anton and Schweigel¹² suggested that CSF may 'seep' into a damaged area of the cord along enlarged perivascular spaces. Savoirdo⁴⁰³ and Nurick et al³³⁴ believed a communication is established between a small initial cyst and the subarachnoid space. Subsequent funnelling of CSF into the cyst would enlarge it. Milhorat et al²⁹⁶ believe that the location of many cavities in the 'watershed zone' between the anterior and posterior spinal arteries suggests a vascular role, but the existence of a watershed zone between the anterior and posterior spinal arteries has recently been questioned (*vide infra*).

Squier and Lehr⁴²³ studied 20 spinal cords obtained at postmortem from patients who had suffered spinal injuries and found cysts in 20% of the cases. Early pathological changes included oedema and necrosis, followed by macrophage infiltration, capillary proliferation and reactive gliosis. Cysts were found at the base of the dorsal columns or between dorsal columns and dorsal horns, which were also the sites of myelomalacic cores. Myelomalacic cores did not always result from direct trauma—they were also seen after arterial damage and infarction. The authors postulated that cysts develop from cavitation of myelomalacic cores rather than from haemorrhage.⁴²³ They also suggested that septated syrinxes develop from independent foci of infarction that later undergo extension.

There is controversy regarding the composition of fluid in posttraumatic syrinxes. Edgar and Quail¹²⁵ argued that the fluid is essentially the same as CSF and that it must therefore not be stagnant. Stevens et al⁴²⁶ analysed the cyst fluid in ten patients: the cellular and protein content exceeded that of CSF in all cases, although only slightly in five. The cells were lymphocytes and lipid macrophages. Davis and Symon¹⁰³ suggest that the osmotic effect of proteinaceous material in the cyst may draw fluid into the cyst, explaining the delayed uptake of contrast.

A second area of controversy is whether the cysts communicate with the subarachnoid space and/or the central canal. Nogués³³³ claimed that a posttraumatic cyst may communicate with the injury site, but not with the subarachnoid space. Hida et al,²⁰⁷ using MRI evaluation of posttraumatic syringomyelia, found the rostral end of the syrinxes was epicentric, with no apparent communication between the syrinx and the fourth ventricle. McLean et al²⁸⁵ used myelography to demonstrate communication between the lumbar subarachnoid space, the syrinx, and the fourth ventricle. At surgery the syrinx they described distended with positive pressure ventilation and collapsed after the cord was opened.²⁸⁵ Davis and Symon¹⁰³ reported a case where pre-operative myelographic contrast rapidly entered the cyst cavity, yet at operation the cyst was clearly under higher pressure than the subarachnoid space. They interpreted this as being a 'tension myelocyst,' and suggested this may have formed as a result of cord compression and arachnoiditis, or that an osmotic effect could be operating, with protein within the cyst drawing in fluid from the subarachnoid space. Intra-operative contrast imaging has demonstrated communication between a syrinx cavity below the level of injury with the cavity above the injury.³⁷⁸ Nogués³³³ claims that there is no communication between the cyst and the central canal. Squier and Lehr⁴²³ reported communication with the central canal in one case, but no communication with CSF spaces in the other three cases they studied, although they did not study serial sections of the cystic spinal cords.

1.11.5 Arachnoiditis

According to McLaurin et al,²⁸⁴ Philippe and Oberthur believed that pachymeningitis was secondary to syringomyelia. Other authors have regarded syringomyelia as a consequence of the arachnoiditis. Many putative mechanisms have been similar to the theories that described arachnoiditis and syringomyelia after trauma; these will not be repeated.

Nogués³³³ proposed a vascular mechanism: due to interference with circulation through the constrictive fibrosis, ischaemia leads to necrosis and subsequent cyst formation. Arteritis may be an additional factor in cases following meningitis.^{333,403} Caplan et al⁶⁹ proposed a second mechanism—spinal block alters CSF flow with increase in pressure in the central canal. According to Williams, arachnoiditis may result in shear injuries, venous congestion and ischaemia to produce the initial cavity, which is then enlarged by ‘slosh.’^{441,495}

There is little evidence for communication between syrinxes associated with arachnoiditis and the subarachnoid space. Nogués claims that no communication exists between the cavity and the central canal or the subarachnoid space.³³³ A case of communication between the spinal subarachnoid space and a post-meningitic syrinx was described by Savoiaro.⁴⁰³

1.11.6 Birth trauma

It has been noted that many patients with syringomyelia were born after a difficult labour.^{330,492,495} Williams⁴⁹⁵ claimed that birth injury is the most common identifiable cause of syringomyelia. In response to a questionnaire, he found a higher incidence of difficult birth among patients with syringomyelia, compared with a control group.⁴⁹² Foetal hydrocephalus may result in difficult labour, but Williams⁴⁹² found no evidence for this among the 104 syringomyelia patients he studied.

Birth difficulty has been cited as a possible cause of hindbrain malformations, which then cause syringomyelia. Williams^{492,495} suggested that moulding of skull bones, basilar invagination, brain compression, brain swelling due to anoxia, intracranial haemorrhage and subsequent hydrocephalus could all be contributory. Prolonged labour, forceps delivery and abnormal birth have been associated with basal arachnoiditis, intraventricular haemorrhage and the Chiari I malformation.³³³ Alternatively, a traumatic birth may increase the cerebellar protrusion in an existing Chiari malformation, it may cause rupture of the ependyma of the

central canal, or haemorrhage may obstruct the flow of CSF and cause, or worsen, arachnoiditis.^{330,333,492}

1.11.7 Immunological

Blagodatsky et al⁵¹ found elevated levels of immunoglobulin (Ig) G, M or A in syrinx fluid in 16 of 26 patients. Higher concentrations of IgG were found in early (up to 3 years) and late (longer than 5 years) stages of the disease, while IgM was elevated only in the early stage. During the early stage of the disease, inflammatory infiltrates were found in the pia mater and immunohistochemical examination revealed specific staining for IgG. They concluded that immunopathological mechanisms played a role in addition to hydrodynamic factors.

1.11.8 Communication with the fourth ventricle

The existence of a communication between the fourth ventricle and the syrinx via the central canal is a crucial element of the theories of Gardner and Williams. Evidence for and against a communication has been accumulated pathologically, at operation and with imaging.

One of the difficulties in resolving the question of whether a communication exists has been the paucity of autopsy studies of syringomyelia. Hinokuma et al²⁰⁹ studied 18 cases and found communication with the fourth ventricle in six cases of Chiari II malformation, but not in the four cases of Chiari I or in the cases of spinal tumour or Dandy-Walker syndrome. Foster and Hudgson¹⁵⁴ reported four autopsy cases and found a communication in one. The case of Banerji and Millar²⁴ had no communication. Of the posttraumatic syringes studied by McLean et al²⁸⁵ at autopsy, operation or myelography, a communication could be demonstrated with the fourth ventricle in only a few cases. Peerless and Durward³⁵¹ state that autopsy and operative observations clearly demonstrate that there is not always a communication between a syrinx and the fourth ventricle. The case of Day et al¹⁰⁷ supports communication with the ventricular system in syringomyelia associated with spina bifida and

Chiari type II malformation. Rice-Edwards³⁷⁷ studied 20 cases, finding no evidence of communication with the fourth ventricle in three and a 'very narrow communication' in the remainder. He argued that these findings are incompatible with Gardner's theory.³⁷⁷ Milhorat et al²⁹⁶ recently reported a series of 105 autopsy cases. Syringes communicating with the fourth ventricle were found in association with hydrocephalus in 47 cases. The series included a large number of severe birth defects that influenced the number of communicating syringes. Cases with Chiari I malformation, basilar impression and arachnoiditis, did not have a communication between the syrinx and the fourth ventricle in 70% of cases.

Gardner and colleagues,^{169,171,175} and Ellertsson and Greitz¹²⁸ believed there was invariably a communication between the syrinx and the fourth ventricle. This view was based on the belief that a communication was always demonstrable at operation, and that postmortem shrinkage made the communication difficult to find. Conway⁹³ found a communication at surgery in six of 12 cases, although he described the cases without proven communication to have 'suspected' communication. In four, a communication was shown by the appearance of dye in the syrinx after injection into the lateral ventricle.⁹³ Krayenbühl and Benini²⁵⁰ reported seven cases with proven communication with the fourth ventricle. These cases responded well to ventricular shunting. Blagodatsky et al⁵¹ reported communication in 14 of 52 patients, demonstrated by contrast or air injected into the syrinx at operation. Hall et al¹⁹³ used radioisotope ventriculography to demonstrate the presence of a communication between the ventricles and the dilated central canal in myelodysplasia. They believed this was confirmatory evidence for Gardner's hypothesis.¹⁹³ Using contrast myelography, James et al²³² demonstrated communication between the fourth ventricle and the syrinx.

Current imaging techniques have not usually shown a communication between the syrinx and the fourth ventricle.¹⁸⁰ Milhorat et al³⁰⁶ found MRI evidence for a communication between the fourth ventricle and the syrinx in nine of 65 patients with syringomyelia. The

communicating group also had hydrocephalus^{302,306} and ventriculoperitoneal shunting in this group reduced syrinx size.³⁰² In a further study, Milhorat et al³¹¹ used MRI to examine 45 patients with hindbrain lesions and syringomyelia. In 33 without hydrocephalus, there was compression or deformity of the upper end of the spinal cord in all cases, and no MRI evidence of communication with the fourth ventricle. There was a communication with the fourth ventricle in all 12 cases with syringomyelia and hydrocephalus (all associated with basilar arachnoiditis or Dandy-Walker cyst). Park et al³⁴⁷ found only one of 13 patients had a communication between the syrinx and the fourth ventricle; other patients had a long segment of intervening normal cord. Aubin et al¹⁶ found evidence of communication with the fourth ventricle in five of 142 patients, but none of these had a Chiari malformation. Syrinx-free cord intervening between the foramen magnum and the syrinx was seen in 90% of the cases studied with MRI by Milhorat et al.³¹¹ Oldfield et al³⁴¹ reported MRI and intraoperative ultrasound studies of seven cases. They found no evidence of communication with the fourth ventricle.

1.12 Animal models

There are numerous animal experimental models of syringomyelia. Each will be reviewed with reference to the relevant pathogenetic theories.

1.12.1 Spontaneous

Syringomyelia has been observed in many animals, but it is a rare occurrence.⁴³² It is usually seen in association with dysraphic disorders. Foster¹⁵² describes reports of cases in the Rex rabbit (autosomal recessive genetics), fox terriers and bulldogs (autosomal dominant), cattle and rhesus monkeys. There are case reports of syringomyelia in a horse¹⁹⁹ and in a panther.¹⁹ A genetically-determined spinal dysraphic syndrome that may be accompanied by hydromyelia and syringomyelia is seen in Weimaraner dogs.⁴³² None of these species has

been used in a systematic study of the pathogenesis of syringomyelia but it is likely that syrinxes form in these animals by mechanisms similar to those in human dysraphic disorders.

1.12.2 Kaolin hydrocephalus

Early models of syringomyelia evolved from studies of hydrocephalus. The first experimental models of hydrocephalus were based on occlusion of the aqueduct of Sylvius either by a cotton pledget or with an inflatable balloon.^{91,290,291,298} Ventricular enlargement and raised intracranial pressure is followed by compensation either by reduction of CSF production or by parenchymal absorption of CSF.^{91,291,298} Experimental hydrocephalus has also been produced by obstructing the outlets of the fourth ventricle with arachnoiditis caused by cisternal injection of kaolin.^{78,126,255,323,409} Kaolin is a fine, hydrated aluminium silicate earth which produces progressive, granulomatous sclerosing arachnoiditis when injected in the subarachnoid space.^{196,409} When it is injected into the cisterna magna, a fibrous subarachnoid cuff forms at the craniocervical junction.³⁷⁰ In animals developing hydrocephalus after cisternal kaolin injection, the intracranial pressure rises and the cerebral ventricles dilate. Within three weeks compensation occurs and the intracranial pressure returns toward the normal range.³²³

It was initially thought that in these models of hydrocephalus, absorption of CSF occurred through the ventricular ependyma and via the parenchymal vessels.^{126,298} However, as the ventricles dilate the central canal also dilates and it was therefore suggested that CSF flows down the central canal to gain access to the subarachnoid space at the dorsal end of the cord.^{255,323} Experiments by Eisenberg et al¹²⁶ demonstrated that CSF does flow down the dilated central canal and is absorbed from the spinal subarachnoid space. It has also been shown that if the spinal cord is ligated in an animal with kaolin-induced hydrocephalus CSF absorption no longer occurs, the intracranial pressure rises, and the animal dies.^{126,191,323} This has been interpreted as evidence against a compensatory absorption of CSF from the

parenchyma or vessels around the central canal, and supporting a flow out into the subarachnoid space at the conus.³²³ In contrast, James et al²³¹ produced a model of communicating hydrocephalus in dogs in which the central canal did not dilate. The authors concluded that dilatation of the central canal was not a compensatory mechanism of CSF absorption in this model.

The first animal model of syringomyelia was reported in 1954 by McLaurin et al.²⁸⁴ They injected kaolin or ethyl iodophenylundecylate (Pantopaque) into the cisterna magna of dogs. Arachnoiditis developed around the base of the brain, posterior fossa and upper spinal cord. In addition to hydrocephalus, 25 of the 39 dogs developed macroscopic cavitation of the spinal cord. Pathological changes included an adhesive collagenous arachnoiditis completely surrounding the cord and necrosis of the posterior portion of the cord, particularly involving the posterior horns. These changes occurred as early as nine days after injection. A dilated central canal was often seen at four to five weeks and cavities separate from the central canal were seen later. The pathological processes were most marked in the cervical region but were also seen in the thoracic cord. These authors concluded that the meningitis caused ischaemia by compressing subarachnoid vessels and that the pattern of necrosis was due to susceptibility of these regions to ischaemia. They did not consider the possibility of central canal enlargement being secondary to hydrocephalus. They did however demonstrate communication between the ventricles and the syrinx by injecting Evans blue prior to sacrificing the animals.

Hall et al^{191,196} produced hydrocephalus and syringomyelia in dogs after injecting kaolin into the cisterna magna, and compared the results with an experimental model of ischaemic myelopathy. In the kaolin group, the central canal dilated and this was followed by extension of fluid-filled spaces dissecting into the surrounding grey matter. In some cases the central canal disappeared and patches of ependyma were scattered in the walls of the cyst.

Cavitation generally extended into the dorsal horns. Neurones were not affected. In comparison, the ischaemic group had loss of neurones, with necrosis of the grey matter, but the central canal remained unchanged. They therefore disputed the hypothesis of McLaurin et al²⁸⁴ that the spinal cord cavities in this model were the result of ischaemia. Instead, they argued that these results supported Gardner's hydrodynamic theory. In a further study, Hall et al¹⁹⁴ used radioisotopes to demonstrate that compensatory enlargement of the central canal occurs rapidly following experimental hydrocephalus and that parenchymal cavities originate from the enlarged central canal. This study also demonstrated a communication between the distal central canal and the lumbar subarachnoid space. Since most experimental syringes develop in grey matter, Hall et al¹⁹¹ suggested that the structural strength of white matter is greater than that of the grey matter.

Hall et al¹⁹⁷ simultaneously measured the pressures in the ventricles, the subarachnoid space and the syrinx in dogs with kaolin-induced hydrocephalus and syringomyelia and found a complete ventriculo-subarachnoid block. In addition, there was a higher baseline pressure in the syrinx than in the ventricle or subarachnoid space. Raising ventricular pressure increased the syrinx pressure but aspiration of ventricular fluid did not acutely lower the syrinx pressure. They concluded that a ventriculo-syrinx valve effect was operating to inflate the syrinx during transient rises of ventricular pressure and that transmission of thoracic pressures to the spinal subarachnoid space with compression of the cord may enlarge syringes in a process similar to Williams' 'slosh.'

Nakamura et al³²³ studied the resistance to flow of CSF at the spinal level in kaolin-induced hydrocephalic cats. They cannulated the dilated central canal of hydrocephalic cats by inserting a blunt needle into the rostral opening of the central canal; in order to access the canal they had to resect parts of the cerebellum and occipital lobes. Evans blue solution was then infused into the canal at various rates. During infusion at 0.022 or 0.043 mL/min (at a

pressure of 8 cm to 15 cm H₂O), dye appeared on the posterior surface of the lumbar spinal cord. Dye was present in the central canal in all specimens, and the dorsal columns were preferentially stained to varying degrees. They concluded that in hydrocephalic cats, the spinal cord central canal dilates to allow flow of CSF into the spinal subarachnoid space.

Becker et al⁴¹ used a cat model of hydrocephalus and syringomyelia produced by injecting kaolin into the cisterna magna. One aim of their study was to determine whether occluding the central canal protected the animal from developing syringomyelia. In one group of animals they plugged the opening of the central canal with cotton soaked in kaolin and coated with dental cement and in another group they 'isolated' the central canal by also occluding the filum terminale by extradural ligation at the sacral level. Hydrocephalus developed as early as two weeks after kaolin injection, while syringomyelia developed later, becoming progressively more marked by eight weeks. Degenerative changes were noted in the dorsal columns of the cervical region. Syrinxes communicating with the central canal were present, usually in the cervical region, but also in the thoracic and lumbar regions. They found syringomyelia in cats with hydrocephalus, but no syringomyelia in cats up to eight weeks after occlusion of the central canal opening. In cats with 'central canal isolation,' no syrinxes developed with or without kaolin hydrocephalus, but the canal remained patent. These cats were followed for a maximum of eight weeks—this may not have been long enough for canal dilatation in the animals with just isolation of the canal, or the isolation may not have been complete. Becker et al⁴¹ believed these experimental results supported Gardner's concepts.

Chakraborty et al⁷⁸ studied the ultrastructural changes in a cisternal kaolin model of syringomyelia in the rabbit. The ventral ependyma of the central canal was flattened and stretched, while the dorsal ependyma was split, with the syrinx extending through the dorsal median plane. There was oedema in the subependymal region and around the posterior

median septum. The perivascular spaces were enlarged, especially near the central canal and in the dorsal white matter.

Williams et al^{496,504} used a kaolin hydrocephalus canine model to measure the cranial and spinal pressures. They found a higher pressure in the ventricle than in the spine, and in some cases isolation of pressure transmission between the cavities. These results may have been influenced by the loss of spinal CSF during cannula insertion in this technique.⁴⁹⁶ Occasionally the syrinxes communicated with the subarachnoid space at the filum terminale. In their initial study,⁵⁰⁴ 11 of 16 dogs developed cord cavitation. Hydrocephalus was sometimes found without cord cavitation, but cavitation was not present without hydrocephalus.

Ikata et al²²² used the lumbo-cisternal infusion technique to study penetration into the cord of various tracer molecules injected into the subarachnoid space of rats with kaolin-induced syringomyelia. They found that tracers diffusely penetrated the spinal cord and were present in the extracellular space, but they did not study routes of tracer movement in any detail. Tracers were perfused in the subarachnoid space for one hour, so any possible rapid movement of tracers into the cord was not studied.

1.12.3 Intramedullary fluid injection

Williams and Weller⁵⁰⁹ repeatedly injected saline or CSF into the spinal cord of dogs, via a catheter inserted into the dorsal substance of the cord at the lower thoracic level. A syrinx was found in each of the four dogs studied. The syrinxes were usually situated between the dorsal horns and dorsal to the grey commissure. The cervical region was more prone to syrinx development. At some level in each of the dogs, the cavity was shown to communicate with the central canal. Around the cavities was oedema producing a spongy appearance within the tissue. The authors claim this is similar to the histological appearance seen in humans who die soon after acute extensions of a syrinx cavity. There was adhesive

arachnoiditis at the site of catheter insertion, but Williams and Weller did not believe this contributed to the syrinx development. Since the fluid was not injected into the central canal, and the canal communicated with each of the syrinxes, they concluded that the central canal is not necessarily opened from within, but that a cavity may lay it open and distend it.

1.12.4 Kaolin spinal subarachnoid block and trauma

Cho et al,⁸⁵ used a rabbit model of syringomyelia. They compared the frequency of syrinx production after spinal trauma alone, and after spinal trauma and subarachnoid kaolin injection. Syrinxes were usually situated in the posterolateral aspect of the spinal cord. All but one syrinx was separate from the central canal; they all were connected to the fibroglial scar at the site of the injury. They found a statistically significant higher incidence of syrinx formation in the kaolin plus trauma group than in the trauma only group. Injection of spinal subarachnoid kaolin only did not produce any syrinxes. Their impression was that arachnoiditis was also present at the levels at which syrinxes formed. They concluded that arachnoiditis favours the production of posttraumatic syringomyelia and suggested that the fluid entered via the dorsal root entry zones in the presence of raised pressure in the subarachnoid space proximal to the block. It was proposed that pressure changes in the venous system via the action of intraspinal veins on CSF from exercise, coughing or straining are transmitted to the cavity and lead to its expansion.

1.12.5 Haematomyelia

In a rat model of haematomyelia produced by injecting blood into the dorsal columns of the spinal cord, Milhorat et al²⁹⁴ found blood within the central canal and extending predominantly rostrally to the fourth ventricle within 2-6 hours of the injection. During the next 15 days, cellular debris, proteinaceous material and fibrin were seen in the central canal rostral to the injection site. They described this as the 'sink action' of the central canal and claimed the rostral direction was not surprising considering the caudal end of the canal was

closed. They postulated that the ependymal cilia may act in directing the rostral flow in the canal.

1.12.6 Intramedullary kaolin

Milhorat et al³¹³ injected kaolin into the dorsal columns of the spinal cord in rats and found rostral drainage of kaolin crystals in the central canal with the production of septa and synechiae and acute dilatation of obstructed segments of the central canal. Syringes gradually developed caudal to the level of obstruction. The experimental technique involved injection of 1.2 to 1.6 µl of kaolin into the dorsal columns of the spinal cord at C6. Kaolin crystals and polymorphonuclear leucocytes entered the central canal within 24 hours and drained rostrally. Ependymal cell proliferation was induced, which formed synechiae and obstruction of the central canal at the level of the injection or above it. The central canal caudal to this obstruction became massively dilated within six weeks. There was no evidence of communication with the fourth ventricle and there was no hydrocephalus. Proposed sources of syrinx fluid included CSF entering via the Virchow-Robin spaces or dorsal roots, inflammatory products, ependymal secretion and interstitial fluid derived from metabolism. They claim the results of this study add further weight to the 'sink action' theory of the central canal.

1.12.7 Focal spinal cord lesions

Focal lesions of the dorsal columns have been used to produce cystic cavities. Yeziarski et al⁵¹⁶ injected quisqualic acid in an attempt to study the effects of this excitatory amino acid on the neurones in the spinal cord. In addition to neuronal death occurring at the site of injection, spinal cord cysts were found in 23 of 25 animals injected with the amino acid. Large cysts also formed at the site of photochemically induced spinal cord injury reported by Bunge et al.⁶⁶ These models have not been used to study the pathogenesis of syringomyelia.

1.12.8 Other models

Hydromyelia, with other CNS malformations, occurs in the offspring of mice infected with haemagglutinating virus of Japan in early pregnancy.³³⁹ Malformations, including hydromyelia, are also seen after injection of various neurotropic drugs in pregnant mice.²³⁶ These animals have not been used to study syringomyelia.

Models of spinal cord compression have not resulted in syringomyelia, or at least syringomyelia was not reported.^{7,439,445}

1.13 Summary

Syringomyelia is an uncommon disease that affects mainly young adults and causes disabling neurological deficits. Various ontogenetic theories have been proposed, but no single theory explains all syrinxes. Each theory either lacks objective supporting evidence or has evidence against it. The animal model most commonly used—cisternal kaolin with hydrocephalus—has been used as evidence for the theories of Gardner and Williams, despite syringomyelia in communication with the fourth ventricle being less common than ‘non-communicating’ syringomyelia. The recent work of Milhorat and his colleagues has suggested that fluid enters the central canal, and presumably syrinxes, from the spinal cord parenchyma. The origin of this fluid remains unknown.

Various surgical treatments have been used; none has been successful in a majority of patients over the long term. This lack of an effective treatment for syringomyelia reflects a lack of understanding of its pathophysiology. Even where treatments are successful, the mechanism of this success is not known. It is self-evident that an improved understanding of the pathophysiology of syringomyelia is necessary to improve its treatment.

2 Anatomy and physiology of CNS extracellular fluid and CSF

The normal physiology of human CNS extracellular fluid and CSF remains largely enigmatic. Not only are these fluids particularly difficult to study in experimental animals, but the results may also be inapplicable to humans.¹¹⁰ Studies in humans are even more difficult since inadequate fixation vitiates the examination of relevant neuroanatomy, such as central canal openings. Nevertheless, much information has been obtained that may have bearing on the pathophysiology of syringomyelia. It is not intended for this review to provide a comprehensive discussion of CNS fluids; rather, their physiology and anatomical spaces will be discussed in relation to the pathophysiology of syringomyelia.

2.1 Development of CSF spaces and the choroid plexuses

Amniotic fluid bathes the developing neuroepithelium until the closure of the neural tube in the fourth or fifth week.^{152,170} CSF then fills the cavity of the neural tube and is an ultrafiltrate of plasma until the choroid plexuses form, when it becomes a secreted fluid.^{133,170} The choroid plexuses develop at sites where the wall of the neural tube is attenuated—in the roof of the third and fourth ventricles and the medial walls of the lateral ventricles. The plexus of the fourth ventricle is the first to develop (as early as four weeks), while the lateral ventricle choroid plexus fills its ventricle by the end of the second month.^{133,399} According to Epstein and Johanson,¹³³ the prominence of the plexuses at this stage is indicative of their importance in brain development. The plexuses undergo notable developmental changes: the primary villi form in the later stages of foetal development and mitochondria, which drive the secretory process, become numerous in the choroidal cells of infants several days old. After birth, the capillary network becomes more intricate and the villi expand.¹³³

The ventricles develop from the neural canal^{258,399} and the developing fourth ventricle roof is initially permeable to CSF.^{152,170,336} The foramen of Magendie appears at approximately the seventh week but it is not known when the foramina of Luschka open.¹³³ It

was traditionally thought that these openings developed as a result of CSF pressure from the actively-secreting choroid plexuses, but there is now evidence that the foramina do not form in this way.¹³³ It is also now apparent that the subarachnoid space forms prior to the choroid plexuses, implying that the space does not form as a result of dissection of the meninx primitiva by CSF flowing out of the fourth ventricle under pressure.¹³³

2.2 The central canal

The neural tube forms with the closure of the neural folds after day 22.²⁵⁸ The lumen, or neural canal, eventually forms the ventricles and the central canal. The neural canal initially opens into the amniotic cavity at each end through neuropores.²⁵⁸ The neuropores diminish in size as neurulation continues and the caudal neuropore closes on day 26. The spinal cord caudal to the caudal neuropore forms by a different mechanism. It forms from the caudal eminence by the process of secondary neurulation,²⁵⁸ where a solid neural cord is formed within the caudal eminence and the central canal forms by cavitation. This process is complete by six weeks.²⁵⁸

At the beginning of the second month the central canal is large relative to the size of the cord, but from then on it decreases in relative and actual size.³²⁹ The ventral ependyma of the central canal is induced by the notochord to form the floor plate, which retains contact with the ventral surface of the spinal cord as the cord grows, forming the ventral median septum.⁴⁰⁰ It appears that foetal central canal ependymal cells also play an active secretory role in the programming of neural developmental events.^{400,401}

2.2.1 Central canal patency

The central canal is present in all vertebrates. It is patent in non-mammalian vertebrates and in almost all mammals, including cats,^{41,223} dogs,^{231,509} monkeys,²²³ rabbits,²²³ and rats,⁶⁵ but apparently not in the house shrew, whale or dolphin.²²⁷ The size of the central canal varies amongst species. According to Bradbury and Latham,⁵⁶ the central canal in

rabbits has an average cross sectional area of 0.025 mm². Ikegami and Morita²²³ found the monkey central canal to be much smaller than the rabbit's.

The normal rat central canal has been studied by Bruni and Reddy.⁶⁵ In the cervical region it is round or oval in shape, and located in the central grey matter with the grey commissure being narrower dorsally than ventrally. From the thoracic to lumbar and conus levels, the central canal is 'collapsed' and assumes a dorsoventrally-elongated shape. The central canal in the filum is variable in shape and occupies a larger cross sectional proportion of the cord than at other levels. The central canal lining usually consists of a single layer of pseudostratified cuboid to columnar ependymal cells, but in some areas the lining is up to four layers thick. Some ependymal cells abut directly on the abluminal basal lamina of capillary perivascular spaces and blood vessels are numerous in the region of the central canal.⁶⁵

Contemporary dogma holds that unlike that in other animals, the central canal in humans does not remain patent throughout life^{41,152,333,337,399,489} and that there is normally no fluid flow within it.^{180,399} The few reports of human central canal morphology usually have not been systematic studies of different cord levels. Foster¹⁵² stated that the normal human central canal is only vestigial in adults, but he also described the frequent finding of a patent canal at autopsy in otherwise normal individuals. Ollivier¹¹⁵ believed that the normal human central canal was obliterated. In contrast, Stilling⁴²⁷ demonstrated that a small central canal is usually present in adults. Conway⁹³ stated that 71% to 80% of normal subjects have a closed, vestigial central canal. Gardner et al¹⁷³ examined the conus medullaris and filum terminale in autopsy specimens of 11 adult humans. The central canal was closed in eight, patent in two and dilated in one.¹⁷³ Netsky examined 24 infant spinal cords; in each case the central canal was round or oval and patent.³²⁹ In his study of 50 adult cords, Netsky³²⁹ found a patency of 20%, while the remainder were partially or totally obliterated. Cornil and Mosinger⁹⁴ examined 66 adult cords and found a patent canal in 19. Newman et al³³⁰ found the central

canal to be patent in children and in adults up to the fifth decade. Kasantikul et al²³⁹ believed the canal to be patent in the first two decades of life but occluded by glial and ependymal proliferation thereafter. The cases of central canal rupture following sudden intracranial hypertension reported by Leramo and Rewcastle²⁶⁴ support the existence of a patent canal; they claim that rupture in these cases is caused by pressure transmission down a patent central canal.²⁶⁴

Milhorat et al^{308,309} recently reported a comprehensive study of the central canal in humans. They studied 232 cords at autopsy, ranging in age from aborted fetuses to 92 yrs. Stenosis of the central canal was more complete and in more levels in older individuals. The highest grades of stenosis involved T2-T8, with relative sparing of the rostral and caudal ends of the canal. The pathological changes of central canal stenosis were similar to those seen in aqueduct stenosis. Occlusion of the entire central canal was present in only four individuals. Four occult syrinxes were found, and each was defined rostrally and caudally by central canal stenosis. These authors concluded that stenosis of the central canal with age may explain why focal syrinxes are seen in adults and holocord syrinxes are seen in children. They also suggested that stenosis of the canal may prevent normal circulation of CSF and impair the functioning of CSF-contacting neurones in the spinal cord, which possibly contributes to constipation, impotence and orthostatic hypotension.

The postulated causes of canal occlusion or stenosis have included viral or bacterial infection, trauma, rupture of the ependymal lining due to raised CSF pressure,²³⁹ haemorrhage, and neoplastic seeding.³⁰⁹ In support of an infectious aetiology, it has been demonstrated that selective involvement of ependymal cells in subclinical measles,²³⁹ influenza A and pox virus,³¹⁴ and parainfluenza 2, mumps and reovirus type 1^{307,309,402} is common. The pathophysiology of central canal occlusion may be similar to aqueduct stenosis, where similar aetiological factors have been cited.^{257,293,307}

A detailed study of ependymal infection with reovirus type I has been reported by Milhorat and Kotzen.³⁰⁷ After inoculating suckling hamsters with reovirus type I, they found inflammatory changes in the ependyma of the cerebral ventricles, aqueduct of Sylvius, fourth ventricle and the central canal. There was no evidence of inflammation in the neural parenchyma or leptomeninges. Healing of the ependyma was associated with obstruction of the angles of the lateral ventricles, the foramen of Monro, the aqueduct of Sylvius, the outlets of the fourth ventricle and the central canal. The central canal ependyma became disorganised and there was proliferation of subependymal astrocytes and capillaries with intracanalicular gliosis. Gliovascular buds were seen adjacent to areas of ependymal ulceration. Ependymal cells eventually became densely packed or divided into islands within an enveloping sea of glial fibres.

2.2.2 The terminal ventricle and caudal openings

The ventriculus terminalis does not form by neurulation. Degeneration and the formation of vacuoles within a cell mass caudal to the posterior neuropore forms the caudal central canal and the terminal ventricle.⁴¹⁵ Retrogressive differentiation forms the filum terminale and shrinks the terminal ventricle.²⁸⁶

The central canal in the conus medullaris and filum has not been extensively studied. In most vertebrates, the central canal dilates in the conus or filum to form the terminal ventricle, which may have a functional relationship to Reissner's fibre⁸⁶ (*vide infra*). In their study of 23 human cords, Choi et al⁸⁶ found the terminal ventricle at the lower end of the conus medullaris. The terminal ventricle reaches maximum size after age two, so it is a cavity that grows along with the rest of the CNS.⁸⁶ Lendon and Emery²⁶³ found forking of the central canal in the conus or filum in 46% of children. There were dorsoventral duplications of the canal that began in the conus medullaris and continued into the terminal ventricle but rarely further. Occasionally there were also outpouchings.

The presence of an opening from the ventriculus terminalis into the subarachnoid space has been demonstrated in several species. Bradbury and Latham found wide connections between the central canal in the filum terminale and the subarachnoid space in rabbits.⁵⁶ Similar communications have also been shown in the guinea pig and rhesus monkey.³²⁴ Nakayama³²⁴ used electron microscopy to demonstrate a dorsal opening of the central canal in rabbits and rats, and a pial-lined communication in guinea-pigs. Becker et al⁴¹ perfused the central canal of cats with trypan blue and found dye flowed from the filum terminale into the subarachnoid space; histological study revealed a single layer of cells separating the central canal and the subarachnoid space. A direct opening into the subarachnoid space was found in crab-eating monkeys by Ikegami and Morita,²²³ who also noted that the region of the opening was highly vascularised. There have been few studies in humans: Suzuki⁴³⁴ reported an opening in the filum terminale and Sakata et al³⁹³ demonstrated a caudal aperture of the central canal in eight human (one a condemned criminal whose body was perfused with fixative within 10 minutes of death) and two macaque monkey spinal cords. According to Sakata et al,³⁹³ ependymal cells at the openings are continuous with the cytoplasmic processes of pial fibroblasts, sharing a common basal lamina.

Study of the human central canal, and in particular its communications with the subarachnoid space, is hampered by: 1) the difficulty in obtaining adequately-fixed spinal cord tissue; and 2) the difficulty in reconstructing the longitudinal morphology of the canal from routine transverse histological sections. Sakata et al³⁹³ used computer three-dimensional (3-D) reconstruction of the central canal in the conus and filum to study the morphology of the central canal, the terminal ventricle and the caudal opening. They did not give details of the computer technique, but it appears they used manual outlining of the central canal and the pia before using a computer to produce 3-D reconstructions. They seem to have been limited to reconstructing a small number of sections, taken from intervals of 100 μm to 250 μm . Using

this technique and light and electron microscopy, they demonstrated a caudal opening of the filum terminale central canal into the subarachnoid space and found that the central canal apparently becomes wider and Y-shaped as it approaches the terminal ventricle.

2.2.3 Imaging

The central region of the spinal cord demonstrates signal characteristics on MRI similar to water (or CSF). This signal has been attributed to the central grey matter, rather than the central canal.⁴⁸⁵ MRI of formalin fixed spinal cords confirms that the central grey matter has a higher signal than the surrounding white matter,^{47,421} but the central canal was not mentioned in these studies. These results must be treated with caution, because in addition to shrinkage with formalin,^{175,262,421} the signal intensity of spinal cord white and grey matter is altered considerably after formalin fixation.^{74,189,420} The central canal of the medulla has been described as an oval tubule on axial magnetic resonance (MR) sections,³¹¹ but these authors did not describe the appearances of the spinal cord. Curtin et al⁹⁸ compared MR images of cadaver spinal cords with histological specimens, but concentrated on technical imaging factors and did not mention the central canal.

Intraoperative ultrasound has been used to image the spinal cord and it has been claimed that the central canal can be demonstrated.^{113,277,424} Nelson et al³²⁸ have shown the central echo complex is actually produced by the interface between the ventral white commissure and the central end of the anterior median fissure.

2.2.4 Ependyma and cilia

Ependymal cells are ciliated in all vertebrates studied. Cilia have been studied in goldfish, frogs, turtles, birds, rats and rabbits,³²⁵ possums,^{454,455} and monkeys.¹³⁵ Cilia are present in human foetal ependymal cells, and these persist in some areas in the adult.³⁸¹ Cilia in different parts of the ventricular system have different frequencies of beating, but unlike tracheal cilia, it appears they are unable to move particles.³⁸⁷ According to several

authors,^{327,387,453} ventricular cilia do not seem to result in bulk flow of fluid, but may have a role in reducing the unstirred layer and thereby improving non-synaptic neurotransmission. Other authors believe that the cilia do cause bulk CSF flow.⁵⁶ Each species has a characteristic number of cilia but in all species the polarity of the central canal cilia is caudad. Nakayama and Kohno³²⁵ believed this is evidence for a cilia-propelled caudad flow of CSF in the central canal. Cifuentes et al⁸⁸ also considered that ciliary movement accounts for a caudad CSF flow in the rat central canal, as did Bradbury and Latham for the rabbit⁵⁶ and Nakayama³²⁴ and Nakayama and Kohno³²⁵ for rabbits and rats. In contrast, Milhorat et al³⁰¹ suggested that cilia in the central canal may direct CSF cephalad and that cilia in the ventricles direct CSF toward the fourth ventricle.

It was once held that the ependymal lining prevents fluid passing from the central canal into the surrounding parenchyma.¹⁵² In adult vertebrates however, the ependymal cells are joined by gap junctions, allowing equilibration of CSF and interstitial fluid.¹³³ In some stages of foetal development, the ependymal cells are joined by tight junctions, suggesting that fluid movement is regulated at these stages.¹³³ The ependyma of the central canal in lower vertebrates (teleosts and urodeles) has tight junctions.⁶⁵

It has been suggested that postnatal ependyma does not have the capacity to regenerate,⁴⁰² but ependyma in immature and adult rats does have a regenerative capacity that can be evoked by local injury.^{64,65,177} Tearing of the ependyma in the ventricles leaves discontinuities that become filled with glial processes.⁴⁰²

2.2.5 CSF-contacting neurones

Tanycytes in the floor of the third ventricle connect hypothalamic nuclei with the ventricular surface.³⁸¹ The ventricular surface of tanycytes has microvilli, rather than cilia³⁸¹ and the ventricular lining in this region is characterised by tight junctions. Tanycytes may function as conduits for hormones from the CSF to the hypothalamic nuclei or vice versa.³⁸¹

There are neurones that project into the lumen of the central canal in many mammals, including primates.^{9,65,135,223,379,468} These CSF-contacting neurones are especially numerous in the terminal ventricle.⁴⁶⁸ It has been suggested that these neurones may interact with Reissner's fibre since they project processes into the canal where they touch Reissner's fibre.³⁷⁹ They may be involved in feedback to the subcommissural organ regarding changes in CSF flow or pressure.^{379,468} Neurones around the central canal also project to the reticular formation in the medulla and pons.⁶⁵

2.2.6 Subcommissural organ and Reissner's fibre

The subcommissural organ (SCO) is an enigmatic, ancient, and persistent structure of the vertebrate brain that consists of two populations of secretory cells.^{344,379} Unlike other circumventricular organs, it does not have fenestrated capillary endothelial cells and appears to have an effective blood-brain barrier.³⁷⁹ In addition, there appears to be a CSF-subcommissural organ barrier,³⁷⁹ isolating the cells within a double barrier system. In humans, the subcommissural organ reaches its maximum size during foetal development and regresses around puberty.^{75,379} In Old and New World monkeys, the subcommissural organ is conspicuous and rich in secretion.³⁷⁹

Reissner's fibre is a glycoprotein thread-like structure produced by the subcommissural organ, remaining attached to it and extending to the caudal end of the central canal.^{75,379,455} There is evidence that CSF hydrodynamics play a role in the formation of Reissner's fibre and that secretion from central canal ependymal cells adds to the fibre.^{379,455} — amorphous flocculent material is found in the CSF of the central canal and it adheres to the surface of the fibre.¹³⁴ Reissner's fibre grows in a caudad direction at a rate of 1% to 10% of its length per day and the distal end of the fibre partially fills the terminal ventricle, where in lower vertebrates it is probably absorbed into blood vessels.³⁷⁹ In higher vertebrates, there is

evidence that the fibre passes through a dorsal opening of the terminal ventricle into the subarachnoid space.^{324,379,455}

Reissner's fibre is present in almost all vertebrates studied, including all non-human primates.¹³⁴ It has been reported to be absent from the spinal cord of bats, camels, and man,³⁷⁹ although some authors have reported the fibre's presence in man.^{224,242} Woollam and Collins⁵¹⁴ reported that Reissner's fibre is not present in the rat spinal cord, but this may have been due to problems in preparing the spinal cord central canal for electron microscopy,⁴⁵⁵ as it has been demonstrated in the rat by other authors.^{246,511} Reissner's fibre is present in sheep.³⁴⁴

The function of the subcommissural organ and Reissner's fibre is unknown. Reissner's fibre may interact with ependymal cells—fibres project from the surface of ependymal cells to Reissner's fibre.³⁷⁹ It has been suggested that Reissner's fibre may act as a detoxifier of the CSF, since it has a great capacity to adhere particulate materials and biogenic amines.³⁷⁹ The fibre may be involved in regulation of CSF production and flow by providing information about CSF flow to CSF-contacting neurones.^{379,468}

2.3 Formation of CSF

The estimated volume of CSF in the human nervous system is 120 mL and daily production is about 500 mL.³⁸¹ The concept that the choroid plexus produces CSF was accepted early in this century, but controversy remains over the relative importance of other sources of production.^{184,266}

2.3.1 Formation of CSF by the choroid plexus

According to many authors, the choroid plexus forms the bulk of the CSF.^{381,435} It is lined by a single layer of cuboidal cells that have apical microvilli and tight junctions joining their apical surfaces.^{282,381} A fenestrated capillary is at the centre of each villus.²⁸² The blood-CSF barrier results from the tight junctions of the epithelial cells, not from the vascular

endothelium.²⁸² Sweet et al⁴³⁵ propose that although the choroid plexuses are responsible for the bulk of CSF production, their main role is to secrete electrolytes into the CSF. Accordingly, water exchange, but not electrolyte exchange, occurs rapidly at other sites in the CSF pathways.⁴³⁵ Levin et al²⁶⁶ postulate that the main function of the choroid plexus may be the active clearance of substances from the CSF, whereas the production and regulation of the fluid composition of the CSF may be reserved for endothelial cells of the cerebral parenchymal vasculature.

2.3.2 Extrachoroidal formation of CSF

There is ample evidence for an extrachoroidal production of 30% to 60% of total CSF^{95,266,282,292,300,361,381,382} The site of origin of extrachoroidal CSF is not known. McComb²⁸² claimed that the brain parenchyma is the major source, while Epstein and Johanson¹³³ suggested that extrachoroidal formation of CSF in adults is probably by persistence of the embryonic pattern of CSF production. Suggested sources for extrachoroidal fluid include capillary endothelial secretion, ependymal secretion and arachnoidal secretion.¹⁰⁶ Cserr and Knopf⁹⁶ proposed that secretion of fluid by cerebral capillary endothelium through the coupled transport of solutes and water, generates a driving force for bulk flow of interstitial fluid from brain to CSF, preferentially via the perivascular spaces.

2.4 Absorption of CSF

2.4.1 Absorption of CSF by arachnoid villi

The hypothesis that CSF is absorbed by the arachnoid villi was first proposed in 1875 by Key and Retzius.²⁴³ Weed⁴⁷⁸ believed the driving force is the difference in osmotic pressure between CSF and blood, while Davson^{104,105} argued that hydrostatic mechanisms must predominate, since proteins and other large molecules are able to pass from CSF into blood. Although it is accepted that CSF does drain through arachnoid villi into venous sinuses,¹⁰⁵ the mechanism and quantity of drainage remain unknown. Theories of the

drainage mechanism include arachnoid villi acting as valves,⁴⁸² villus cells phagocytosing CSF and CSF passing through villus cells by vacuolation.¹⁰⁵

Following the demonstration in 1964 by Di Chiro¹⁰⁹ that isotope injected into the lumbar subarachnoid space flows up to the vertex, standard teaching has been that CSF drainage is primarily via the cranial arachnoid villi.^{104,282,381} Several authors have challenged the interpretation of these findings. Greitz¹⁸⁴ claimed that the concentration of isotope at the vertex is due to dilution of the tracer in the subarachnoid spaces around the base of the brain by fresh CSF pouring out of the fourth ventricle. Schossberger and Touya⁴⁰⁷ argue that the apparent accumulation of tracer toward the parasagittal area is due to filling of large CSF spaces in the fissures and sulci, rather than flow to the arachnoid villi. In some experimental animals (cats and dogs), the bulk flow over the cerebral hemispheres seems to be very slow, indicating that drainage of CSF into the superior sagittal sinus may not be important, at least in these species.²⁸²

2.4.2 Absorption of CSF at other sites

Aside from cranial arachnoid villi, possible sites for CSF absorption* include lymphatics around meninges and nerve roots, blood vessels in the meninges, and arachnoid villi in spinal nerve roots. In at least some species (sheep, rabbits, cats and dogs), it appears that a significant proportion of CSF drains via the distal ends of cranial nerves, including the optic¹³⁷ and olfactory,¹³⁶ into the extracerebral lymphatics.^{136,282,381} Experimental studies in animals indicate that 25% to 67% of the CSF drains into the lymphatic system with the olfactory pathway being the prime route.^{96,136,282,520} The movement of tracers from the subarachnoid space to cervical lymph nodes occurs rapidly—India ink was found in lymph nodes five minutes after injection in one rat study.⁵²⁰ Zenker et al⁵¹⁹ used ferritin tracer studies in rats to present evidence for widespread absorption of CSF into the lymphatics and

* Leonardo da Vinci believed that "...the sieve (cribriform plate)...discharges the superfluous humours of the head into the nose."⁴⁴⁸

blood vessels of the meninges. Finally, arachnoid villi are present around spinal nerve roots in experimental animals and humans, and may therefore be a source of drainage.^{244,390,422} None of these possible pathways has been confirmed in humans.³⁸¹

2.5 Flow of CSF and extracellular fluid

2.5.1 CSF flow in the subarachnoid space

CSF from the cerebral ventricles exits via the foramina of the fourth ventricle into the cisterna magna. Its subsequent flow within the subarachnoid space is a matter of some controversy. Dyes injected into the lateral ventricles appear in the cisterna magna within a few minutes and reach the lumbar CSF within 40 minutes.¹¹⁰ According to Rosenberg³⁸¹ and Davson et al,¹⁰⁵ CSF leaving the fourth ventricle follows one of two patterns: it can move up over the convexities to the arachnoid villi, or it can mix with the CSF in the spine subsequently to be removed via spinal vascular structures or to pass back up to the hemispheres. Based on experiments in rhesus monkeys, Di Chiro et al¹¹¹ claimed that CSF exits the fourth ventricle and separates into two components: 1) exiting only through the foramen of Magendie and descending posterior to the cord, and 2) exiting from all foramina of the fourth ventricle to ascend around the brain stem. Milhorat and Clark²⁹⁷ reported that a large proportion of the CSF flows up through the perimesencephalic cisterns and then over the hemispheres to the parasagittal arachnoid villi. From studies using gated MRI and radionuclide cisternography, Greitz¹⁸⁴ claimed that most of the CSF leaving the ventricles does not reach the arachnoid villi. He postulated that the CSF is absorbed by brain capillaries as it passes over the cerebral hemispheres, most of it being absorbed before it reaches the arachnoid villi.¹⁸⁴ Di Chiro¹¹⁰ used ¹³¹I-albumin injected into the lumbar subarachnoid space to trace CSF flow in humans. He found activity in the basal cisterns one hour after injection, in the Sylvian fissures at three hours and over the cerebral convexities at 12 hours. At 24 hours the activity was along the superior sagittal sinus. The lumbar dural sac remains high in

activity for at least 48 hours. Di Chiro¹¹⁰ found no alteration in the upward spread of a radiolabelled tracer injected in the lumbar subarachnoid space either with change in position or barbotage.

Substances injected into the lumbar CSF do not usually enter the ventricles.^{106,381} In experimental animals with communicating hydrocephalus, dye injected into the cisterna magna does reflux into the ventricles, whereas in normal animals, the dye remains in the subarachnoid space.²⁹⁷

The driving force for the circulation of CSF is thought to be a combination of the continuous production and drainage of CSF, arterial pulsations, and the activity of the ependymal cilia.⁸⁹ The relative contribution of each of these remains controversial.⁸⁹ Arterial and CSF pulsations as mechanisms of flow had hardly been considered until noninvasive investigation of CSF flow was made possible with the introduction of cardiac gated cine MRI.^{184,185,447} After using this technique, Greitz et al¹⁸⁵ claimed that extraventricular CSF flow is caused mainly by pulsatile movements and not by bulk flow. Greitz¹⁸⁴ used MR phase images from a cardiac gated standard spin echo sequence. CSF flow velocity was determined from phase images by subtracting the local phase of the CSF from the phase of the background stationary tissue, or using the quotient between the phase values. Net flow of fluid was calculated on axial scans by calculating the velocity of CSF flow and multiplying it by the cross-sectional area of moving CSF.¹⁸⁴ Greitz et al¹⁸⁵ found a net flow only in the aqueduct, stating that other net flows, if present, were too small to measure. They claimed that since the error of the technique in measuring bulk flow is 10%, net bulk flow in the spinal canal cannot be reliably measured.¹⁸⁵ Armonda et al¹⁴ point out that gated cine-mode MRI measures flow velocity, and that pulsatile movement of CSF does not necessarily result in bulk flow. Phase-contrast cine MRI has been used by Enzmann and Pelc.¹³¹ They concluded that there is pulsatile movement of CSF related to the changes of brain volume during systole

and diastole. Compression of the lateral ventricles (rather than the third) appeared to be the driving force for CSF movements. To allow for this oscillation, there needs to be capacitance in the system. This appeared to be in the lumbar dural sac, where there was little pulsatile flow. The fourth ventricle and cisterna magna seemed to be 'mixing chambers.'

Following the conclusions of investigators early this century, it was thought that there is no true flow of CSF in the spinal canal; substances spread into the subarachnoid space only by simple diffusion.¹¹¹ More recent investigations, particularly with gated MRI, have demonstrated CSF movements in the spinal subarachnoid space. It is now claimed that CSF descends posterior to the spinal cord and ascends anterior to it.¹¹⁰ Enzmann and Pelc¹³¹ found oscillatory movements of CSF around the cervical and thoracic spinal cord, but little or no movement in the lumbar spine. Drugs infused into the lumbar theca gradually decrease in concentration rostrally in the spinal canal; Kroin et al²⁵² believe this may be due to the pulsatile flow of CSF. According to Kroin et al,²⁵² cats have a very slow rate of spinal CSF movement, but a high rate of absorption in the spinal nerve roots.

2.5.2 CSF flow in perivascular spaces

Weed,^{478,479} in 1914, described the perivascular spaces as channels carrying fluid from the brain to the subarachnoid space. Cushing⁹⁹ injected ferrocyanide solution in the subarachnoid space and precipitated Prussian blue in the perivascular and perineuronal spaces and from these experiments proposed that fluid flows from the perivascular spaces into the subarachnoid space. In 1950, Brierley⁵⁹ reported the results of a CSF flow study. He injected India ink into the cisterna magna of rabbits and found extensive penetration of ink particles into the perivascular spaces of the brain and spinal cord. This was contrary to the contemporary belief that fluid flowed from the interstitial space into the perivascular space then into the subarachnoid space. There was also penetration into the central canal, and a

dilatation of the central canal in the lower sacral region, containing ink. He postulated that the experimental technique may have been responsible for the 'apparent' perivascular flow.

The next report of perivascular flow was by Lee and Olszewski²⁶¹ in 1960. They injected ¹³¹I-albumin into the cisterna magna and found radioactivity in a pattern suggesting transpial diffusion, but also found radioactivity around superficial and deep vessels, after as little as 20 minutes. This was followed in 1964 with a study by Klatzo et al,²⁴⁵ who used fluorescein-labelled serum proteins to examine CSF flow. They found tracer outlining the blood vessels that penetrate the spinal cord from the surface. Wagner et al⁴⁷³ injected HRP into the ventricles of rats and found rapid (10 to 30 minutes) spread of the tracer to the perivascular spaces, extending down to the level of the capillaries.

Borison et al⁵⁴ injected HRP into the cisterna magna of cats and examined the brain stem after varying intervals. Rapid and deep penetration via the perivascular spaces was observed. The results were identical whether the animal was perfuse-fixed intravascularly or ventriculo-cisternally, a fact which demonstrates that the movement of HRP into the perivascular spaces was not an artifact of intravascular fixation. In addition, HRP was injected into a cat immediately after its death and there was no evidence of perivascular HRP movement.⁵⁴ They concluded that the subarachnoid CSF should be regarded as being in dynamic chemical communication with all parts of the CNS.

Rennels et al^{373,374} injected HRP into the CSF of cats and found transpial and transependymal penetration of the marker into the extracellular space, but more striking was a rapid passing of tracer into the perivascular spaces, not only into the spaces around large vessels, but also into a sleeve of capillary basal lamina, which they believe to be an extension of the perivascular space. The rapid influx of tracer into these spaces (HRP permeated throughout cats' brains via perivascular spaces within 4 minutes of a cisternal injection) could not be explicable on the basis of diffusion alone.³⁷⁴ They proposed that CSF passes

continually from perivascular spaces of arterioles and capillaries, into the extracellular space and then either across ependyma or into perivascular spaces of venules. They believed the driving force for this flow is the pulsations of penetrating arterioles, as evidenced by the lack of tracer influx into the perivascular space with aortic or brachiocephalic ligation to dampen arterial pulsations. This flow through the extracellular space would allow clearance of substances not able to cross the blood brain barrier; the CSF therefore functioning as the lymphatic system of the brain.³⁷³

Ichimura et al²²¹ have argued against a rapid flow in perivascular spaces. Complex microinjection techniques were used to infuse large molecular weight tracers (labelled albumin and India ink) into the perivascular space of rat cortical vessels; video-densitometric measurements of fluorescently labelled albumin were used to assess the *in vivo* flow of fluid and gold-labelled albumin was used to study the ultrastructural location of tracers. They recorded slow movement of the tracers and the direction of movement was unpredictable. They believed that flow in the perivascular space is slow and moving 'backwards and forwards.'

2.5.3 CSF flow in the central canal

The flow of CSF in the central canal has been studied in rabbits, rats, guinea pigs, monkeys, cats and lampreys.^{56,88,89} Bradbury and Lathem,⁵⁶ in 1965, were the first to study CSF flow in the central canal. They studied rabbits, infusing Evans blue, ¹³¹I-albumin or colloidal graphite into the lateral ventricle or cisterna magna. Two hours after ventricular infusion the markers had extended along the entire length of the central canal. In addition, Evans blue had extended into the sacral subarachnoid space through the dorsal surface of the filum terminale. In animals receiving cisternal infusions, no dye entered the central canal. They also studied rhesus monkeys, cats, rats, guinea-pigs and sea-lampreys by injecting dye into the lateral ventricle. No dye was found in the central canal in any of these animals except

in one of two guinea-pigs and two of three rats (faint staining only). They calculated that the rate of CSF flow along the central canal was 0.5 $\mu\text{L}/\text{min}$, or 5% of CSF production. They concluded that there was a caudad flow of CSF within the central canal of rabbits, but that this probably had no function since flow could not be demonstrated in other species. They believed the motive power for the flow was the ependymal cilia. They argued that the flow could not be due to rises in hydrostatic pressure because no increase in intraventricular pressure was measured. Other investigators have demonstrated central canal CSF flow in other species. Nakayama,³²⁴ in 1976, injected India ink into the lateral ventricles of rabbits, guinea-pigs, cats and rats. He observed the dye passing down the central canal and out a dorsal opening of the canal in the filum terminale in each of the species. Klatzo et al²⁴⁵ infused fluorescein-labelled proteins into the ventricles of cats and subsequently found staining of the central canal.

Cifuentes et al⁸⁸ perfused 30 μL of 3% HRP into the lateral ventricle of rats and studied the distribution of the marker in the spinal CSF compartments. Tracer was found throughout the central canal 13 minutes after injection. It was no longer present in the canal 2 hours after injection. The marker penetrated through the ependymal lining (to a maximum of 10 μm) and was found in the basement membrane of subependymal capillaries and appeared in the lumen of endothelial pinocytotic vesicles. They also found vessels entering the spinal cord through the ventral median sulcus and approaching the central canal. These vessels were the only ones to have HRP along their course through the grey matter. They concluded that a communication existed between the central canal and the outer CSF space, via the intercellular spaces and the perivascular basement membrane at the ventral median sulcus. They believed the absence of tracer at two hours indicated rapid clearance into the parenchymal capillaries. They also stated that the presence of the communication between the

outer CSF space and the subependymal labyrinths would allow free flow of CSF from the spinal subarachnoid space into the labyrinths.

Cifuentes et al⁸⁹ studied CSF flow in the central canal of normal and RF-deficient rats using HRP injected into the lateral ventricle. They found a decrease in the amount of tracer in the central canal in RF-deprived rats. They attributed this to turbulence at the opening of the central canal, which reduced the bulk flow of CSF down the canal. They suggested that RF was necessary to guide the normal caudad flow of CSF in the central canal.

Milhorat et al³⁰¹ used different techniques to study flow within the central canal of rats. They injected Evans blue dye into various CSF compartments (lateral ventricle, cisterna magna, and the lumbar subarachnoid space) and found no evidence of dye entering the central canal. This is in contrast to previous similar experiments. Their ventricular injection was of 1.0 μ L of 2% Evans blue dye over a period of 30 seconds. Injection of Evans blue dye into the dorsal columns of the spinal cord was followed by movement of the dye into the central canal and drainage cephalad to exit through the outlets of the fourth ventricle within 90 minutes. To counter any argument that this movement of dye resulted from the pressure of the injection, they repeated the experiment with intravenous dye entering the dorsal columns at the site of a freeze lesion; the same results were obtained. Dye injected into the spinal CSF penetrated into the spinal cord but did not reach the central canal in the 90 minutes of the study. They concluded that there is an intraluminal cephalad fluid flow in the central canal and that this may represent a 'sink action.' Milhorat et al³¹¹ claim that this flow is consistent with fluid movement within a tube open at one end and receiving transmitted pulsations from the spinal arteries and veins.

One possible explanation for the contrasting findings of Milhorat and the other investigators with regard to the direction of flow within the central canal is the volume of tracer injected. Most investigators have used relatively large volumes of tracer. Compared to

the adult mouse brain (0.4 mL), 0.03 mL is a very large volume (an equivalent volume in humans would be 100 mL).³¹⁴

2.5.4 Flow of neural interstitial fluid

The flow of fluid in the interstitial space and across the ependyma potentially has great significance in the pathophysiology of syringomyelia. Much of the work to date has concentrated on fluid movement within the brain. The main controversy centres on whether fluid moves by simple diffusion or by bulk flow. Diffusion is the non-energy dependent spreading of a substance and is dependent on the molecular weight and concentration of the substance. Bulk flow depends on hydrostatic and osmotic pressure and is independent of molecular weight.³⁸¹ The mooted functions of a flow of interstitial fluid are also controversial.

Electron microscopic studies of the brain in the 1950s failed to demonstrate an interstitial space,³⁸¹ but this has since been shown to be an artifact of fixation.^{381,451} It is now estimated that the extracellular space comprises 15% to 20% of the brain volume^{17,282,381} and is 15 nm to 20 nm in width.^{61,62,97,299} The channels of the extracellular space are capable of transporting large molecules.^{61,62} Cells of other organs are supported by a connective tissue matrix containing collagen, but neurones are embedded in a matrix of glial cells with very little collagen.³⁸¹ Complex carbohydrates (glycosaminoglycans, hyaluronic acid, dermatan sulfate, chondroitin sulfate and heparan sulfate) are present in the extracellular space but their function is unknown.^{50,122,381}

The blood-brain barrier prevents movement of large molecules from the capillaries to the interstitial fluid, but there is no such barrier across the pia or ependyma where there are gap junctions rather than tight junctions.^{65,203,299,510} Water and molecules up to 1600 daltons cross the pia readily¹⁴⁹ and there is even greater permeability across the ependyma—molecules as large as 5000 daltons pass via the intercellular route.¹⁴⁹ Water can therefore

diffuse readily between the subarachnoid space, the CNS parenchyma and the ventricular or central canal CSF. Although diffusion between these compartments has been demonstrated, questions remain regarding the possibility of bulk fluid flow. If interstitial bulk flow does exist, it may be due to CSF passing through the parenchyma or due to production of fluid from metabolism and the vasculature. Formation of interstitial fluid is thought to occur by active transport processes at the capillary,³⁸¹ and accounts for 30-60% of the CSF production.^{300,382}

Investigators earlier this century accepted the concept of a slow flow of fluid from the neural extracellular fluid into the CSF acting to remove excess fluid and metabolites from the brain.^{97,99,100,480,481} This hypothesis was subsequently abandoned, partly because of early electron microscopic studies which suggested there was no extracellular fluid space.³⁸¹ It is now often claimed that substances move in the neural extracellular space only by simple diffusion.^{143,332,510} Hayman and Hinck²⁰⁵ argue that there is no bulk flow of CSF in normal brain, for the following reasons: 1) hydrostatic pressure in the interstitial space is lower than systemic blood pressure and spinal CSF pressure; and 2) CSF pressure changes do not affect the entry of CSF tracers into brain. They believe the entry of CSF tracers is determined by diffusion alone, and call this a 'sink action.'

Other authors argue in favour of the existence of bulk flow. Preferential flow¹⁷ or clearance into the ventricles^{97,301} of injected intracerebral extracellular markers is allegedly evidence of bulk flow. It is thought that flow into the ventricles contributes to the clearance of solutes from the interstitial spaces of the brain.^{97,301} Rosenberg et al^{382,383} studied the movement of various tracers in the extracellular space of cats' brains. They concluded that transport in grey matter extracellular spaces is primarily by diffusion, whereas transport in white matter is by diffusion and bulk flow towards the ventricle. This direction of flow is reversed in experimental hydrocephalus, where CSF moves from the ventricles into the surrounding brain.^{297,315,383} Rosenberg³⁸¹ proposed that the driving force for bulk flow is the

formation of interstitial fluid, possibly aided by the pulsations of blood vessels.³⁸¹ In contrast, Cifuentes et al⁸⁸ believe there is a flow of fluid essentially in the opposite direction: from the ventricles across the ependyma and into the capillaries of the brain parenchyma. It has been shown by some investigators that molecules of different molecular weight move at the same rate through the extracellular space, indicating that the movement is by convection, or bulk flow, rather than diffusion.²⁸² Ohata and Marmarou³³⁸ found contradicting results using molecules of differing sizes and concluded that diffusion was the main mode of interstitial fluid movement. Bach-y-Rita¹⁷ proposes that 'non-synaptic neurotransmission' occurs in the CNS; this would rely on movement of neurotransmitters in the intercellular space.

Some authors have reported studies of spinal cord interstitial fluid movements. Milhorat et al³¹² injected HRP into the dorsal columns of rats and there was no apparent barrier to the molecule passing between ependymal cells and entering the central canal. They interpreted this as evidence for a 'sink action' of the central canal. Water-soluble iodinated contrast media penetrate the brain and spinal cord after injection into the subarachnoid space.^{118,205,216,222,390,391} Where the surface contacting the CSF is white matter, the penetration is diffuse, whereas high concentrations are achieved in cortical areas.²⁰⁵ Dubois et al¹¹⁸ found a gradual increase in the attenuation of the spinal cord after subarachnoid injection of water-soluble contrast; they concluded that this was due to simple diffusion from the CSF into the spinal cord extracellular space. Ikata et al²²² came to the same conclusion. They infused different tracers in the subarachnoid space of rats, using a lumbar-cisternal perfusion technique. After one hour, fluorescein (molecular weight 376 daltons) was observed throughout the spinal cord parenchyma. After a similar infusion protocol, Evans blue (molecular weight 789) was seen only in the peripheral white matter. In rats examined 24 hours after the perfusion, fluorescein was absent and Evans Blue was seen throughout the

spinal cord. Ultrastructural studies using HRP (after 2 hours of perfusion) and lanthanum demonstrated tracer in the extracellular space and the basement membrane of glial cells.²²²

There is some evidence that interstitial fluid flows into extracranial lymphatics. Krisch et al²⁵¹ have demonstrated trabeculae containing collagen bundles and blood vessels connecting the subpial space to the subdural mesothelium. These authors found tracers passing from the brain into these trabeculae and suggested that this was a pathway of extracellular fluid flow out of the brain.²⁵¹ According to Krisch et al,²⁵¹ subendothelial clefts of brain capillaries communicate with the intercellular clefts of the brain. They argue that the perivascular space is not in free communication with the subarachnoid space and that there may be two drainage systems for CSF: 1) arachnoid villi for subarachnoid space CSF, and 2) lymphatic drainage of interstitial fluid of superficial cortical layers. This is supported by the finding of Bradbury et al⁵⁵ that lymphatic drainage of an injected radioisotope was greater after intracerebral injection than after CSF injection. It has been suggested that in rabbits, dogs and sheep the interstitial fluid drains across the cribriform plate into the nasal mucosa and into the cervical lymphatics.^{55,381} Whether significant drainage by this route occurs in humans is not known.³⁸¹

2.6 Pressure and pulsations in the nervous system

2.6.1 Pulsatile movement of the brain and spinal cord

Pulsatile movements of the brain and spinal cord have been demonstrated. Cine-MRI has been used to demonstrate pulsatile movement of the brain. The brain apparently has a 'piston-like' movement, causing the brain stem to move caudally during systole.^{131,186} During systole there is also caudal movement of the spinal cord with the highest velocities recorded in the upper cervical cord.²⁶⁷ Spinal cord movement is decreased in tethered cord and compressive lesions.²⁶⁷

2.6.2 CSF pressure pulsations

Bering's⁴³ theory that pulsations of the CSF are due to vascular expansion of the choroid plexus has been questioned. Du Boulay et al¹¹⁷ believe the pulse has two components: 1) a ventricular pulse due to compression of the third ventricle by brain expanding with arterial blood; and 2) a basal cistern pulse, also due to expansion of the cerebral hemispheres. Greitz et al¹⁸⁶ also argued that arterial expansion of the brain is responsible for the movement of CSF.

Hamer et al¹⁹⁸ studied intracranial CSF pulsations in dogs. They found that the pulsations are mainly arterial in origin, and that the pulse amplitude increases significantly in raised intracranial pressure. Adolph et al⁶ used dogs to study the arterial and venous contributions to cisternal CSF pulsations. They concluded that under normal conditions, the major pulsations were arterial, but that under some conditions venous pulsations could prevail. They did not study pulsations at other sites. CSF pulse waveforms measured in the lateral ventricle and cisterna magna are nearly identical, while the lumbar CSF pulse wave is normally damped.⁴³⁸ This damping is diminished by infusion of saline into the subarachnoid space to raise the CSF pressure.^{121,438} Studies of lumbar CSF pressure indicate that spinal pulsations result from a combination of transmitted pulsations from the brain and from spinal arterial pulsations.^{121,438,459} Using system analysis, Urayama⁴⁵⁹ determined that the relative components of the lumbar pressure pulse were: transmitted cranial pulsations 23% and spinal vessel pulsations 77% (arterial 39% and venous 38%). Dunbar et al¹²¹ studied the CSF pulse wave in dogs. After several manipulations including spinal block and aortic occlusion, they concluded that arteries supplying the spinal cord make a major contribution to the formation of the CSF pulse wave. In addition, they believed the cerebral arterial structures and the choroid plexus are not the site of origin of the lumbar CSF pulse wave.¹²¹

2.6.3 CSF pressure and spinal cord blood flow

The relationship between CSF pressure and spinal cord blood flow has been investigated with regard to paraplegia occurring after thoracic aortic surgery.^{4,241,273,356} CSF is often withdrawn prior to aortic cross clamping in an attempt to increase spinal cord perfusion pressure and prevent ischaemia. In a canine experiment, Kazama et al²⁴¹ found no significant increase in spinal cord blood flow after CSF withdrawal during aortic cross clamping. There was a reduction in flow when the CSF pressure was elevated to 20 and to 40 mm Hg. There was a small increase in CSF pressure with aortic clamping but the authors did not believe this was sufficient to decrease spinal cord perfusion pressure to ischaemia-producing levels.²⁴¹ In contrast, Aadahl et al,⁴ using laser Doppler measurements in pigs, recorded significant increases in spinal cord blood flow following withdrawal of CSF. They were able to inhibit spinal cord blood flow totally by raising CSF pressure above 15 mm Hg.⁴ Piano and Gewertz³⁵⁶ measured CSF pressure and compliance in dogs undergoing aortic clamping. CSF pressure was increased by thoracic aortic clamping, but compliance was not changed. They concluded that during aortic clamping CSF pressure increases due to volume changes in venous capacitance beds within the dural space, and that draining CSF improves spinal cord blood flow by enhancing patency of thin-walled intradural veins.³⁵⁶

2.7 Function of CSF

For most of this century, the CSF has been considered mainly as a mechanical buffer for the brain and spinal cord.^{282,292} Its possible role as a lymph system of the brain was initially proposed by Weed in 1914,⁴⁷⁸⁻⁴⁸⁰ and supported by Cushing,¹⁰⁰ but this hypothesis was gradually relegated to obscurity.^{184,292} Clearly there is a need for the nervous system to remove potentially toxic substances, including neurotransmitters and electrolytes. There is ample evidence that the ionic composition of CSF and interstitial fluid is tightly regulated, since plasma changes in potassium, calcium and sodium have little effect on brain levels.^{240,381} CSF no doubt has a role in regulating the composition of the neural extracellular

fluid.^{240,374,510,513} Milhorat²⁹² also argued that the CSF provides a chemical environment for the CNS, has a role in intracerebral transport and acts as the lymphatic system of the CNS. Evidence for this includes the clearance of many lipid-insoluble substances from the brain by net transport into the CSF.²⁹²

The CSF probably acts as a 'sink.' Since the concentration of substances is lower in the CSF than in the brain, the substances move down the concentration gradient into the CSF.^{381,513} The rate of transfer of most substances across the capillary-brain interface is slower than their rate of removal from the CSF.⁵¹³ Therefore, their concentration in the interstitial fluid of the brain is higher than in CSF.⁵¹³ Hochwald et al²¹² believe the sink action of the CSF limits excess water accumulation in the brain during water intoxication.

2.8 Neural vasculature and barrier structures

2.8.1 Barriers in the nervous system

The blood-brain barrier is formed by capillary and venule endothelial cells with tight junctions.^{234,299,381,418} These junctions allow passage of molecules up to 0.6 nm to 0.8 nm.²⁹⁹ Molecular charge and lipid solubility are other factors influencing the passage of molecules across the barrier.²⁹⁹ The blood-brain barrier is lacking in the circumventricular organs (median eminence, organum vasculorum of the lamina terminalis, subfornical organ, subcommissural organ, neural lobe, pineal gland and area postrema).³⁸¹

The blood-CSF barrier is formed by the apical tight junctions of the choroid plexus^{282,381} and the tight junctions of the arachnoid barrier layer.³²¹ The CSF-blood barrier is formed by arachnoid cells (which have tight junctions) and arachnoid villi.³⁸¹

The brain-CSF barrier is formed by ependyma and pia with gap junctions.^{299,381} The only type of intercellular contacts in the external glial limiting membrane is gap junctions.⁴⁷² The intercellular clefts leading from the CSF into the brain are therefore patent. The ependyma overlying the infundibular recess, median eminence, area postrema and choroid

plexus has tight junctions and is impermeable to macromolecules.^{149,297} Other ependymal surfaces are not a barrier to many substances including large proteins.^{60,62,282,368}

2.8.2 Neural vasculature

Seven to eight large radicular branches participate in the arterial supply of the spinal cord.²⁵⁹ The afferent supply varies considerably, but the intramedullary vasculature is constant.²⁵⁹ The intramedullary arterial supply consists of peripheral arteries, branching from radicular arteries, that supply the white matter, while central arteries from the anterior median sulcus supply the grey matter and the inner white matter.^{259,456} In the cervical and thoracic enlargements, the central arteries are large and numerous.²⁵⁹ Capillary networks are more dense in the grey matter than in the white and veins parallel the arteries.⁴⁵⁶ The pial arterial plexus has been traditionally reported to give rise to intramedullary arteries that supply the ventral and lateral white matter, however recent evidence suggests that branches of the sulcal arteries extend to the pial surface in the white matter and that there is no pial arterial plexus.²⁴⁹

Koyanagi et al²⁴⁹ have studied the arterial supply of the rat spinal cord. Sulcal arteries run straight and usually give no branches in the anterior median sulcus; sulcal veins are undulating and receive branches. The sulcal arteries curve alternately to the right and left to supply the rich capillary network of the central grey matter. The posterior spinal arteries give off branches that curve rostrally then enter the posterior grey matter. Other branches of the posterior spinal arteries enter the posterior columns. The grey matter contains a rich butterfly-shaped capillary network, whereas the lateral and anterior columns contain radially oriented vessels. Radial veins drain the anterior and lateral white columns and the lateral and ventral aspects of the anterior and lateral grey matter.

2.8.3 Structure of the perivascular space

Unlike vessels in other tissues, penetrating arteries and emerging veins of the CNS have a surrounding fluid-filled space.³⁷³ The traditional view of the perivascular space is that

it is a continuation of the subarachnoid space around blood vessels entering neural tissue.^{139,381} Hutchings and Weller²²⁰ used scanning electron microscopic studies of human autopsy material to suggest a different structure. They found a continuous sheet of pial cells separating the CSF in the subarachnoid space from the perivascular and subpial spaces. The single layer of pial cells separating the spaces is joined by gap junctions and desmosomes.¹⁴⁵ According to Esiri and Gay,¹³⁹ the perivascular and subpial spaces are continuous. Other investigators claim that the perivascular spaces extend around vessels in the subarachnoid space.²²¹ The intraparenchymal perivascular spaces are lined by the basement membrane of the glia limitans.¹³⁹

Capillaries do not have a true perivascular space. They have a sleeve-like basal lamina composed of a macromolecular meshwork between the capillary endothelium and the end feet of astrocytes, formed by fusion of the glial and vascular basal laminae at the arteriolar origins of capillaries.^{373,374} This basal lamina communicates with the extracellular space via gaps between adjacent astrocytic endfeet.³⁷³ Brightman's electron microscopy studies revealed perivascular spaces reaching as far as the capillaries in some instances.⁶¹ Usually, the space ended with fusion between the basement membrane of the glial parenchymal border and the blood vessel wall.^{61,139} According to Rosenberg,³⁸¹ the basal lamina of brain capillaries is a continuation of the perivascular cell layers around the vessels in the perivascular space.

The central canal of the rat is surrounded by labyrinthine structures formed by basement membranes associated with subependymal capillaries, similar to the labyrinths found in the ventricular subependymal regions.⁸⁸

2.9 CSF tracers

It has been assumed that studies of water-soluble foreign substances injected into the CSF give information about the flow and absorption of CSF.²¹⁸ The distribution of water soluble substances in the CSF is determined by: 1) the rate of diffusion; 2) bulk flow; and 3)

the rate and pathway of elimination.⁵¹⁰ Di Chiro¹¹⁰ claimed that molecular size of tracers is critical; those with a small molecular size leave the CSF at the closest point and do not follow the CSF flow. Holtas et al²¹⁶ reported considerable variation in the concentration of tracers in the CSF and the spinal cord after a single injection. They attribute this to variations in the size of the subarachnoid space and the spinal cord and claim that the quantitative assessment of tracer movement is therefore difficult. Leakage of tracer is reduced and its spread within the CSF is enhanced when the injection needle (even if percutaneous) is left in place after injection.⁴⁰⁷ Some investigators also seal around the needle or cannula with cyanoacrylate.²¹⁶

The advantages and disadvantages of the more common CSF tracers will be reviewed.

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2.9.1 Vital dyes

The use of vital dyes such as Evans blue injected into the CSF is said to be a reliable means of examining bulk flow.^{97,292,301} Evans blue dye has a molecular weight of 789 daltons,²²² which makes it susceptible to rapid diffusion and therefore vitiates the study of bulk flow.

2.9.2 Blue dextran 2000

Blue dextran 2000 has a molecular weight of 1×10^6 daltons; it therefore has a low diffusion coefficient and low permeability into cells.⁹⁷ The diffusion coefficient is $9.7 \pm 0.36 \times 10^{-8} \text{ cm}^2 \text{ sec}^{-1}$ and is independent of diffusion time for test periods ranging from one to six days.⁹⁷ It is so large that it does not move appreciably by diffusion.²⁹² It may be too large to pass readily between the gap junctions of ependymal and pial surfaces.

2.9.3 Horseradish peroxidase

Horseradish peroxidase (HRP) is a glycoprotein with a diameter of 5 nm to 6 nm^{89,122,299,312} and a molecular weight of 43,000 daltons.^{122,222} It is a relatively non-toxic,⁸⁹ readily diffusible protein.¹²² Fixation with perfused paraformaldehyde occurs with sufficient rapidity to prevent postfixation diffusion artifact.¹²² Rapid fixation of HRP is critical to avoid

artifactual distortion of CSF flow profiles by post-perfusion diffusion. Dunker et al¹²² found perfusion with 2.5% paraformaldehyde and 2% glutaraldehyde provided the optimum combination of rapid fixation and minimal brain shrinkage.

HRP is not completely inert. Some of the pial cells may be 'resting macrophages' and they become reactive in the presence of HRP.³²¹ Free HRP is endocytosed by neurones on the basis of concentration gradients.¹⁷⁹ It does not undergo adsorptive endocytosis unless bound to lectins.¹⁷⁹

Tetramethylbenzidine is a more sensitive localiser of HRP than is diaminobenzidine, and provides better visibility of the reaction product under light microscopy with less background noise.^{288,289,373} The best results have been obtained with long incubations and moderate concentrations of substrate and chromogen.²⁸⁸ The HRP-tetramethylbenzidine reaction product is unstable, so that counterstaining may cause loss of the reaction product and the reaction product may fade with time.³⁸⁸ The reaction product therefore needs to be stabilised. Diaminobenzidine may be superior to TMB for electron microscopy.²⁸⁸

Cifuentes et al⁸⁸ digitised images of sections and measured the relative optic density of each point in a 100 × 100 matrix, but the variability of the HRP reaction product density would seem to make such objective analysis unreliable.

2.9.4 Other tracers

Many other substances have been used as CSF tracers. Phenosulfonphthalein was used by Dandy in his work on hydrocephalus and also by others.²⁹⁷ It is water soluble, and becomes pink when alkalisied.²⁹⁷ Fluorescein-labelled serum proteins have been used,²⁴⁵ but the relationship between the intensity of the fluorescence and the concentration of the fluorophore is not linear.³⁶⁸ Lanthanum (molecular weight 138.9 daltons) is liable to be lost from tissue during experiments²²² and as a colloid is probably too toxic for *in vivo* experiments.²⁹⁹ Colloidal graphite was used by Bradbury and Lathem,⁵⁶ but it is very large

with a maximum particle size of 5 μm . India ink also has large particles (0.4 μm to 1.5 μm).⁵⁹ Ferritin has been used; it has a molecular weight of 400,000 daltons and a diameter of 10 nm.^{62,299} Prussian blue was used by Weed⁴⁸⁰ and Howarth and Cooper.²¹⁸ It has the advantage of being precipitated by perfusion fixation with hydrochloric acid and formalin, which indicates the antemortem location of the tracer. Unfortunately the mixture is toxic, causing epileptic fits and muscle twitching when administered into the subarachnoid space.²¹⁸ Its physiological effect is therefore too great to be a reliable indicator of normal CSF flow. Greitz¹⁸⁴ used ⁹⁹Tc-DTPA to study bulk flow in humans; Bradbury and Latham used ¹³¹I-albumin to study flow in rabbits. These tracers are not ideal for structural localisation. Calcium ions have been used. Although too small to be seen directly with the electron microscope, they can be precipitated with a phosphate-containing fixative.²⁹⁹ Calcium has many physiological reactions, so its distribution in tissue may not reflect the usual physiological distribution.²⁹⁹ Trypan blue has also been used but it is toxic.⁵⁹

2.10 Summary

The anatomy and physiology of CSF and its spaces are poorly understood. CSF is formed by the choroid plexuses and probably other sites. It is absorbed by the arachnoid villi and possibly by other structures. Little is known of CSF circulation within cranial and spinal subarachnoid spaces. The spinal cord central canal is patent in almost all vertebrates and there is evidence that it remains patent in humans unless pathological stenosis occurs. Many investigators consider that fluid flows in a caudad direction in the central canal, but there is also evidence suggesting a cephalad flow. Whether movement of substances in the CNS interstitial fluid occurs by bulk flow or by simple diffusion remains controversial. There has been little study of spinal cord extracellular fluid movement; there is some evidence that fluid flows from the spinal cord interstitial space into the central canal, but the source of this fluid is not known. It has been shown that CSF flows rapidly from the subarachnoid space into

arterial perivascular spaces, but its subsequent destination has been a matter of speculation.

The CSF probably acts as a 'sink,' clearing metabolites and excess neurotransmitters from the nervous system, but the precise mechanism of this function remains obscure. The physical characteristics of HRP make it currently the best molecule for CSF flow studies.

AIMS AND HYPOTHESES

Hypotheses

1. CSF flows from the subarachnoid space into the perivascular spaces and into the interstitial space.
2. Fluid in the interstitial space normally enters the central canal.
3. The flow of fluid from the subarachnoid space and ultimately into the central canal is driven by arterial pulsations.
4. Flow into the central canal continues even when the outflow of the central canal is occluded.
5. Arterial pulsation-driven flow into isolated segments of central canal is the driving force for the enlargement of non-communicating syrinxes.

Aims

1. To determine whether fluid flows from the subarachnoid space into the central canal via the perivascular spaces.
2. To examine the effect of altering arterial pulsations and CSF pressure on this flow.
3. To determine whether this flow continues into isolated segments of central canal and whether the flow continues while the isolated segments are enlarging to form syrinxes.
4. To determine whether rapid perivascular fluid flow occurs in the cerebellum as it does in the cerebrum and spinal cord.
5. To develop a method of studying the three-dimensional morphology of the human central canal.

GENERAL METHODS

1 Animals used and ethics approval

All animals used in these experiments were supplied by and housed in the Institute of Medical and Veterinary Science, Adelaide. Animals had water and food pellets available *ad libitum* and were kept in cages with a day/night cycle of 11/13 hours. Ethics approval was obtained for each experiment from the Animal Ethics Committees of the Institute of Medical and Veterinary Science and the University of Adelaide. Male Sprague-Dawley rats (Table 4) and male wethers (Table 5) were used. Rats were kept in cages in groups of up to six before use; after survival experiments, rats were housed in individual cages. Sheep were always housed individually. Animals were weighed at the first anaesthetic.

Table 4. *Details of rats used in experiments described in this thesis.*

Experiment	Number of	Average	<u>Ethics Approval Nos.</u>	
	Animals	Weight $\pm$ SD	IMVS*	U of A†
Reproduction of rat syringomyelia model	34	396 $\pm$ 110 g	47/93 49/94	M/060/95
Development of methods for studying CSF flow	24‡	335 $\pm$ 36 g	47/93 49/94	M/060/95
CSF flow in normal rats	20	382 $\pm$ 90 g	49/94	M/060/95
CSF flow in rats with isolated central canal	3	416 $\pm$ 6 g	49/94	M/060/95
CSF flow in a rat model of syringomyelia	77	333 $\pm$ 39 g	49/94	M/060/95
TOTAL	137	351 $\pm$ 55 g		

* Institute of Medical and Veterinary Science

† University of Adelaide

‡ 17 of these rats were also included in the 'Reproduction of rat syringomyelia model'

Table 5. *Details of sheep used in experiments described in this thesis.*

Experiment	Number of	Average	Ethics Approval Nos.	
	Animals	Weight $\pm$ SD	IMVS*	U of A†
Development of methods for studying CSF flow	3	41 $\pm$ 3 kg	50/94	M/059/95
Normal CSF flow in sheep	5	45 $\pm$ 5 kg	50/94	M/059/95
CSF flow in sheep with reduced arterial pulsations	4	42 $\pm$ 8 kg	50/94	M/059/95
CSF flow in sheep with reduced spinal pressure	2	40 kg	50/94	M/059/95
Development of a sheep model of syringomyelia	16	41 $\pm$ 3 kg	47/93 38/95 3/95	M/010/95
TOTAL	30	42 $\pm$ 5 kg		

* Institute of Medical and Veterinary Science

† University of Adelaide

2 Neurological examination

Animals surviving after experiments had neurological examinations performed daily until there were no neurological deficits and weekly thereafter. Neurological function was evaluated with a grading system modified from those of Tarlov⁴³⁹ and Delamarter et al¹⁰⁸ (Table 6).

Table 6. *Grading of neurological function in experimental animals.*

Grade	Function
1	Complete paraplegia, no motion of the hind extremities
2	Slight motion of the hind extremities
3	Able to stand
4	Able to walk
5	Able to run

3 Anaesthesia

3.1 Anaesthesia of rats

Rats were induced in an anaesthetic chamber with 4% halothane in oxygen (1.5 L/min). General anaesthesia was continued with the animal self-ventilating 2% halothane in oxygen at the same flow rate through a nose cone. Each animal was placed on pads to support the thorax and with the hind limbs flexed to support the pelvis so that there were free abdominal respiratory movements. The depth of anaesthesia was monitored with noxious stimuli (pinch) to a hind limb. In survival experiments, each animal was left in position breathing 100% oxygen for 1 min to 2 min before being placed in an individual cage in the lateral position. Rats used in the first experiment (reproduction of the intraparenchymal kaolin syringomyelia model) were given an intraperitoneal injection of thiopentone prior to sacrifice with intra-aortic fixative perfusion. This was found to be unnecessary and was not given in subsequent experiments.

3.2 Anaesthesia of sheep

Anaesthesia was induced in sheep with 1 g thiopentone injected intravenously then maintained with 2% to 2.5% halothane in oxygen via an endotracheal tube. Each animal was

either mechanically ventilated or allowed to breathe spontaneously, depending on the requirements for each experiment. In survival experiments, each animal was given 100% endotracheal oxygen at the completion of the experiment until it was satisfactorily self-ventilating. Ventilation with halothane and oxygen was continued in non-survival experiments until the fixative perfusion was commenced. Maintenance intravenous normal saline or Hartmann's solution was administered during prolonged experiments.

4 Perfusion-fixation

4.1 Perfusion-fixation of rats

The fixation procedure was performed rapidly at the completion of each experiment. The animal was turned supine and a transverse subcostal incision was made to expose the anterior abdominal wall musculature. These muscles were opened transversely and the peritoneal cavity was entered. The xiphoid process was retracted and the diaphragm opened circumferentially. The thoracic cage was then opened longitudinally on each side and the anterior thoracic wall was retracted to expose the heart and aorta. A blunt 19 gauge needle was introduced through the apex of the heart into the ascending aorta. Sodium heparin (2000 IU in 2 mL) was injected into the aorta and the heart was clamped transversely to keep the needle in place. The right atrium was transected. A 30 second flush with 0.1 mol/L phosphate buffer (pH 7.4) at 120 mm Hg was followed by perfusion with 750 mL to 1000 mL 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at 120 mm Hg. Aldehyde perfusion was commenced in all animals within 2 min of the completion of the experiment. Perfusion pressures were generated with compressed air in a closed system and monitored with a sphygmomanometer.

4.2 Perfusion-fixation of sheep

At the completion of each experiment, the animal was turned supine to allow perfusion-fixation. If not already exposed, the common carotid arteries and internal jugular

veins were dissected out. The animal was then anticoagulated with 10000 IU sodium heparin in 10 mL saline injected intravenously. The common carotid arteries were cannulated to allow fixative inflow directed proximally in the left artery and distally in the right artery. The internal jugular veins were cannulated to allow fluid efflux from the proximal left vein and distal right vein. The stump of each vessel was ligated. Each animal was perfused with 15 L of 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at a pressure of 120 mm Hg. Perfusion was always commenced in less than 10 minutes after turning the animal supine. Perfusion pressures were generated with compressed air in a closed system and monitored with a sphygmomanometer. Drainage from the jugular veins was via a closed system into a collection container (Figure 3).

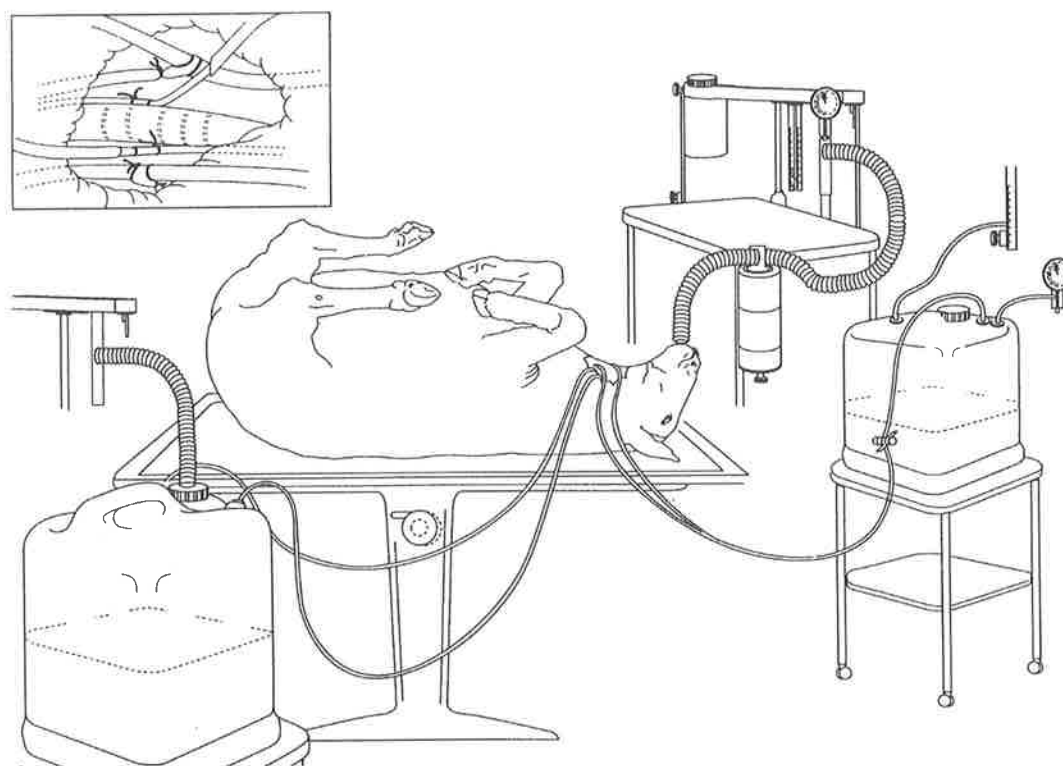


Figure 3. *Perfusion-fixation of sheep with a closed drainage system to minimise leakage of formalin fumes. Inset: cannulation of the carotid arteries and jugular veins.*

5 Tissue processing

5.1 Tissue removal from rats

The spinal column and skull were removed from each rat immediately after perfusion-fixation and postfixed in either 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for up to 4 hours, or in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for up to 24 hours. The brain and spinal cord were then dissected out and blocks were cut from the cerebral hemispheres, midbrain, medulla, cervical cord, thoracic cord, lumbar cord and conus medullaris. Four 3 mm blocks from the cervical cord were cut for transverse sectioning in all rats: C2, C4, C6 and C8. In rats undergoing kaolin injection and in control rats, four cervical blocks were also cut for longitudinal paraffin-embedded sectioning. These encompassed the spinal cord segments immediately caudad to the blocks taken for transverse sectioning, so that in these rats the entire cervical spinal cord was taken for either transverse or longitudinal sectioning. The caudal end of each longitudinal block was marked with a small oblique cut. The blocks taken for longitudinal sectioning were kept in either paraformaldehyde or paraformaldehyde and glutaraldehyde fixative until embedding in paraffin. The other blocks were taken immediately for vibratome sectioning.

5.2 Tissue removal from sheep

Spinal cords were removed from the sheep immediately after perfusion-fixation, by performing cervical, thoracic, lumbar and sacral laminectomies with an oscillating power saw. The entire cervical cord was removed and blocks were taken from T8, L3 and the conus medullaris. These specimens were postfixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer for up to 24 hours prior to TMB processing and up to 6 weeks prior to paraffin section processing. Blocks were taken from each spinal cord segment C1 to C8 and from T8, L3 and the conus medullaris. Each block was cut in transverse section; some additional blocks were taken for longitudinal sectioning.

5.3 Paraffin sections

Tissue blocks destined for paraffin sectioning were processed through a graded series of alcohol to paraffin with xylene as a clearing agent. Blocks were then embedded in paraffin for either longitudinal or transverse sectioning. Seven micrometre sections were cut using a microtome and mounted on glass slides. Sections were then stained with haematoxylin and eosin (H&E) in a standard manner.

5.4 Vibratome sections and localisation of HRP with TMB

Blocks cut for HRP localisation were sectioned with a Lancer vibratome (Technical Products International Inc., St. Louis). The blocks were mounted with cyanoacrylate and immersed in a bath of Tris buffer (pH 7.6). Sections of 50 μm to 100 μm thickness were cut and washed in Tris buffer (pH 7.6) before mounting on uncoated glass slides and air-drying overnight. Some sections were kept in Tris buffer (pH 7.6) for floating section processing with TMB.

The sections were soaked for 20 minutes in 0.01 mol/L sodium acetate buffer (pH 3.3) containing 0.005% 3,3',5,5' TMB and 0.1% sodium nitroprusside. Hydrogen peroxide was added to achieve a final concentration of 0.01% and the sections were incubated for a further 20 minutes. The reaction product was stabilised in a 5% solution of ammonium molybdate in 0.01 mol/L sodium acetate buffer (pH 3.3). Sections were then dehydrated through graded alcohols to xylene and coverslips were applied with xylene-based mounting medium. Details of the TMB processing technique are given in Appendix 2, *TMB processing*, page 245.

Sections were studied using light microscopy and HRP reaction product was identified as a dark blue granular stain on a light blue background. Because the HRP/TMB technique does not use embedded sections, HRP not fixed to tissue is lost during processing. HRP in the central canal lumen is therefore not seen directly; HRP was assumed to have reached the lumen when reaction product was present on the luminal surface of ependymal cells.

6 Photography

Macroscopic photographs were taken with a Nikon F601 using Agfa 100 ASA colour slide film; intraoperative photomicrographs were taken with an Olympus OM10 on a Zeiss microscope; high power photomicrographs were taken with an Olympus CA 35 mounted on an Olympus microscope; and low power photomicrographs were taken with a Wild Photomacroscope system. Film used for microscopic photography was Kodak EPT-160 Tungsten Ektachrome.

EXPERIMENTS

1 Preliminary experiments

1.1 Intraparenchymal kaolin model of syringomyelia

This experiment was performed as a pilot study to develop the rat model of syringomyelia described by Milhorat et al.³¹³ It was also done to develop the skills required for rapid fixation of the experimental animal and to cut longitudinal sections of the spinal cord demonstrating the central canal. A further aim was to determine the appropriate spinal cord levels for further study.

1.1.1 Methods

Thirty-four male Sprague-Dawley rats, weighing 280 g to 585 g were used (Table 4). Kaolin was injected into the spinal cord of 32 rats; two rats received no injection and were used as controls. One rat died due to anaesthetic complications and another was sacrificed because the spinal cord was damaged during the laminectomy. These two rats were excluded from further analysis. Seventeen of the remaining rats were also used to examine methods of studying CSF flow (see *Development of methods for studying spinal fluid flow*, page 108).

1.1.1.1 Kaolin injection

Each rat was anaesthetised as described previously. Aseptic microsurgical technique was used for the entire procedure. A midline incision was made over the cervical spinous processes and the paraspinal muscles were stripped from the spinous processes and laminae of C5 to C7. A laminectomy of C6 was performed to expose the translucent dura and the spinal cord. A 10 μ L Hamilton syringe with a 30 gauge point style 4 needle mounted on a stereotaxic frame was brought into the operative field. The bevel of the needle was arranged to face laterally. The needle was advanced vertically through the translucent dura and into the spinal cord to a depth of approximately 1.5 mm, ensuring that the opening of the needle was completely within the dorsal columns of the spinal cord. Over a period of 30 seconds, 1.5 μ L

of 250 mg/mL kaolin in normal saline was injected into the dorsal columns. The needle was then withdrawn and the wound was closed in layers with non-absorbable sutures.

1.1.1.2 Tissue processing

Rats were killed 1 day, 3 days, 1 week or 6 weeks after the kaolin injection. They were anaesthetised and perfused with paraformaldehyde and glutaraldehyde in phosphate buffer as described previously, except that some animals were injected with 1000 IU sodium heparin rather than 2000 IU. The brain and spinal cord were removed and postfixed as described above.

Blocks were cut from the cerebrum and midbrain for cutting in coronal section. The medulla and entire spinal cord were cut into blocks 4 mm to 5 mm long, that were allocated alternately for cutting in transverse section or longitudinal section. The tissue blocks were embedded in paraffin and sections 7 μ m thick were cut using a microtome. Sections were mounted on glass slides and stained with H&E.

1.1.2 Results

There was unilateral lower limb weakness (grade 2) in one animal that was sacrificed 24 hours after kaolin injection. No neurological deficits were observed in other animals.

1.1.2.1 Problems encountered

Several technical difficulties were encountered during this pilot study. Intraoperative localisation of the sixth cervical lamina proved difficult until intraoperative reference was made to the vertebra prominens. It was initially difficult to achieve a kaolin injection into the spinal cord without also injecting kaolin into the subarachnoid space. This problem was solved by sharpening the 30 gauge needle tips under a microscope (even when new) so that the needle would penetrate the pia more easily and the needle opening would be completely within the cord. Anticoagulation prior to fixative perfusion was initially achieved with 1000 IU heparin. Although this gave adequate fixation for morphological examination, there were

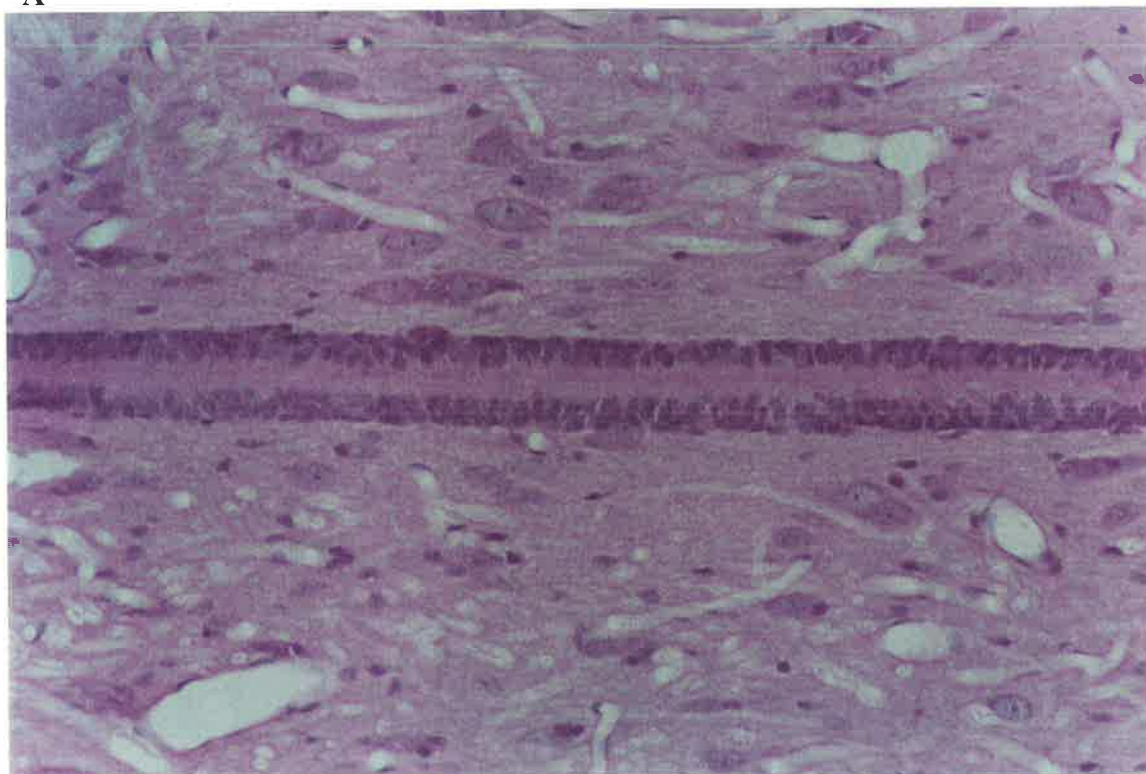
many residual erythrocytes. Injection with 2000 IU heparin gave superior results both in quality of fixation and in numbers of residual erythrocytes. Demonstrating the central canal in longitudinal sections proved to be a difficult technical exercise that required experience and careful embedding of the tissue.

1.1.2.2 Controls

The central canal in various parts of the spinal cord is shown in longitudinal section in Figure 4. In the cervical region, the central canal has a definite lumen, whereas in other parts of the cord, the ependymal surfaces are in close apposition.



A

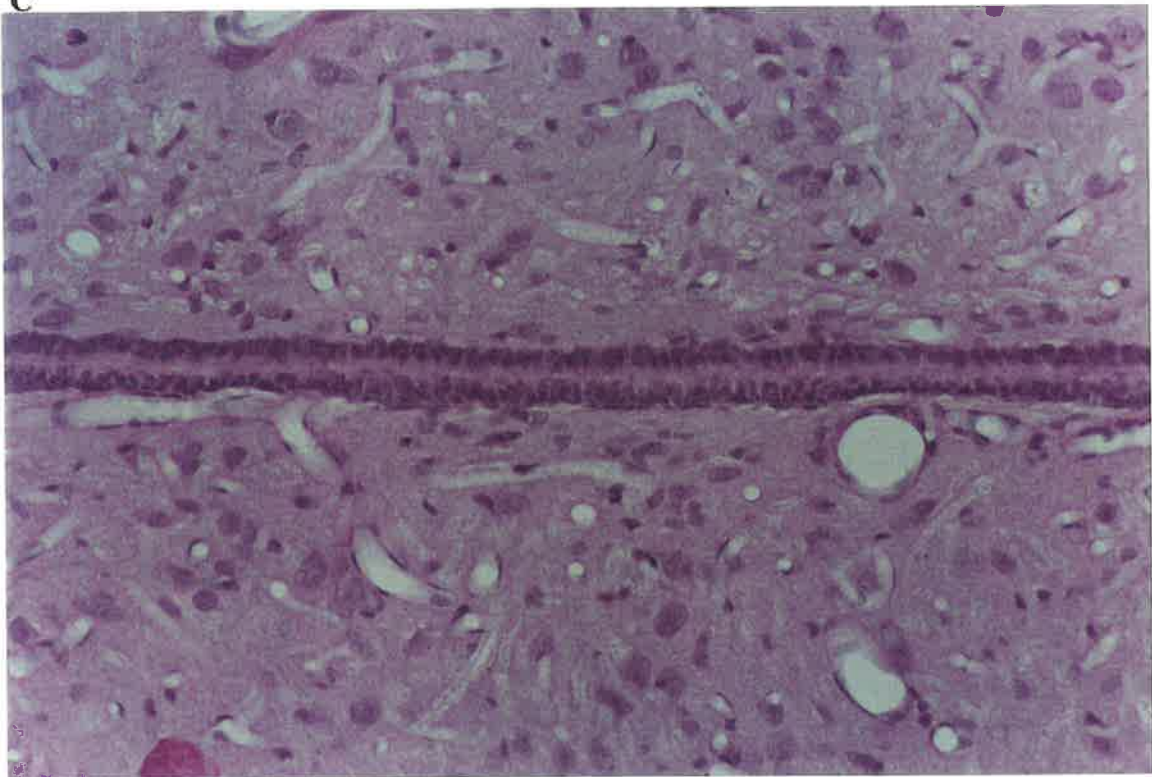


B

Figure 4. Photomicrographs of longitudinal sections of spinal cord from a normal rat, demonstrating the central canal in the cervical (A) and thoracic (B) spinal cord. H&E, $\times 200$. Figure 4 continued on next page.



C



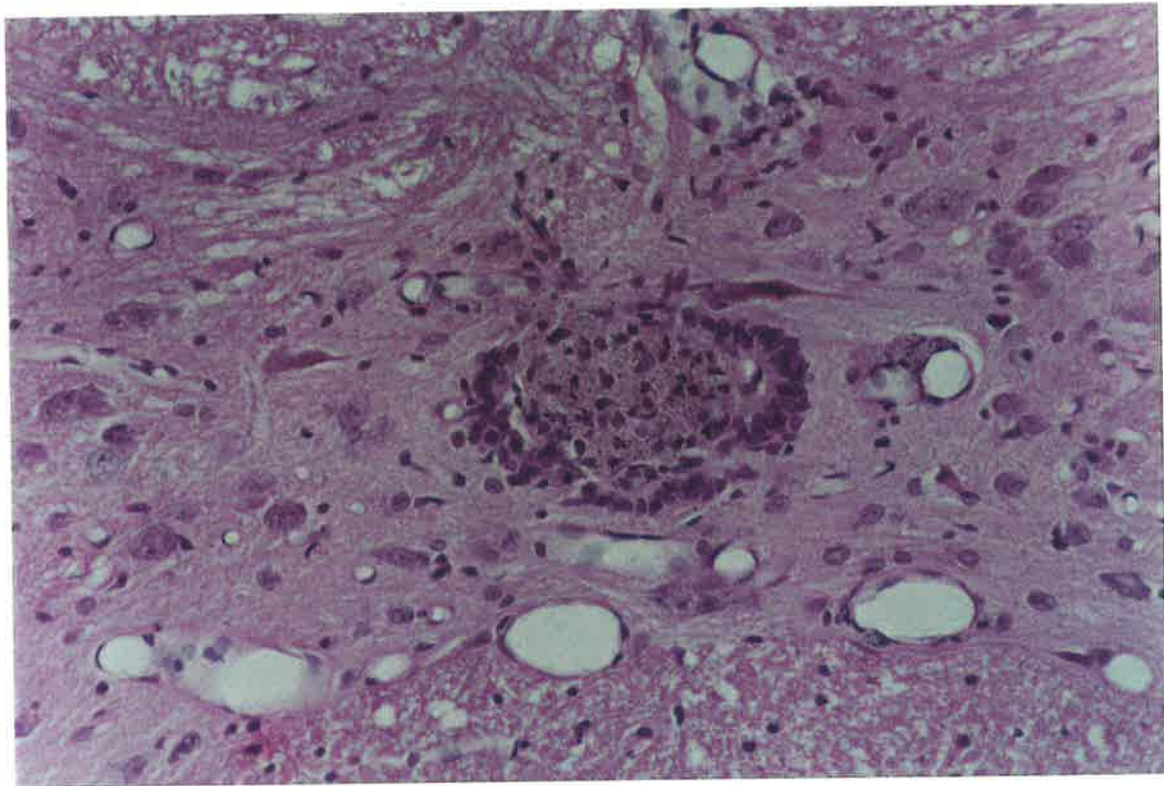
D

Figure 4 (cont). *C: lumbar spinal cord; D: conus medullaris. H&E, $\times 200$.*

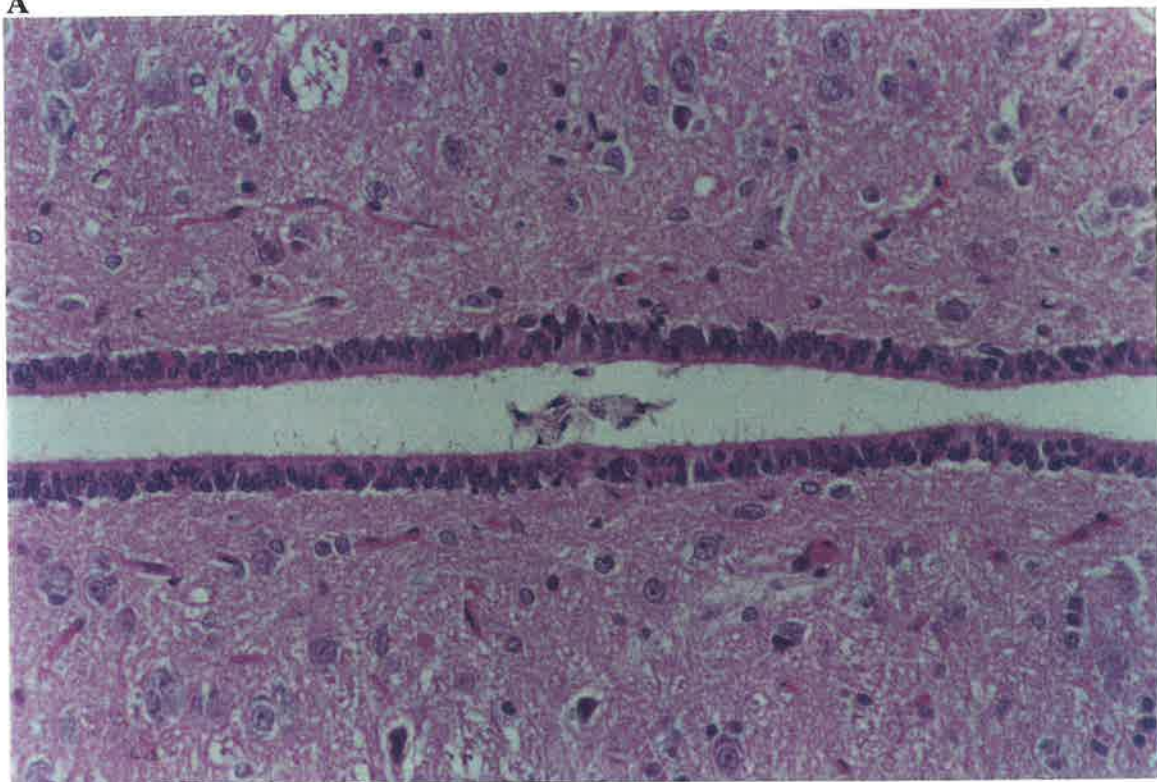
1.1.2.3 Kaolin injection

At the injection site the dorsal columns contained a clump of kaolin crystals that progressively became invaded with inflammatory cells. The inflammatory cells were

predominantly polymorphonuclear leucocytes at 1 day and 3 days after injection, then predominantly macrophages. Inflammatory cells were also in the central canal at all time intervals after injection. Inflammatory cells were always seen rostral to the injection point, but in some animals there were also cells within the central canal caudal to the injection point (Figure 5). The central canal became obstructed by collections of kaolin crystal-laden inflammatory cells and ependymal proliferation at the point of injection and at various levels in the cervical cord rostral to the injection (Figure 5). Occlusion of the canal usually was by a dense cellular mass; thin trabeculae formed by ependymal cells were only occasionally seen. Subependymal astrocytes contributed processes to the cellular mass within the central canal (Figure 6). Macrophages containing kaolin crystals were also seen in central grey matter perivascular spaces close to the point of injection and in the more rostral cervical spinal cord (Figure 6). The lumen of the central canal enlarged between segments of occlusion. This was first seen by 3 days after the kaolin injection and was most marked by 6 weeks (Figure 7). Although the central canal was unequivocally enlarged, it did not become massively dilated in any of the animals.

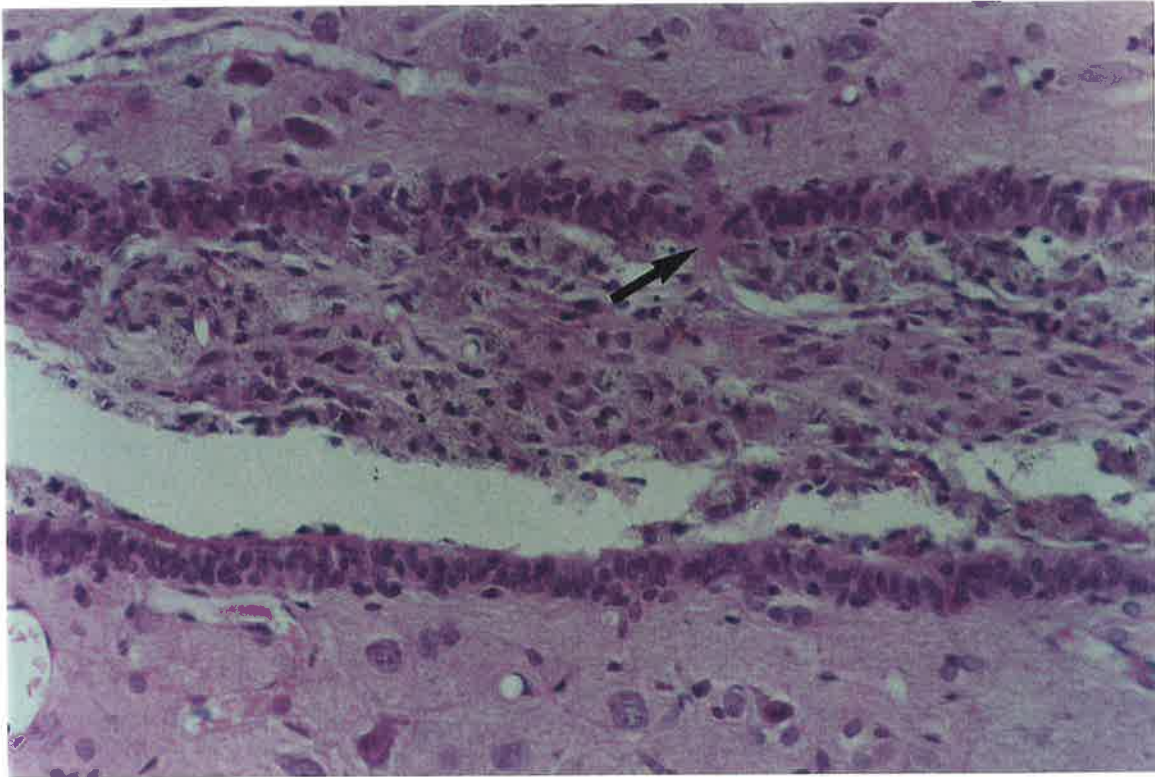


A

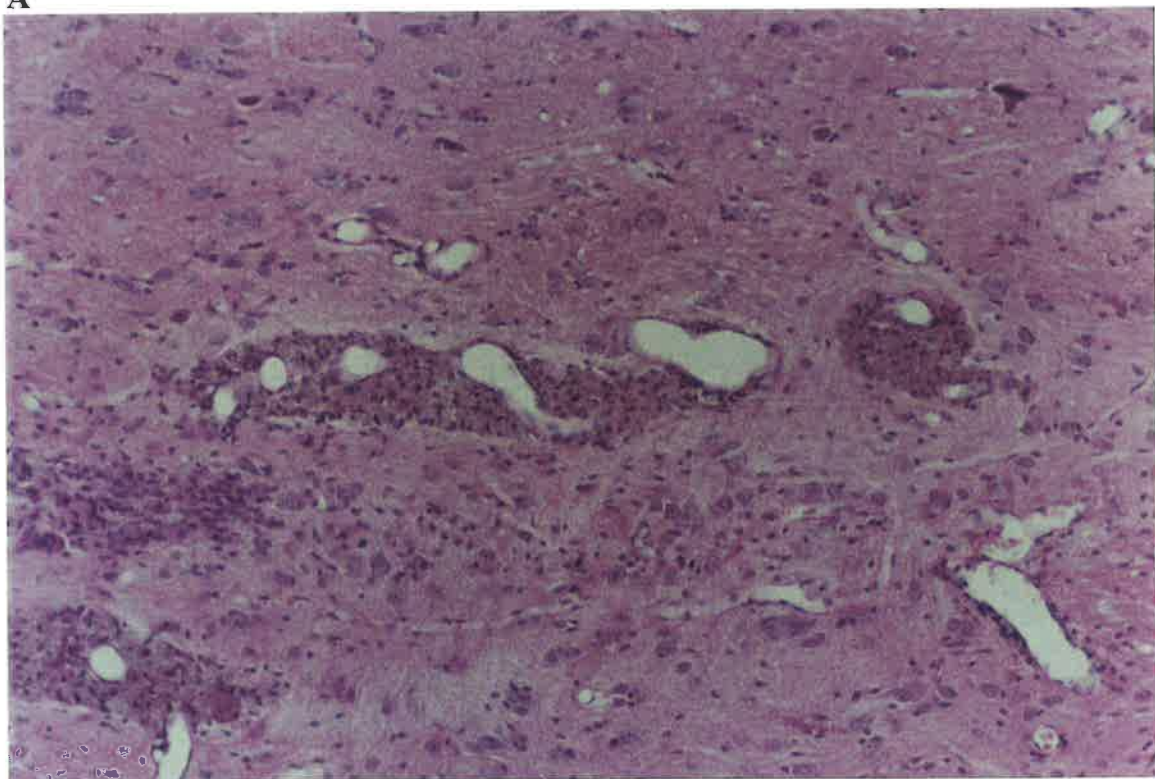


B

Figure 5. Photomicrographs of spinal cord sections from a rat sacrificed 6 weeks after kaolin injection. *A*: Transverse cervical cord section rostral to the injection point, with kaolin-laden inflammatory cells occluding the central canal. H&E, $\times 200$. *B*: Longitudinal section of conus medullaris (caudal to the injection point), demonstrating kaolin-laden macrophages in the central canal. H&E, $\times 200$.



A



B

Figure 6. Photomicrographs of longitudinal spinal cord sections from a rat sacrificed 6 weeks after kaolin injection. *A:* There is a florid fibrous inflammatory response in the cervical central canal lumen and glial processes (arrow) from subependymal cells are passing through the ependyma to reach the lumen. H&E, $\times 200$. *B:* Kaolin-laden macrophages are present within perivascular spaces in the central grey matter. H&E, $\times 100$.

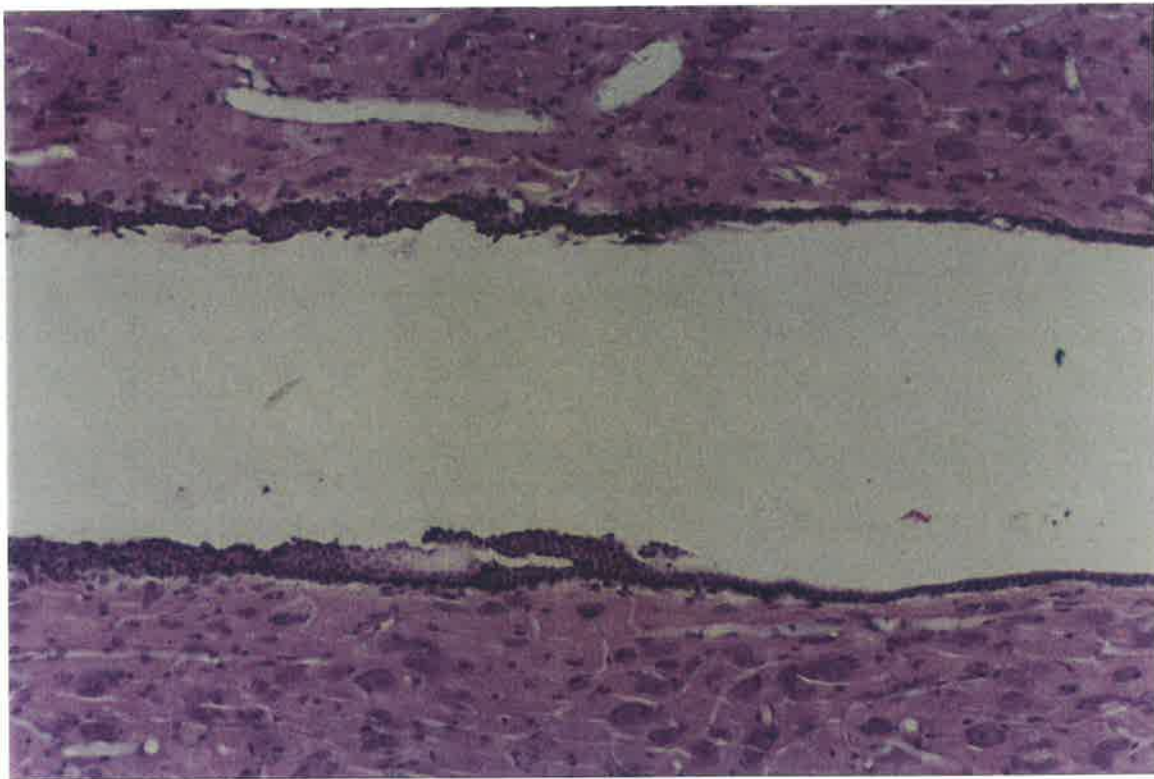


Figure 7. *Photomicrograph of a longitudinal cervical spinal cord section from a rat sacrificed 6 weeks after kaolin injection. The central canal lumen is enlarged. H&E, $\times 100$.*

1.1.3 Discussion

This study was necessary to develop the skills required to produce the intraparenchymal model of syringomyelia and to identify problems that might impair the study of CSF flow in this model. The central canal dilates in this model presumably as a result of occlusion of the rostral lumen by kaolin-induced inflammation and ependymal proliferation.³¹³ This occlusion results from kaolin injected into the cord, but any kaolin that spilled into the subarachnoid space could also cause arachnoiditis that may interfere with CSF flow in the subarachnoid space. It is therefore preferable to achieve an intraparenchymal injection without subarachnoid spillage; this was facilitated by sharpening the needle tip so that pial penetration was achieved more easily and the needle hole was entirely within the cord parenchyma during the injection. Anticoagulation prior to fixative perfusion enhances tissue fixation. The technique initially used gave adequate fixation for morphological examination, but there were residual erythrocytes. Large numbers of residual erythrocytes may cause

slower fixation in parts of the spinal cord tissue by obstructing intravascular fixative flow. This may interfere with studies of CSF flow because tracers need to be rapidly fixed in place for accurate assessment of their location. A larger dose and volume of heparin resulted in fewer erythrocytes and was therefore used for all subsequent experiments.

After kaolin injection, the central canal became occluded at the point of injection and more rostrally by ependymal proliferation and by inflammatory cells containing kaolin. The central canal became progressively dilated between segments of occlusion, although the massive dilation at 6 weeks after kaolin injection reported by Milhorat et al³¹³ was not seen. The reason massive dilation was not seen in this experiment is not clear. It may be that a different concentration or preparation of kaolin was used that produced the more florid inflammatory cellular response seen in this experiment. Nevertheless, there was unequivocal and reproducible dilation that is sufficient to use in studies of CSF flow in an enlarging central canal.

Macrophages containing kaolin crystals were present in central grey matter perivascular spaces. Kaolin crystals may have entered the perivascular spaces via an interstitial route from the original injection site, or they may have first entered the central canal then passed between ependymal cells to reach the perivascular spaces. Alternatively, the crystals may have been taken up by macrophages at the injection point; the macrophages then migrating to the perivascular spaces via the same routes. The first mechanism appears most likely given the proven ability of the crystals to move in the interstitial space. Whatever the mechanism, the presence of these cells in the perivascular spaces provides further evidence of a communication between the extracellular space, the central canal and the perivascular spaces.

In the model developed by Milhorat et al,³¹³ the central canal caudal to the injection point did not contain kaolin crystals or inflammatory cells. Although the rostral section of the

central canal in animals in this study did contain large numbers of cells, the caudal section of the central canal did contain inflammatory cells in some animals. This may occur because fluid flowing into the central canal caudal to a blockage flows caudally to exit the central canal via the opening in the conus medullaris.³²⁴

1.2 Development of methods for studying spinal fluid flow

This pilot study was performed to determine the most appropriate CSF tracer to use in subsequent studies and to determine the optimal dose and routes of injection.

1.2.1 Methods

Twenty-four male Sprague-Dawley rats weighing 280 g to 385 g (Table 4) and three merino wethers weighing 38 kg to 44 kg (Table 5) were used.

1.2.1.1 Injection of tracer into the cisterna magna of rats

Sixteen rats were used. With the animal anaesthetised, a midline incision was made to display the occipital bone, foramen magnum and posterior arch of the atlas. The atlanto-occipital membrane was left intact. A 30 gauge point style 4 needle on a Hamilton syringe mounted on a stereotaxic frame was introduced obliquely through the atlanto-occipital membrane into the cisterna magna with the bevel of the needle directed dorsally. The needle was left in place until fixation.

1.2.1.2 Injection of tracer into the spinal subarachnoid space in rats

Eight rats were used. In each animal tracer was injected into the spinal subarachnoid space either at the lumbar or thoracic level. A midline incision was made to expose the spinous processes of the third to fifth lumbar vertebrae for the lumbar injection or the ninth to twelfth thoracic vertebrae for the thoracic injection. The laminae were exposed by reflecting the paraspinal muscles. For the thoracic injection, the spinous process and laminae of T10 were removed. The T11 and T12 spinous processes were also removed to allow the needle to be positioned, but the laminae at these levels were left intact. Similar bone removal was

performed at L3 to L5 for the lumbar injection. A 30 gauge needle on a Hamilton syringe mounted on a stereotaxic frame was introduced into the subarachnoid space at the rostral limit of the laminectomy. The needle was curved and the syringe introduced obliquely so that the bevel of the needle, directed dorsally, was positioned in the subarachnoid space under the posterior arch of the next intact rostral vertebra.

1.2.1.3 Injection into the spinal subarachnoid space of sheep

After the animal had been anaesthetised and placed in the right lateral position, a midline posterior cervical incision was made to expose the spinous processes of the second to sixth cervical vertebrae. A C4/5 laminotomy was made to expose the underlying dura. A tuberculin syringe with a 26 gauge needle was used to inject tracer into the subarachnoid space. The needle was left in place after the injection.

1.2.1.4 CSF tracers

Evans blue (1% in normal saline) was used as the CSF tracer in eight rats; HRP (3% or 4% in normal saline) was used in the other animals. The tracers were injected with the needle positioned in the subarachnoid space either in the cisterna magna (Evans blue or HRP) or the spine (HRP only). Volumes ranging from 5 μ L to 10 μ L, injected at a rate of 2 μ L/min were used in the rats; volumes of 100 μ L to 500 μ L injected over 30 seconds were used in the sheep.

1.2.1.5 Tissue processing

The animals were perfused with fixative as described previously and the brains and spinal cords of the rats and the spinal cords of the sheep were removed. Sections were processed with TMB if HRP was used as the tracer. Nervous system tissue from the animals injected with Evans blue was studied macroscopically and under the dissecting microscope; blocks were subsequently processed and embedded in paraffin for histological study.

1.2.2 Results

Evans blue injected into the cisterna magna stained the pia of the brain and the spinal cord. The depth of staining varied with the time from injection to fixation. Examination of tissue slices under the dissecting microscope did not reveal sufficient detail to determine the relationship of the dye to perivascular spaces or the central canal. Histological analysis of tracer location was not possible.

HRP reaction product was detected as a dark blue granular staining against the light blue endogenous peroxidase activity. Reaction product was present in perivascular spaces and stained the surface of the brain and spinal cord in rats and the cervical spinal cord of sheep, regardless of the volume used. Labelled vessels were more numerous when greater volumes of HRP were used.

Injection of HRP into the lumbar subarachnoid space of rats without causing CSF leakage proved to be technically very difficult. The subarachnoid space at the thoracic level was better defined and injection at this level without CSF leakage was performed more easily. Injection into the subarachnoid space in the sheep was performed without causing CSF leakage.

1.2.3 Discussion

The aim of this study was to determine the appropriate type and dose of CSF tracer to use for subsequent experiments on fluid flow. Because the area of interest is flow in the perivascular spaces and in the interstitial space of the central grey matter, the tracer needs to be localised at a histological level. Evans blue dye gives a good indication of fluid movement in the subarachnoid space, but does not allow histological localisation. HRP reacted with TMB provides good histological localisation of the tracer.

Injection of tracer has the potential to interfere with normal CSF flow. The aims of injection should therefore be to minimise the impact on the volume and pressure of CSF in the

subarachnoid space. The conventional technique has been to use a lumbo-cisternal infusion of tracer with concomitant drainage of CSF,^{110,183} but this undoubtedly interferes with CSF flow in the subarachnoid space. The alternative of a single injection of tracer has the potential to raise the CSF pressure and volume significantly. An aim of this study was therefore to develop a technique of tracer injection that would provide adequate information about CSF movement, but would not add a significant volume to the CSF. A slow injection of a small volume without CSF drainage was chosen as the most appropriate mechanism to achieve these aims. The volume injected (10 μ L in rats and 100 μ L to 500 μ L in sheep) is small compared to the total CSF volume in these animals. The needle was left in place after HRP injection to prevent CSF leakage. Injecting through the atlanto-occipital membrane in rats rather than opening the membrane prior to injection was also an important technique to prevent leakage. The maximum volume that can be injected without significantly interfering with CSF flow is a matter of conjecture, however it was thought that a volume of 10 μ L would not add significantly to the rat CSF volume and that a volume of 250 μ L would not add significantly to the CSF volume in sheep. After injecting these volumes of HRP, the perivascular spaces were well labelled and movement of tracer into the interstitial space was easily identifiable. Injecting a larger volume of tracer may have resulted in a more diffuse labelling of the interstitial space and impaired the assessment of interstitial fluid movement.

A spinal route of subarachnoid tracer injection was used in addition to the cisternal route in rats because it is possible that tracer injected into the cisterna magna could pass directly into the central canal, making the detection of flow from perivascular spaces into the central canal impossible. Thoracic injection of tracer without causing CSF leakage was technically easier than injection in the lumbar space, so the thoracic site was chosen for the spinal route of injection for subsequent studies. A midcervical injection was chosen in sheep

because this delivers tracer to the area of interest and in the time periods studied it is unlikely that significant flow into the fourth ventricle and down the central canal would occur.

1.3 Comparison of mounted and floating TMB processing

This experiment was performed to determine whether there were any differences in the results obtained with processing sections for HRP localisation using the floating section method compared with the mounted section method.

1.3.1 Methods

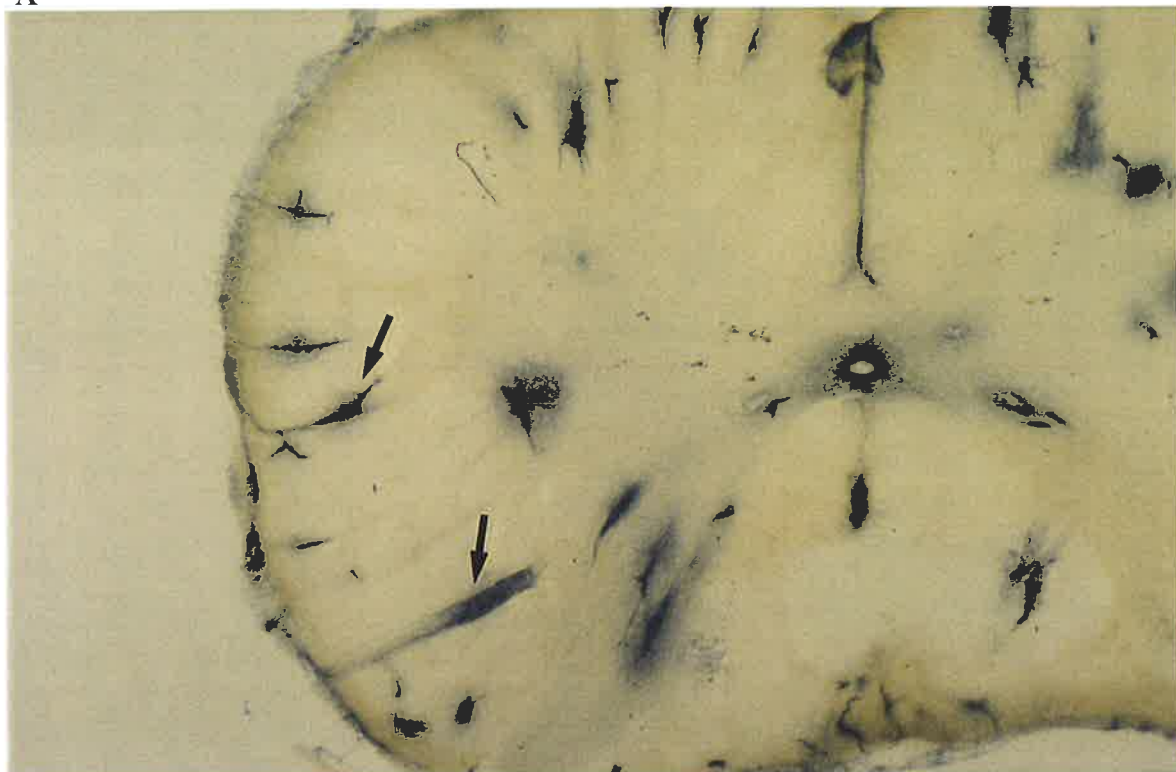
A Sprague-Dawley rat weighing 305 g was anaesthetised and positioned as described above (see *Anaesthesia of rats*, page 92). This rat was also used in the *Spinal fluid flow in normal rats* study. The animal was perfuse-fixed 10 min after the HRP injection and the spinal column and skull were post-fixed as previously described. After removing the brain and spinal cord, sections from the cerebrum, medulla and cervical, thoracic and lumbar cord were cut with a vibratome to a thickness of 50 μm to 100 μm . Half the sections from each level were immediately processed using the floating-section TMB method, the other half were mounted on glass slides and allowed to air-dry overnight before the mounted-section TMB processing method was used.

1.3.2 Results

Representative sections from each of the methods are shown in Figure 8. Endogenous peroxidase activity was seen in ependymal cells and remaining erythrocytes. This was a uniform light blue staining in the ependymal cells and a dark staining in the red cells, but these appearances were quite distinct from the granular dark blue HRP reaction product. Floating section processing resulted in a higher level of endogenous peroxidase activity than did mounted section processing, but the patterns and intensity of HRP activity were not discernibly different (Figure 8).



A



B

Figure 8. Comparison of floating and mounted TMB processing. Floating section processing (A) results in a higher background (endogenous) level of peroxidase activity than mounted section processing (B), but HRP activity (arrows) is unaffected. Section size and shape is better preserved using the mounted processing method (B). HRP/TMB, $\times 25$.

1.3.3 Discussion

The original descriptions of the TMB method for HRP localisation were for floating section processing and this method has been used by most investigators,^{183,288,289,373,374,384} although mounted section processing is an accepted alternative (W. Blessing, personal communication, 1994). The mounted section method is preferable when dealing with sections from many different levels of the neuraxis because it allows simultaneous processing of all sections. This experiment was done to ensure that the results of this method are comparable to those of the floating section method. The results confirm that the pattern and distribution of HRP reaction product are similar with the two methods. The floating section method produces a higher level of endogenous peroxidase activity, but this does not obfuscate the interpretation of HRP activity.

2 Spinal fluid flow in normal rats

This experiment was performed to test the hypothesis that CSF normally flows from the subarachnoid space into the perivascular spaces and then across the interstitial space to the central canal.

2.1 Methods

2.1.1 Animals

Twenty adult male Sprague-Dawley rats weighing 350-500 g were used (Table 4).

2.1.2 Surgical procedures

The animals were anaesthetised as described previously. There were two groups of ten: in one group tracer was injected into the cisterna magna, the second group received an injection into the subarachnoid space at the level of the 10th thoracic vertebra. All operative procedures were performed using microsurgical technique without electrocautery.

In nine animals, normal saline containing 3% HRP was injected into the cisterna magna as described previously. A volume of 10 μL was injected at 2 $\mu\text{L}/\text{min}$ for 5 min. One control animal received an injection of normal saline without HRP. Three of the HRP-injected animals were sacrificed at each of the following times after completing the injection: 0 min, 10 min, and 30 min. The control animal was sacrificed 10 min after injection.

In nine animals, normal saline containing 3% HRP was injected into the spinal subarachnoid space at 2 $\mu\text{L}/\text{min}$ for 5 min for a total of 10 μL . One control animal received an injection of normal saline without HRP. Animals were sacrificed after injection as in the cisternal injection group.

2.1.3 Tissue processing and HRP localisation

After perfusion-fixation, the skull and spine were removed and postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer before removing the brain and spinal cord.

Blocks were taken from the cerebral hemispheres, midbrain, medulla, C2, C4, C6, C8, T8, L3 and conus medullaris. Transverse sections of 50-100 μm were cut from each block using a vibratome and washed in Tris buffer (pH 7.6) before mounting on glass slides and air-drying. Longitudinal sections were also cut from some blocks. The sections were soaked for 20 min in 0.01 mol/L sodium acetate buffer containing 0.005% 3,3',5,5' tetramethylbenzidine and 0.1% sodium nitroprusside. Hydrogen peroxide was added to achieve a final concentration of 0.01% and the sections were incubated for a further 20 min. The reaction product was stabilised in a 5% solution of ammonium molybdate in 0.01 mol/L sodium acetate buffer.

2.2 Results

2.2.1 Controls

Endogenous peroxidase activity was seen in ependymal cells and remaining erythrocytes. This was a uniform light blue staining in the ependymal cells and a dark staining in the red cells, but these appearances were quite distinct from the granular dark blue reaction product seen after HRP injection.

2.2.2 Cisternal injection

2.2.2.1 0 min

HRP reaction product was detectable in the pia and the underlying extracellular space in all sections except the conus medullaris. The depth of this transpial HRP diffusion varied according to the distance from injection (Figure 9). Reaction product was seen in perivascular spaces in the basal portions of the cerebral hemispheres and in the midbrain, medulla and all spinal levels. Labelled vessels were more numerous in the more rostral spinal cord sections. All white matter columns, dorsal and ventral horns and central grey matter contained labelled vessels. Vessels traversing the white matter entered the dorsal or ventral horns, but their perivascular spaces were labelled only in the white matter. Vessels entering the grey matter

from the ventral median fissure contained reaction product throughout their length, as did vessels entering the dorsal horns from the posterior surface of the cord.

In all rostral cervical sections, the central canal contained reaction product and the central grey matter often contained diffuse interstitial labelling and numerous labelled perivascular spaces (Figure 10A). The central canal was labelled in some caudal cervical, thoracic, lumbar and conus sections. The central canal was labelled in some sections that were caudal to unlabelled sections. In some thoracic and conus sections, reaction product was seen in perivascular spaces near the central canal, and extending through the interstitial space and the ependymal lining into the central canal (Figure 10B). In these sections, HRP reaction product in the ependymal lining was often only in that section of ependyma closest to the perivascular space, and there was no reaction product in the interstitial space in other regions adjacent to the central canal.

Although perivascular spaces close to the lateral, third and fourth ventricles and the cerebral aqueduct were labelled and there was spread of reaction product into the surrounding interstitial space, there was no evidence of preferential flow from the perivascular spaces towards the ependymal lining of the ventricles or aqueduct.

2.2.2.2 10 min

Transpial HRP diffusion depth was greater in this group than in the previous group (Figure 9). Compared with the 0 min group, there were more perivascular spaces labelled in the cerebral hemispheres, midbrain and medulla. There was denser labelling of the pia over the convexities of the cerebral hemispheres than in the 0 min group. The central canal contained reaction product at all spinal levels except at the conus in one animal. The cervical central grey matter usually contained diffuse reaction product, but in some sections a distinctive pattern of labelling from a perivascular space to the central canal was seen (Figure 11A). This was particularly seen in relation to vessels entering the cord from the ventral

median fissure. A similar pattern occurred in some lumbar and thoracic sections (Figure 11B).

2.2.2.3 30 min

The depth of transpial HRP diffusion increased further in this group (Figure 9). Numerous perivascular spaces were labelled at all levels. There were numerous perivascular spaces labelled in the cerebral hemispheres, but no evidence of a flow from these spaces towards the cerebral ventricles or aqueduct. The central canal contained dense reaction product at all spinal levels. The grey matter surrounding the central canal was diffusely labelled and no distinct pattern of label between perivascular spaces and the central canal could be identified.

Trans-Pial HRP Diffusion After Cisternal Injection

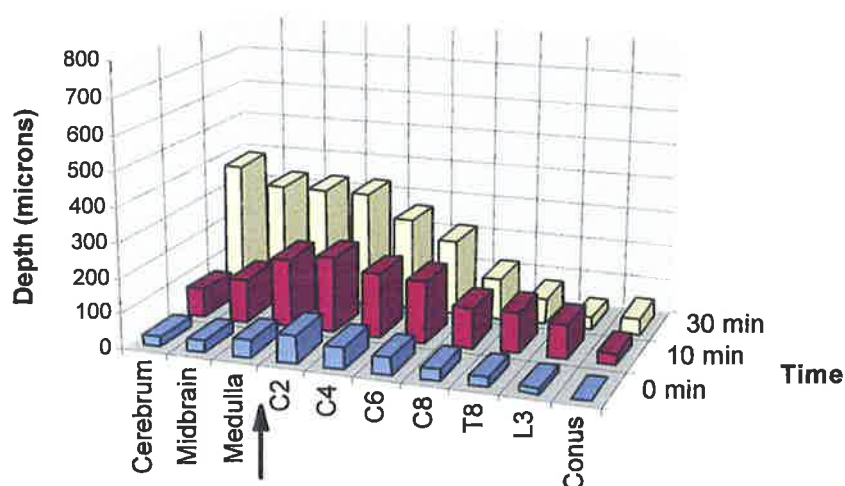
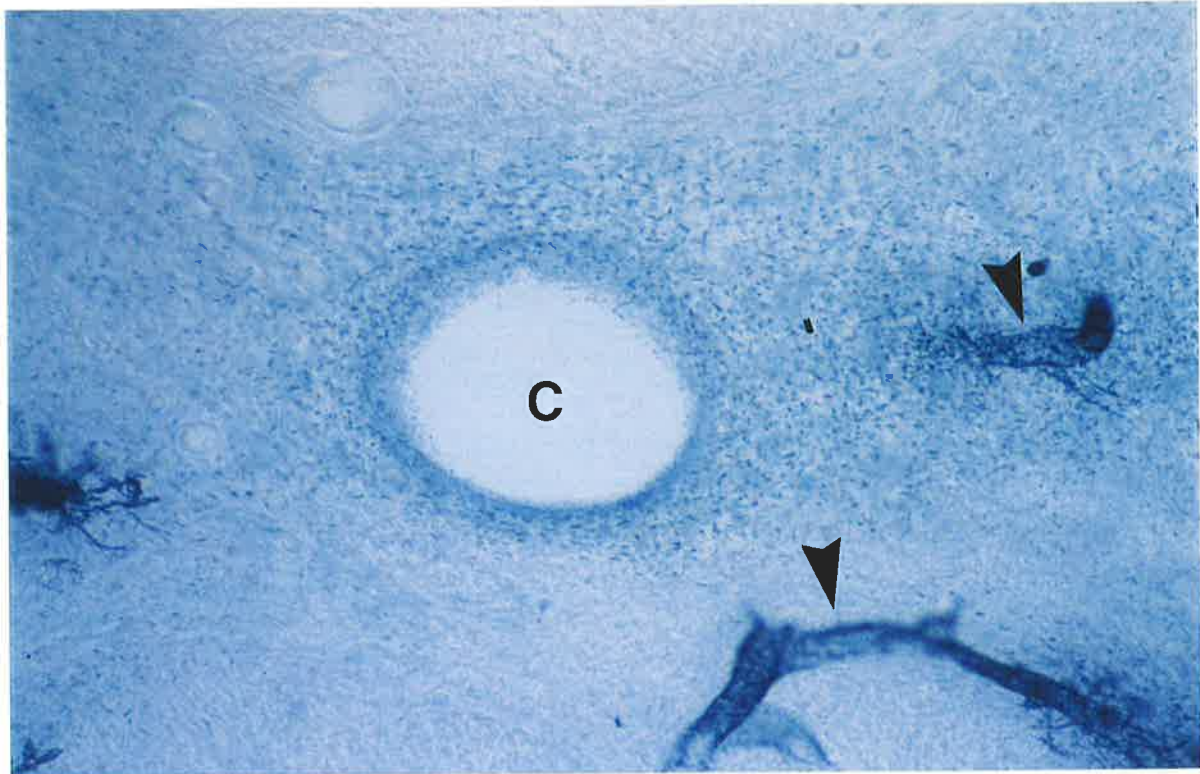
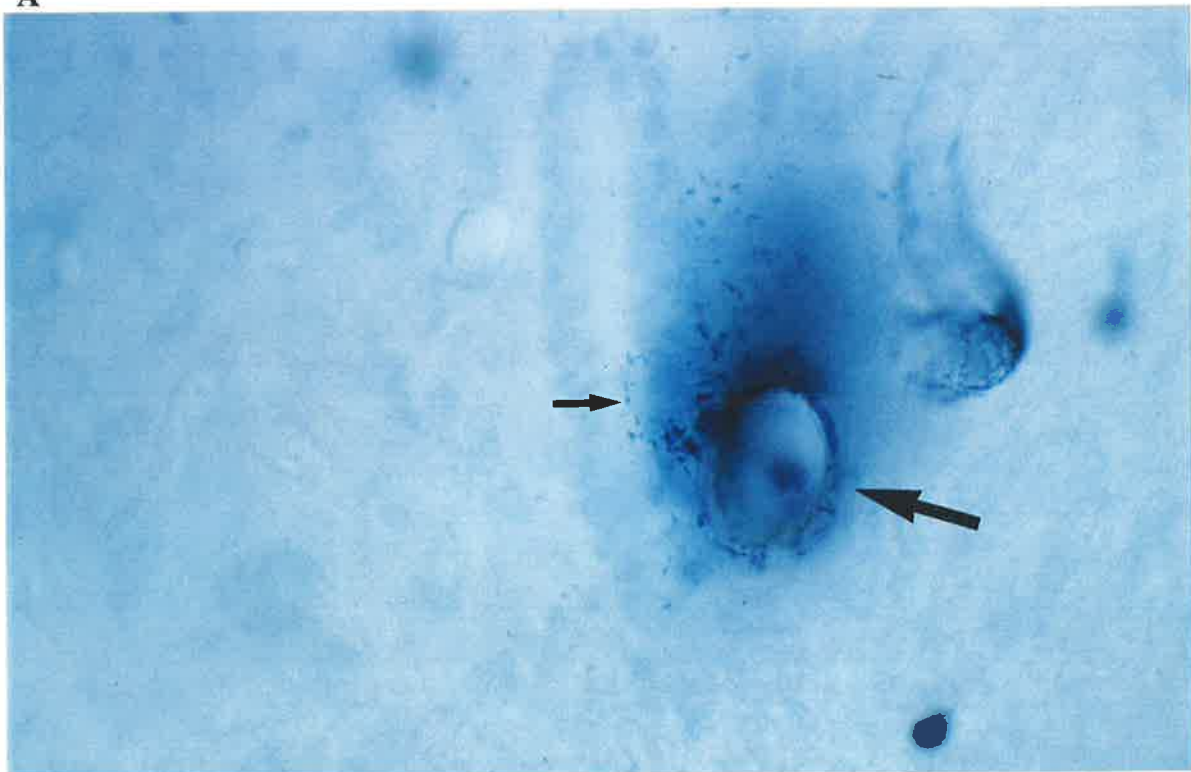


Figure 9. Mean transpial penetration of HRP at each level in rats sacrificed at various times after cisterna magna HRP injection. Arrow: injection site.

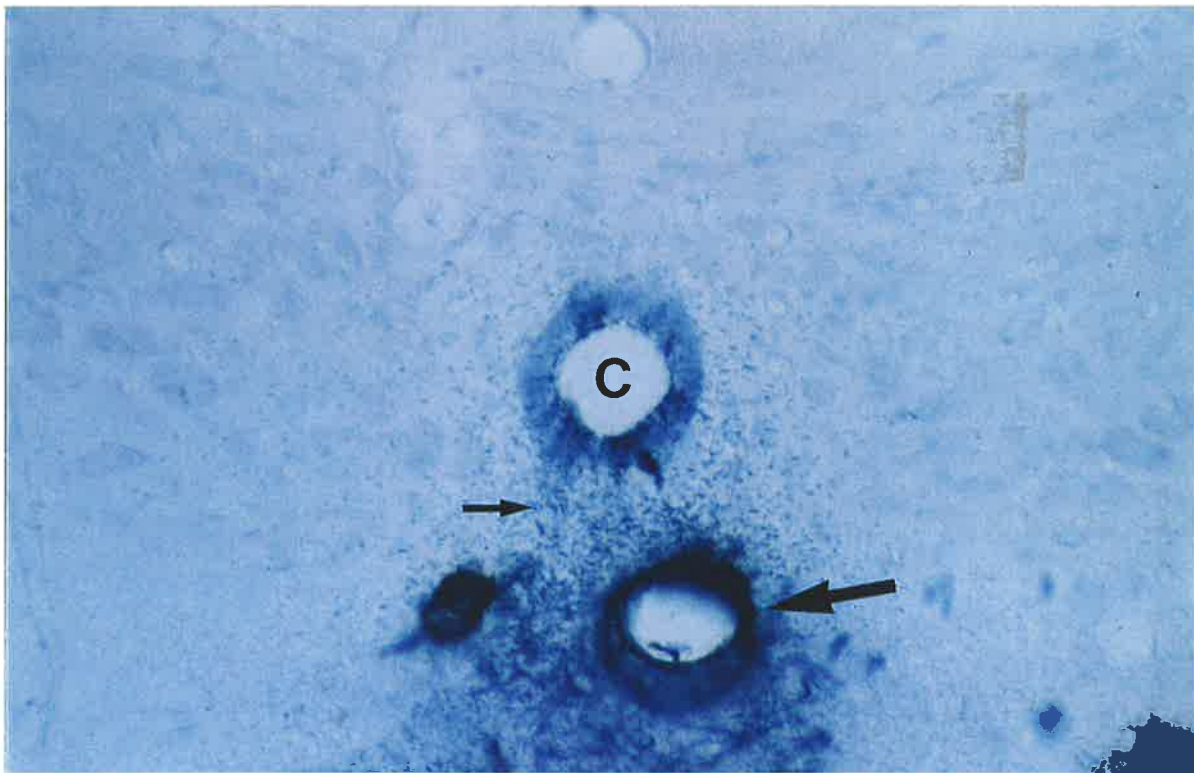


A

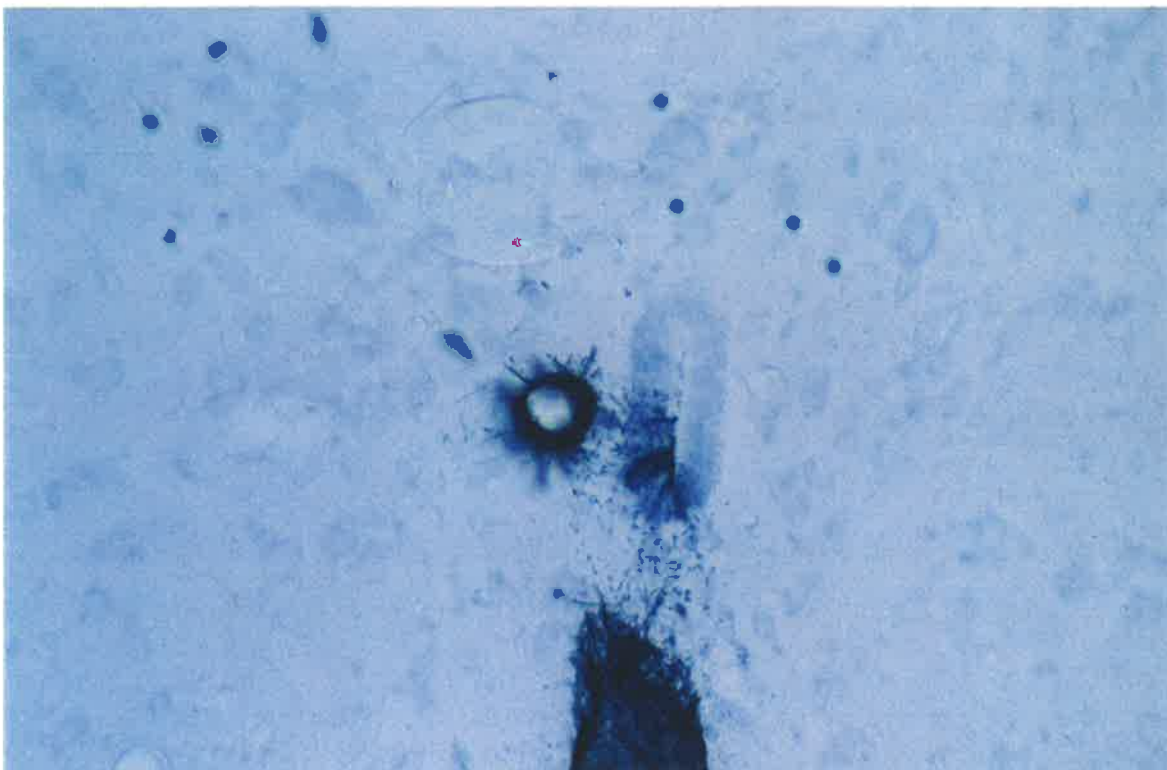


B

Figure 10. Photomicrographs of transverse spinal cord sections 0 min after cisternal HRP injection. A: C8. HRP reaction product is lightly labelling the central canal (C) and the extracellular space of the central grey matter. Numerous perivascular spaces in the central grey matter are heavily labelled (arrows). HRP/TMB, $\times 200$. B: conus medullaris. Reaction product is in a perivascular space (arrow) close to the central canal and is streaming between ependymal cells to reach the central canal lumen (small arrow). There is no label in the ependymal lining or interstitial space adjacent to the central canal except near the labelled perivascular space. The central canal in the lumbar cord rostral to this section did not contain HRP reaction product. HRP/TMB, $\times 400$.



A



B

Figure 11. Photomicrographs of transverse spinal cord sections 10 min after cisternal HRP injection. A: C2. HRP reaction product is densely staining central grey matter perivascular spaces (large arrow) and spreading through the interstitial space (small arrow) and ependyma to reach the central canal (C). HRP/TMB, $\times 200$. B: L3. HRP reaction product is spreading from perivascular spaces in the central grey matter into the central canal. HRP/TMB, $\times 200$.

2.2.3 Spinal injection

2.2.3.1 0 min

The depth of transpial diffusion varied according to the distance from injection (Figure 12). Few perivascular spaces were labelled in the cerebral hemispheres, midbrain and medulla. There was a gradation of perivascular labelling in cervical sections. In the most caudal cervical section (C8), there were many perivascular spaces labelled in all white matter columns. In the more rostral cervical sections, there were fewer labelled perivascular spaces. The reaction product was limited to the white matter segments of these vessels, even where the vessels could be seen to enter the grey matter. Label was seen around many vessels in the central grey matter. The thoracic sections in all animals were heavily labelled with reaction product. At this level, there was perivascular reaction product in many vessels of the white matter, central grey matter and ventral and dorsal grey matter. In addition, there was diffuse interstitial labelling of all white matter columns. There was labelling of numerous vessels in the grey and white matter in the lumbar sections, but fewer labelled vessels in the sections of conus medullaris.

The central canal was heavily labelled with reaction product in the thoracic and lumbar sections, and lightly labelled in the conus. In the cervical cord, label was seen only in the lower one or two segments (C6 and C8), except in one animal where label was present in the central canal of some C2 sections. In some blocks, label in the central canal was seen only in sections where a labelled vessel was close to the canal and reaction product appeared to be spreading from the perivascular space, through the ependymal lining and into the canal (Figure 13). This pattern of reaction product spreading from vessels in the central grey matter into the central canal was also seen in other blocks where all sections had label in the central canal.

2.2.3.2 10 min

Pial staining was seen in all spinal cord sections and the depth of staining was less in sections further from the injection point (Figure 12). Some perivascular spaces in the basal parts of the cerebral hemispheres and the midbrain were labelled. Numerous vessels in the medulla were outlined and there was pial and ependymal labelling at this level. Perivascular spaces were labelled in the white and grey matter at all levels of the cord; sections closer to the injection point contained greater numbers of labelled vessels. The central canal contained reaction product at all spinal levels, although in the most rostral cervical blocks only sections with a labelled vessel close to the central canal had label in the canal. In many sections, reaction product appeared to spread from the perivascular space, through the ependyma and into the central canal.

2.2.3.3 30 min

Transpial diffusion of reaction product was seen in all spinal cord sections. The depth of diffusion varied with the distance from injection (Figure 12). Reaction product was seen in perivascular spaces throughout the brain and spinal cord. Although numerous perivascular spaces in the grey and white matter were labelled in all cervical sections, there were more vessels labelled in the caudal sections than in the rostral sections. In the thoracic and lumbar regions, there was diffuse labelling of virtually the entire white matter, and of the grey matter in the dorsal horns. The central canal contained reaction product at all levels except in some mid-cervical blocks. Sections from some rostral cervical blocks contained reaction product only if there was label in a perivascular space in the adjacent central grey matter and between this vessel and the central canal. Spread of HRP from perivascular spaces into the central canal was seen in many cervical and some lumbar sections (Figure 13). The central grey matter in other sections contained diffuse interstitial HRP reaction product.

Trans-Pial HRP Diffusion After Spinal Injection

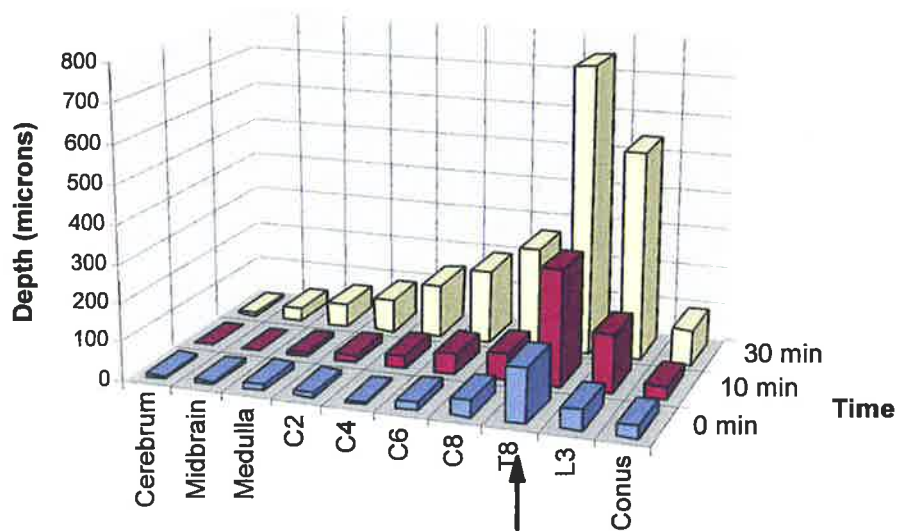
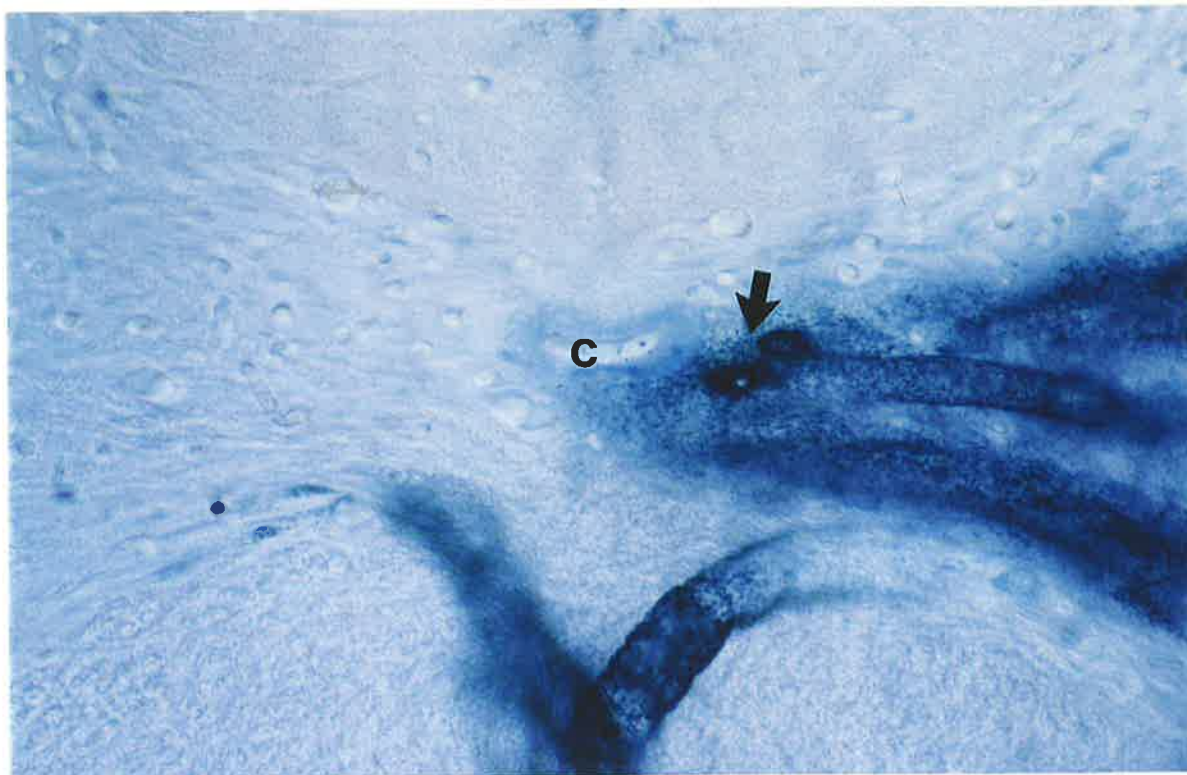
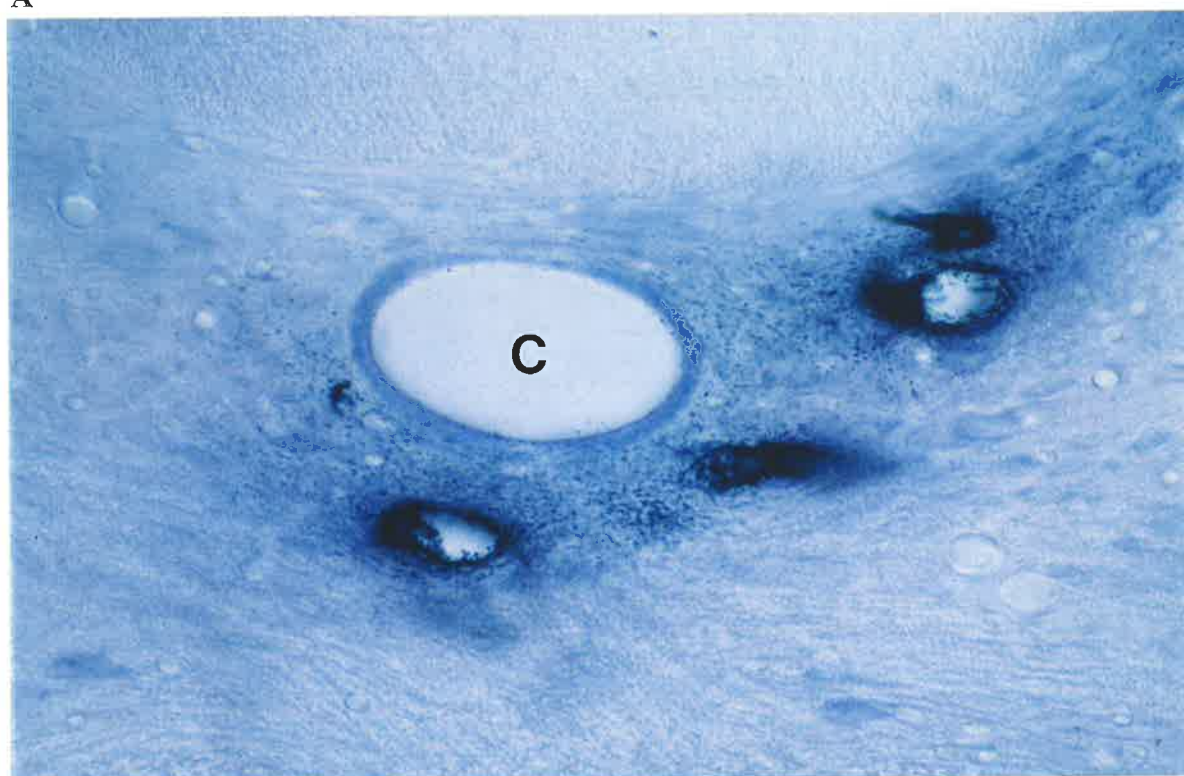


Figure 12. Mean transpial penetration of HRP at each level in rats sacrificed at various times after spinal HRP injection. Arrow: injection site.



A



B

Figure 13. Photomicrographs of transverse spinal cord sections after thoracic subarachnoid injection of HRP. A: C8, 0 min after injection. HRP is present in perivascular spaces (arrow) near the central canal and is spreading from the perivascular spaces into the central canal (C), the lumen of which is lightly labelled with reaction product. HRP/TMB, $\times 200$. B: C4, 30 min after injection. HRP appears to be spreading from perivascular spaces, through the interstitial space and ependymal lining to reach the central canal (C). HRP/TMB, $\times 200$.

2.3 Discussion

2.3.1 CSF flow in the subarachnoid space

HRP spread rostrally and caudally within the subarachnoid space, as indicated by transpial HRP staining. The caudal spread of HRP after cisternal injection was more marked than the rostral spread after spinal injection, suggesting that CSF is flowing predominantly in a caudal direction in the spinal subarachnoid space.

2.3.2 Perivascular fluid flow

Perivascular labelling by HRP is most unlikely to be a postmortem diffusion artifact. In addition to the reasons cited by Rennels et al^{373,374} and Borison et al,⁵⁴ this study showed many vessels outlined in sections where there was little transpial diffusion of HRP. There was rapid movement of HRP from the subarachnoid space into the perivascular spaces of vessels in the brain and spinal cord, as previously demonstrated by Rennels et al.^{373,374} The rapid fluid flow demonstrated in this experiment and by Rennels et al^{373,374} is at variance with the results of Ichimura et al²²¹ who found only slow flow using fluorescently-labelled albumin injected directly into the perivascular space. The findings in the latter study may have suffered from the fact that the relationship between the intensity of the fluorescence and the concentration of the fluorophore is not linear.³⁶⁸ An alternative explanation is that the source of the rapid fluid flow is not the extension of the perivascular space around vessels in the subarachnoid space (the site of the microinjection performed by Ichimura et al²²¹) but that it is CSF in the subarachnoid space entering the perivascular space through the pia.

Cifuentes et al⁸⁸ have described different types of vessels in the spinal cord. After HRP injection, circumferential penetrating 'Type-A' vessels have reaction product in their perivascular spaces as they traverse the white matter but not where they enter the grey matter. 'Type-B' vessels enter from the ventral median fissure and contain HRP throughout their course in the grey matter. 'Type-A' and 'Type-B' vessels were identified in this study, but in

addition, there were vessels entering the dorsal horns from the periphery that were labelled in their grey matter course. The anatomical or functional basis for these differences is not known.⁸⁸

HRP reaction product spread from the perivascular spaces into the surrounding interstitial space. In the spinal cord there was preferential flow towards the central canal (*vide infra*). In the cerebrum, midbrain and medulla there was also flow from the perivascular spaces into the surrounding interstitial space, but there was no evidence of preferential flow toward the ventricles or cerebral aqueduct. It may therefore be that the fluid dynamics of the brain and spinal cord are different—with a flow toward the central canal in the spinal cord and a flow away from the ventricles in the brain. The direction of fluid flow in the brain has been a matter of controversy.^{17,88,97,301} This study does not support the existence of a normal fluid flow into the ventricles from the interstitial space.

2.3.3 Fluid flow into the central canal from perivascular spaces

The results of this study strongly suggest that CSF flows from the perivascular spaces, across the interstitial space and into the central canal. Evidence for this includes: 1) the patterns of HRP reaction product seen between perivascular spaces and the central canal; 2) the presence of reaction product in regions of central canal caudal to unlabelled segments of central canal; 3) the presence of reaction product in the central canal in some blocks only in those sections that had a labelled vessel near the central canal; and 4) the rapid diffuse labelling of the central canal and the surrounding grey matter.

The patterns of HRP reaction product seen in many sections were quite distinctive. From heavily labelled perivascular spaces, HRP reaction product was distributed in the interstitial space towards, and into, the central canal. There was a more limited spread of HRP from perivascular spaces in directions away from the central canal. In many cases, the only reaction product visible in the central canal or ependyma, was that apparently coming from a

nearby perivascular space. In some cases, the reaction product was clearly visible between only those ependymal cells on the side of the central canal nearest a labelled perivascular space. Unless there was diffuse labelling of the grey matter around the central canal, the only reaction product visible in the interstitial space near the central canal was that between the canal and a labelled perivascular space.

A theoretical possibility is that after injection, HRP entered the fourth ventricle and moved caudally in the central canal, then passed through the ependymal lining to the surrounding interstitial space. However, HRP reaction product was seen in segments of central canal caudal to unlabelled segments of central canal. This finding was seen both after cisternal and spinal injection. In the more caudal segments, HRP could be seen between the central canal and a perivascular space in the pattern described above. The possibility that HRP entered the caudal central canal opening³²⁴ is also unlikely, given that HRP appeared first in the rostral central canal after cisternal injection, and in the lumbar and thoracic cord after spinal injection. Further evidence against a central canal to perivascular flow is that reaction product in the central canal of some segments of spinal cord was seen only in sections where a labelled vessel was close to the canal and reaction product could be seen between the vessel and the canal. Other sections from the same segments of cord did not have reaction product in the central canal. This is strongly supportive of the concept that HRP (and CSF) is entering the central canal from the perivascular spaces rather than HRP moving along the central canal either from the fourth ventricle or the caudal opening. Finally, the rapid labelling of the central grey matter is more consistent with flow from the perivascular spaces than diffusion from the central canal.

The anatomical pathway for fluid communication between the perivascular spaces and the central canal has been demonstrated by Cifuentes et al.⁸⁸ There are subependymal labyrinths around the central canal that may represent differentiation of the basement

membrane of subependymal vessels.⁸⁸ HRP moves freely from the subarachnoid space, follows the perivascular spaces of vessels entering the cord from the ventral median fissure and enters the subependymal labyrinths. In turn, the labyrinths are in communication with the ependymal intercellular space and the central canal lumen.⁸⁸

Although Cifuentes et al⁸⁸ considered that this anatomical arrangement allowed 'free transfer' of CSF, Rennels et al³⁷⁴ have shown that flow along the perivascular spaces is dependent on arterial pulsations. HRP injected into the subarachnoid space rapidly outlined the vessels of the spinal cord, but this did not occur if arterial pulsations were diminished by ligating the aorta without reducing mean arterial pressure.³⁷⁴ It is therefore possible that rather than 'free transfer,' there is a net unidirectional flow of fluid, driven by arterial pulsations. Perivascular spaces in the spinal cord are covered at the surface by pia mater,²²⁰ are lined by the basement membrane of the glia limitans¹³⁹ and extend as far as capillaries.⁶¹ Capillaries have a sleeve-like basal lamina that is composed of a macromolecular meshwork which communicates with the extracellular space via gaps between adjacent astrocytic foot processes.³⁷³ Systolic expansion of arteries in the perivascular space may force fluid through the surrounding basement membrane, while in diastole fluid may enter the perivascular space from the subarachnoid space through gap junctions in the covering pia. The function of such a unidirectional flow may be to clear metabolites, neurotransmitters and breakdown products from the extracellular space—the 'lymphatic' system of the spinal cord. Alternatively, it may have a role in non-synaptic neurotransmission.¹⁷ It would certainly account for the accumulation of contrast media in the spinal cord after myelography.^{118,216,222,390}

The existence of a one-way fluid flow into the central canal would add considerable support to the theory that non-communicating syringomyelia develops in segments of central canal isolated by stenosis or occlusion. The source of fluid in such cases has long been an enigma.^{175,313,499} Most theories have suggested that an alteration of CSF flow in the

subarachnoid space results from craniospinal pressure differentials, however the mechanism by which fluid enters the cysts has been obscure.^{175,499} The continuation of an arterial-pulsation driven flow along perivascular spaces into the central canal would explain the accumulation of fluid and development of cysts in such cases.

3 Spinal fluid flow in normal sheep

This experiment was performed to determine whether the fluid flow from perivascular spaces into the central canal demonstrated in rats also exists in sheep. If so, it would add support to this being a normal physiological flow in vertebrates, including man. The use of a larger animal than the rat also allows study of fluid flow after varying arterial pulsations and spinal subarachnoid pressure.

3.1 Methods

Five merino wethers weighing 44 kg to 54 kg were used (Table 5). Each animal was anaesthetised as described previously and mechanically ventilated. At the beginning of each experiment, the common carotid arteries and internal jugular veins were dissected out in preparation for rapid perfusion-fixation, but these vessels were not ligated at this stage. Fluid-coupled pressure transducers (Ohmeda DTX) were used to monitor arterial pressure in the right axillary artery and the central venous pressure via the right axillary vein. After these catheters were placed, the animal was turned to the right lateral position for the remainder of the experiment. Intracranial pressure was monitored using a fibre-optic probe (Camino Laboratories, San Diego) inserted in the left parietal region. The spinal pressure was monitored using a fluid-coupled transducer connected to a 23 gauge catheter placed in the cervical subarachnoid space via a laminotomy at the C2 level and secured with cyanoacrylate to prevent CSF leakage. Pressures were recorded in digital form using a MacLab/8s (AD Instruments, Castle Hill, Australia) and analysed using the MacLab Chart software running on a Macintosh Quadra 605 (Apple Computer Inc).

CSF tracer was administered into the subarachnoid space at the C4/5 level as described previously (see *Injection into the spinal subarachnoid space of sheep*, page 109) with a tuberculin syringe and a 26 gauge needle. One control animal was injected with 250 μ L normal saline; all other animals received 250 μ L 4% HRP in normal saline injected over 30

seconds. The needle was left in place to prevent CSF leakage. One animal was sacrificed immediately after injection; the other four, including the control animal, were sacrificed 10 minutes after injection. Immediately on completion of each experiment, the animal was perfused with paraformaldehyde (see *Perfusion-fixation of sheep*, page 93).

3.2 Results

Endogenous peroxidase activity was seen as a light blue background staining and a darker staining in ependymal cells and the few remaining erythrocytes. The control animal (injected with normal saline) had no other visible reaction product; HRP reaction product was seen in the other animals as a granular dark blue staining distinct from the endogenous activity. The animal sacrificed immediately after injection had HRP reaction product in perivascular spaces of the grey and white matter of each cervical spinal cord level. Staining was more marked in the sections close to the injection point. Although reaction product was visible in central grey matter perivascular spaces and in some cases was spreading toward the central canal (Figure 14), the central canal itself did not contain reaction product in any of the sections. No HRP reaction product was seen in the thoracic, lumbar or conus sections.

Perivascular HRP reaction product was more dense in the animals sacrificed 10 minutes after injection. Numerous perivascular spaces were labelled throughout the grey and white matter of the cervical cord and some were labelled in the thoracic cord. No label was seen in the lumbar or conus sections. In the central grey matter, HRP reaction product was seen spreading from the perivascular spaces into the interstitial space. Reaction product was present in the central canal of many sections throughout the cervical spinal cord. This reaction product appeared to have reached the central canal by spreading from adjacent perivascular spaces in two distinct patterns: from perivascular spaces of central grey matter vessels and from perivascular spaces of vessels in the anterior white commissure (Figure 15). The latter formed a distinctive pattern of reaction product extending from the depths of the

anterior median fissure into the central canal via the embryologically-derived connection between the basal plate and the pial surface. The two patterns were often seen in the same section. The central canal in the thoracic cord did not contain reaction product.

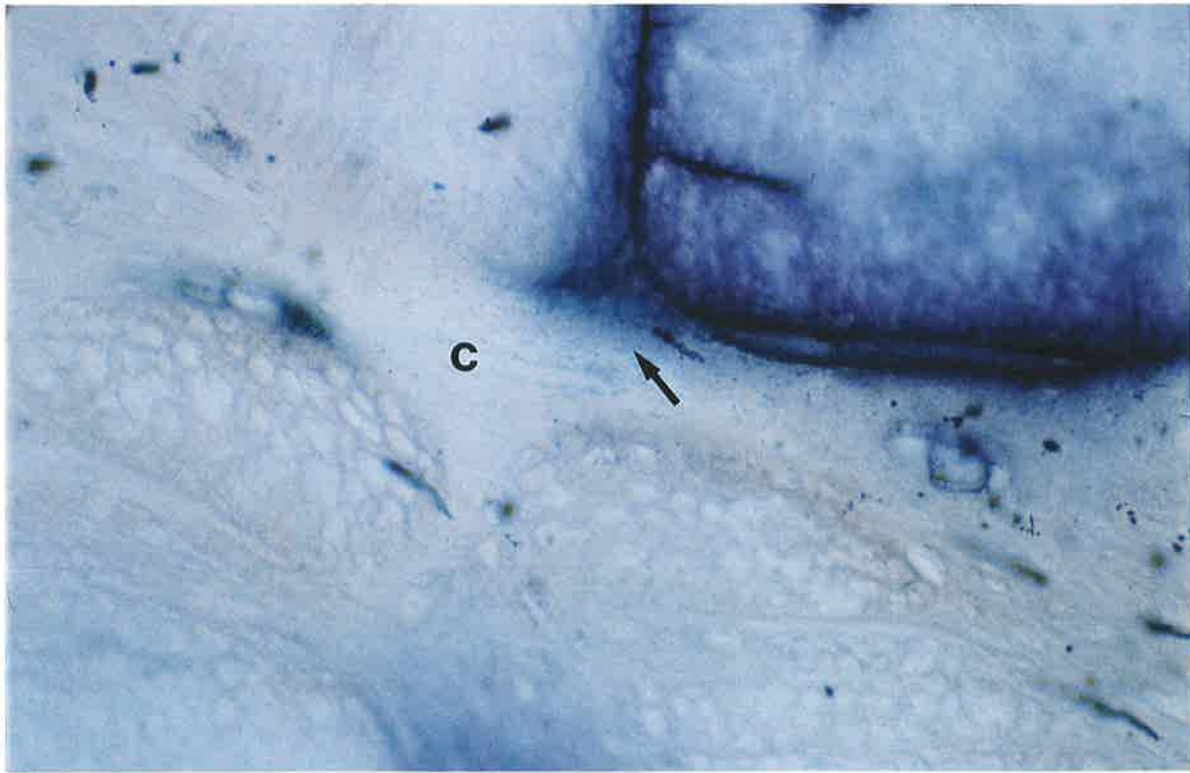
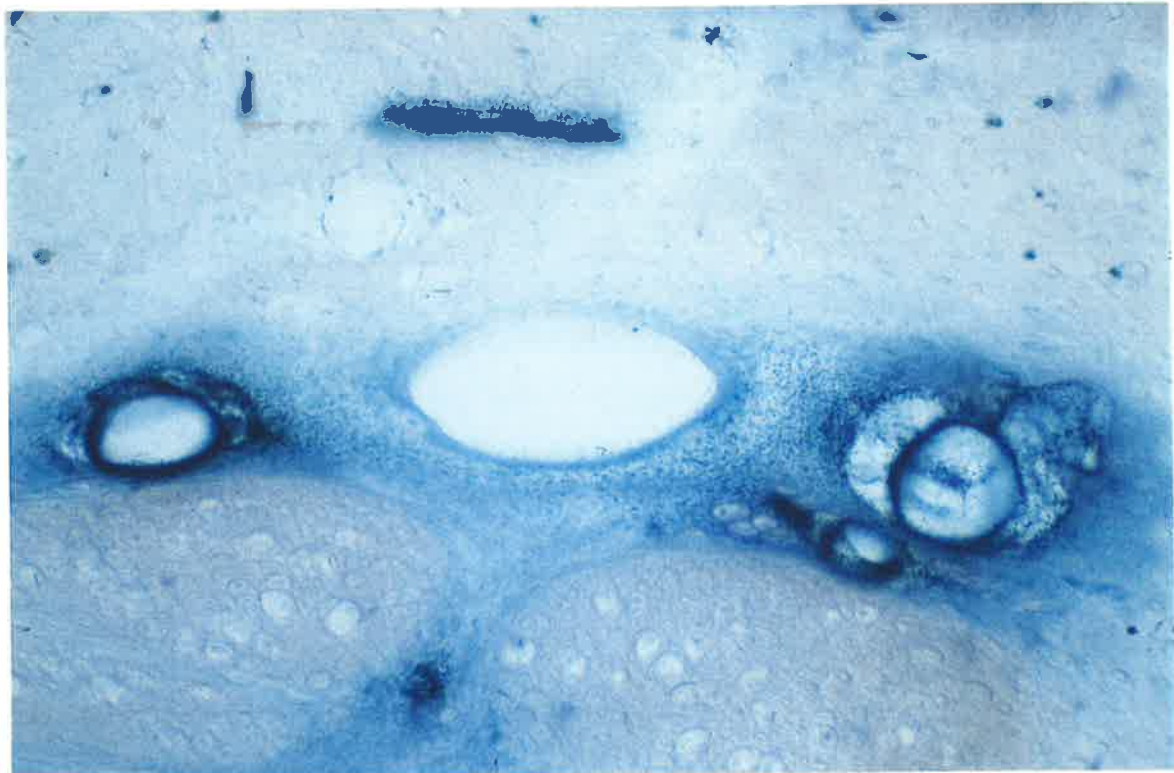
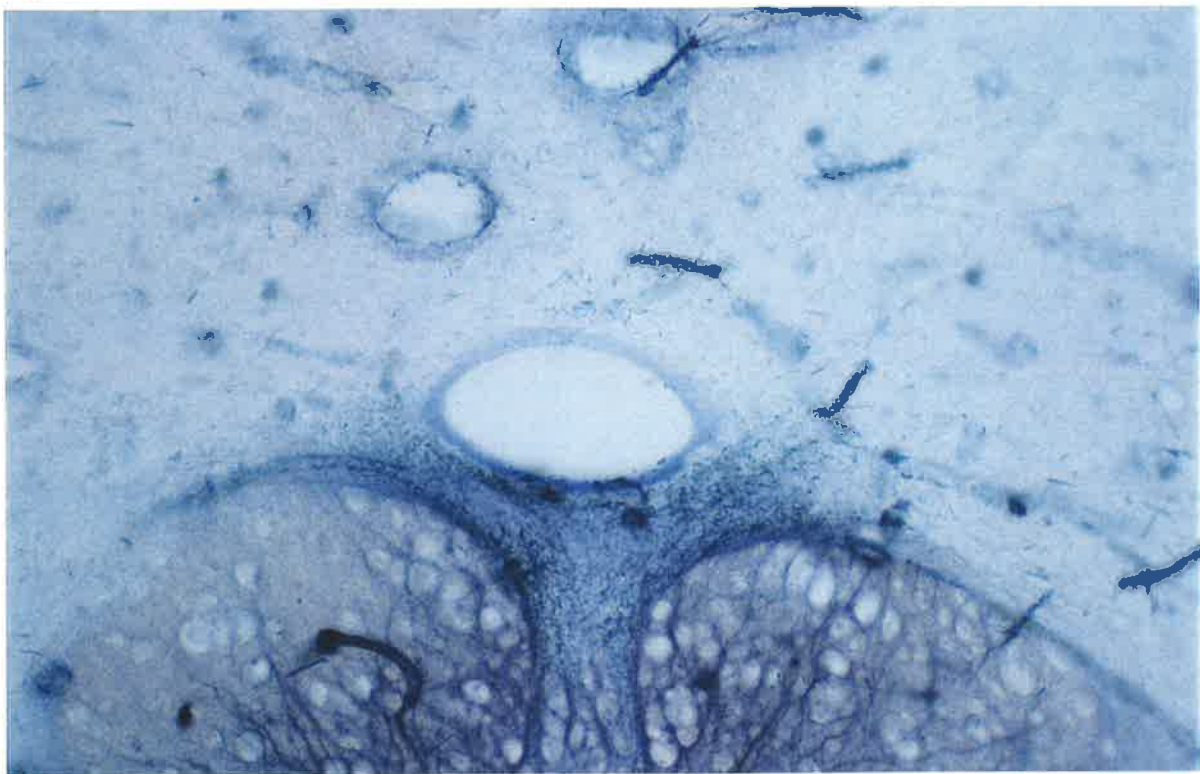


Figure 14. *Photomicrograph of cervical cord section from the sheep sacrificed immediately after HRP injection. HRP reaction product is present in perivascular spaces and is spreading into the interstitial space toward the central canal (arrow). The central canal (C) does not contain reaction product. HRP/TMB, $\times 100$.*



A



B

Figure 15. Photomicrographs of cervical cord sections from sheep sacrificed 10 min after HRP injection. A: Reaction product is in perivascular spaces of central grey matter vessels and is spreading into the central canal. B: HRP reaction product is present in perivascular spaces in vessels entering the cord from the ventral median fissure and is spreading into the central canal in a distinctive pattern. HRP/TMB, $\times 100$.

3.3 Discussion

This study was performed to establish whether the fluid flow from perivascular spaces into the central canal that had been demonstrated in rats also exists in sheep. The sheep was chosen because it is a large animal that would allow manipulation of arterial and spinal pressures in subsequent experiments. It is possible that the experimental technique itself could have artificially altered CSF physiology, for example anaesthesia and mechanical ventilation may have altered the intraspinal pressure characteristics. However, the CSF flow demonstrated here in normal anaesthetised sheep is similar to what was observed in self-ventilating rats (see *Spinal fluid flow in normal rats*, page 115) and in self-ventilating sheep in preliminary experiments. Most of the animals in this study were sacrificed 10 minutes after HRP injection because preliminary studies indicated that this gives the earliest and clearest pattern of HRP movement from perivascular spaces into the central canal.

This study has provided further evidence that CSF flows rapidly from the subarachnoid space into perivascular spaces. Rennels et al³⁷⁴ proposed that a 'paravascular' fluid circulation exists in the nervous system, with an active flow of CSF from the subarachnoid space into the perivascular spaces of arteries and arterioles and continuing through the basal lamina around capillaries. Without direct evidence, they suggested that fluid then perfused the surrounding extracellular space to return to the basal lamina around venules or into the perivascular space of emerging veins. It was proposed that this flow may serve as a 'lymphatic' function of the nervous system. Milhorat et al^{294,301,312} and Cifuentes et al⁸⁸ have previously demonstrated that fluid is capable of moving from the spinal cord interstitial space into the central canal. Milhorat et al^{301,312} have suggested that a normal fluid flow from the spinal cord interstitial space into the central canal constitutes the 'lymphatic' function of the spinal cord. The flow from perivascular spaces into the central canal demonstrated in sheep and in rats, suggests that the source of the normal interstitial fluid flow

is CSF from the subarachnoid space flowing into the interstitial space via the perivascular spaces.

4 Spinal fluid flow in rats with isolation of the central canal from the subarachnoid space

The previous experiments have provided evidence of a fluid flow from the perivascular spaces of the spinal cord, across the interstitial space and into the central canal. The tracer used for these experiments was injected into the subarachnoid space. There is a theoretical possibility that the tracer reached the central canal via the fourth ventricle or the caudal opening of the central canal, rather than from the perivascular spaces. This experiment was performed to test the hypothesis that fluid flow from perivascular spaces into the central canal could be demonstrated even when the central canal is isolated from the subarachnoid space by occlusion of the central canal in two places.

4.1 Methods

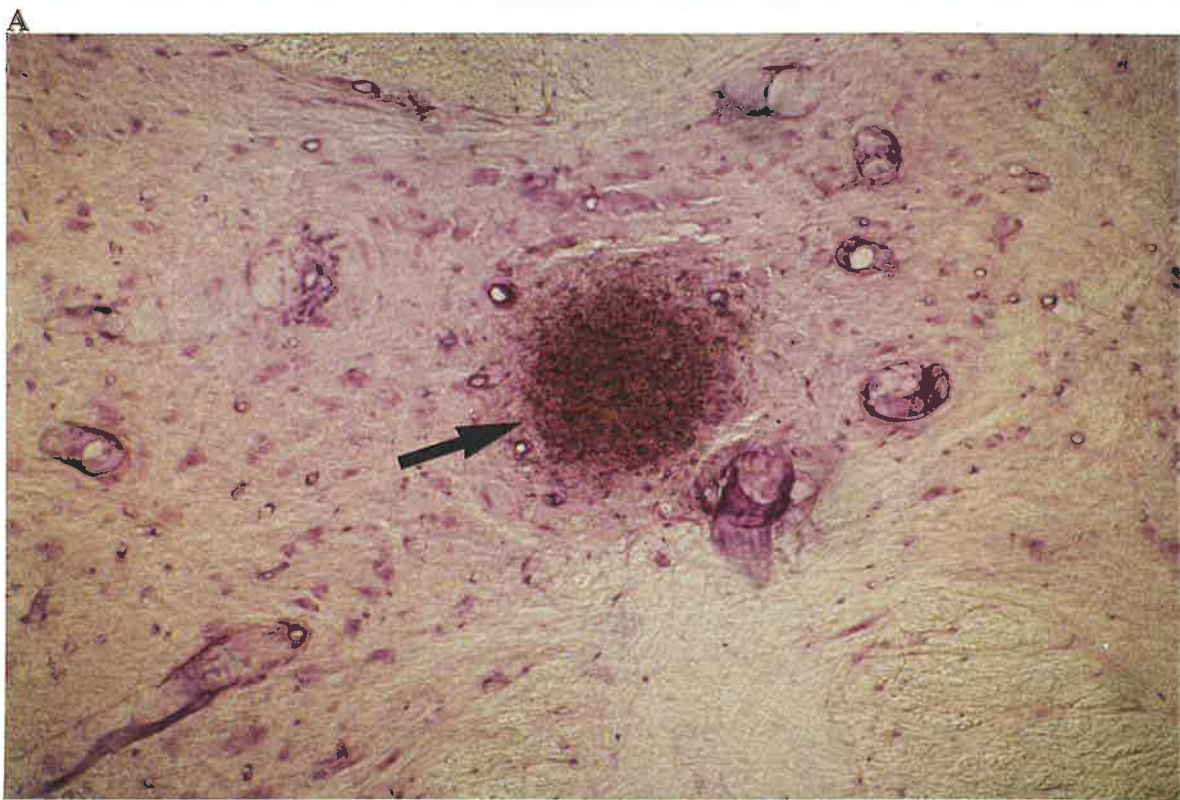
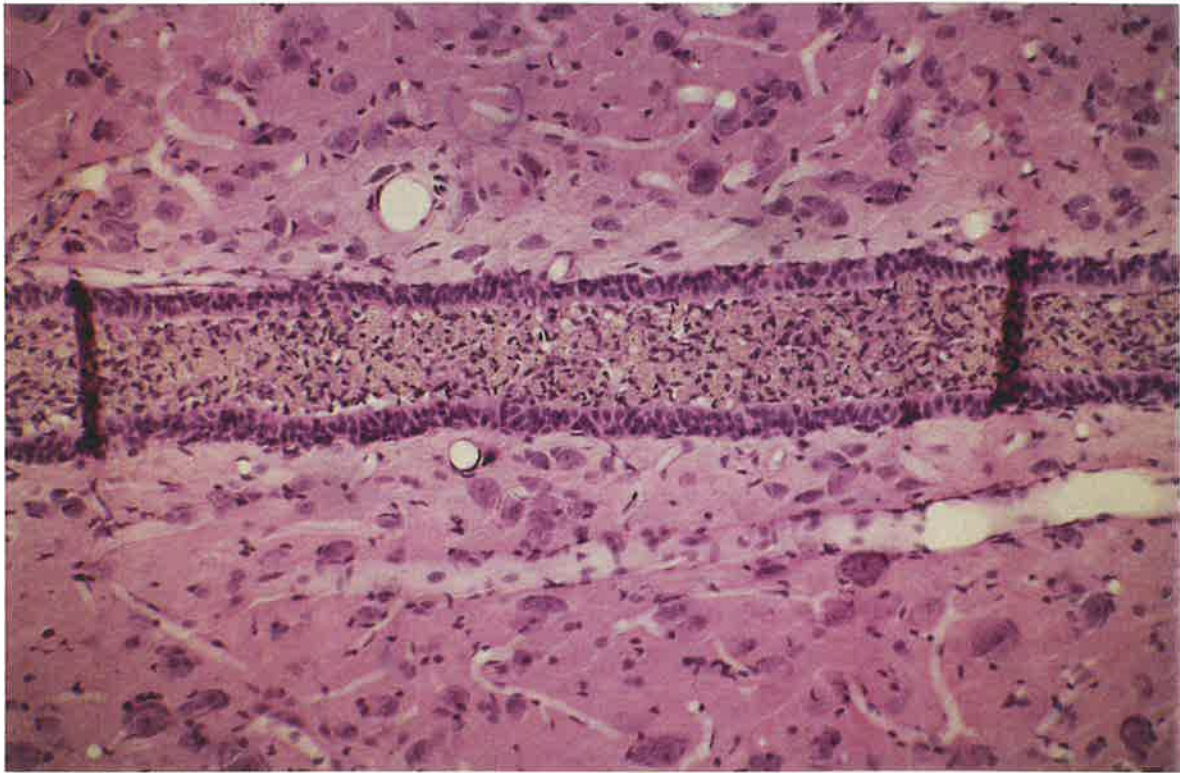
Three male Sprague-Dawley rats weighing 410 g to 420 g were used (Table 4). Isolation of the central canal was achieved by injection of kaolin into the dorsal columns of the spinal cord. This was performed at two levels: at C6 as described previously and at T12, in a similar procedure after laminectomy of T12. The rats were then allowed to recover from the anaesthetic and were returned to their cages. One week later, they were anaesthetised and HRP was injected into the spinal subarachnoid space between the points of kaolin injection. The previously described method for injecting HRP into the thoracic spinal subarachnoid space was used, except that the volume of HRP injected was 5 μ L. One rat was sacrificed immediately after the HRP injection, one 10 min after the injection and one 30 min after the injection. The spinal cord and brain were removed and processed as described previously, except that additional thoracic blocks were cut for TMB processing of transverse sections.

4.2 Results

There were no postoperative neurological deficits observed in any of the rats. Kaolin crystals and inflammatory cells completely filled the central canal at the levels of injection

(Figure 16). The central canal remained patent at other levels of the cord, including the thoracic cord between the injection points (Figure 16).

In all of the rats, there was dense pial and transpial reaction product in the thoracic sections. There was also dense reaction product in the perivascular spaces in the white and grey matter of the thoracic sections. There was less dense staining with reaction product in the surface of the spinal cord and the perivascular spaces at all other levels. In the 0 min and 10 min rats there was reaction product between perivascular spaces and the central canal in the thoracic cord in the pattern described previously. In the 30 min rat the thoracic cord was diffusely labelled with reaction product (Figure 17).



B

Figure 16. Photomicrographs of spinal cord sections from a rat injected with kaolin in the dorsal columns at C6 and at T10. *A*: longitudinal section rostral to the injection point demonstrating occlusion of the central canal with kaolin crystals and inflammatory cells. *B*: transverse section close to the lumbar injection. The central canal is occluded with kaolin crystals and inflammatory cells (arrow). The central canal between the injection points was patent. H&E, $\times 100$.

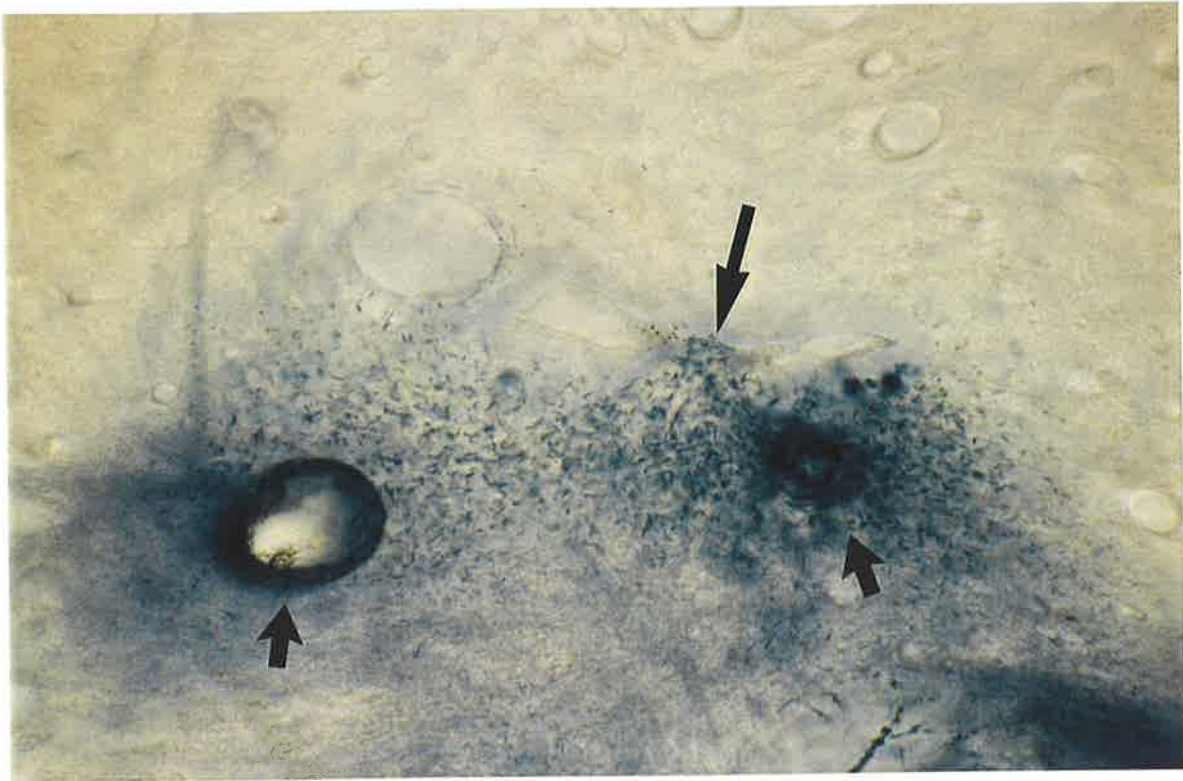


Figure 17. *Photomicrograph of thoracic spinal cord section from the rat sacrificed 10 min after HRP injection. HRP reaction product is in central grey matter perivascular spaces (small arrows) and is spreading from the perivascular spaces into the central canal, which is patent but has an adhesion (large arrow). HRP/TMB, $\times 250$.*

4.3 Discussion

This experiment was performed to confirm that HRP was entering the central canal from the perivascular spaces rather than from the fourth ventricle or the caudal opening of the central canal. The use of the intraparenchymal kaolin injection technique gave clear histological evidence for occlusion of the central canal. Occlusion was seen at each of the levels of kaolin injection and the central canal remained patent between the occlusions. HRP was injected into the spinal subarachnoid space between the occlusions so that HRP could not enter the central canal from the fourth ventricle or from the caudal opening. HRP reaction product was demonstrated in the isolated segment of central canal. In the animals sacrificed 0 min and 10 min after the HRP injection, the distinctive pattern of labelling from the central grey matter perivascular spaces towards and into the central canal was seen. These results

provide very strong support for the existence of a normal flow of fluid from the perivascular spaces into the interstitial space and into the central canal.

HRP was injected into the subarachnoid space between the levels of kaolin injection. The marked difference between the density of reaction product in sections taken from between the kaolin injections and in those taken more rostrally and more caudally suggests that there was a relative block of fluid flow in the subarachnoid space. This obstruction to flow may have been caused by arachnoiditis resulting from the operative trauma of the injection or from small amounts of kaolin that may have spilled into the subarachnoid space at the time of injection. This obstruction to flow may alter the dynamics of fluid flow and may even contribute to the formation of syrinxes in the intraparenchymal kaolin model of syringomyelia. Nevertheless, the patterns of HRP reaction product between perivascular spaces and the central canal were very similar to those seen after HRP injection in normal rats.

5 Spinal fluid flow in sheep with reduced arterial pulsations

The previous experiments have provided strong evidence that there is normally a flow of fluid from the perivascular spaces into the central canal. This experiment was performed to test the hypothesis that this flow is driven by arterial pulsations.

5.1 Methods

Four merino wethers weighing 30 kg to 47 kg were used (Table 1). Each animal was anaesthetised and mechanically ventilated throughout the experiment. At the beginning of each experiment, the common carotid arteries and internal jugular veins were dissected out in preparation for rapid perfusion-fixation, but these vessels were not ligated at this stage. Arterial, central venous, intracranial and spinal subarachnoid pressures were monitored as in the experiments in normal sheep (see *Spinal fluid flow in normal sheep*, page 130).

With each animal in the right lateral position, a left intercostal thoracotomy was performed. In one animal the aortic arch was ligated within the pericardium to abolish arterial pulsations completely. The brachiocephalic artery was partially ligated in two animals. While the axillary artery pressure was monitored, a ligature passed around the brachiocephalic artery was gradually tightened until the pulsatile component of the pressure tracing was diminished. The fourth animal had a 'sham brachiocephalic ligation,' where a ligature was passed around the brachiocephalic artery but was not tightened. Each animal received a subarachnoid injection of 4% HRP in normal saline at the C4/5 level and was sacrificed 10 minutes after injection.

5.2 Results

Ligating the aorta abolished arterial, intracranial and spinal pulsations. HRP reaction product stained the pial surface and some superficial white matter perivascular spaces at each cervical spinal level. At the level of the HRP injection (C4), one section contained reaction product in central grey matter perivascular spaces, but this did not extend into the central canal and no grey matter perivascular spaces were labelled in any other section. There was no HRP reaction product visible in the thoracic, lumbar or conus sections.

Ligating the brachiocephalic artery reduced arterial, intracranial and intraspinal pulse pressures (Figure 18) but mean pressures were maintained close to the pre-ligation levels (Table 7). There was no effect on central venous pressure. The pial surface of each cervical spinal cord section was stained with HRP reaction product and there was labelling of the superficial segments of some white matter perivascular spaces (Figure 19A). Labelled vessels were most numerous close to the point of HRP injection. Reaction product did not reach grey matter perivascular spaces or the central canal at any level. There was no labelling in thoracic, lumbar or conus sections.

Passing a ligature around the brachiocephalic artery without tightening it ('sham brachiocephalic ligation') did not alter arterial, venous, intracranial or intraspinal pressure pulsations. Perivascular spaces were labelled throughout cervical spinal cord grey and white matter and reaction product was seen spreading from perivascular spaces in the central grey matter to the central canal (Figure 19B). There was reaction product in some thoracic superficial white matter perivascular spaces but none in lumbar or conus sections.

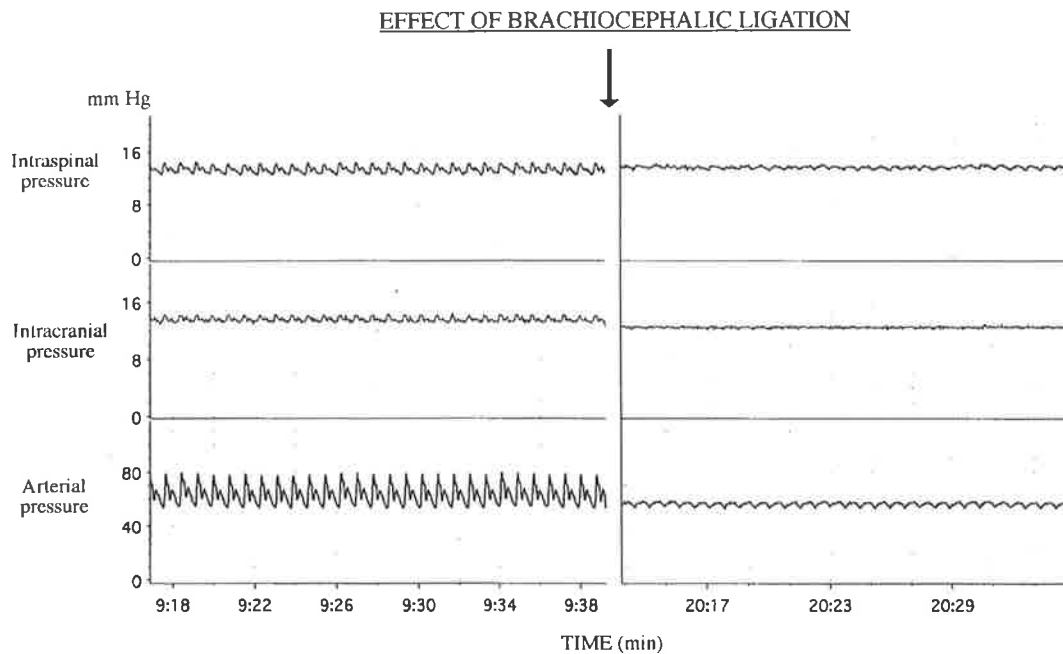
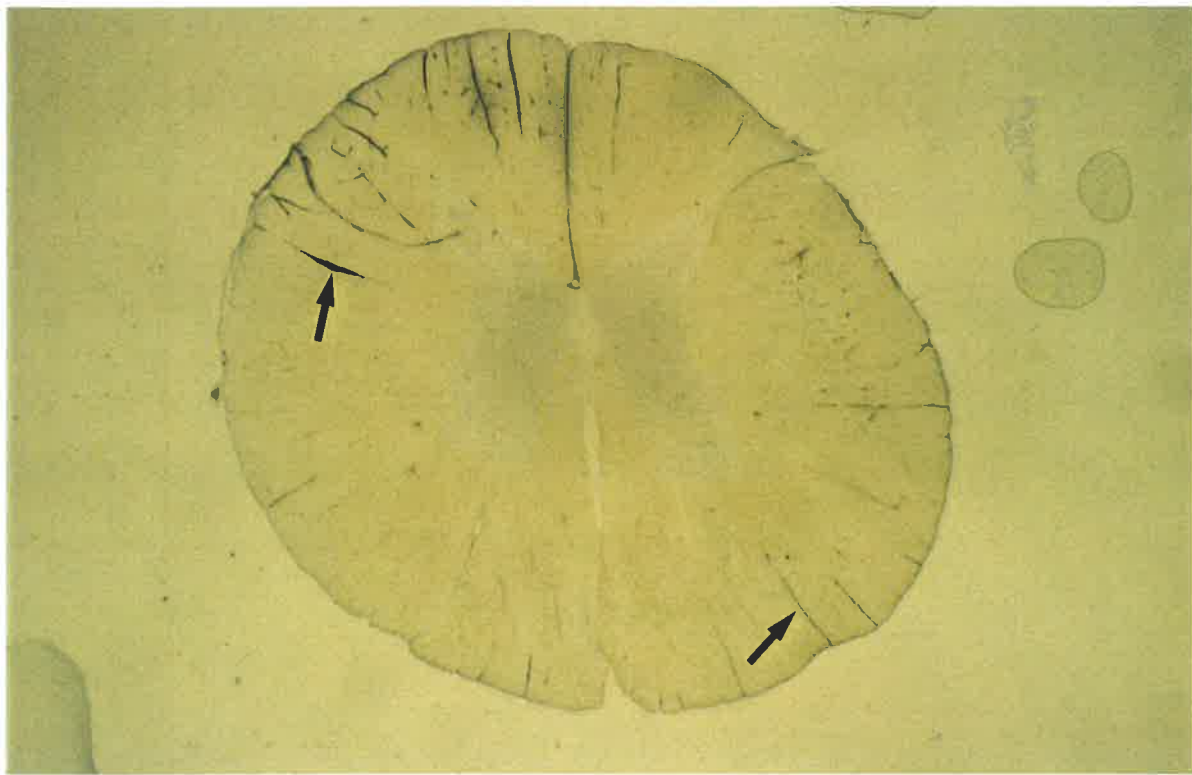
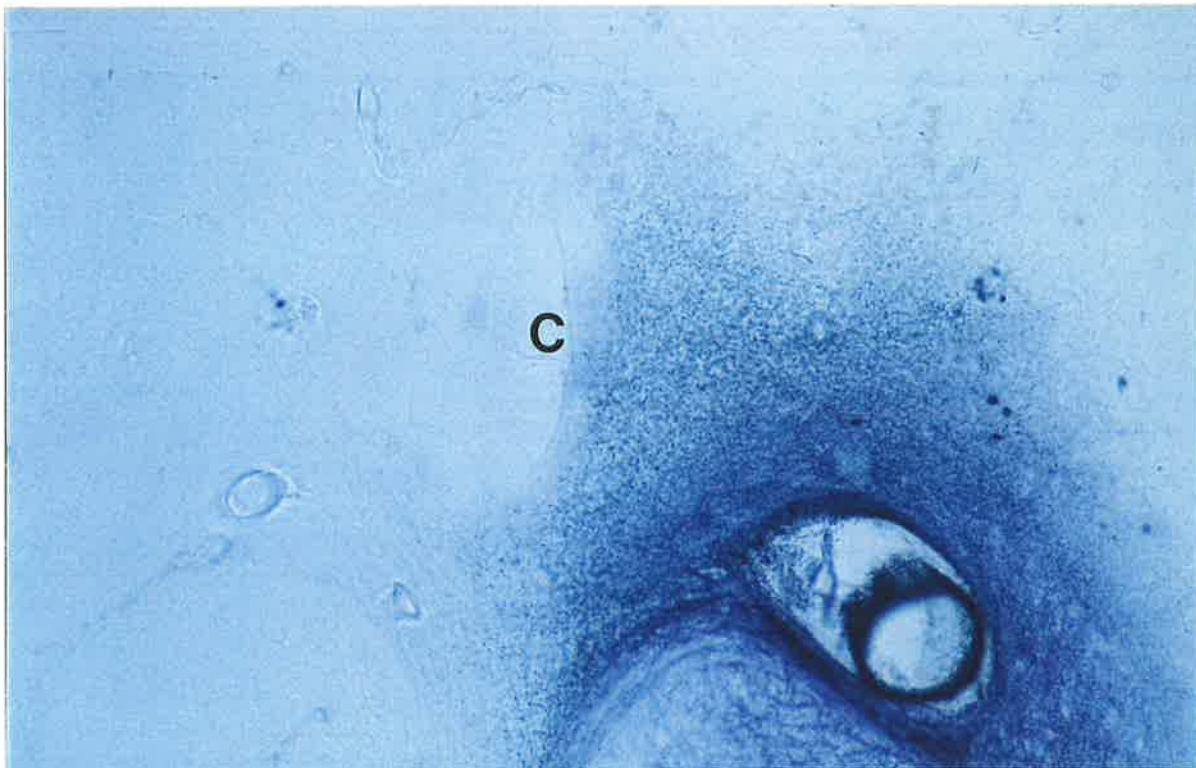


Figure 18. Pressure recordings from a sheep before and after ligation of the brachiocephalic artery. The arrow represents a break in the pressure recordings during which the brachiocephalic artery was ligated. Arterial, intracranial and spinal subarachnoid pulse pressures were reduced after the ligation, without markedly altering the mean pressures.



A



B

Figure 19. *A: Photomicrograph of cervical cord section from a sheep with brachiocephalic artery ligation, sacrificed 10 min after HRP injection. Reaction product is present in superficial segments of some white matter perivascular spaces (arrows). HRP/TMB, $\times 15$. B: Photomicrograph of section of cervical cord taken from the 'sham brachiocephalic ligation' sheep, sacrificed 10 min after HRP injection. HRP reaction product is spreading from perivascular spaces into the central canal (C). HRP/TMB, $\times 100$.*

Table 7. *Spinal subarachnoid and arterial pressures for the sheep undergoing partial ligation of the brachiocephalic artery.*

<u>ANIMALS</u>	<u>PRESSURES (mm Hg)</u>			
	Mean spinal	Spinal pulse	Mean arterial	Arterial pulse
Brachiocephalic ligation (1)				
Pre-ligation	14	4	44	30
Post-ligation	13	2	34	11
Brachiocephalic ligation (2)				
Pre-ligation	13	2	63	23
Post-ligation	13	1	50	4

5.3 Discussion

The sheep was chosen for this experiment because a large animal was needed to enable effective manipulations of arterial pulsations and spinal subarachnoid pressure. The brachiocephalic artery in the sheep gives rise to both carotid arteries and the right vertebral artery.²⁸¹ Partially ligating the brachiocephalic artery therefore reduces arterial pulsations in the CNS but has little effect on other systemic vessels. In this experiment, partial ligation reduced pulse pressure but did not have a marked effect on the mean arterial, spinal or intracranial pressures. Performing a thoracotomy did not appear to alter the CSF physiology because in the 'sham brachiocephalic ligation' animal there were no changes in the pressure tracings and no change in the distribution or pattern of HRP reaction product. The animals in this study were sacrificed 10 minutes after HRP injection because this gives the earliest and clearest pattern of HRP movement from perivascular spaces into the central canal.

Rennels et al³⁷⁴ proposed that a 'paravascular' fluid circulation exists in the nervous system, with an active flow of CSF from the subarachnoid space into the perivascular spaces of arteries and arterioles and continuing through the basal lamina around capillaries. Milhorat et al^{294,301,312} and Cifuentes et al⁸⁸ have previously demonstrated that fluid is capable of moving from the spinal cord interstitial space into the central canal. Milhorat et al^{301,312} have

suggested that a normal fluid flow from the spinal cord interstitial space into the central canal constitutes the 'lymphatic' function of the spinal cord. The flow from perivascular spaces into the central canal demonstrated here in sheep, and previously in rats, suggests that the source of the normal interstitial fluid flow is CSF from the subarachnoid space flowing into the interstitial space via the perivascular spaces. It has not been clear what the driving force for such a unidirectional fluid flow is. Rennels et al³⁷⁴ have provided evidence that the flow of fluid from the subarachnoid space into the perivascular spaces is dependent on arterial pulsations; the flow was reduced when arterial pulsations were reduced by partially ligating the brachiocephalic artery in dogs. However, they did not measure spinal subarachnoid pulsations and did not specifically look for flow into the central canal. This study has also demonstrated that reducing arterial pulsations reduces flow into the perivascular spaces. In addition, reducing arterial pulsations abolishes flow into the central canal. These results support the hypothesis that a unidirectional flow of fluid from perivascular spaces across the interstitial space and into the central canal is driven by arterial pulsations. Systolic expansion of arteries in the perivascular space may force fluid through the surrounding basement membrane, while in diastole fluid may enter the perivascular space from the subarachnoid space through gap junctions in the covering pia.

This study has provided further evidence for the existence of an arterial-pulsation driven fluid flow from the subarachnoid space, into the perivascular spaces, across the interstitial space and into the central canal. The function of this flow may be a 'lymphatic' one, to clear the interstitial space of metabolites and neurotransmitters. Alternatively, it may have a role in non-synaptic neurotransmission.¹⁷ The existence of a one-way fluid flow into the central canal would add considerable support to the theory that non-communicating syringomyelia develops in segments of central canal isolated by stenosis or occlusion. The source of fluid in such cases has long been an enigma.^{175,313,499} The continuation of an

arterial-pulsation driven flow along perivascular spaces into the central canal would explain the accumulation of fluid and enlargement of cysts.

6 Spinal fluid flow in sheep with reduced spinal CSF pressure

This experiment was performed to determine whether lowering the spinal subarachnoid pressure has any effect on the flow of fluid from perivascular spaces into the central canal.

6.1 Methods

Two merino wethers each weighing 40 kg were used (Table 5). Each animal was anaesthetised and mechanically ventilated throughout the experiment. At the beginning of each experiment, the common carotid arteries and internal jugular veins were dissected out in preparation for rapid perfusion-fixation, but these vessels were not ligated at this stage. Arterial, central venous, intracranial and spinal subarachnoid pressures were monitored as in the experiments in normal sheep (see *Spinal fluid flow in normal sheep*, page 130).

CSF was withdrawn via the cannula used to monitor the subarachnoid pressure. In one animal, CSF was withdrawn so that the mean subarachnoid pressure was halved; in the second animal sufficient CSF was removed to keep the subarachnoid pressure below 5 mm Hg. Each animal received an injection of 250 μ L 4% HRP into the subarachnoid space at C4/5 and was sacrificed 10 minutes after injection.

6.2 Results

Ten millilitres of CSF were withdrawn from the spinal subarachnoid space in the first animal; this reduced the subarachnoid pressure to approximately half the baseline level without changing arterial pressure (Table 8). Sixteen millilitres of CSF were withdrawn from the second animal to lower the mean spinal subarachnoid pressure to less than 5 mm Hg (Table 8, Figure 20). In each case the spinal subarachnoid pulse pressure changed from 2 mm Hg to 1 mm Hg.

There was transpial staining with HRP reaction product at all cervical spinal cord levels. Perivascular spaces were labelled throughout the cervical cord white and grey matter. Reaction product spread from perivascular spaces in the central grey matter and the ventral white commissure into the central canal in the two patterns described above (Figure 21). This was seen in sections distant from the injection point as far as C1. Some perivascular spaces were also labelled in the thoracic cord but no reaction product was seen in lumbar or conus sections.

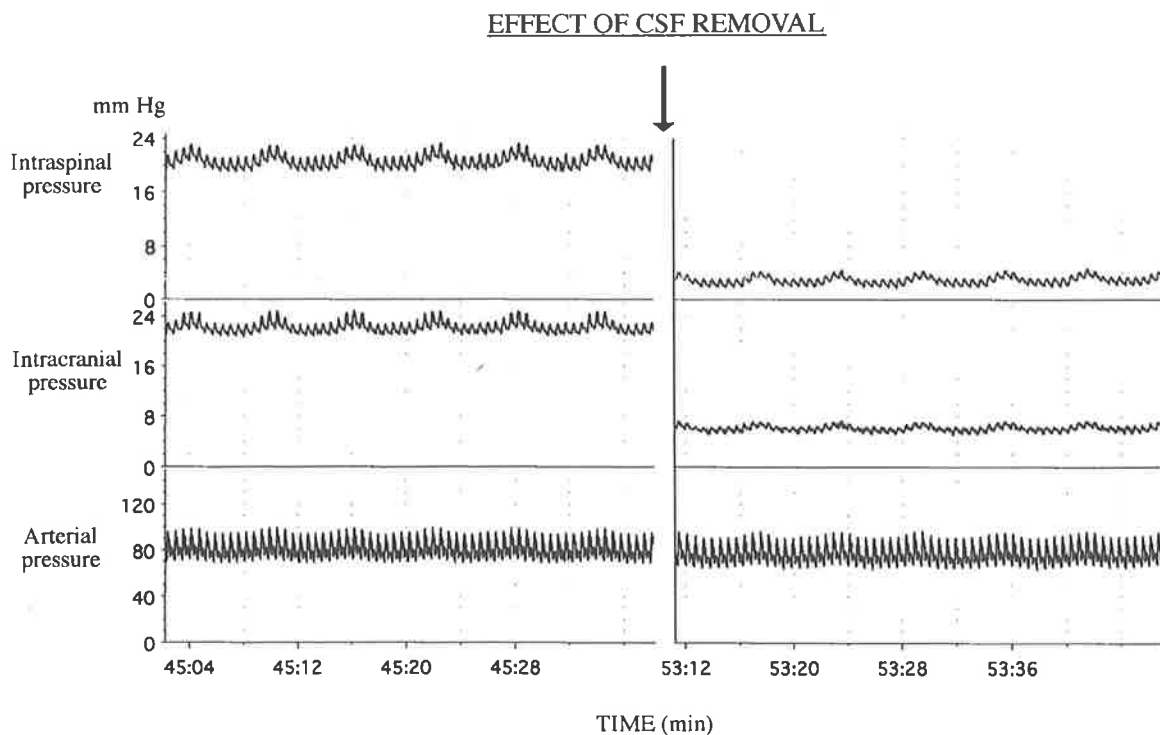


Figure 20. Pressure recordings from a sheep before and after removal of 16 mL CSF from the spinal subarachnoid space (arrow), lowering the spinal subarachnoid pressure to less than 5 mm Hg.

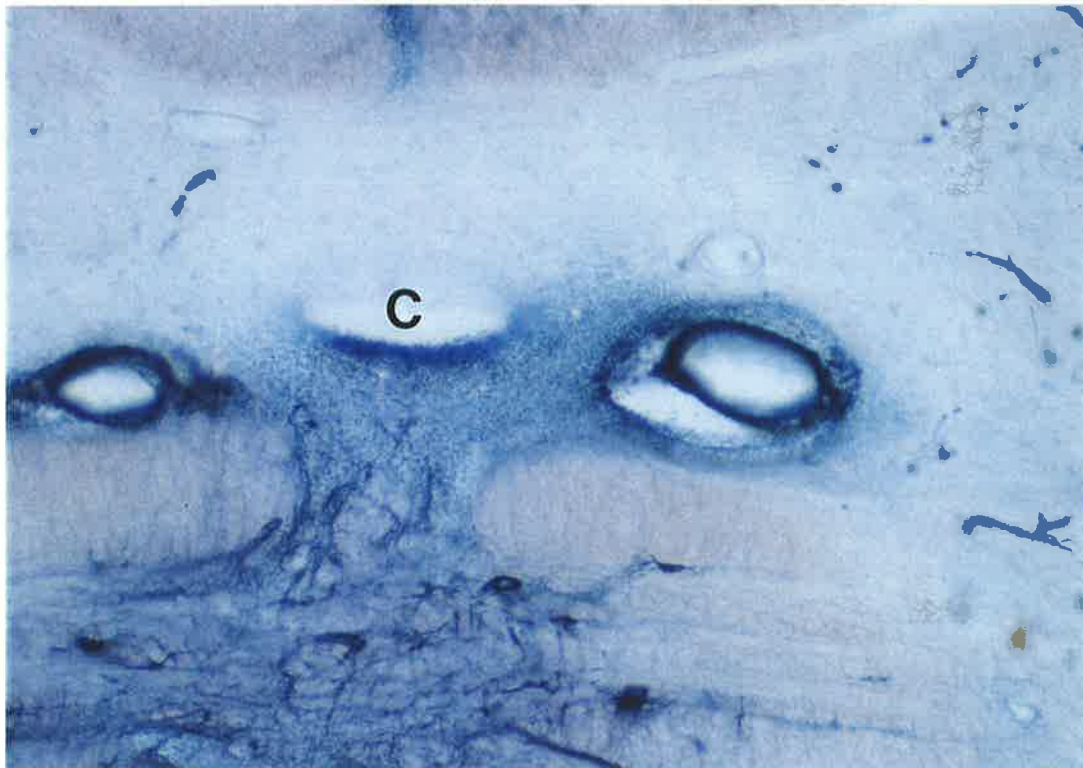


Figure 21. Photomicrograph of a section of cervical cord taken from a sheep with reduced spinal subarachnoid pressure, sacrificed 10 min after HRP injection. Reaction product is present in central grey matter perivascular spaces and is spreading into the central canal (C). HRP/TMB, $\times 100$.

Table 8. Spinal subarachnoid and arterial pressures for the sheep undergoing CSF removal from the spinal subarachnoid space.

<u>ANIMALS</u>	Mean spinal	<u>PRESSURES (mm Hg)</u>			
		Spinal pulse	Mean arterial	Arterial pulse	
CSF removal (10 mL)					
Pre-removal	18	2	69	26	
Post-removal	7	1	69	29	
CSF removal (16 mL)					
Pre-removal	21	2	81	25	
Post-removal	4	1	76	27	

6.3 Discussion

There is some evidence that posterior fossa decompression may result in resolution of syrinxes by altering subarachnoid pressure and compliance.²⁰⁶ In this experiment, CSF was removed prior to the HRP injection in an attempt to examine the effect on perivascular flow of

lowering spinal subarachnoid pressure. Lowering the pressure in this way did not appear to affect the perivascular or interstitial fluid flow. It may be that removing CSF lowers the pressure in the spinal subarachnoid space but does not lower the pressure in perivascular spaces. It would be interesting to examine the effect of increasing spinal subarachnoid pressure by increasing central venous pressure, as raised venous pressure has been cited in the pathogenesis of syringomyelia.^{283,413,444,495} Increased spinal subarachnoid pressure may increase the flow of fluid in perivascular spaces and across the interstitial space.

7 Spinal fluid flow in rats with non-communicating syringomyelia

The aim of this study was to determine whether fluid flow from perivascular spaces into the central canal continues during enlargement of the central canal in an animal model of syringomyelia.

7.1 Methods

Seventy-eight male Sprague-Dawley rats, weighing 255 g to 450 g (Table 4) had kaolin injected into the dorsal columns at C6 as described above (see *Kaolin injection*, page 98). The CSF flow was studied using HRP injected into the cisterna magna or the spinal subarachnoid space (see *Development of methods for studying spinal fluid flow*, page 108) at 1 day, 3 days, 1 week or 6 weeks after kaolin injection. Each of these groups was further subdivided for study at 0 minutes, 10 minutes or 30 minutes after HRP injection (Table 9). The brains and spinal cords were processed as described previously.

Each rat was studied in detail. The central canal was examined in serial sections from the sagittal paraffin-embedded blocks and in the transverse vibratome sections. These sagittal and transverse sections were used to determine the morphology of the central canal in the entire cervical cord and in the segments of the thoracic and lumbar cord and conus medullaris. Patterns of fluid flow were then examined at each level and related to the morphology of the canal at that level, including whether the canal at that level was isolated by occlusions or trabeculae and whether the canal was dilated (greater than 100 μm diameter). The depth of transpial HRP diffusion at each level was measured using an eyepiece micrometer and compared with the measurements made in normal rats (see *Spinal fluid flow in normal rats*, page 115).

Table 9. Rats used to study CSF flow in the intraparenchymal kaolin model of syringomyelia

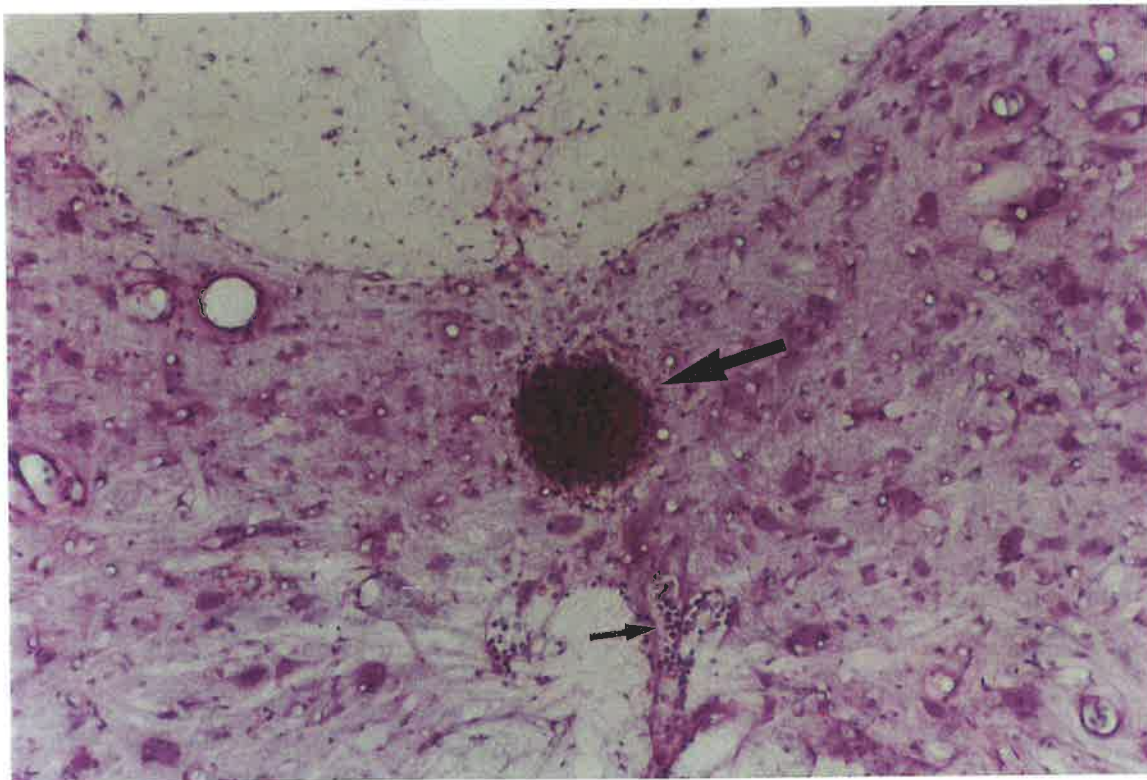
Time after kaolin injection	Time after HRP injection	Number of rats	
		Cisternal injection	Spinal injection
1 day	0 min	3	3
	10 min	2	3
	30 min	2	3
	<i>All times</i>	7	9
3 days	0 min	3	3
	10 min	4	3
	30 min	4	3
	<i>All times</i>	11	9
1 week	0 min	3	3
	10 min	3	3
	30 min	3	3
	<i>All times</i>	9	9
6 weeks	0 min	5	3
	10 min	5	4
	30 min	4	3
	<i>All times</i>	14	10
TOTALS	0 min	14	12
	10 min	14	13
	30 min	13	12
	<i>All times</i>	41	37

7.2 Results

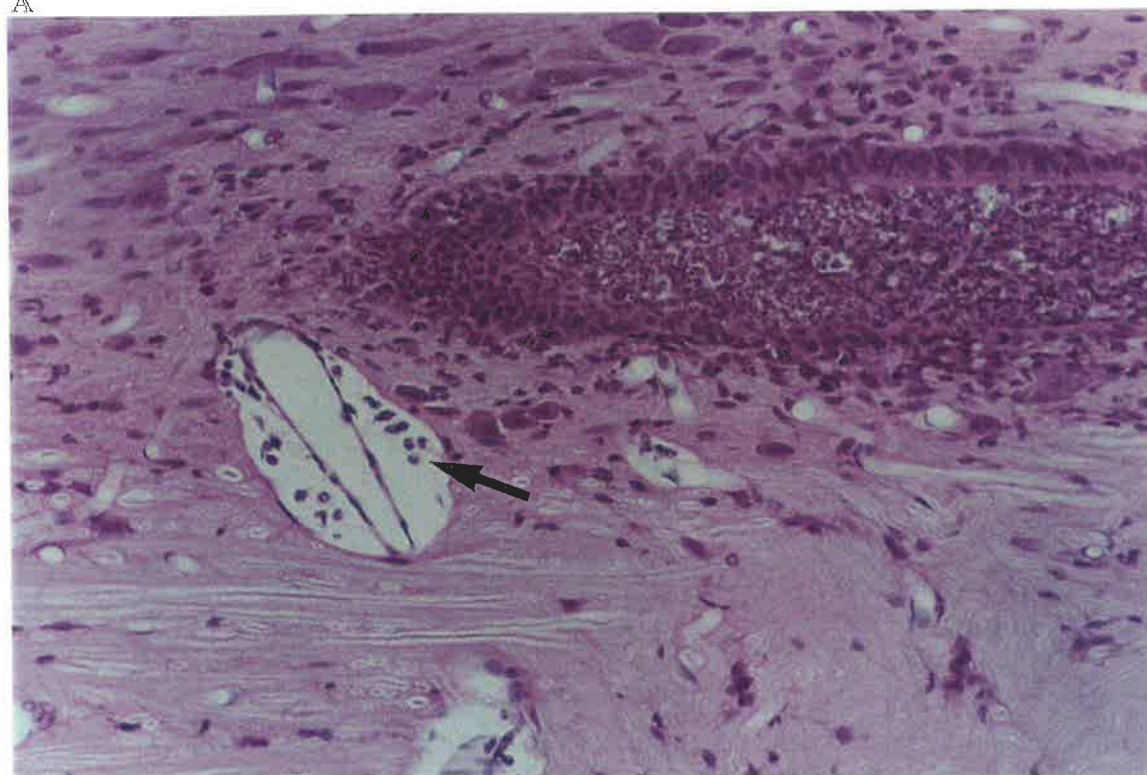
The results will be presented for each time interval after kaolin injection and for each HRP study time at that interval.

7.2.1 *1 day post kaolin injection*

At 1 day after intraparenchymal kaolin injection, there was an acute inflammatory response at the injection site. Kaolin crystals and neutrophils drained into the central canal and formed a column of cells filling the central canal rostral to the injection point (Figure 22). In some areas the central canal was slightly enlarged and packed with cells and crystals. There were also neutrophils and kaolin crystals in the central canal caudal to the injection point in some animals, but these did not fill the canal nor enlarge it. Perivascular spaces in the cervical cord were dilated and often contained inflammatory cells (Figure 23). In some animals there was evidence of ependymal proliferation and the formation of septa and synechiae (Figure 23). The central canal was not dilated except for the areas packed with cells and crystals.

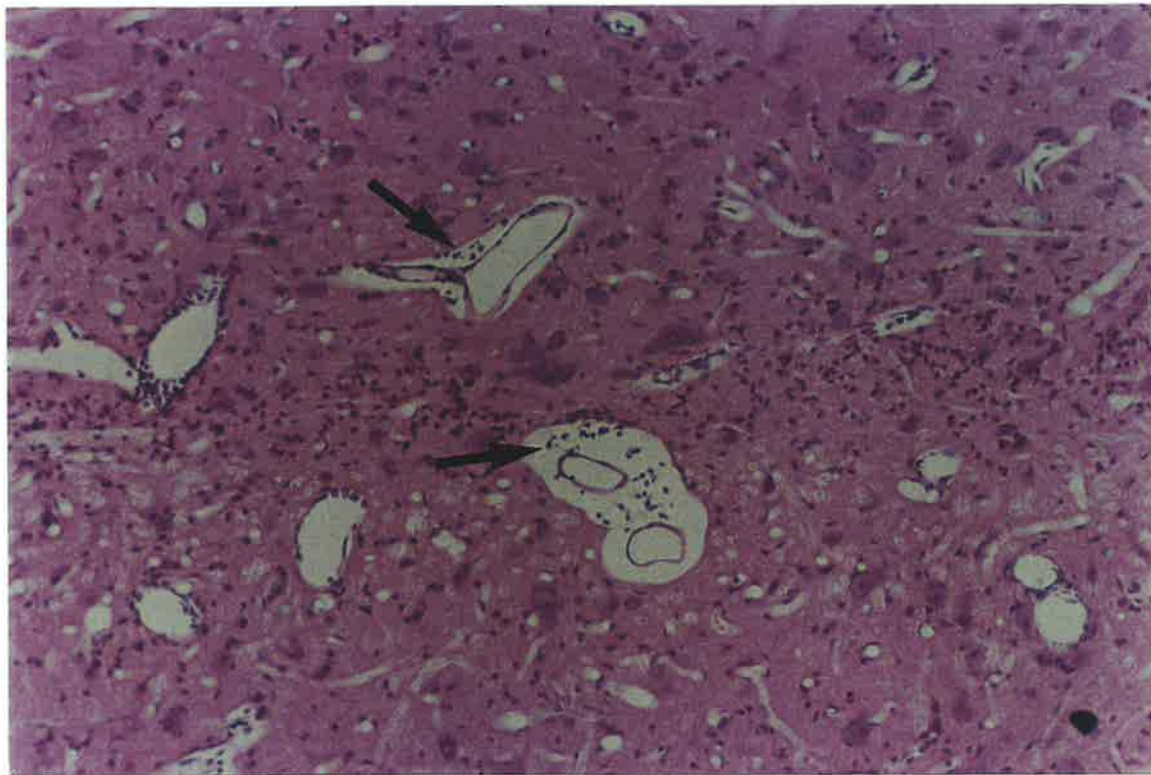


A

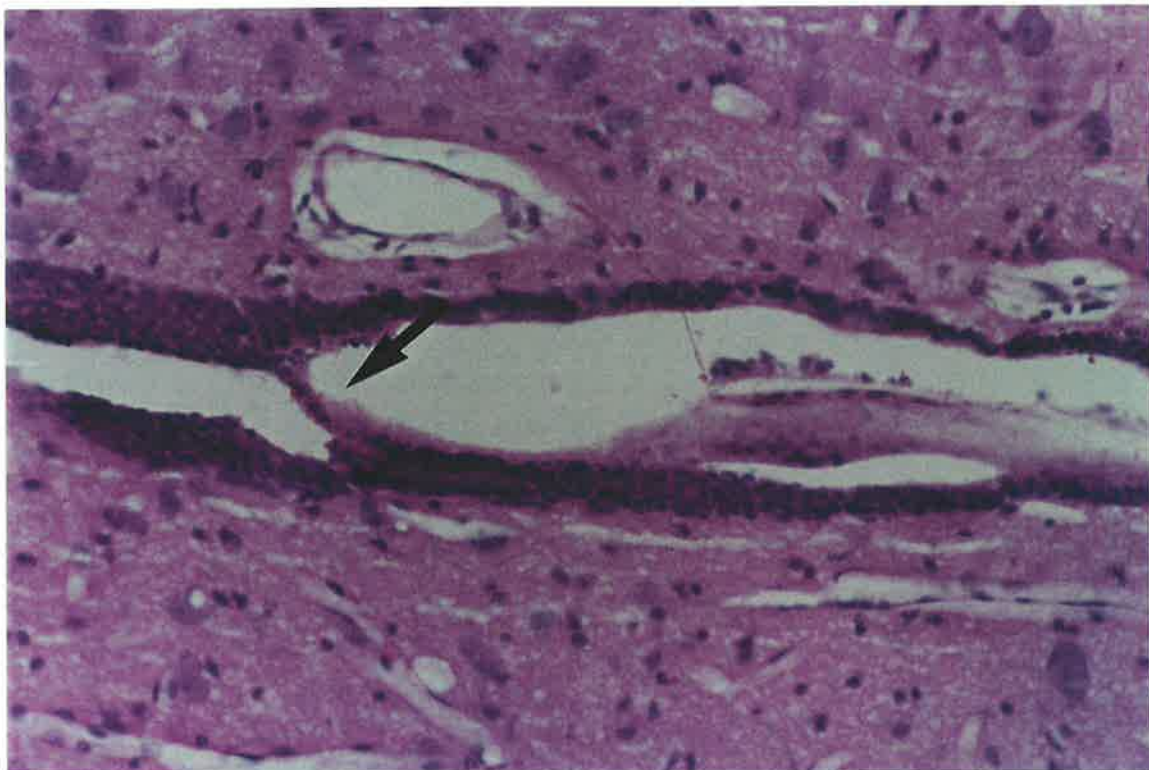


B

Figure 22. Photomicrographs of spinal cord sections from a rat sacrificed 1 day after kaolin injection. *A*: Transverse section. The central canal (large arrow) is packed with kaolin crystals and neutrophils and there are also neutrophils in the surrounding central grey matter and in perivascular spaces (small arrow). Vibratome section, H&E, $\times 100$. *B*: Longitudinal section. A column of kaolin crystals and inflammatory cells is filling the central canal and there are neutrophils in perivascular spaces (arrow). H&E, $\times 200$.



A



B

Figure 23. Photomicrographs of longitudinal cervical cord sections from a rat sacrificed 1 day after kaolin injection. *A: Neutrophils are present in dilated perivascular spaces (arrows) within the central grey matter. H&E, $\times 100$. B: Inflammatory cells are in the central canal and there is evidence of septum formation (arrow). H&E, $\times 200$.*

7.2.1.1 0 minutes

7.2.1.1.1 Cisternal HRP injection

The depth of transpial HRP diffusion at each level of the neuraxis compared with the depth measured in normal rats is shown in Figure 24. The caudal spread of HRP, indicated by the depth of diffusion, was less in the kaolin-injected rats than in the normal rats. The depth of diffusion in rostral segments was increased. There were numerous neutrophils throughout the cervical spinal cord parenchyma that exhibited endogenous peroxidase activity (Figure 25). This was distinguishable from the granular HRP reaction product. Perivascular spaces were labelled at all levels of the spinal cord, but these were fewer than the number of vessels labelled at the same interval in normal rats. There were vessels labelled in all white matter columns, in the central grey matter and the dorsal and ventral horns. The pattern of reaction product spreading from perivascular spaces towards the central canal that was seen in normal rats was also seen at many spinal cord levels (Figure 25, Figure 26). The central canal did not always contain reaction product. In some blocks, there was reaction product in the central canal only in sections where there was a labelled central grey matter perivascular space and reaction product was spreading from this space into the central canal (Figure 26). In some animals, the central canal in the conus medullaris was unlabelled and the cervical central canal was occluded with crystals and cells, yet the thoracic and lumbar sections had labelled central canals with reaction product spreading from central grey matter perivascular spaces into the canal. Reaction product was present between perivascular spaces and segments of central canal filled with cells and crystals (Figure 25).

Trans-Pial Diffusion After Cisternal HRP Injection

0 min post HRP

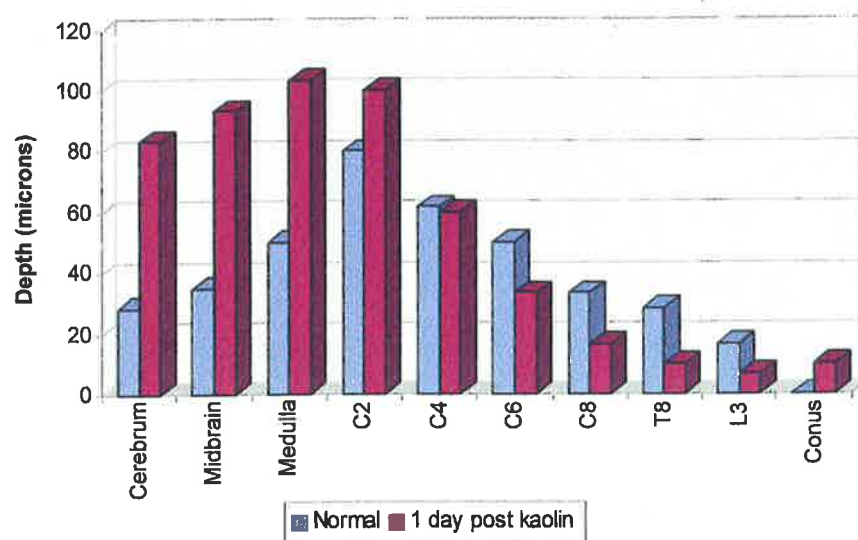
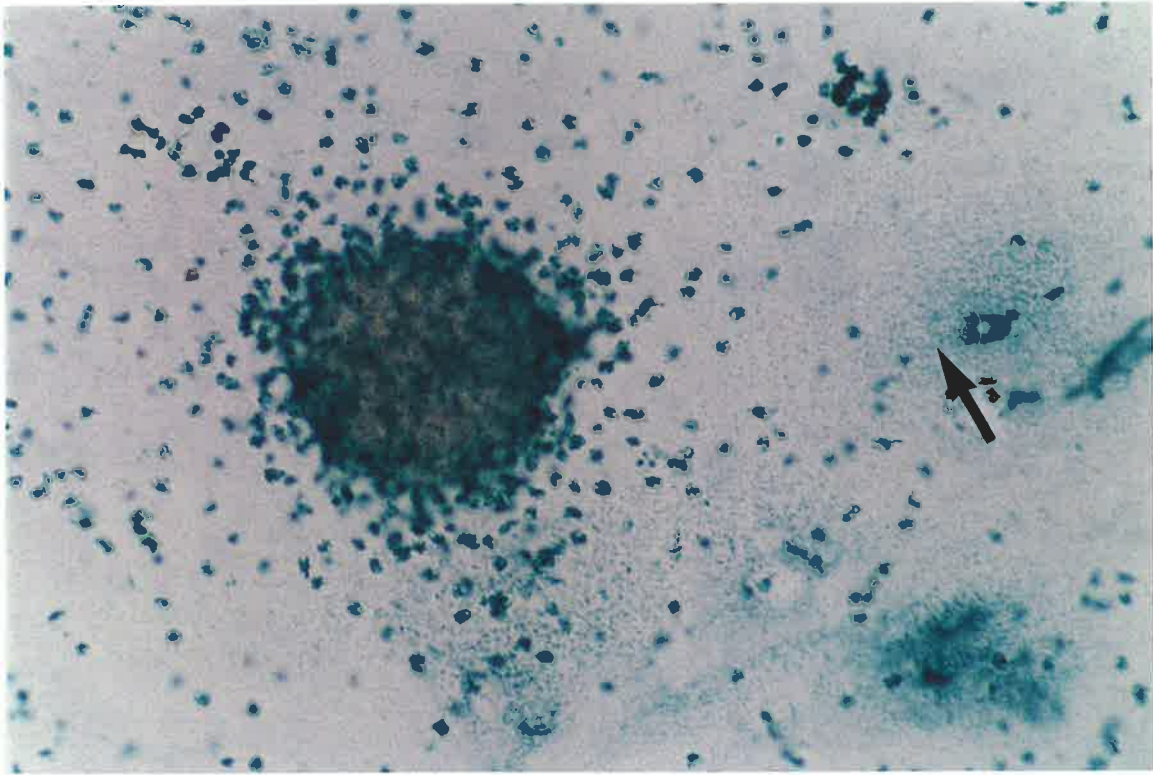
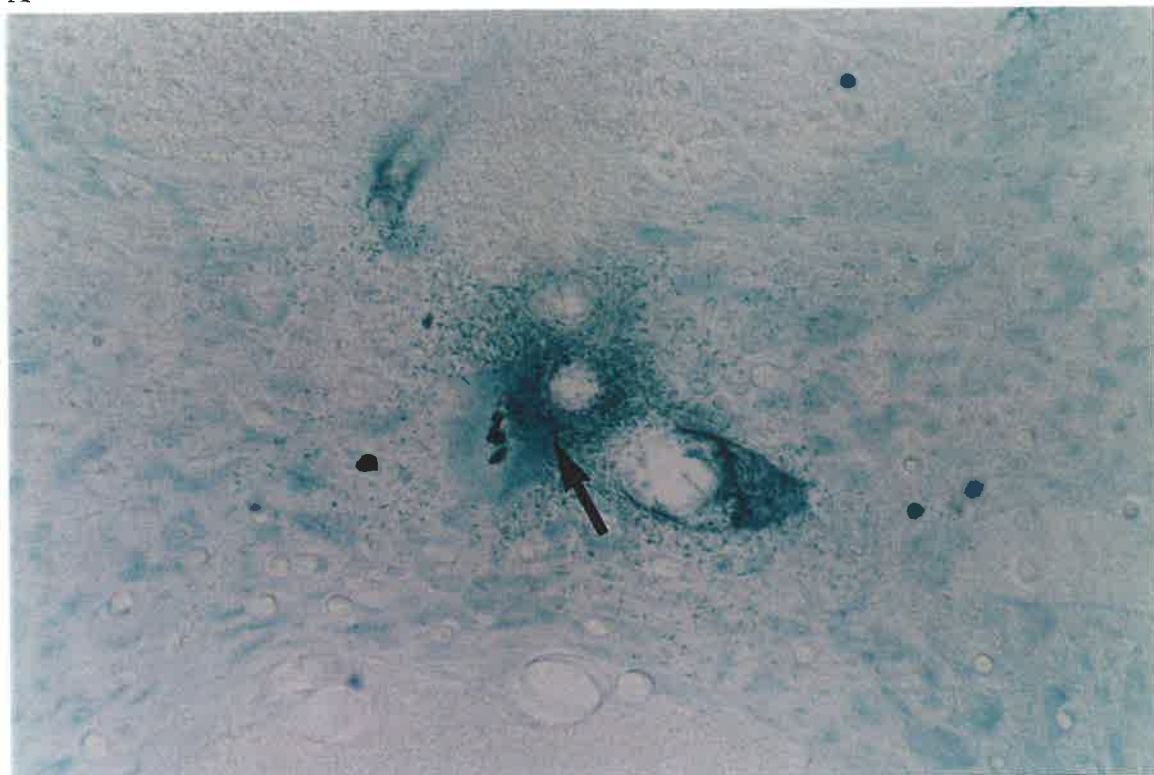


Figure 24. *Average depth of transpial HRP diffusion at each level in normal rats and in rats sacrificed 1 day after kaolin injection and 0 minutes after cisternal HRP injection.*

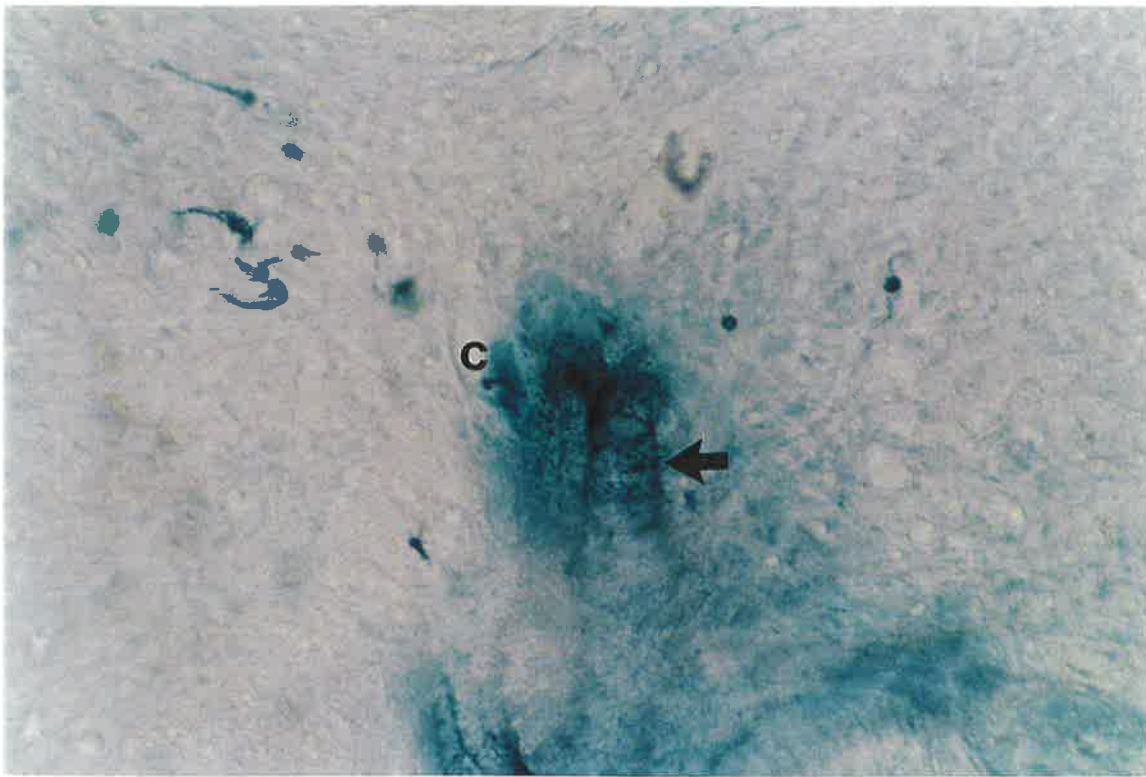


A

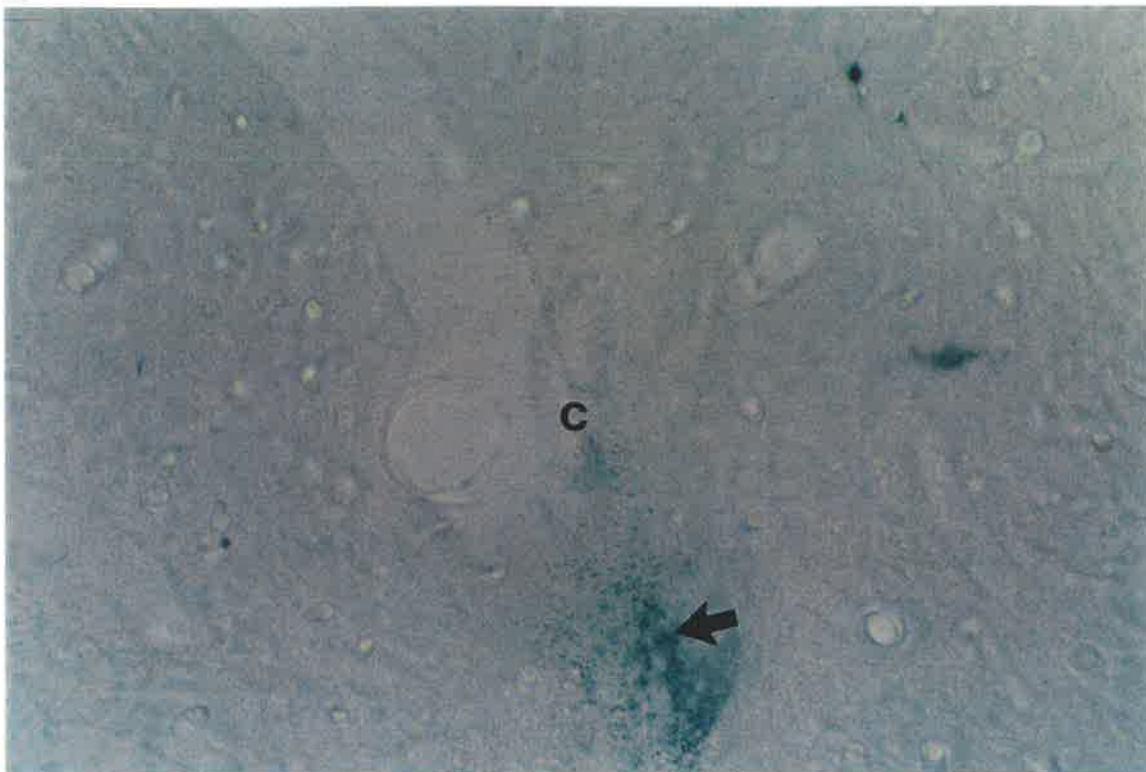


B

Figure 25. Photomicrographs of transverse cervical cord sections from a rat sacrificed 1 day after kaolin injection and 0 minutes after cisternal HRP injection. A: C6. Kaolin crystals and neutrophils fill the central canal and neutrophils within the parenchyma display endogenous peroxidase activity. Granular HRP reaction product is labelling central grey matter perivascular spaces and is spreading (arrow) towards the central canal. HRP/TMB, $\times 100$. B: C8. Reaction product is spreading (arrow) from a labelled central grey matter perivascular space towards the central canal. HRP/TMB, $\times 100$.



A



B

Figure 26. Photomicrographs of transverse lumbar spinal cord sections from different rats sacrificed 1 day after kaolin injection and 0 minutes after cisternal HRP injection. Reaction product is spreading from labelled central grey matter perivascular spaces (arrows) towards the central canal (C). The section in A was the only section from the lumbar cord in this rat that had reaction product in the central canal. HRP/TMB, $\times 200$.

7.2.1.1.2 Spinal HRP injection

Transpial HRP diffusion at 0 minutes after spinal HRP injection in rats sacrificed 1 day after kaolin injection is compared with the diffusion in normal rats in Figure 27. The rostral spread of HRP was more limited in the kaolin-injected rats than in the normal rats. There was also a more limited distribution of labelled perivascular spaces in the kaolin rats. In one animal, one white matter perivascular space was labelled at the C2 level. The most rostral labelled vessel in the other animals was at the C4 level and the most rostral labelling of the central canal was at the C6 level, immediately rostral to the kaolin injection point. There was preferential spread of reaction product from central grey matter perivascular spaces into the central canal at the thoracic, lumbar and conus medullaris levels. This pattern was also seen in the cervical sections where central grey matter perivascular spaces were labelled (Figure 28). In one animal, the lower cervical, thoracic and lumbar central canal was heavily labelled. Adjacent perivascular spaces were also labelled, but in this animal it was possible that the HRP had spread from the central canal to the perivascular spaces rather than in the usual pattern (Figure 29).

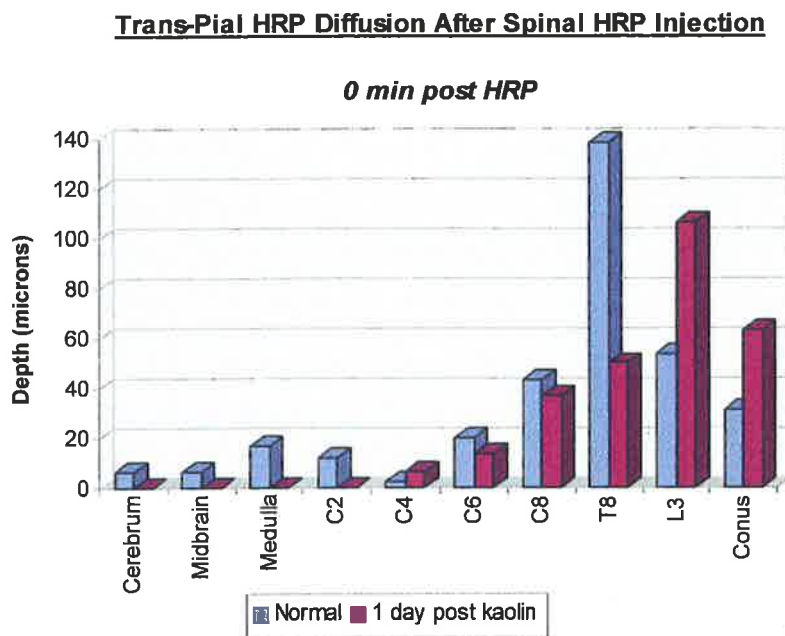
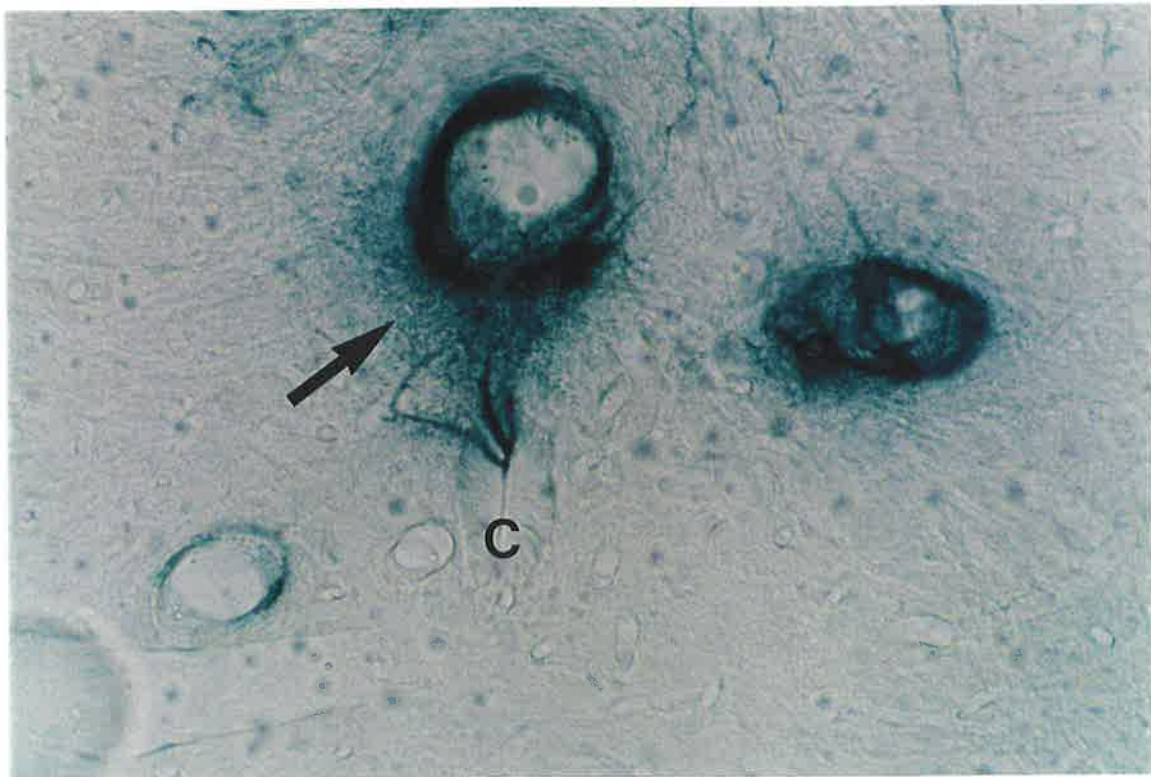
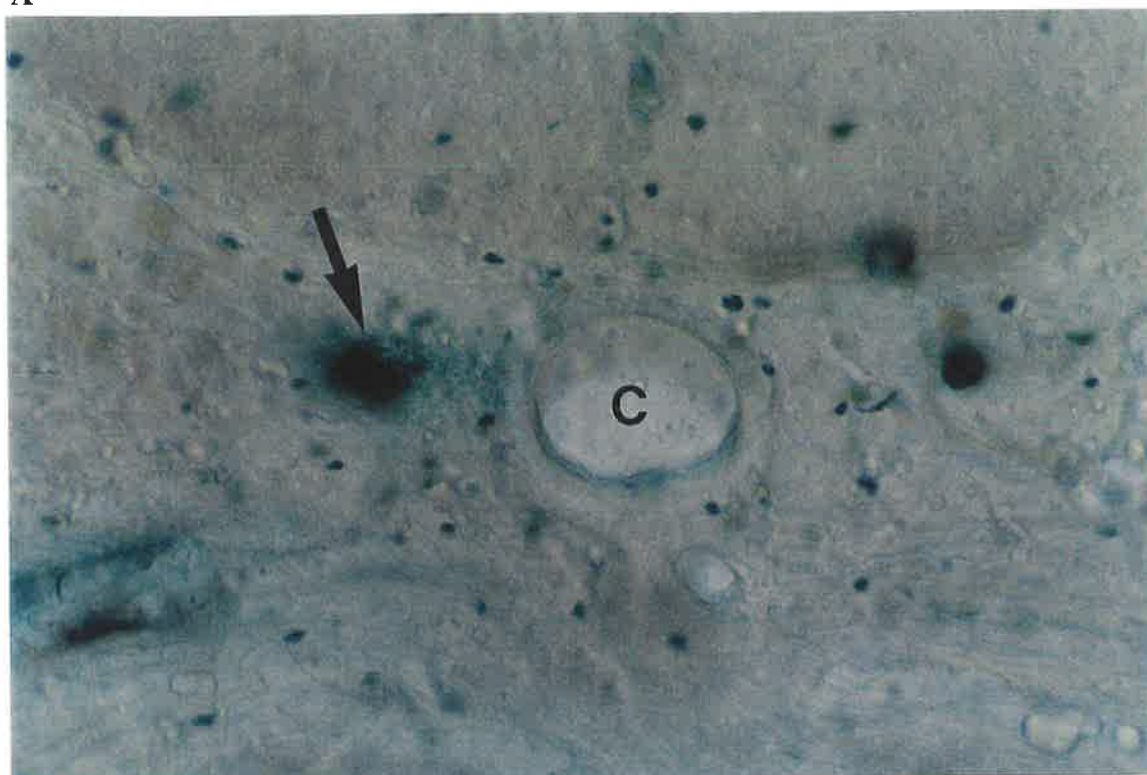


Figure 27. Average depth of transpial diffusion in normal rats and in rats sacrificed 1 day after kaolin injection and 0 minutes after spinal HRP injection.



A



B

Figure 28. Photomicrographs of transverse spinal cord sections from rats sacrificed 1 day after kaolin injection and 0 minutes after spinal HRP injection. Reaction product is labelling perivascular spaces (arrows) in the central grey matter and is spreading into the central canal (C). A: L3, B: C6. HRP/TMB, $\times 200$.



Figure 29. *Photomicrograph of transverse C6 section from a rat sacrificed 1 day after kaolin injection and 0 minutes after HRP injection. The central canal (C) is heavily labelled and there is reaction product between the central canal and grey matter perivascular spaces (arrows). HRP/TMB, $\times 250$.*

7.2.1.2 10 minutes

7.2.1.2.1 Cisternal HRP injection

A comparison of transpial diffusion at 10 minutes after cisternal HRP injection in normal rats and rats sacrificed 1 day after kaolin injection is shown in Figure 30. The depth of diffusion in the spinal cord in kaolin-injected rats was less than that in normal rats.

Perivascular spaces in white and grey matter were labelled at all levels of the neuraxis. The number of vessels labelled and the density of labelling were greater than in the kaolin-injected rats studied 0 minutes after cisternal HRP injection. There were numerous examples in cervical and thoracic cord sections of HRP reaction product spreading from central grey matter perivascular spaces into the central canal (Figure 31). The central canal contained reaction product at all levels.

Trans-Pial Diffusion After Cisternal HRP Injection

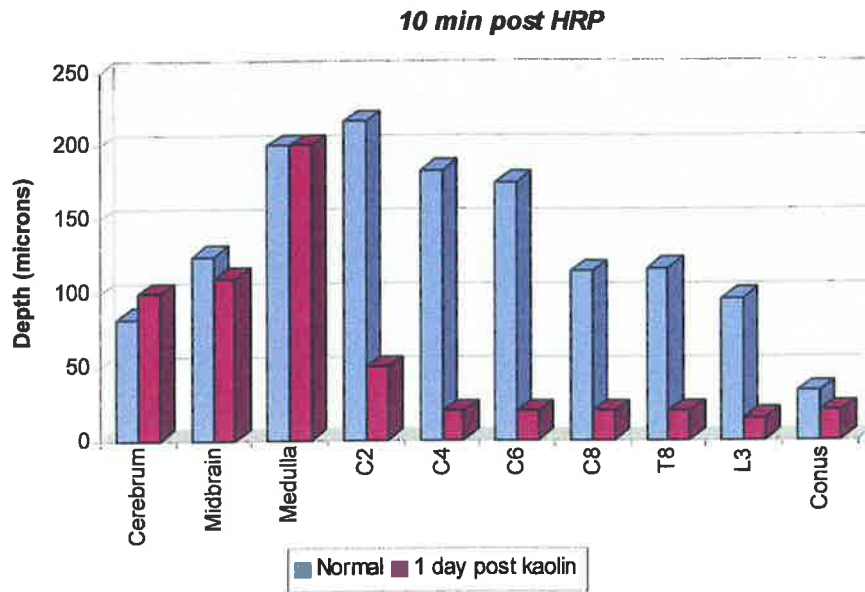


Figure 30. Average depth of transpial diffusion in rats sacrificed 1 day after kaolin injection and 10 minutes after cisternal HRP injection, compared with diffusion in normal rats.

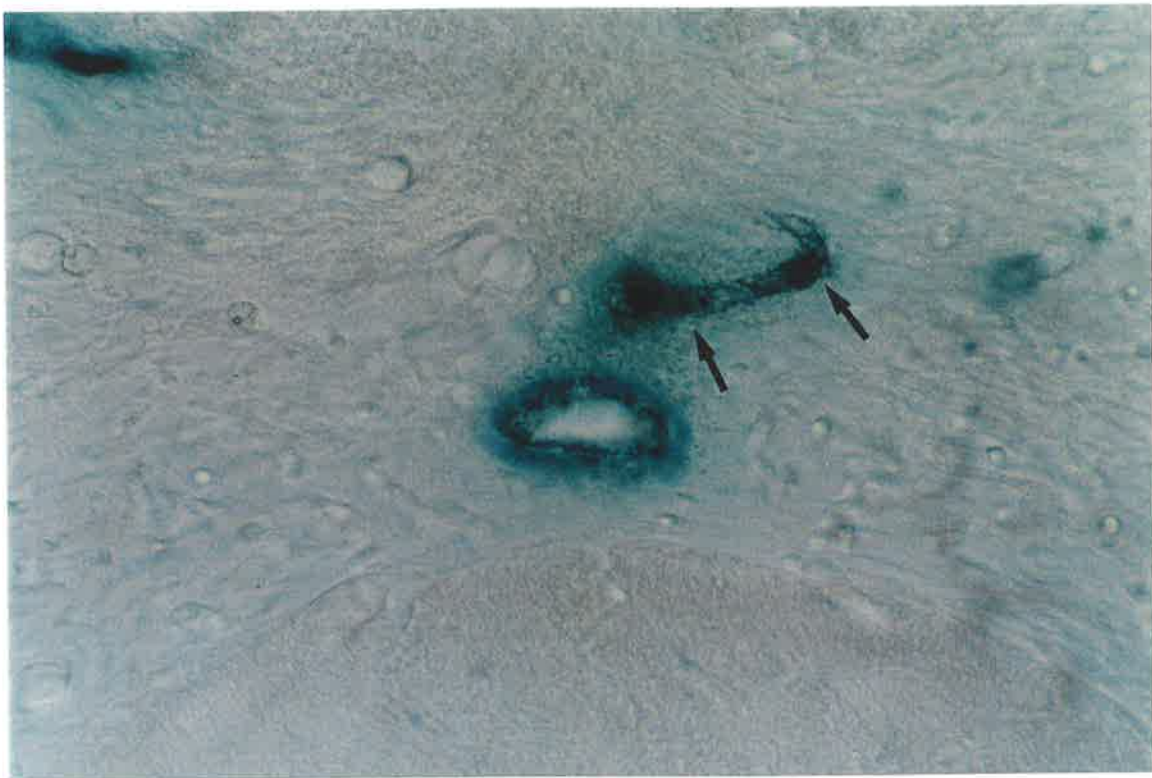


Figure 31. Photomicrograph of a transverse cervical cord section from a rat sacrificed 1 day after kaolin injection and 10 minutes after cisternal HRP injection. Reaction product is labelling central grey matter perivascular spaces (arrows) and is spreading into the central canal. HRP/TMB, $\times 200$.

7.2.1.2.2 Spinal HRP injection

Transpial diffusion 10 minutes after HRP injection is compared in normal rats and rats 1 day after kaolin injection in Figure 32. The depth of transpial diffusion in the cervical cord in the kaolin rats was less than in the normal rats. In the kaolin-injected rats, perivascular spaces were labelled at all levels of the spinal cord and occasionally in the cerebrum, midbrain and medulla. There was a greater number of labelled vessels in the more caudal spinal cord sections. The central canal was labelled only if central grey matter perivascular spaces were also labelled at the same level. The pattern of reaction product spreading from perivascular spaces into the central canal was seen in caudal cervical, lumbar and conus sections (Figure 33).

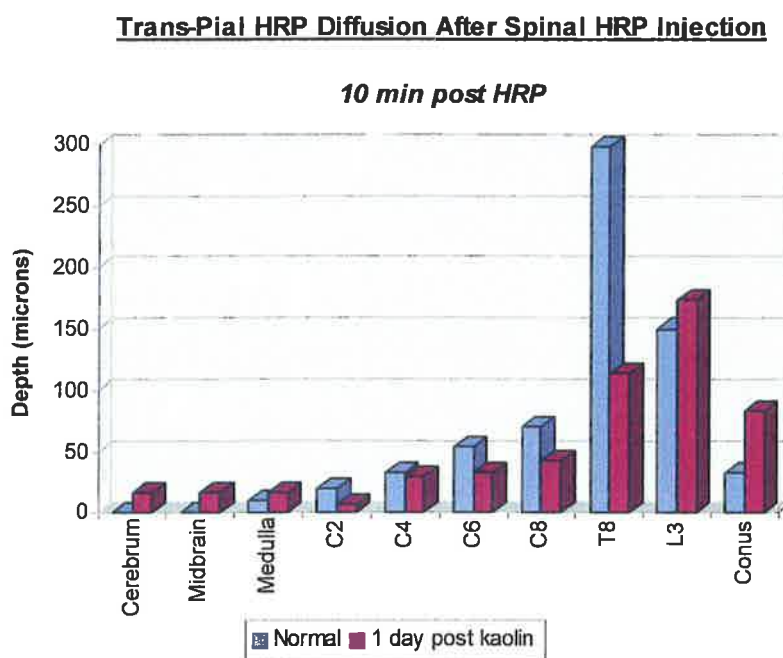


Figure 32. Comparison of transpial diffusion 10 minutes after spinal HRP injection in normal rats and rats 1 day after kaolin injection.

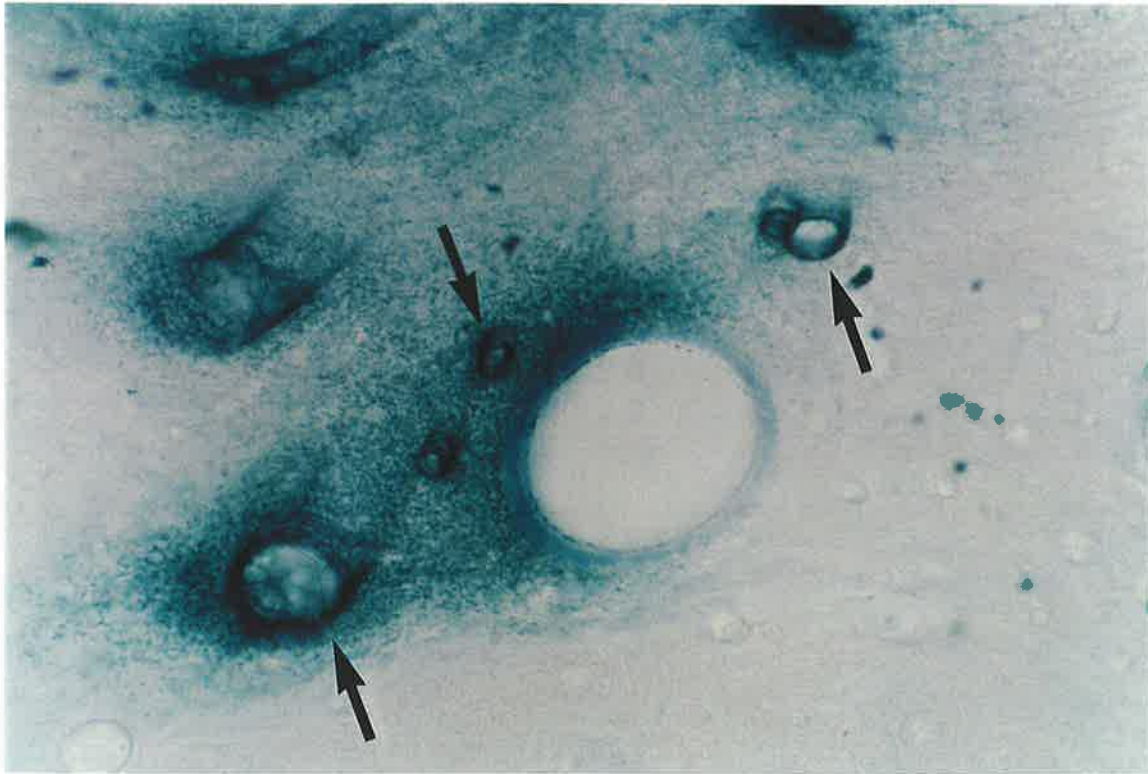


Figure 33. *Photomicrograph of a transverse cervical cord section from a rat sacrificed 1 day after kaolin injection and 10 minutes after spinal HRP injection. Reaction product is labelling central grey matter perivascular spaces (arrows) and is spreading into the central canal. HRP/TMB, $\times 200$.*

7.2.1.3 30 minutes

7.2.1.3.1 Cisternal HRP injection

The depth of transspial diffusion in this group was less than in normal rats (Figure 34). Labelled perivascular spaces were more numerous at each level than in the kaolin-injected 0 minute or 10 minute cisternal HRP groups. Perivascular spaces were labelled in all white matter columns and the grey matter. In some cervical, thoracic and lumbar sections there was reaction product spreading from perivascular spaces into the central canal. The central canal was labelled in cervical, thoracic and lumbar sections.

Trans-Pial Diffusion After Cisternal HRP Injection

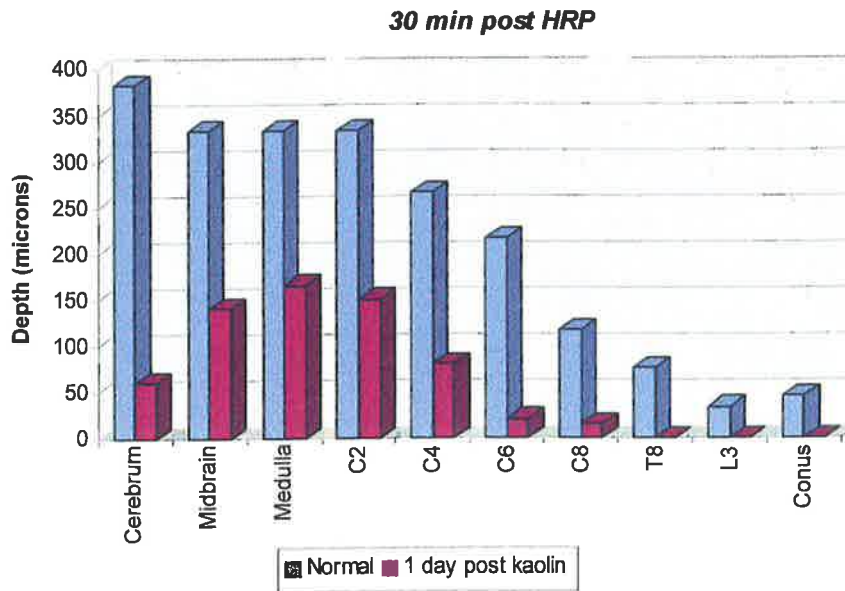


Figure 34. *Transpial diffusion 30 minutes after cisternal HRP injection in normal rats and in rats 1 day after kaolin injection.*

7.2.1.3.2 Spinal HRP injection

The depth of transpial diffusion in the rats studied 30 minutes after spinal HRP injection and 1 day after kaolin injection is compared with diffusion in normal rats in Figure 35. The depth of transpial diffusion was greater than in the 0 minute and 10 minute spinal HRP groups and there were also more labelled perivascular spaces at all levels. The central grey matter was often diffusely labelled with reaction product, but there were some sections in which the reaction product was spreading preferentially from perivascular spaces into the central canal (Figure 36). This preferential flow pattern was seen in some cervical, thoracic, lumbar and conus sections.

Trans-Pial HRP Diffusion After Spinal HRP Injection

30 min post HRP

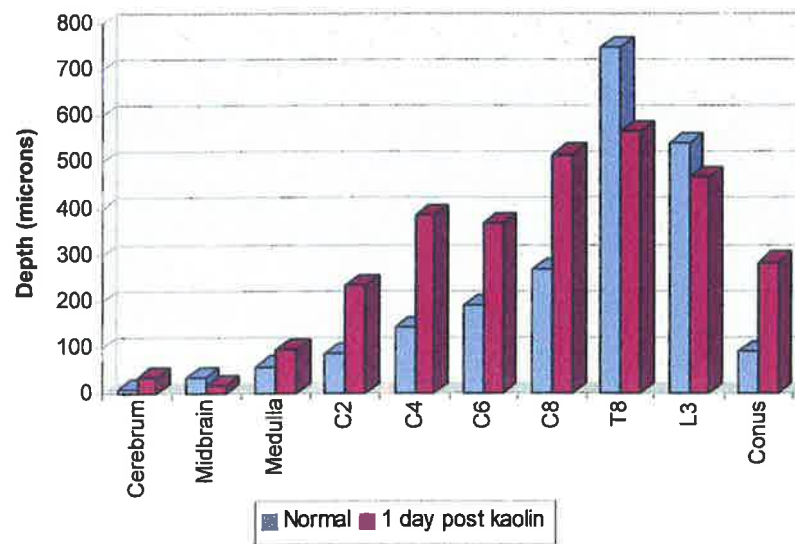
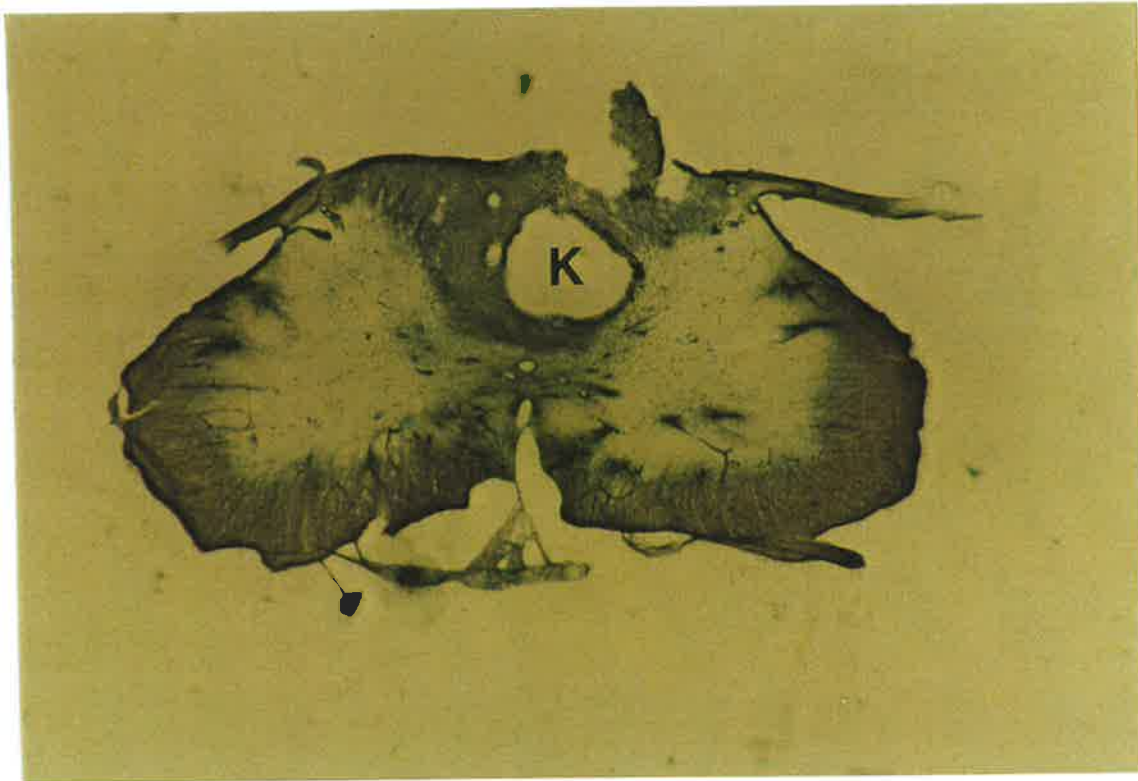
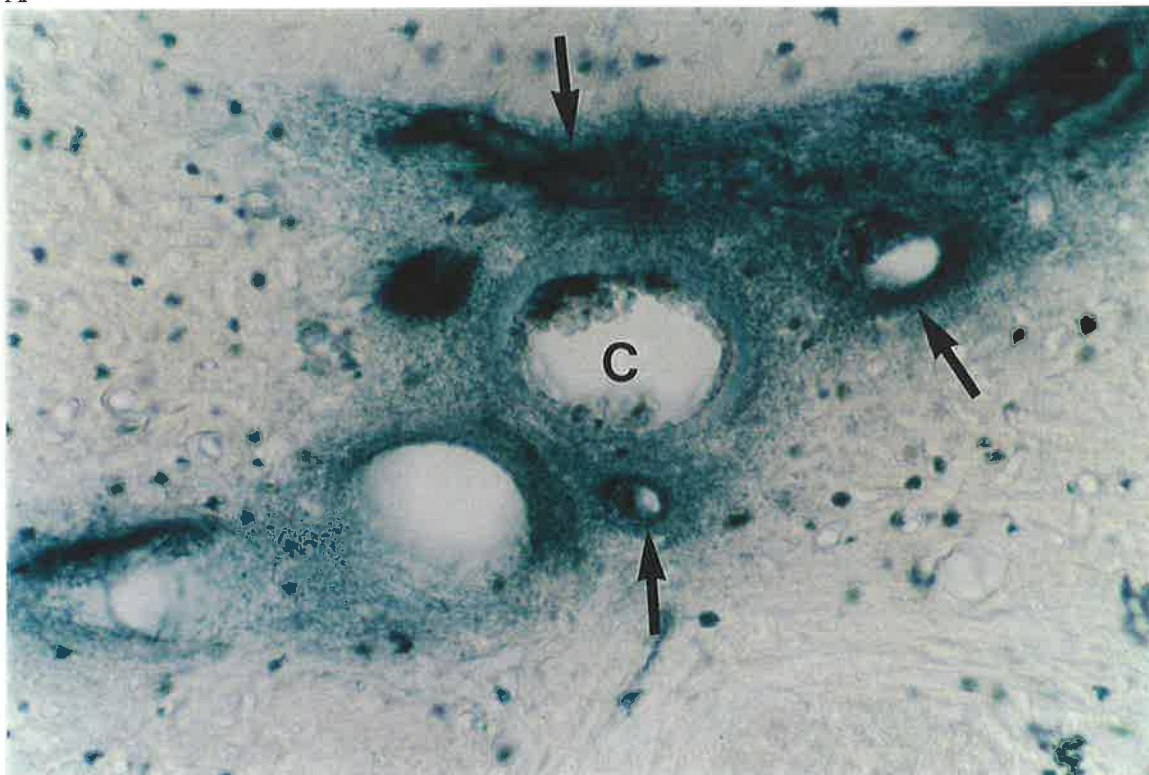


Figure 35. Comparison of transpial diffusion 30 minutes after spinal HRP injection in normal rats and in rats 1 day after kaolin injection.



A

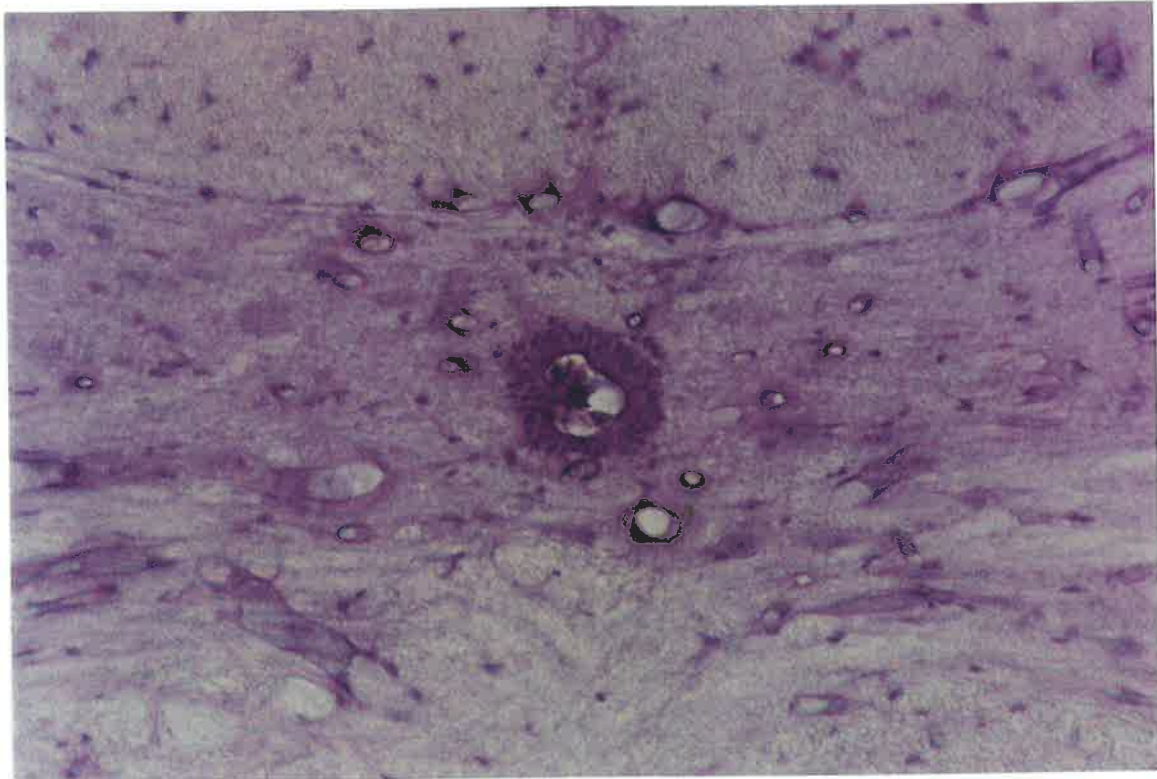


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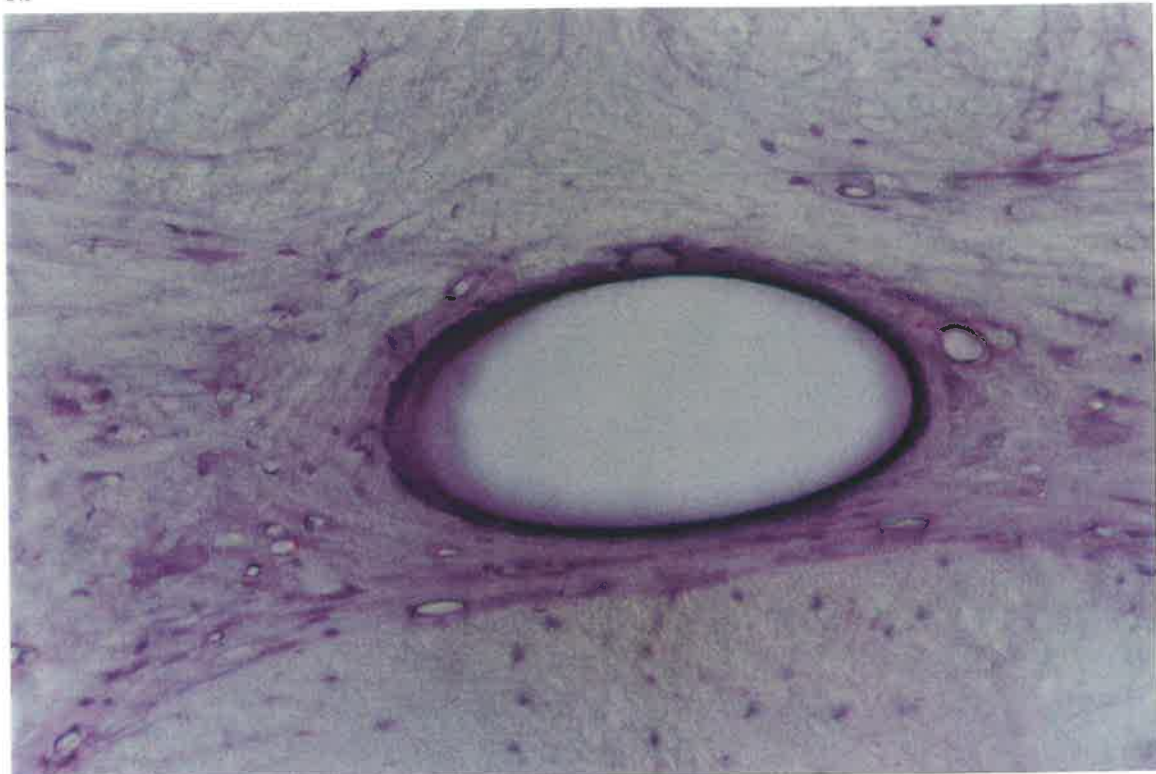
Figure 36. Photomicrographs of transverse cervical spinal cord sections from a rat sacrificed 1 day after kaolin injection and 30 minutes after spinal HRP injection. A: C6. The kaolin injection site (K) appears empty because the kaolin has come out during processing. There is diffuse HRP reaction product labelling the white matter and central grey matter. HRP/TMB, $\times 20$. B: C2. HRP reaction product is spreading from perivascular spaces (arrows) into the central canal (C). Other sections at this level had diffuse labelling of the central grey matter and central canal. HRP/TMB, $\times 200$.

7.2.2 3 days post kaolin injection

The kaolin injection site at 3 days contained kaolin crystals, neutrophils and some macrophages. The central canal contained inflammatory cells (Figure 37), but these were not as numerous as in the 1 day group. There were adhesions in the cervical central canal and trabeculae formed by ependymal proliferation (Figure 38). The central canal was sometimes dilated between occlusions formed by adhesions or trabeculae (Figure 37). Cervical cord perivascular spaces often contained inflammatory cells. These cells were not as numerous as in the 1 day group and the perivascular spaces were not dilated. The central canal caudal to the kaolin injection point was not dilated and only occasionally contained inflammatory cells and kaolin crystals.

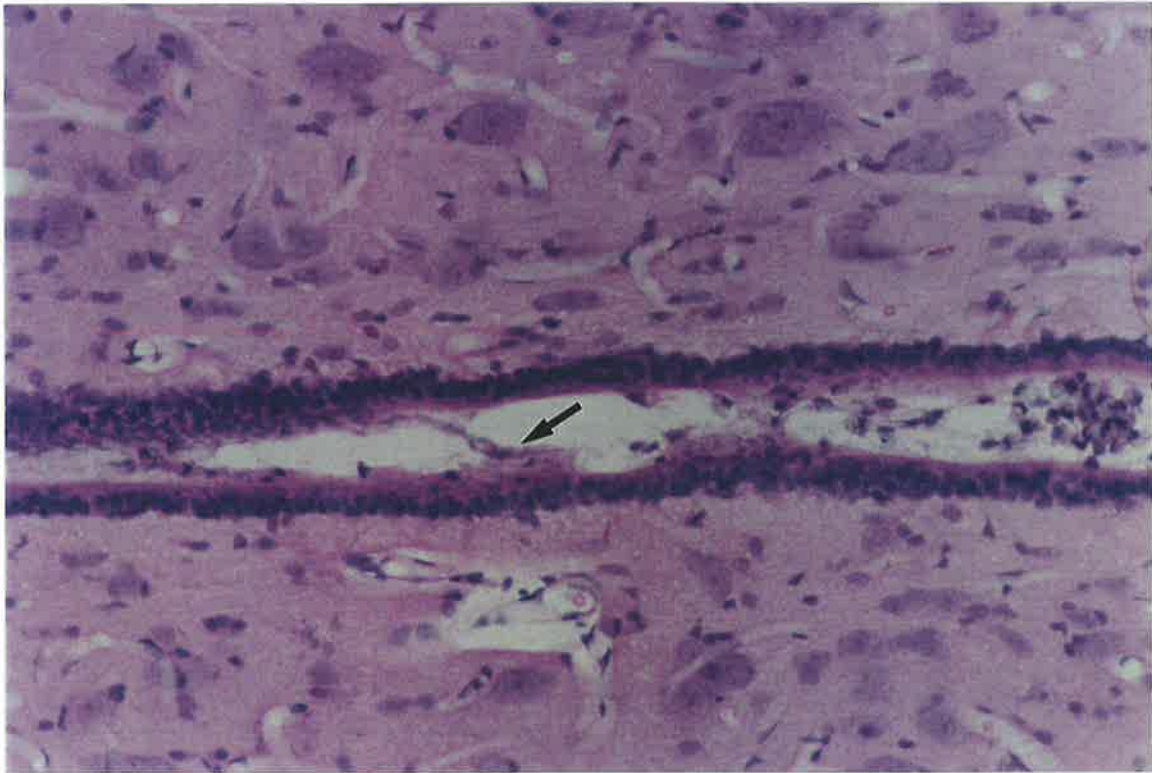


A

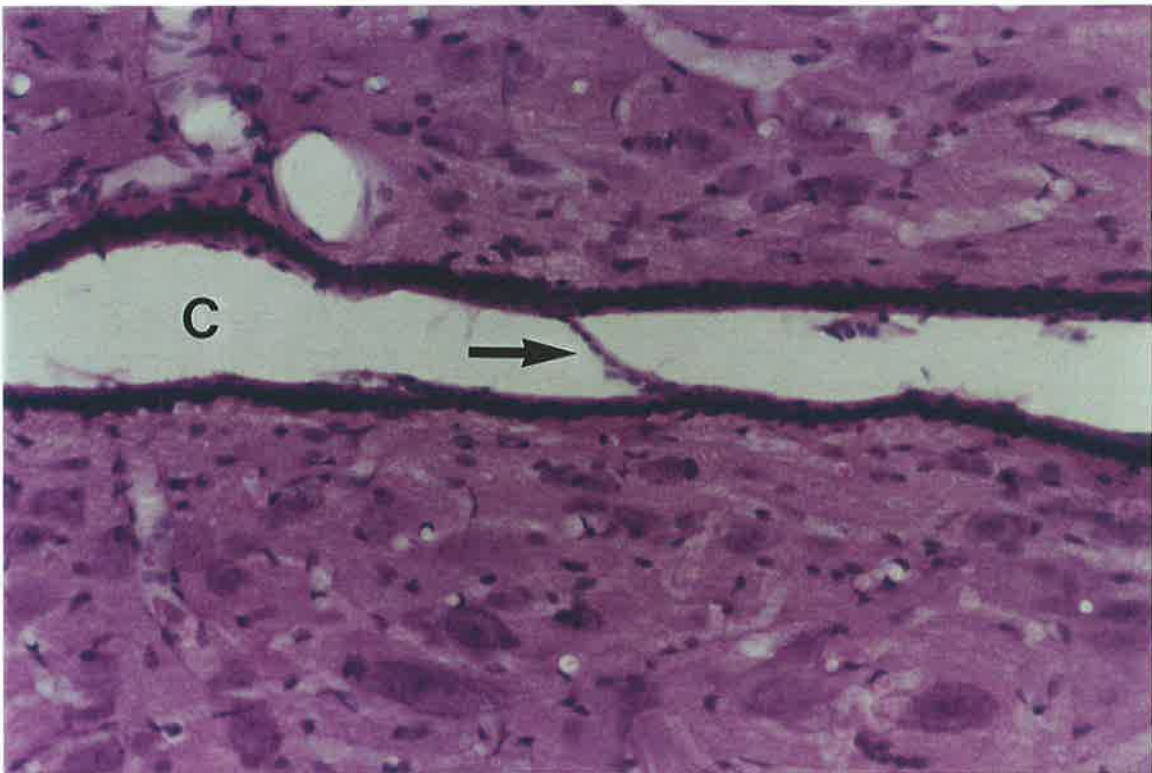


B

Figure 37. Photomicrographs of transverse cervical cord sections in rats sacrificed 3 days post kaolin injection. A: The central canal contains inflammatory cells and there is adhesion formation. H&E, $\times 200$. B: The central canal is dilated. H&E, $\times 200$.



A



B

Figure 38. Photomicrographs of longitudinal cervical spinal cord sections from rats sacrificed 3 days after kaolin injection. A: There is budding of ependymal cells to form a trabecula (arrow). H&E, $\times 200$. B: There is a dilated segment of central canal (C) isolated by an adhesion (not seen) and a trabecula (arrow). H&E, $\times 200$.

7.2.2.1 0 minutes

7.2.2.1.1 Cisternal HRP injection

Transpial diffusion in normal rats is compared with diffusion in rats 3 days after kaolin injection in Figure 39. The depth of diffusion was greater in the kaolin-injected rats in the brain and rostral cervical cord. There was a similar number of perivascular spaces labelled in the kaolin-injected rats in this group and in the corresponding 1 day post kaolin group. Central grey matter perivascular spaces were labelled at most spinal cord levels and sections from all animals had reaction product spreading from these vessels to the central canal (Figure 40). This occurred even if the central canal appeared to be occluded by adhesions or cells. In the case of the central canal appearing occluded by adhesions, forming a 'slit,' reaction product could be seen between ependymal cells and in the slit between ependymal cell luminal surfaces.

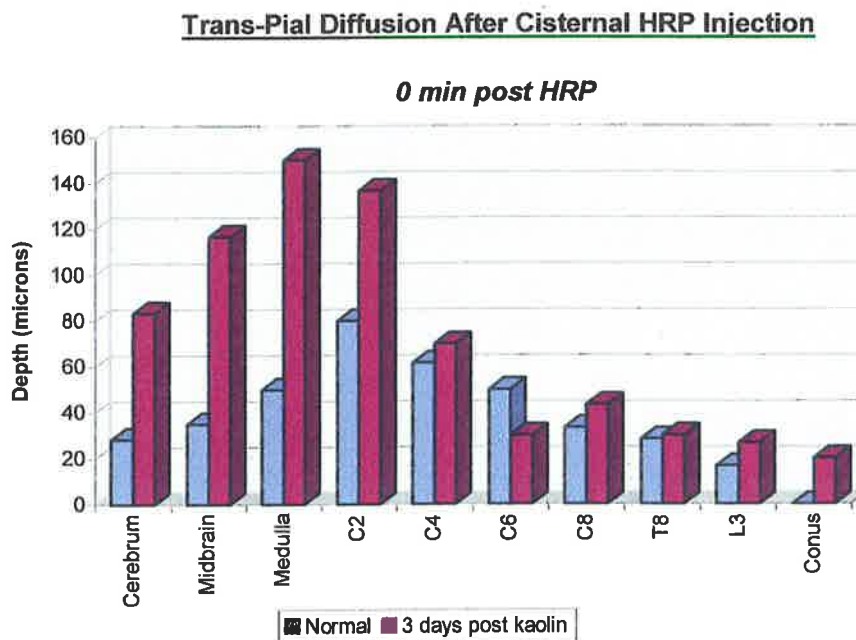


Figure 39. Average depth of transpial diffusion 0 minutes after cisternal HRP injection in normal rats and in rats 3 days after kaolin injection.

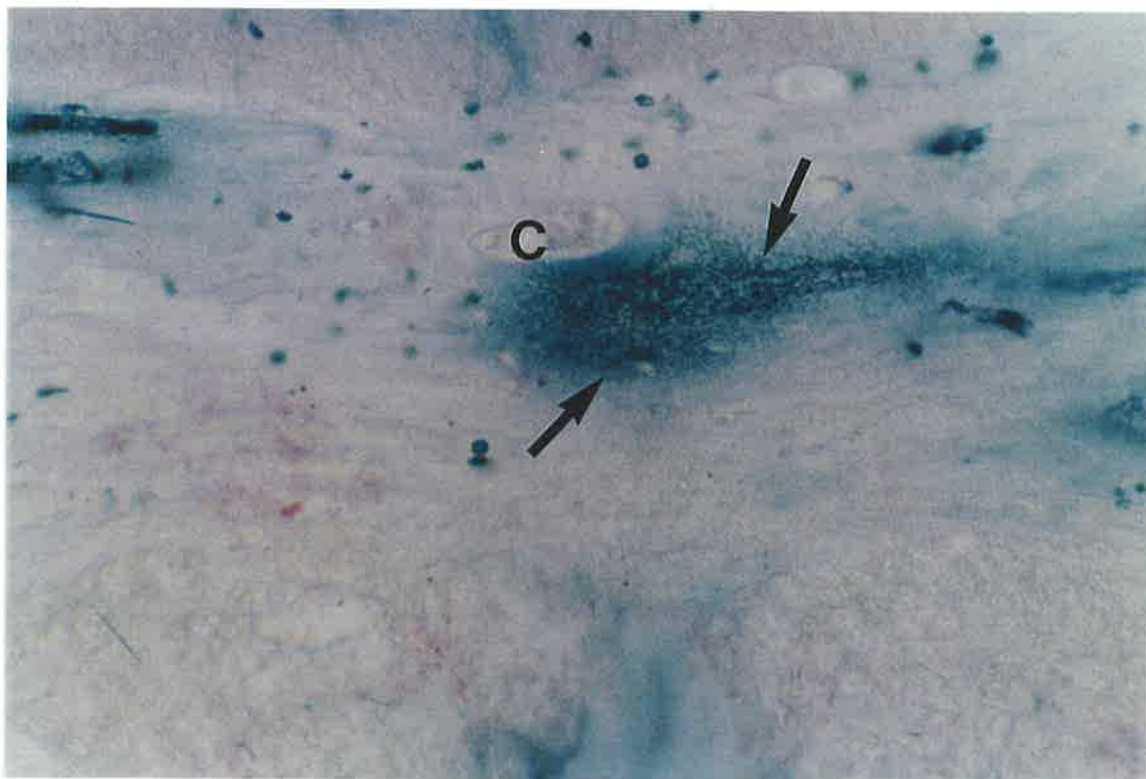


Figure 40. *Photomicrograph of transverse C6 section from a rat sacrificed 3 days post kaolin injection and 0 minutes post HRP injection. Reaction product is spreading from central grey matter perivascular spaces (arrows) into the central canal (C), which contains inflammatory cells. HRP/TMB, $\times 200$.*

7.2.2.1.2 Spinal HRP injection

Transspial diffusion was greater in rats sacrificed 3 days after kaolin injection than in normal rats (Figure 41). There were labelled perivascular spaces at all levels of the neuraxis. There was a gradation of labelling, with more vessels being labelled in the sections closer to the HRP injection point. Central grey matter perivascular spaces were labelled and at many levels reaction product was spreading into the central canal. This occurred even in sections through segments of isolated and dilated central canal (Figure 42). At some levels, reaction product was in the central canal only if a labelled perivascular space was nearby and reaction product was spreading from the perivascular space into the canal.

Trans-Pial HRP Diffusion After Spinal HRP Injection

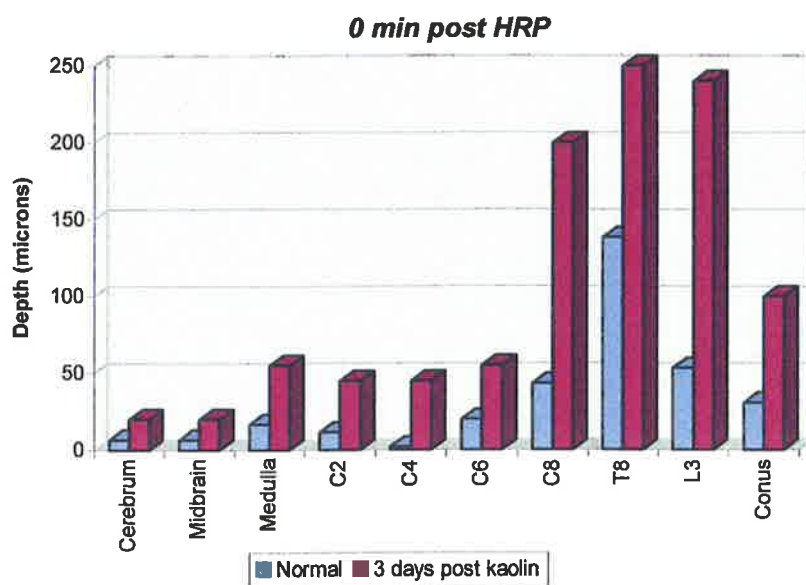


Figure 41. *Transpial diffusion 0 minutes after spinal HRP injection in normal rats and in rats 3 days after kaolin injection.*

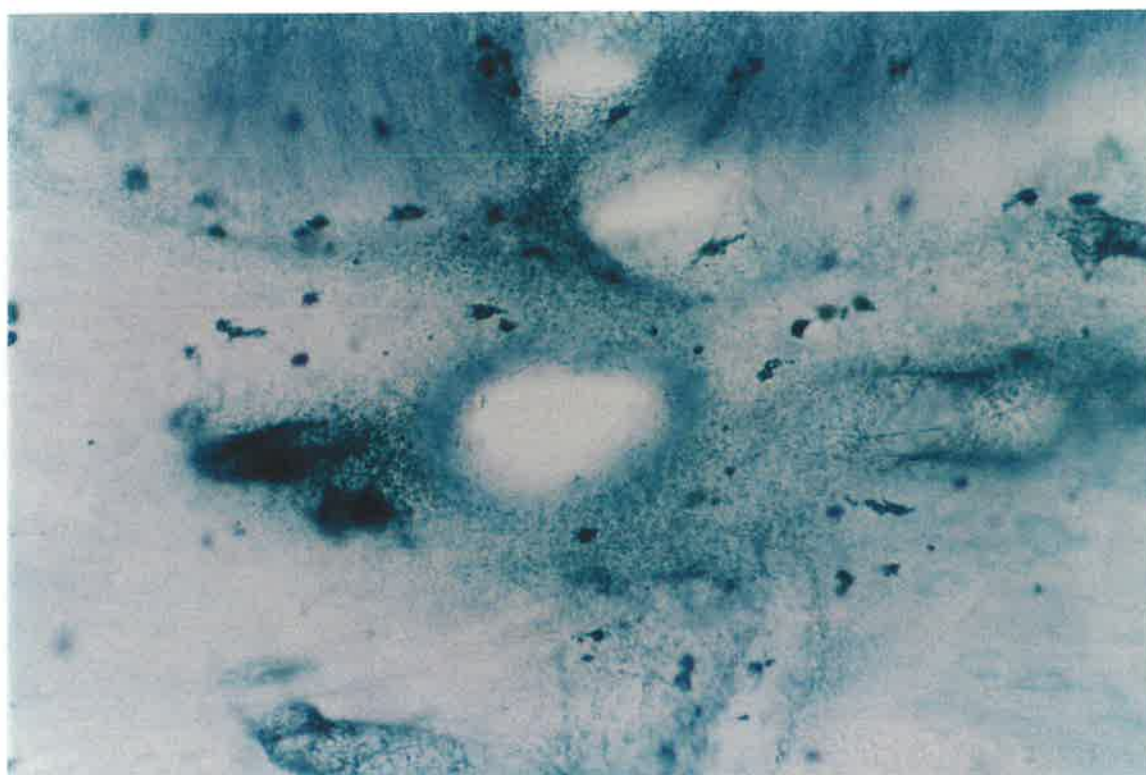


Figure 42. *Photomicrograph of a transverse section through C6 in a rat sacrificed 3 days after kaolin injection and 0 minutes after spinal HRP injection. Reaction product is spreading from central grey matter perivascular spaces into the central canal. This section is from a segment of central canal that was isolated by adhesions and trabeculae and was diluted. HRP/TMB, $\times 200$.*

7.2.2.2 10 minutes

7.2.2.2.1 Cisternal HRP injection

The HRP reaction product intensity was markedly reduced in two rats in this group. They were both sacrificed on the same day and appeared to have been perfused with excess fixative solution. These rats were excluded from the CSF tracer part of the study. Transpial diffusion in the remaining rats is compared with normal rats in Figure 43. The depth of diffusion in the kaolin-injected rats was less than in the normal rats at all spinal cord levels, particularly those caudal to the kaolin injection point. The number of perivascular spaces labelled in this group was greater than the number labelled in the corresponding 0 minute group. The density of labelling was also greater. The central grey matter was diffusely labelled so that no specific patterns of tracer movement were discernible. The central canal was labelled at all spinal cord levels, even where the canal was isolated by adhesions or trabeculae.

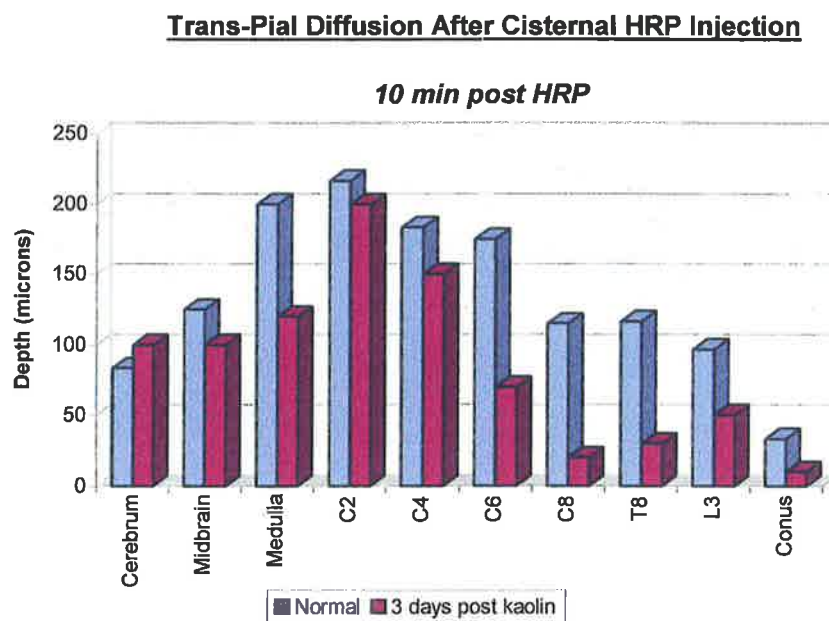


Figure 43. *Transpial diffusion 10 minutes after cisternal HRP injection in normal rats and rats studied 3 days after kaolin injection.*

7.2.2.2.2 Spinal HRP injection

Transpial diffusion in kaolin-injected rats was seen only in spinal cord levels caudal to the kaolin injection site (Figure 44). Perivascular spaces were labelled only at levels caudal to the kaolin injection point. No reaction product was detectable in sections rostral to the kaolin injection point. The central grey matter and central canal in thoracic, lumbar and conus sections were diffusely labelled and patterns of spread from perivascular spaces could not be detected.

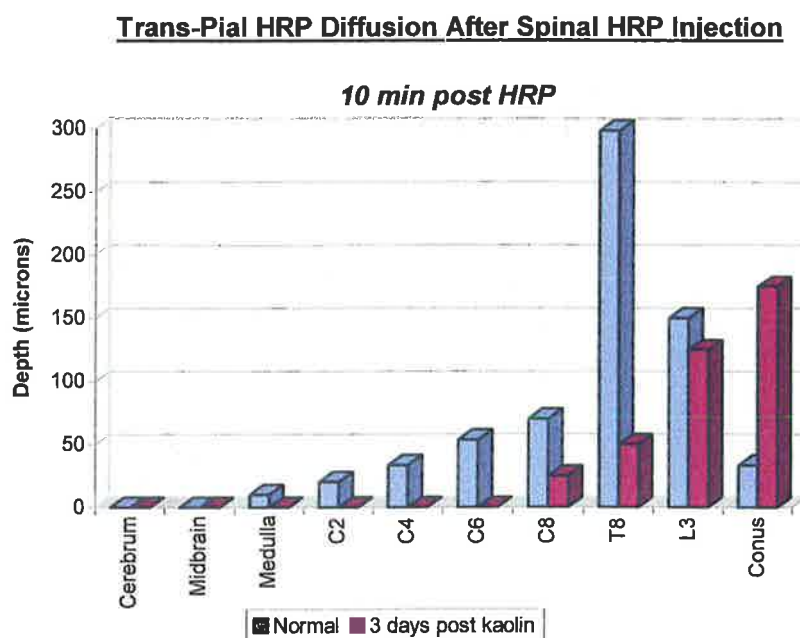


Figure 44. *Transpial HRP diffusion 10 minutes after spinal HRP injection in normal rats and in rats 3 days after kaolin injection.*

7.2.2.3 30 minutes

7.2.2.3.1 Cisternal HRP injection

There was less transpial diffusion in the spinal cord levels in this group than in the normal rats (Figure 45). Numerous perivascular spaces were labelled in rostral cervical cord, cerebrum, midbrain and brainstem sections. Very few labelled vessels were present in other sections, especially those caudal to the kaolin injection point. Sections that did have reaction product in the central grey matter perivascular spaces also had reaction product spreading from these spaces into the central canal. Reaction product was present in this pattern even at levels where the central canal was isolated and dilated.

Trans-Pial Diffusion After Cisternal HRP Injection

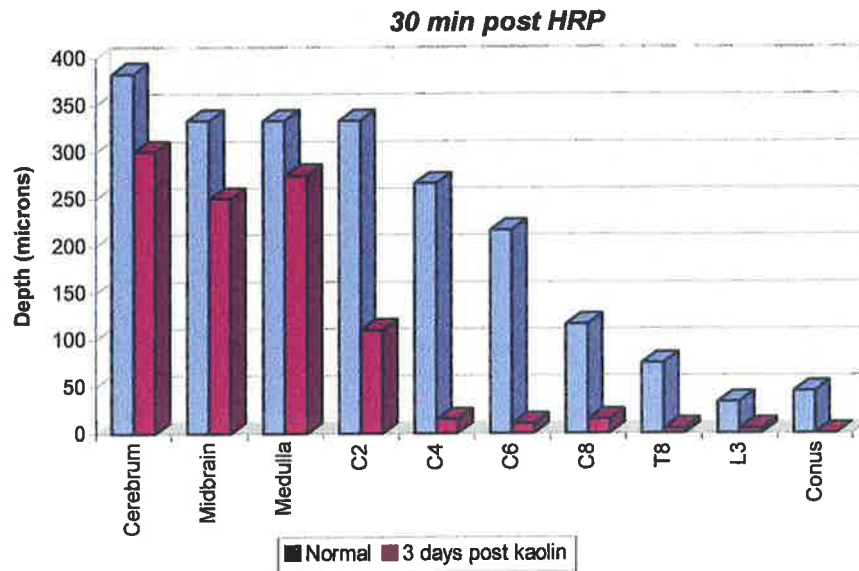


Figure 45. *Transpial diffusion 30 minutes after cisternal HRP injection in normal rats and in rats 3 days after kaolin injection.*

7.2.2.3.2 Spinal HRP injection

The depth of transpial HRP diffusion in this group was less than in the normal rats (Figure 46). No HRP was detected in the cerebrum or midbrain. Perivascular spaces were labelled in the medulla and rostral cervical spinal cord, but there were fewer than in the corresponding group 1 day after kaolin injection. Many perivascular spaces were labelled in the thoracic, lumbar and conus sections. The central grey matter and the central canal in these sections were diffusely labelled and reaction product spreading from perivascular spaces to the central canal could be detected only occasionally. The central canal was not labelled in rostral cervical sections. It was labelled in caudal cervical sections and this occurred in sections where the central canal was dilated.

Trans-Pial HRP Diffusion After Spinal HRP Injection

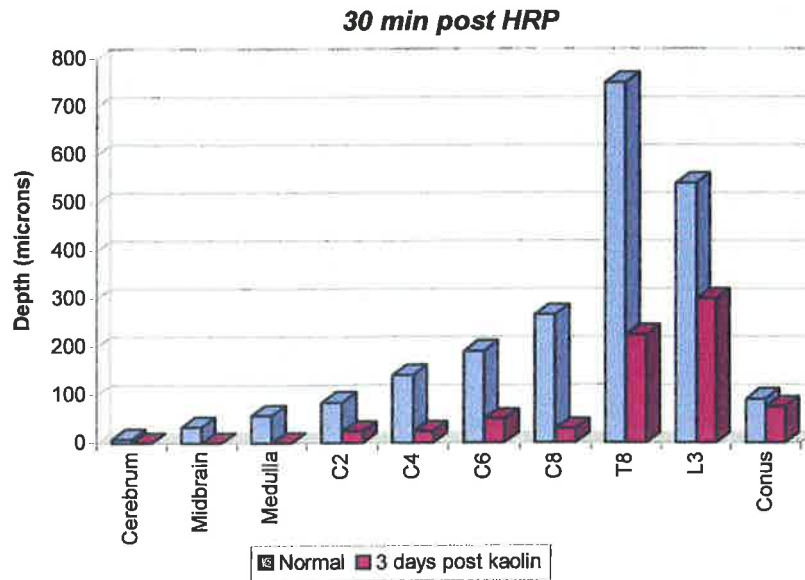


Figure 46. *Transpial HRP diffusion 30 minutes after spinal HRP injection in normal rats and rats 3 days after kaolin injection.*

7.2.3 1 week post kaolin injection

The kaolin injection site 1 week after injection was surrounded and invaded by inflammatory cells, including neutrophils and macrophages. Macrophages containing kaolin crystals were present in perivascular spaces near the kaolin injection site. The perivascular spaces also occasionally contained neutrophils but were not enlarged. There were occlusions of the central canal at various levels in the cervical cord rostral to the injection point and occasionally immediately caudal to it. These occlusions were occasionally formed by inflammatory cells and kaolin crystals packing the central canal but were more often the result of trabeculae or adhesions of the ependyma (Figure 47). The central canal between levels of occlusion was often dilated (Figure 47). The central canal in thoracic, lumbar and conus regions was also dilated in some animals. The central canal contained some inflammatory cells; these were fewer in number than in rats sacrificed at 1 or 3 days post kaolin and were predominantly in the canal rostral to the kaolin injection point.

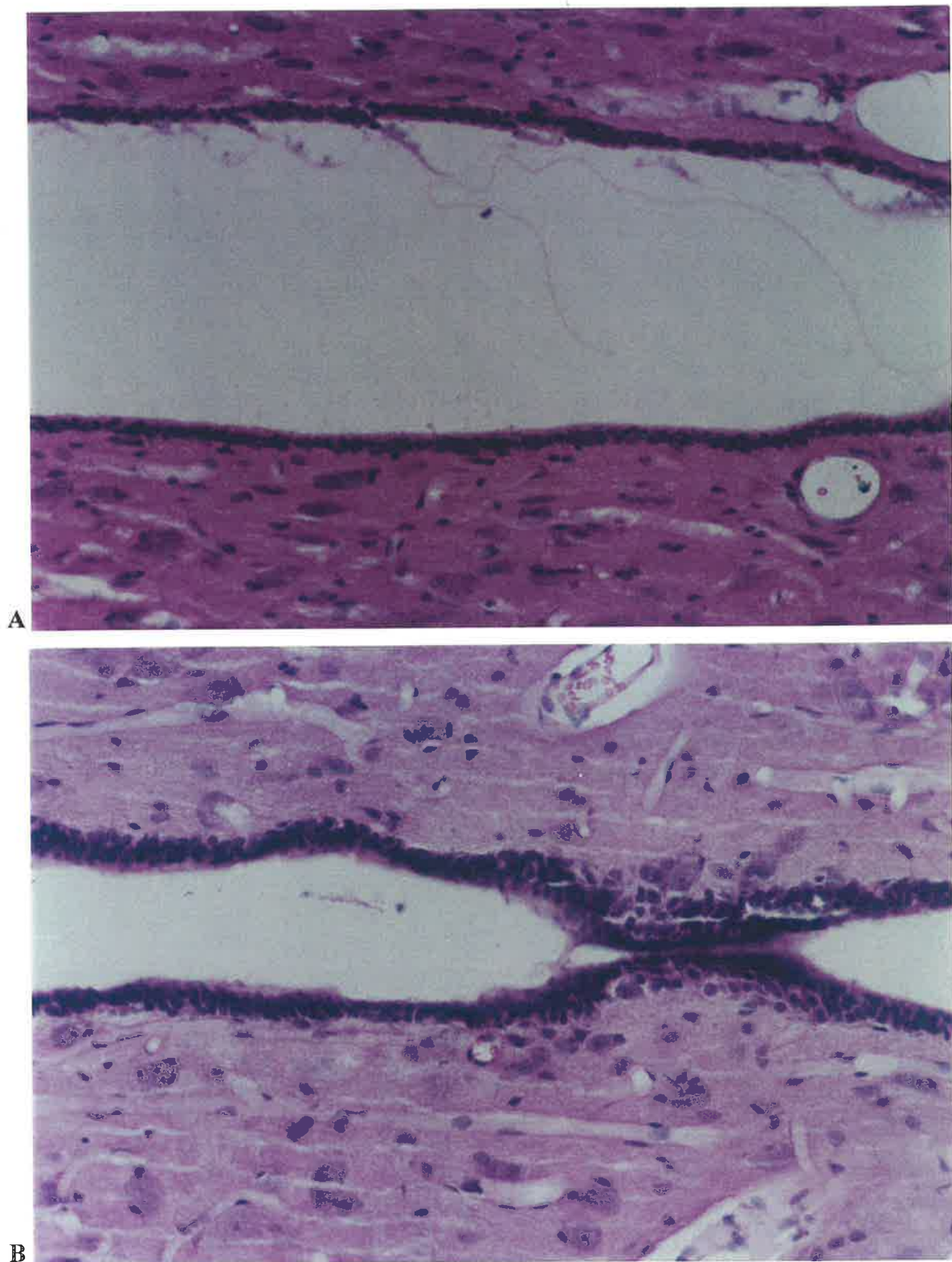


Figure 47. Photomicrographs of longitudinal cervical cord sections from rats sacrificed 1 week after kaolin injection. *A: Enlarged central canal. H&E, $\times 200$. B: Central canal adhesion with dilatation of the canal rostral and caudal to the adhesion. H&E, $\times 200$.*

7.2.3.1 0 minutes

7.2.3.1.1 Cisternal HRP injection

The depth of transpial diffusion 0 minutes after cisternal HRP injection in normal rats is compared with the diffusion in rats 1 week after kaolin injection in Figure 48. Perivascular spaces were labelled at all levels of the neuraxis. Labelled vessels were present in all white matter columns and in the grey matter. The central canal was labelled in most spinal cord sections, including those segments of central canal that were isolated by adhesions or trabeculae. There were many sections in which reaction product was spreading from central grey matter perivascular spaces into the central canal (Figure 49).

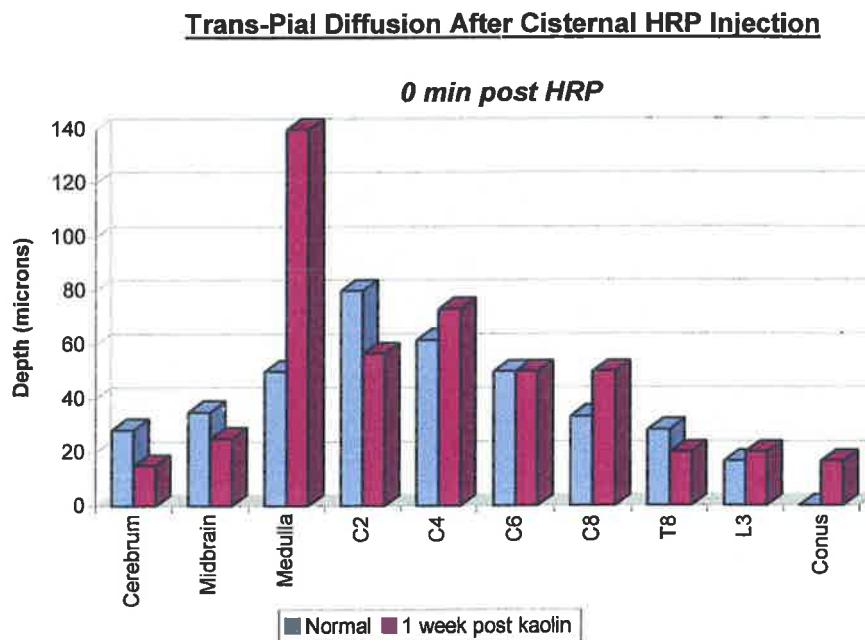


Figure 48. Comparison of transpial diffusion 0 minutes after cisternal HRP injection in normal rats and in rats 1 week after kaolin injection.

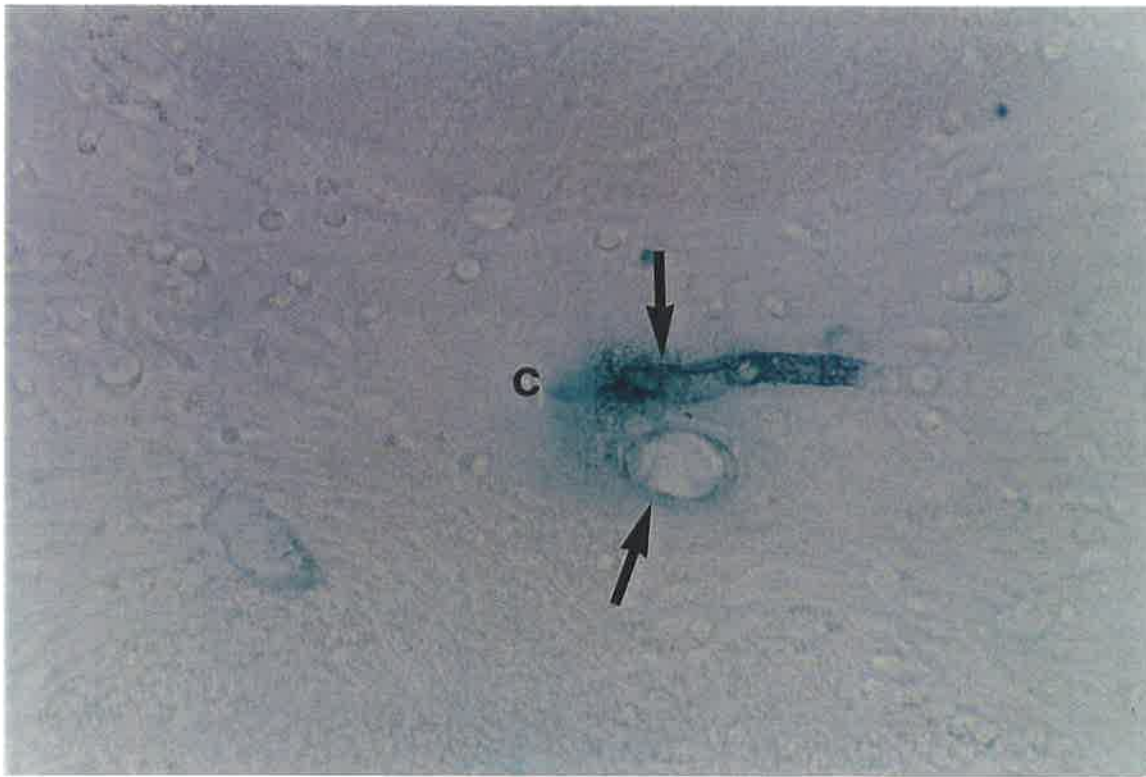


Figure 49. *Photomicrograph of a cervical cord section from a rat sacrificed 0 minutes after cisternal HRP injection and 1 week after kaolin injection. Reaction product is spreading from central grey matter perivascular spaces (arrows) into the central canal (C). This part of the central canal was isolated by adhesions and trabeculae. HRP/TMB, $\times 200$.*

7.2.3.1.2 Spinal HRP injection

The depth of transpial diffusion in this group compared with normal rats studied 0 minutes after spinal HRP injection is shown in Figure 50. Transpial diffusion was greater in the cervical cord in the kaolin-injected rats than in the normal rats. The central canal was labelled at all spinal cord levels. There were numerous perivascular spaces labelled at all levels; these were more numerous closer to the HRP injection site. In many sections the central grey matter was diffusely labelled, but in some cases reaction product was spreading from perivascular spaces into the central canal (Figure 51). This was seen in sections that contained a dilated central canal.

Trans-Pial HRP Diffusion After Spinal HRP Injection

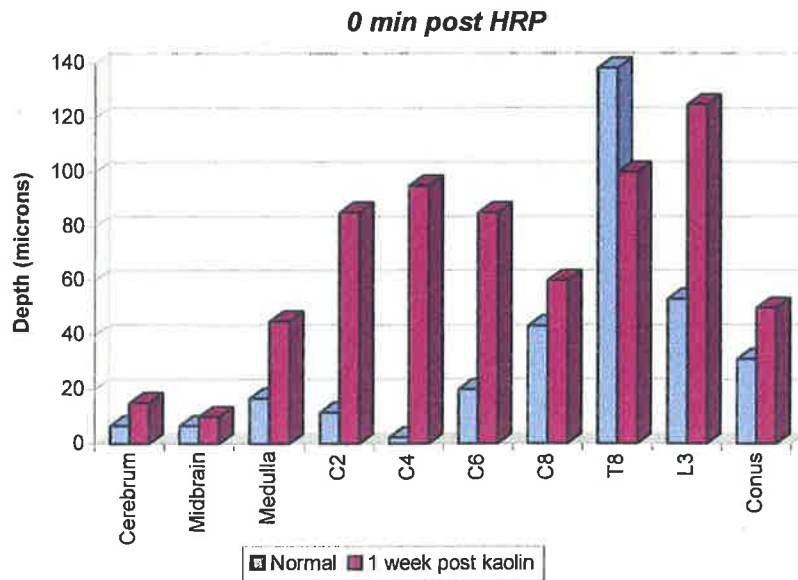


Figure 50. Transpial diffusion 0 minutes after spinal HRP injection in normal rats and rats 1 week after kaolin injection.

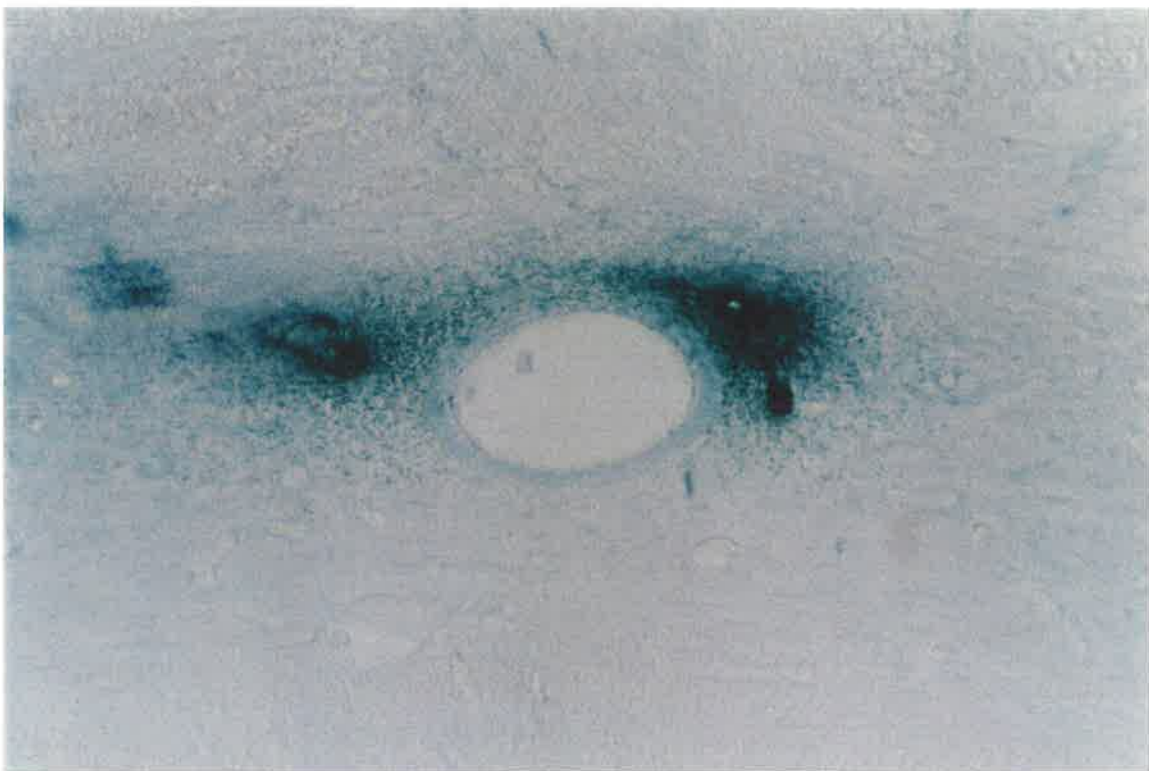


Figure 51. Photomicrograph of cervical cord section from a rat sacrificed 1 week after kaolin injection and 0 minutes after spinal HRP injection. HRP reaction product is spreading from central grey matter perivascular spaces into the central canal. HRP/TMB, $\times 200$.

7.2.3.2 10 minutes

7.2.3.2.1 Cisternal HRP injection

The depth of transpial diffusion in this group is compared with that in normal rats in Figure 52. Transpial diffusion was less in the kaolin-injected rats at all levels. The difference was more marked in spinal cord levels caudal to the kaolin injection site. Perivascular spaces were labelled at all levels of the neuraxis and were present in all white matter columns and the grey matter of the spinal cord. The central canal contained reaction product in all cervical spinal cord levels, but not in the thoracic, lumbar or conus sections. The cervical central grey matter was often diffusely labelled. In many cases, however, reaction product was spreading from central grey matter perivascular spaces into the central canal. The central canal contained reaction product in segments where it was isolated by adhesions or trabeculae.

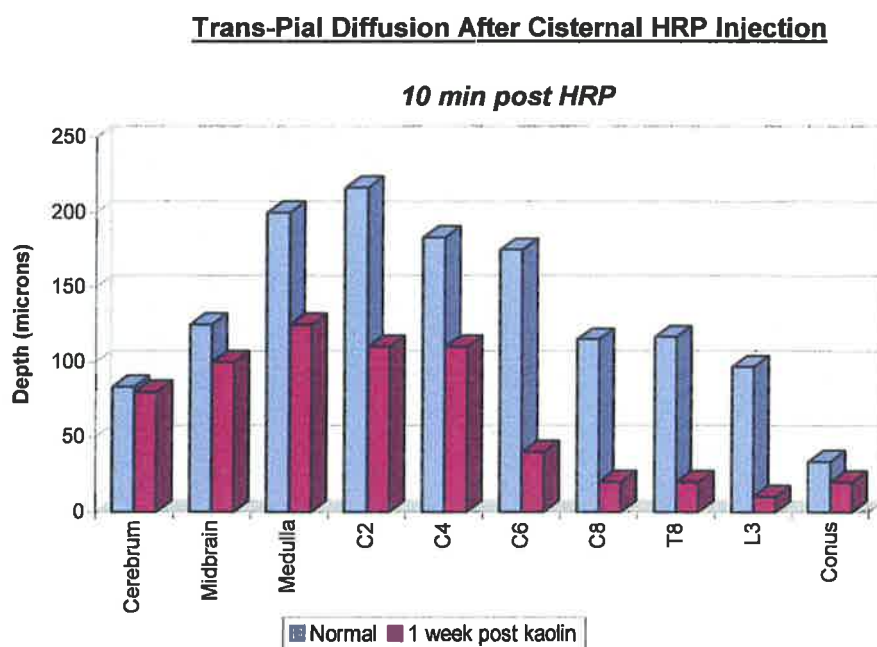


Figure 52. Comparison of transpial diffusion 10 minutes after cisternal HRP injection in normal rats and rats 1 week after kaolin injection.

7.2.3.2.2 Spinal HRP injection

Figure 53 compares the transpial diffusion 10 minutes after HRP injection in normal rats and in rats 1 week after kaolin injection. The central canal contained reaction product in many segments that were isolated or dilated and reaction product was spreading from perivascular spaces into these segments. Numerous perivascular spaces were labelled in the

sections caudal to the kaolin injection point. In some animals there were also labelled vessels in sections rostral to the kaolin injection. In these spinal cord sections the central grey matter was diffusely labelled and there was no directional pattern of reaction product. In one animal however, the central canal and central grey matter were densely labelled in the cord rostral to the kaolin injection point (Figure 54) without many labelled perivascular spaces. This pattern was consistent with HRP having entered the central canal caudal to the kaolin injection site, rather than having entered from perivascular spaces rostral to it.

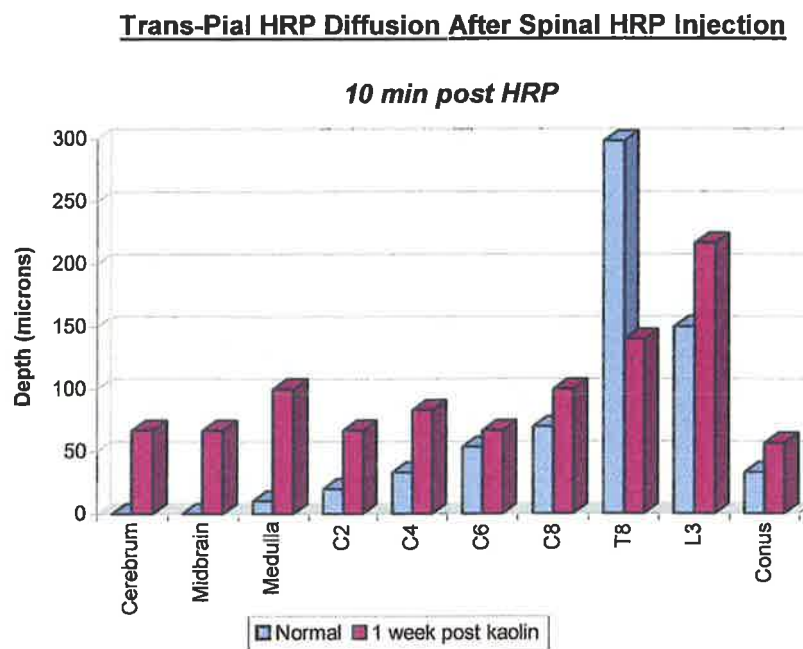


Figure 53. *Transpial diffusion 10 minutes after spinal HRP injection in normal rats and in rats 1 week after kaolin injection.*

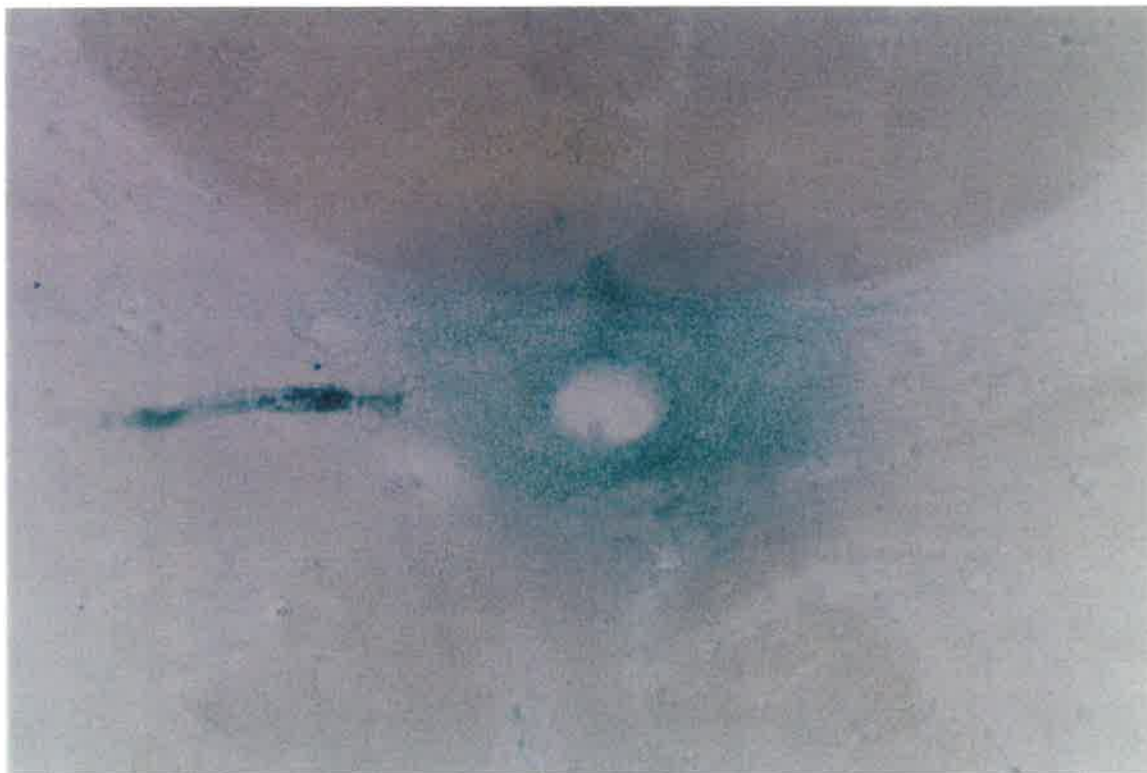


Figure 54. *Photomicrograph of transverse cervical cord section from a rat sacrificed 1 week after kaolin injection and 10 minutes after HRP injection. The density of central canal and central grey matter labelling is disproportionate to the label density in surrounding perivascular spaces, suggesting that HRP did not reach the central canal from these spaces. HRP/TMB, $\times 100$.*

7.2.3.3 30 minutes

7.2.3.3.1 Cisternal HRP injection

Transspial diffusion in the cervical cord in this group was greater than the diffusion in normal rats (Figure 55). Many perivascular spaces were labelled in the brain and cervical cord. There were fewer labelled perivascular spaces in the thoracic, lumbar and conus sections. The central canal was labelled in cervical, thoracic and lumbar sections. This was often in association with diffuse labelling of the surrounding central grey matter, but in some sections reaction product was spreading from perivascular spaces into the central canal (Figure 56).

Trans-Pial Diffusion After Cisternal HRP Injection

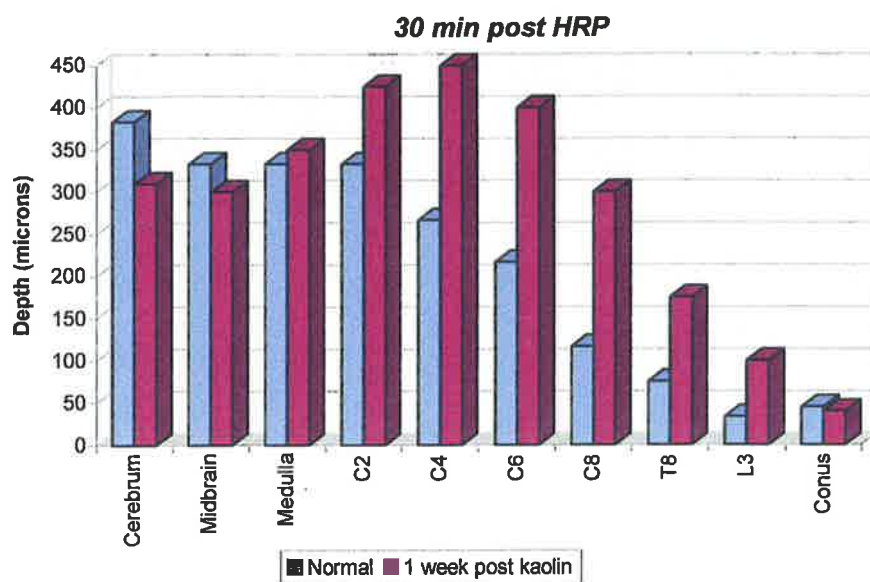


Figure 55. *Transpial diffusion 30 minutes after cisternal HRP injection in normal rats and rats 1 week after kaolin injection.*

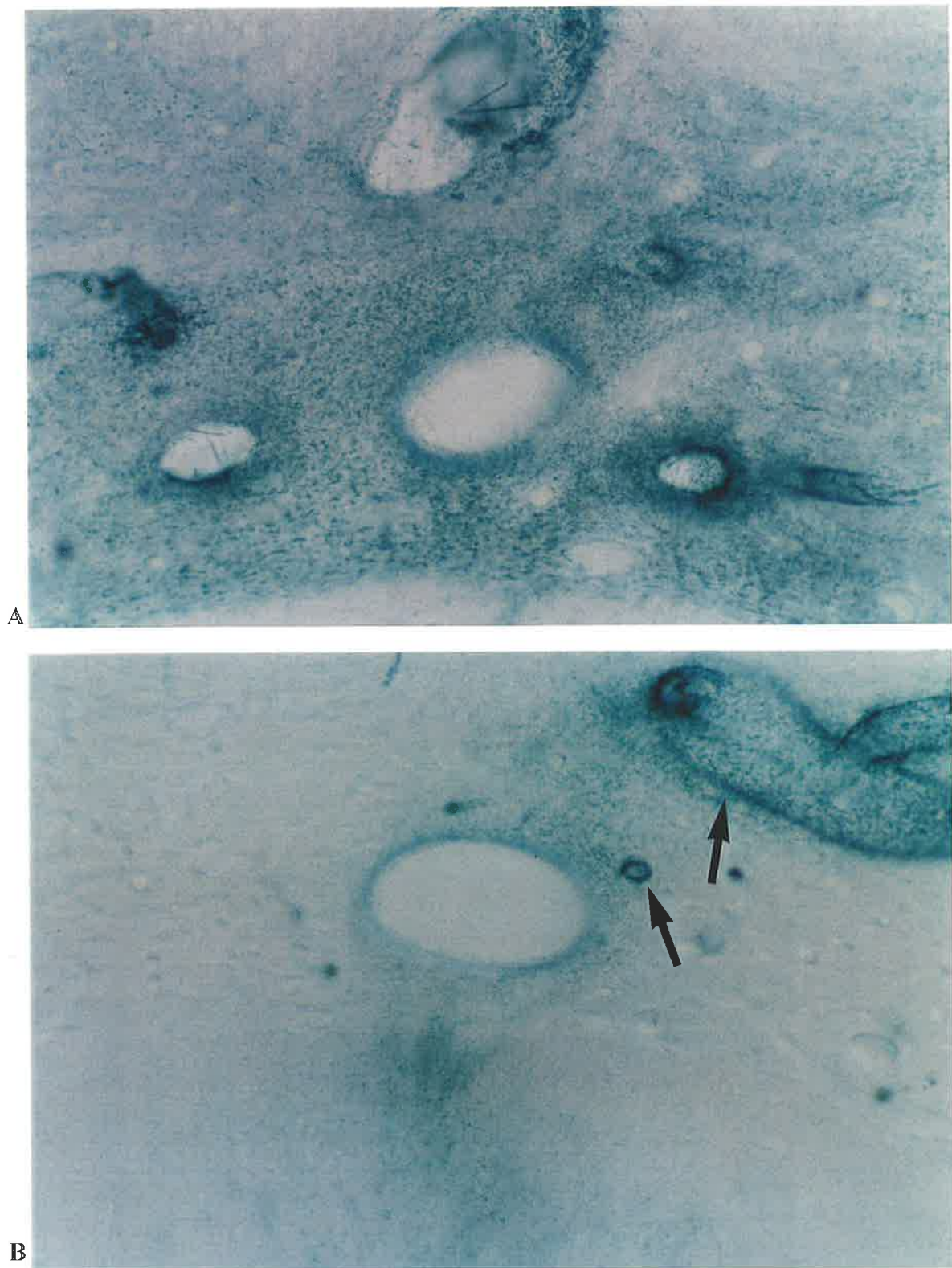


Figure 56. Photomicrographs of transverse cervical sections from a rat sacrificed 1 week after kaolin injection and 30 minutes after cisternal HRP injection. A: C2. There is diffuse labelling of the central grey matter and central canal. B: C4. Reaction product is spreading from perivascular spaces (arrows) into the slightly dilated central canal. Other sections at this level had diffuse labelling of the central grey matter. HRP/TMB, $\times 200$.

7.2.3.3.2 Spinal HRP injection

The depth of transpial diffusion 30 minutes after spinal HRP injection in normal rats and in rats 1 week after kaolin injection is shown in Figure 57. There was diffuse parenchymal labelling in the sections caudal to the kaolin injection point and perivascular spaces were heavily labelled at these levels. There were fewer labelled perivascular spaces in the rostral sections and the density of labelling was less than in the caudal sections. The central grey matter and central canal were labelled at all levels of the spinal cord. In one animal the density of this central grey matter labelling in cervical cord sections was disproportionate to the number and density of labelled perivascular spaces; this pattern had the appearance of HRP having reached the central canal at these levels by entering the canal at more caudal levels (Figure 58). In the other animals, reaction product could be seen spreading from perivascular spaces into the central canal in some cervical sections (Figure 58).

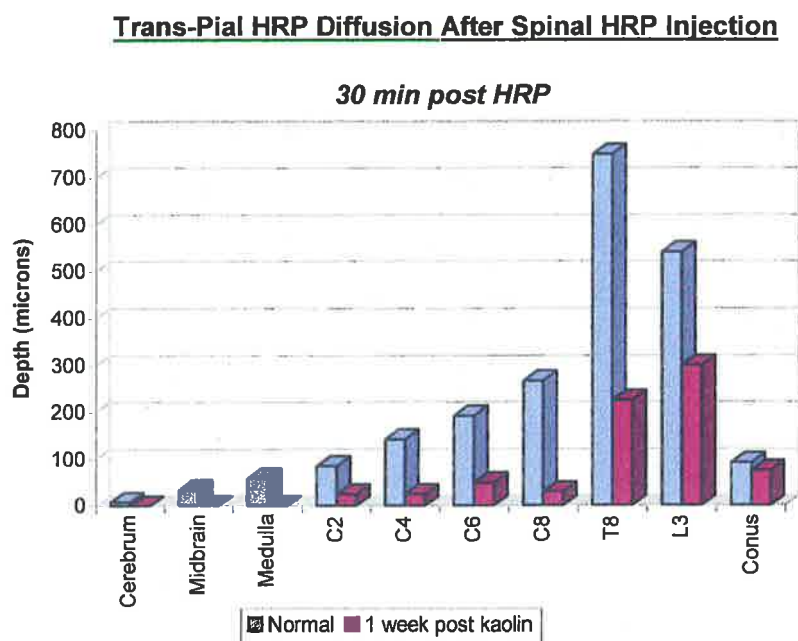


Figure 57. Transpial diffusion 30 minutes after spinal HRP injection in normal rats and rats 1 week after kaolin injection.

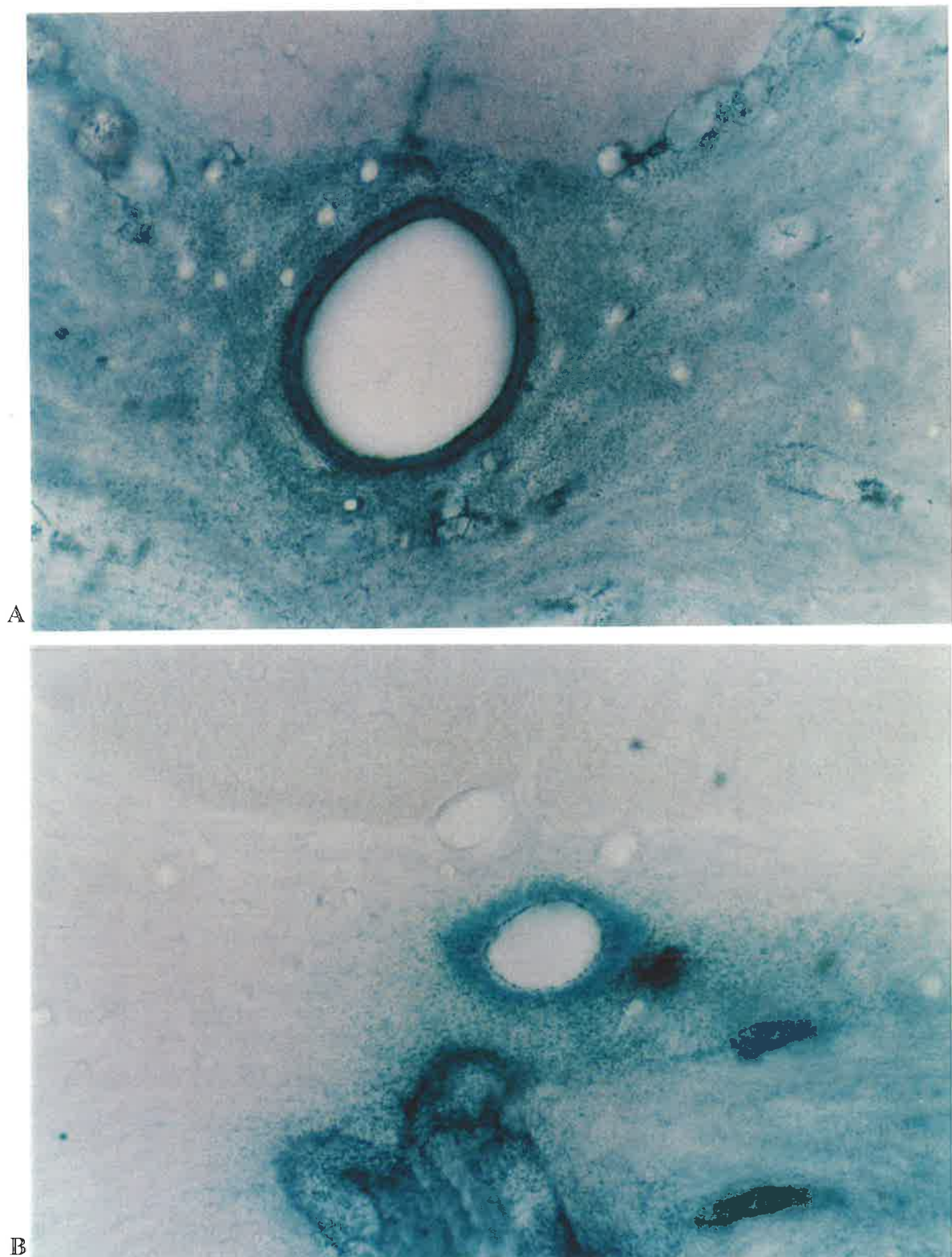


Figure 58. *Photomicrographs of transverse C6 sections from different animals sacrificed 1 week after kaolin injection and 30 minutes after spinal HRP injection. A: The central grey matter and central canal are diffusely labelled and the density of labelling is disproportionate to the number and density of labelling of perivascular spaces. B: Reaction product is spreading from central grey matter perivascular spaces into the central canal. HRP/TMB, $\times 200$.*

7.2.4 6 weeks post kaolin injection

By 6 weeks after kaolin injection the cervical central canal had dilated more than in the 1 week group. The entire canal was not dilated in all animals; in some animals there were segments of canal that had become a solid mass of glial and ependymal proliferation around a collection of inflammatory cells and kaolin crystals (Figure 59). The canal was dilated in segments between these occlusions or between adhesions and trabeculae (Figure 60). There were occasional inflammatory cells and kaolin crystals in the canal. The tissue around dilated segments of central canal was oedematous and showed signs of axonal damage (Figure 60). The kaolin injection site was surrounded by a chronic inflammatory response (granuloma). There were macrophages containing kaolin crystals in some perivascular spaces near the kaolin injection site.

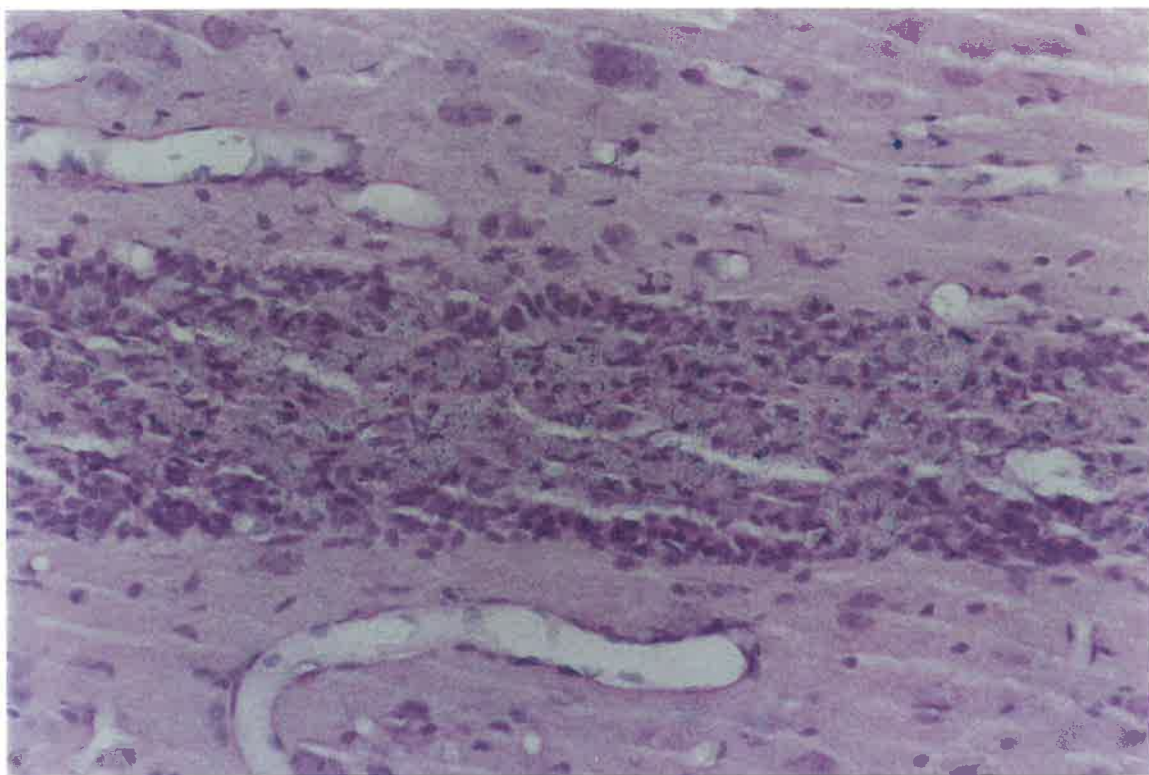


Figure 59. *Photomicrograph of cervical cord section from a rat sacrificed 6 weeks after kaolin injection. The central canal has been completely occluded by inflammatory cells, kaolin crystals and ependymal and glial proliferation. H&E, $\times 200$.*

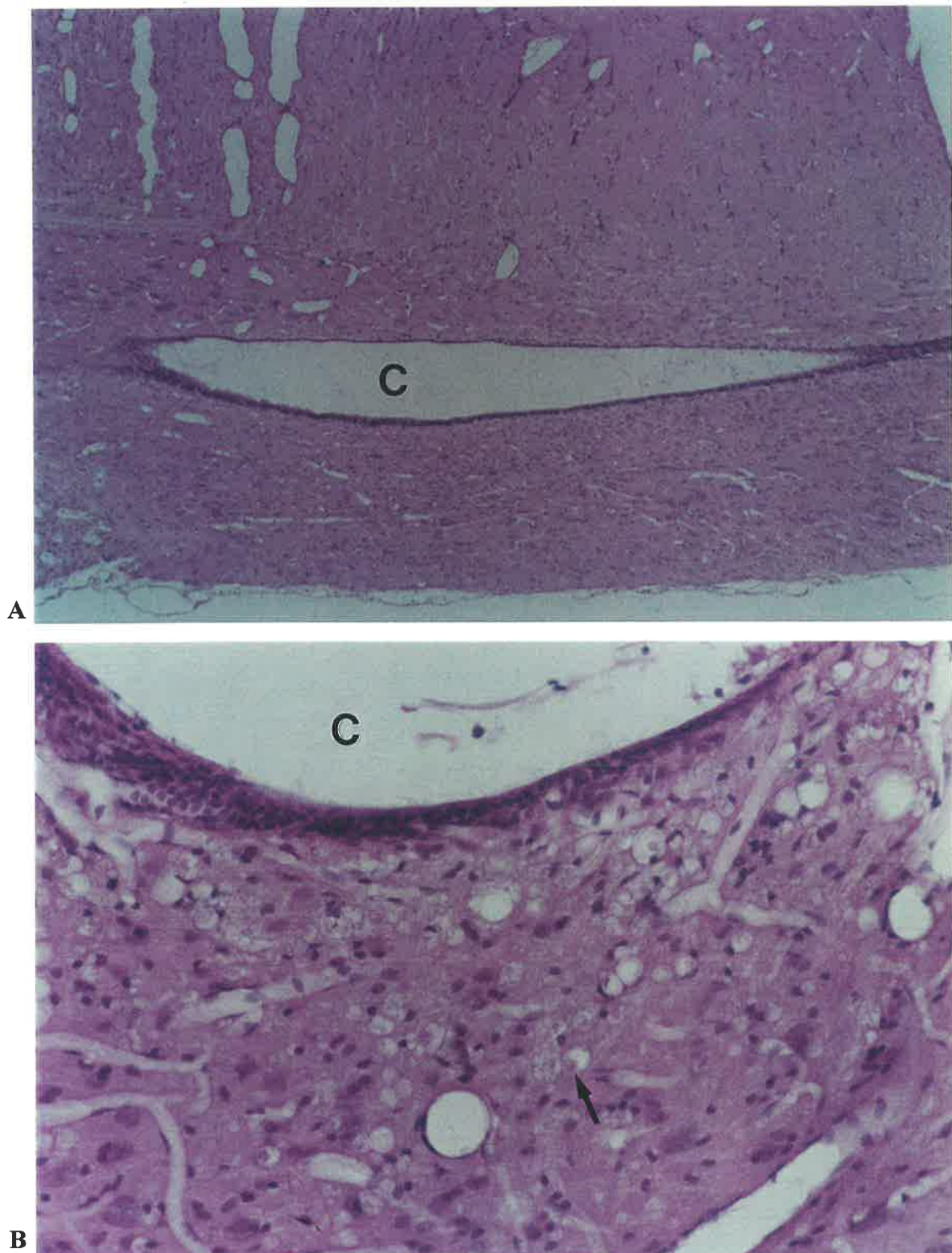


Figure 60. Photomicrographs of cervical cord sections from rats sacrificed 6 weeks after kaolin injection. A: C2, longitudinal section. The central canal (C) is dilated. H&E, $\times 40$. B: Transverse section demonstrating a dilated central canal (C) and changes in the surrounding tissue including oedema and axonal retraction balls (arrow). H&E, $\times 200$.

7.2.4.1 0 minutes

7.2.4.1.1 Cisternal HRP injection

Two animals in this group were excluded from the CSF flow study; one because of inadequate fixation and the other because of a syringe malfunction during HRP injection. The depth of transpial diffusion in the remaining animals is compared with the diffusion in normal rats in Figure 61. Perivascular spaces were labelled at all levels of the neuraxis. In some animals, the central canal contained reaction product throughout the cervical, thoracic and lumbar segments. In other animals only some cervical and lumbar sections had a labelled central canal. The central grey matter was diffusely labelled in some sections, while in others there was a preferential spread of reaction product from perivascular spaces into the central canal. This preferential flow into the central canal was demonstrated in some segments of central canal that were isolated by adhesions and trabeculae.

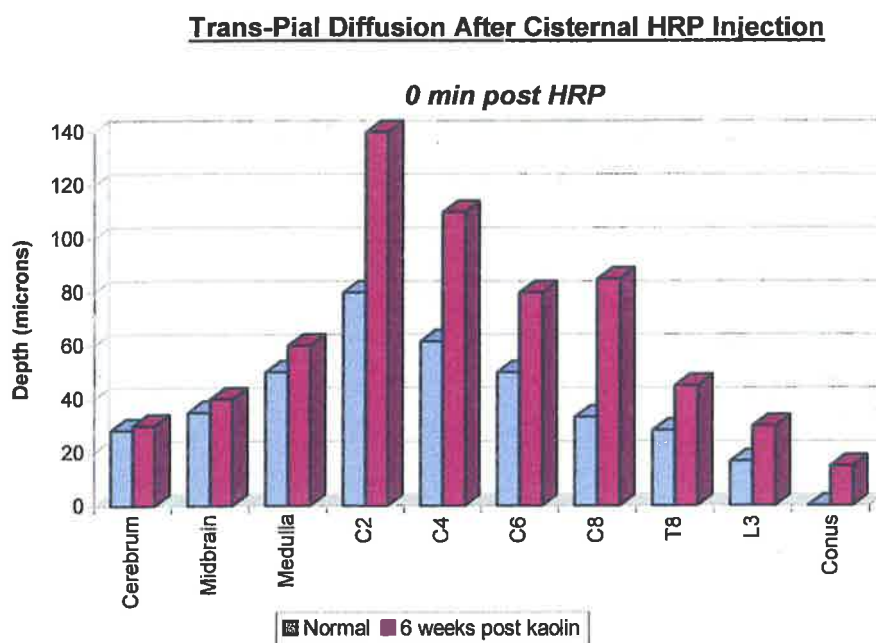


Figure 61. *Transpial diffusion 0 minutes after cisternal HRP injection in normal rats and in rats 6 weeks after kaolin injection.*

7.2.4.1.2 Spinal HRP injection

The depth of transpial diffusion 0 minutes after spinal HRP injection in normal rats and in rats 6 weeks after kaolin injection is shown in Figure 62. Perivascular spaces were labelled at all levels of the neuraxis except the cerebrum. Labelled vessels were more numerous closer

to the HRP injection point. Reaction product did not reach the central grey matter perivascular spaces or the central canal in rostral cervical sections. In the more caudal sections, perivascular spaces were labelled in the central grey matter and reaction product was spreading from these vessels to the central canal (Figure 63). This pattern was also seen in thoracic, lumbar and conus sections. Reaction product was not present in dilated segments of central canal in the rostral cervical cord, but was present in more caudal sections.

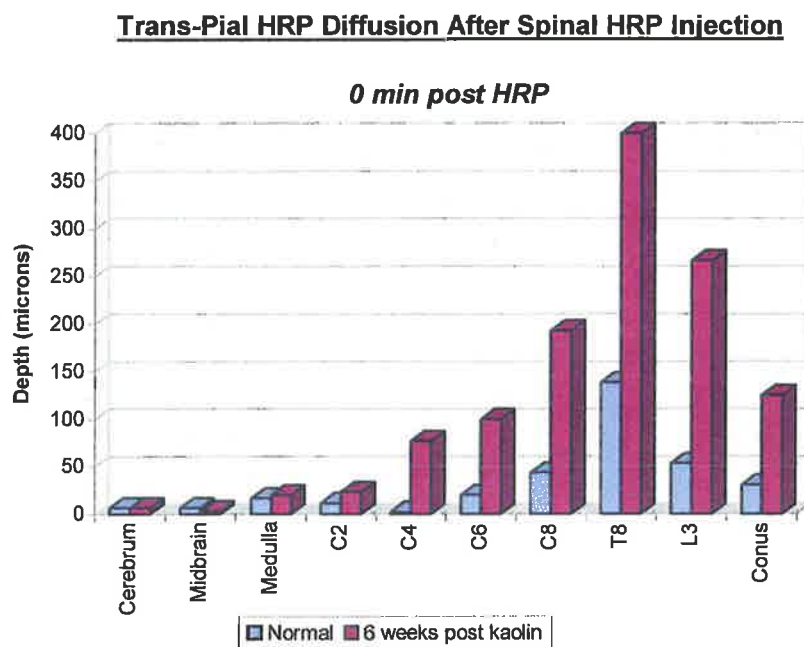


Figure 62. *Transpial diffusion 0 minutes after spinal HRP injection in normal rats and in rats 6 weeks after kaolin injection.*



Figure 63. *Photomicrograph of a transverse cervical cord section (C8) from a rat sacrificed 6 weeks after kaolin injection and 0 minutes after spinal HRP injection. Reaction product is spreading from central grey matter perivascular spaces (arrows) into the central canal (C). HRP/TMB, $\times 200$.*

7.2.4.2 10 minutes

7.2.4.2.1 Cisternal HRP injection

The depth of transspial diffusion in this group is compared with diffusion in normal rats in Figure 64. There were numerous perivascular spaces labelled in the sections rostral to the kaolin injection point. The central canal and the central grey matter were diffusely labelled in the rostral sections and also in the sections caudal to the kaolin injection. The central grey matter labelling was disproportionate to the number and density of perivascular spaces labelled in the caudal sections, consistent with HRP movement in the central canal from more rostral sections. In some cervical sections reaction product was spreading from central grey matter perivascular spaces preferentially into the central canal (Figure 65).

Trans-Pial Diffusion After Cisternal HRP Injection

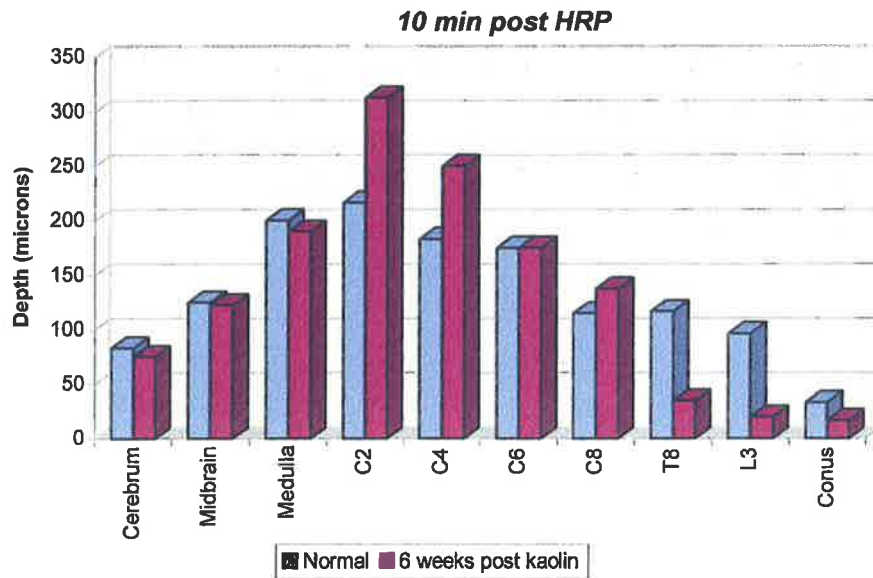


Figure 64. *Transpial diffusion 10 minutes after cisternal HRP injection in normal rats and rats 6 weeks after kaolin injection.*

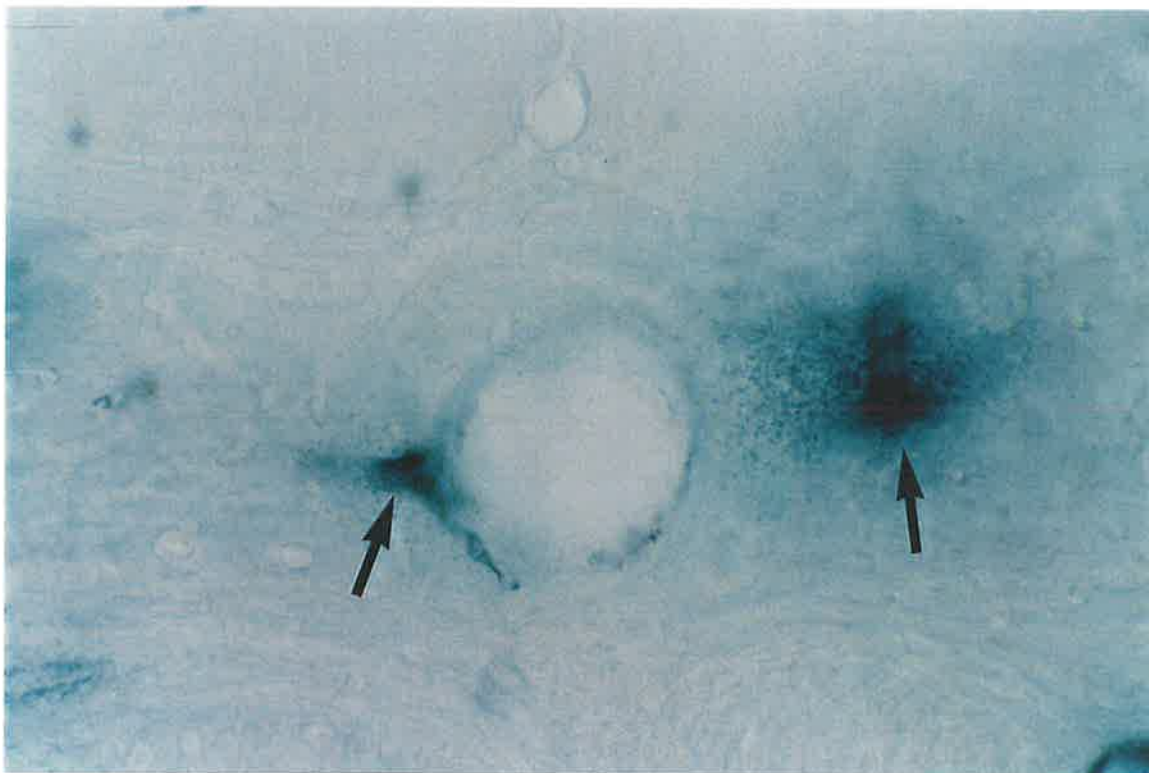


Figure 65. *Photomicrograph of a cervical cord section (C2) from a rat sacrificed 6 weeks after kaolin injection and 10 minutes after cisternal HRP injection. HRP reaction product is labelling perivascular spaces (arrows) in the central grey matter and is spreading into the central canal. HRP/TMB, $\times 200$.*

7.2.4.2.2 Spinal HRP injection

The depth of transpial diffusion was greater at all spinal cord levels in this group than in normal rats (Figure 66). There were very few labelled perivascular spaces rostral to the kaolin injection point, while numerous perivascular spaces were labelled in sections caudal to the kaolin injection point. In the caudal sections there was usually also diffuse labelling of the central grey matter, although in some sections a preferential spread of reaction product from perivascular spaces into the central canal was detectable. This pattern was present in cervical sections immediately rostral to the kaolin injection point, but not in the more rostral cervical levels, in which there were no labelled vessels in the central grey matter and no labelling of the central grey matter extracellular space.

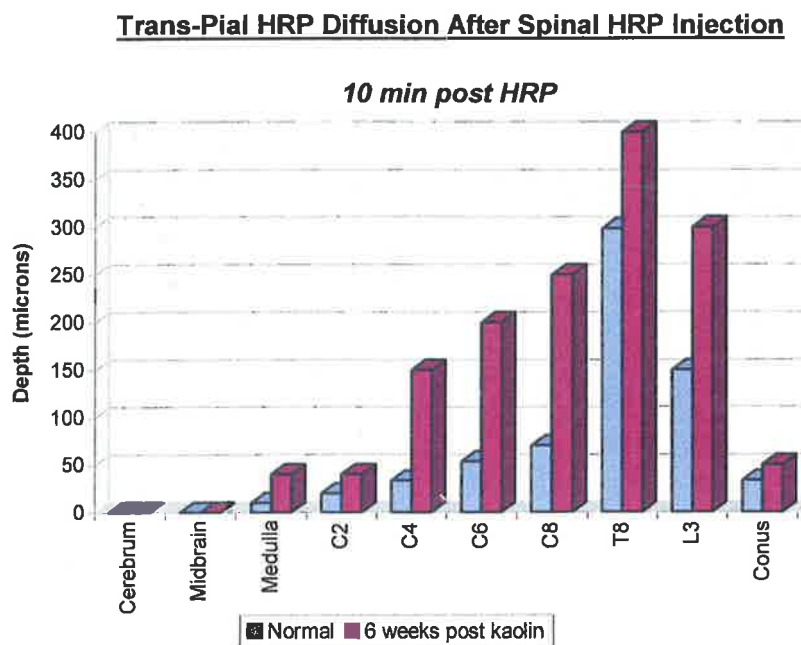


Figure 66. *Transpial diffusion 10 minutes after spinal HRP injection in normal rats and in rats 6 weeks after kaolin injection.*

7.2.4.3 30 minutes

7.2.4.3.1 Cisternal HRP injection

The depth of transpial diffusion 30 minutes after cisternal HRP injection in rats 6 weeks after kaolin injection is compared with the diffusion in normal rats in Figure 67. Many perivascular spaces were labelled throughout the neuraxis and the extracellular space was often diffusely labelled. There was a gradation of labelling; perivascular spaces in the more rostral

sections were more often labelled and the density of labelling was greater. Although the central grey matter was often diffusely labelled, in some sections a preferential flow into the central canal could be detected (Figure 68).

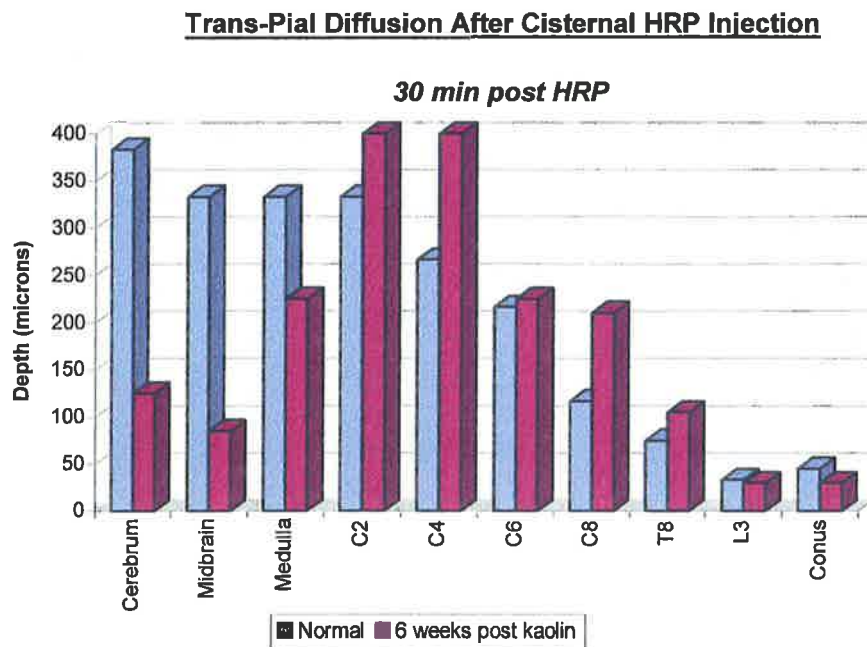


Figure 67. Transpial diffusion 30 minutes after cisternal HRP injection in normal rats and in rats 6 weeks after kaolin injection.

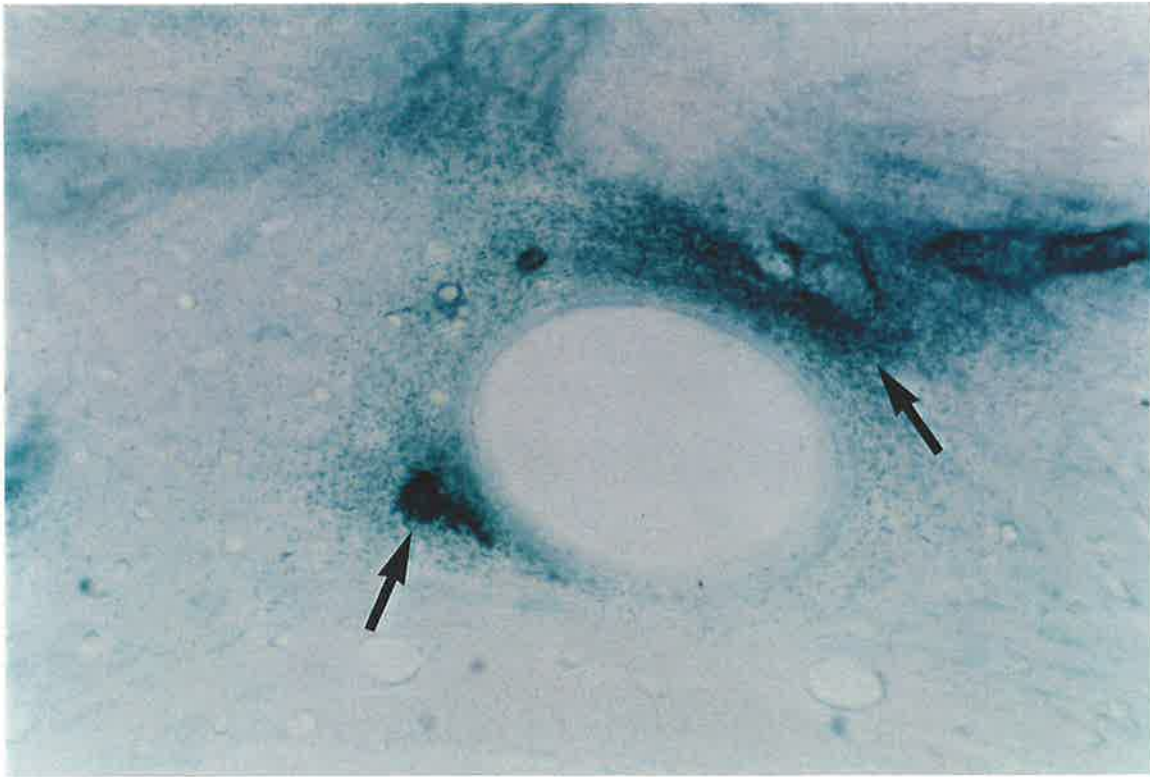


Figure 68. *Photomicrograph of a cervical cord section from a rat sacrificed 6 weeks after kaolin injection and 30 minutes after cisternal HRP injection. There is a preferential spread of reaction product from central grey matter perivascular spaces (arrows) into the central canal. There was diffuse labelling of the central grey matter in other sections from this level. HRP/TMB, $\times 200$.*

7.2.4.3.2 Spinal HRP injection

The depth of transspial diffusion in this group is compared with the diffusion in normal rats in Figure 69. There was diffuse labelling of the extracellular space and perivascular spaces in the cord caudal to the kaolin injection point. Rostral to the kaolin injection point, there were very few labelled vessels. When these rostral labelled perivascular spaces were in the central grey matter, there was preferential spread of the reaction product into the central canal in some cases and diffuse labelling of the central grey matter and central canal in others. The cerebrum did not contain reaction product in any of the animals in this group.

Trans-Pial HRP Diffusion After Spinal HRP Injection

30 min post HRP

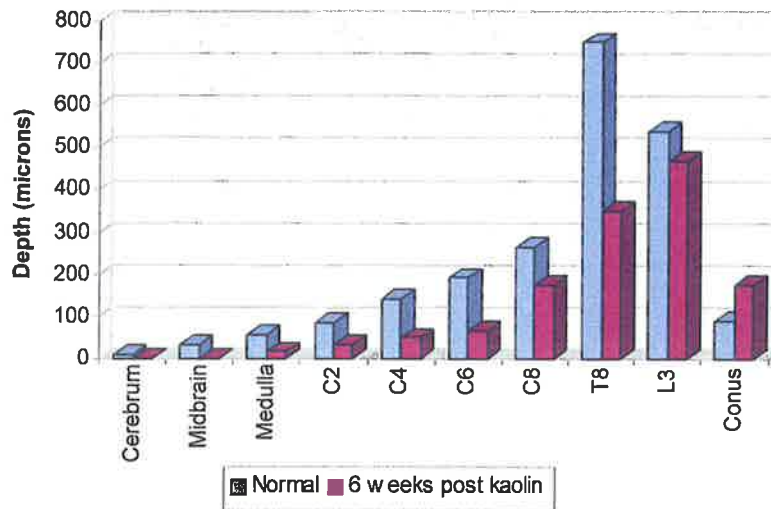


Figure 69. *Transpial diffusion 30 minutes after spinal HRP injection in normal rats and in rats 6 weeks after kaolin injection.*

7.3 Discussion

When referring to the site of HRP injection in this discussion, ‘proximal’ will be used to describe the segments of spinal cord on the same side of the kaolin injection point as the HRP injection and ‘distal’ will be used to describe segments of spinal cord on the other side of the kaolin injection point. That is, after cisternal injection, ‘proximal’ will refer to the cerebrum, medulla, C2 and C4 segments and ‘distal’ will refer to C8, thoracic, lumbar and conus segments.

7.3.1 *Animal model*

This study reproduced the animal model of syringomyelia developed by Milhorat et al³¹³ and described above (see *Intraparenchymal kaolin model of syringomyelia*, page 98). One day after kaolin injection, the central canal rostral to the injection point contained kaolin crystals and inflammatory cells. Occlusions of the central canal then developed by the formation of synechiae and trabeculae and by masses of inflammatory cells and kaolin crystals. There was progressive dilatation of the central canal between occlusions. The perivascular spaces were dilated at each interval after kaolin injection. In the 1 day and 3 day

groups, these dilated spaces contained neutrophils; in the later groups there were macrophages containing kaolin crystals. In the 6 week group, the white matter surrounding syrinxes was oedematous and there were axonal retraction balls, a fact providing evidence that the syrinxes were causing pressure effects on the surrounding tissue.

7.3.2 Subarachnoid CSF flow

Flow of CSF within the subarachnoid space can be studied by examining the depth of transpial diffusion of HRP. The depth of diffusion is dependent partly on the time that HRP in the subarachnoid space is in contact with the pial surface. In the CSF flow study in normal rats (see *Spinal fluid flow in normal rats*, page 115), there was rapid distribution of tracer throughout the subarachnoid space and the depth of diffusion varied according to the distance from the injection point. In this study, the depth of diffusion in most groups indicated that CSF flow in the subarachnoid space was limited at or near the point of kaolin injection (C6). Diffusion in segments of spinal cord on the proximal side of C6 as the HRP injection was equal to or greater than the diffusion in normal animals. On the side of C6 distal to the HRP injection point, the depth of diffusion was often much less than in the normal animals and in some cases there was no transpial HRP diffusion. These results indicate that at the point of kaolin injection there was a block to CSF flow in the subarachnoid space. This is likely to be due to arachnoid adhesions formed by the inflammatory reaction caused by kaolin leaking from the injection site into the subarachnoid space. Verification of an arachnoid reaction was not possible in this study because the dura is removed during vibratome processing. It is conceivable that a blockage would reduce compliance rostral to the kaolin injection because the damping effect of the lumbosacral dural sac is lost. A reduction in compliance may then lead to an increase in perivascular and central canal CSF flow. This increase may be important in the development of syrinxes in isolated segments of central canal. It may also explain the increased rate of syrinxes seen in an experimental model of syringomyelia with

arachnoiditis.⁸⁵ Arachnoiditis may be important in the pathogenesis of posttraumatic syringomyelia.

7.3.3 *Perivascular flow*

HRP reaction product was seen in perivascular spaces in all groups in this study. On the proximal side of the HRP injection, the number and density of perivascular space labelling were comparable to that seen in normal rats. On the distal side of the HRP injection, there were fewer perivascular spaces labelled and the density of labelling was less than in the same segments in normal rats. The distribution and density of labelling was similar at all intervals after kaolin injection. These results provide further evidence for a block to CSF flow in the subarachnoid space at the level of the kaolin injection. There was no definite evidence for an increase in perivascular flow in segments rostral to the kaolin injection point.

7.3.4 *Flow into the central canal*

The previously described pattern of HRP reaction product spreading from central grey matter perivascular spaces into the central canal (see *Spinal fluid flow in normal rats*, page 115) was seen in all groups in this study. Evidence for flow into the central canal rather than flow from the central canal into the perivascular spaces included: 1) in some blocks only those sections with reaction product in the central grey matter perivascular spaces contained reaction product in the central canal; 2) label was present in sections between segments where the central canal was not labelled; and 3) the central canal was labelled in segments of central canal that were isolated by synechiae and trabeculae. Whenever the central canal contained reaction product, perivascular spaces in the adjacent central grey matter were also labelled.

The pattern of flow from perivascular spaces into the central canal was seen at all spinal cord levels, but there were some differences compared with the normal rats. In normal rats, flow into the central canal was identified at all levels of the spinal cord in the 0 min animals. In the 0 min animals in this study, flow into the central canal was identified at all

spinal cord levels proximal to kaolin injection point, but not always in the segments distal to the kaolin injection point. In the animals in the later post HRP groups, flow into the central canal was identified at all spinal cord levels. Although this finding could be interpreted as demonstrating a slower flow into the central canal, a more likely explanation is simply that the CSF block in the subarachnoid space was delaying the HRP reaching the distal spinal cord segments. This is reinforced by the fact that rapid flow into the central canal was always seen in segments proximal to the kaolin injection point whether this was after cisternal or spinal injection.

In some animals there was evidence that reaction product in the central canal distal to the kaolin injection point had reached that point from the central canal proximal to the kaolin injection rather than from perivascular spaces. This occurred in animals with evidence of a complete or near-complete block of CSF flow in the subarachnoid space at the level of the kaolin injection. Presumably in these animals fluid flow in the central canal continued (or was even enhanced) in the presence of a block in the subarachnoid space. Rostral movement of tracer in the central canal in these animals did not extend beyond central canal occlusions.

Rapid flow into the central canal was seen in segments of central canal that were isolated by occlusions of cellular debris, synechiae or trabeculae. Flow into isolated segments of central canal was identified at all time intervals in all groups after kaolin injection. This occurred even in segments that were enlarged and where there was evidence of pressure effects in the surrounding white matter. Assuming that the pressure in these enlarged segments of central canal was higher than in the subarachnoid space, this study provides evidence that perivascular fluid flow into the central canal continues against a pressure gradient and is therefore actively driven.

The results of this study have added further support to the hypothesis that perivascular fluid flow continues during syrinx formation and is the driving force for the enlargement of non-communicating syringes.

8 Human central canal morphology

The aim of this study was to develop a method for studying human central canal 3-D morphology. In other vertebrates the central canal communicates with the subarachnoid space in the region of the conus medullaris (see *The terminal ventricle and caudal openings*, page 60). One of the reasons for developing this technique was to determine whether such openings exist in the human spinal cord. The conus medullaris was therefore examined in this pilot study.

8.1 Methods

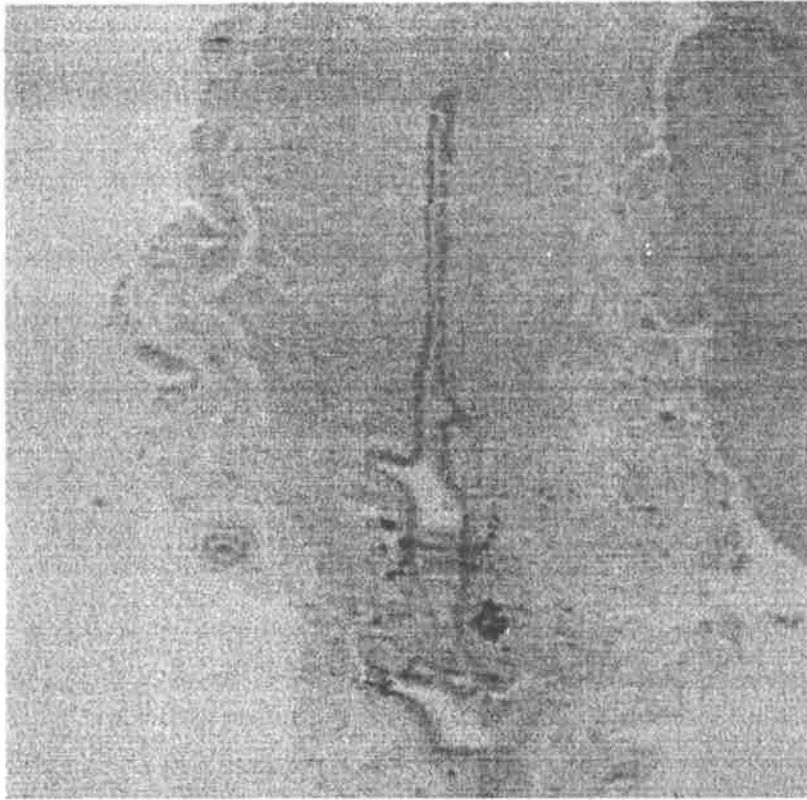
The spinal cords used in this study were from a sheep used in a study of CSF physiology (perfuse-fixed with formalin) and from human cords removed at autopsy at the Royal Adelaide Hospital mortuary and fixed by immersion in formalin. Two human cords were used: one from a five year old boy killed in a motor vehicle accident and one from a 19 year old woman who also died of trauma. A 40 mm block of conus medullaris and filum terminale was removed from each spinal cord. The specimen of sheep spinal cord was entirely of conus medullaris since the spinal cord extends to the caudal limit of the spinal canal in this animal. Each block was then subdivided into four 10 mm blocks that were dehydrated and embedded in paraffin wax. The four separate blocks of spinal cord were then cut serially with a microtome into 10 μm thick sections. Every tenth section was mounted on a glass slide and stained with H&E.

8.1.1 Computer Imaging

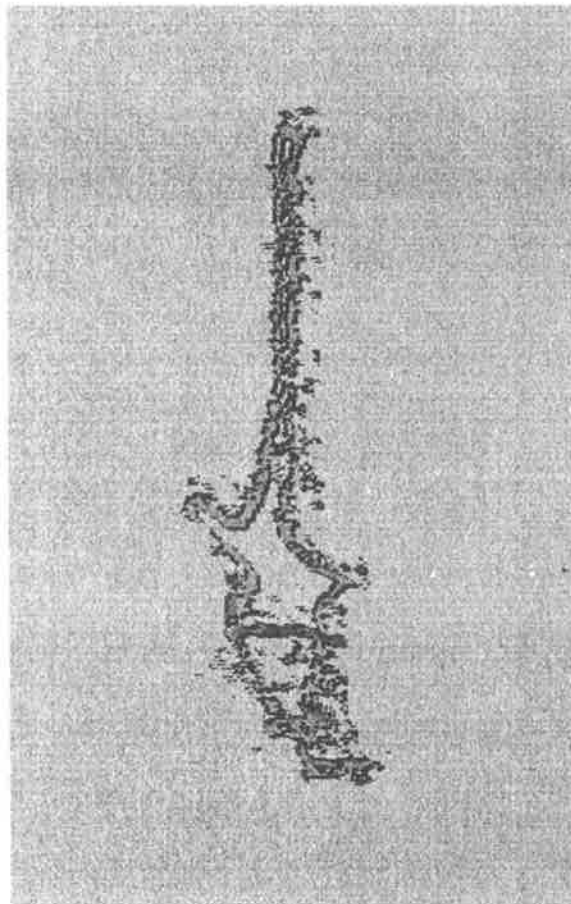
The histological sections were viewed with a light microscope at magnifications up to $\times 100$ to determine the location and extent of the central canal lumen and ependymal lining. They were then photographed and digitised through a light microscope at $\times 4$ magnification using a charge-coupled device (CCD) camera and image capturing software (Q500MC, Leica Cambridge Ltd, Cambridge). Image data were transferred to a Silicon Graphics Indy (Silicon

Graphics Inc, Mountain View, California) workstation for processing. Before the digitised sections could be used to compose a 3-D image of the conus and filum, the sections had to be aligned. This was necessary because each digitised section had been rotated or translated by differing amounts during the mounting and digitisation process. Special software was written* to align and centre each image using the mid-point of the longest axis of the central canal lumen as an alignment point. Pre-processing of the aligned images was necessary to enhance the ependymal cells so that the central canal could be displayed (Figure 70). This image enhancement was achieved using purpose-written C++ software that detected the darker staining of the ependymal cells. Before these enhanced sections could be used in the 3-D image, further editing of the section was required to remove non-ependymal features that remained after enhancement. The 3-D image of the central canal was composed using Persona 1.1 software on the Silicon Graphics workstation. This software had previously been used to generate 3-D images of the skull from CT head scans⁴²⁸ and required some modification for the purposes of this project. Each aligned and digitised section was converted into a contour map consisting of one contour for the surface of the spinal cord and a second contour for the lumen of the central canal. Two methods were used to produce 3-D images of the cord. The contour maps were processed by the software to create a polygon mesh representing the central canal and spinal cord. This polygon mesh was displayed as a 3-D image that could be viewed from any angle using the workstation monitor and stereoscopic glasses. The second method used ray casting to produce 'solid' images of the cord and central canal.

* The software was written by Mr K. Storer using C++ (Silicon Graphics).



A



B

Figure 70. *Image processing. The digitised image of the spinal cord (A) was processed by special software to produce an outline of the central canal by enhancing the contrast of the ependymal cells to the surrounding parenchyma (B).*

8.1.2 Assessment of images

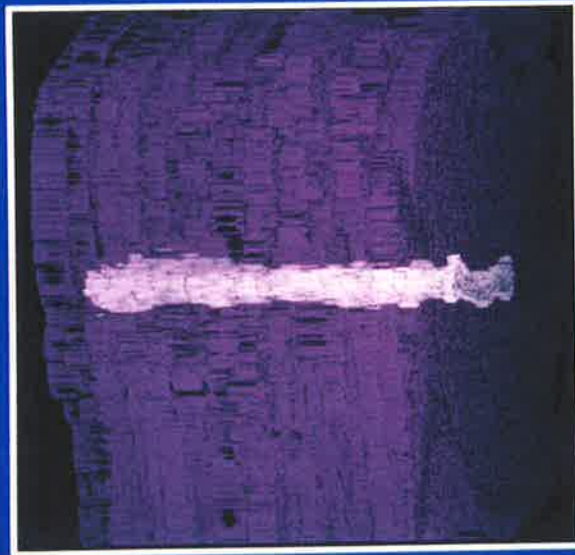
The histological sections were used to determine the extent of the central canal and ependymal cell lining in individual sections and assess the degree of continuity of the ependymal lining. The 3-D images were used to assess the patency, size and shape of the central canal and the presence or absence of openings of the central canal into the subarachnoid space.

8.2 Results

8.2.1 Sheep spinal cord

The histological sections of the sheep spinal cord revealed a central canal that was uniform in size and shape. The 3-D images confirmed that the central canal was patent throughout and there was no evidence of an opening into the subarachnoid space (Figure 71).

Ray Casting 3-D Reconstruction



A

Ray Casting 3-D Reconstruction



B

Figure 71. Three-dimensional reconstruction of sheep conus medullaris. A: Conus medullaris and central canal demonstrating uniformity of the central canal and no evidence of a central canal opening at the surface of the cord. B: Central canal from the same segment of cord, enlarged to demonstrate the canal patency.

8.2.2 *Human spinal cords*

The histological sections of the spinal cord from the five year old boy demonstrated that the central canal was patent throughout the conus medullaris and filum terminale. The ependymal lining of the central canal was continuous throughout the specimen. For most of the cranial segment of the conus, the central canal was regular in shape and size. In the lower part of the conus the central canal appeared to be duplicate (Figure 72). Within the filum, the central canal became irregularly enlarged to form the terminal ventricle (Figure 72). The central canal approached the pia mater in the filum terminale, being separated only by a thin band of connective tissue (Figure 73).

The 3-D images of the spinal cord from the five year old boy (Figure 74) demonstrated that the central canal was patent and continuous throughout the conus medullaris and the filum terminale. The central canal was elliptical and uniform for most of the length of the conus medullaris. Towards the caudal end of the conus, the central canal forked for a distance of 700 μm and then rejoined at the junction of the conus medullaris and the filum terminale. At this point, the lumen became large and irregular and remained patent throughout the segment of filum terminale that was studied.

The histological sections of the spinal cord taken from the 19 year old woman revealed clumps of ependymal cells with some rosettes. There was no definite lumen, and no distinct morphological pattern could be identified (Figure 75). The 3-D reconstructions revealed that these clumps of ependymal cells were in fact arranged in two discrete columns (Figure 76). No lumen could be identified on these images and the ependymal cells did not abut the surface of the cord.

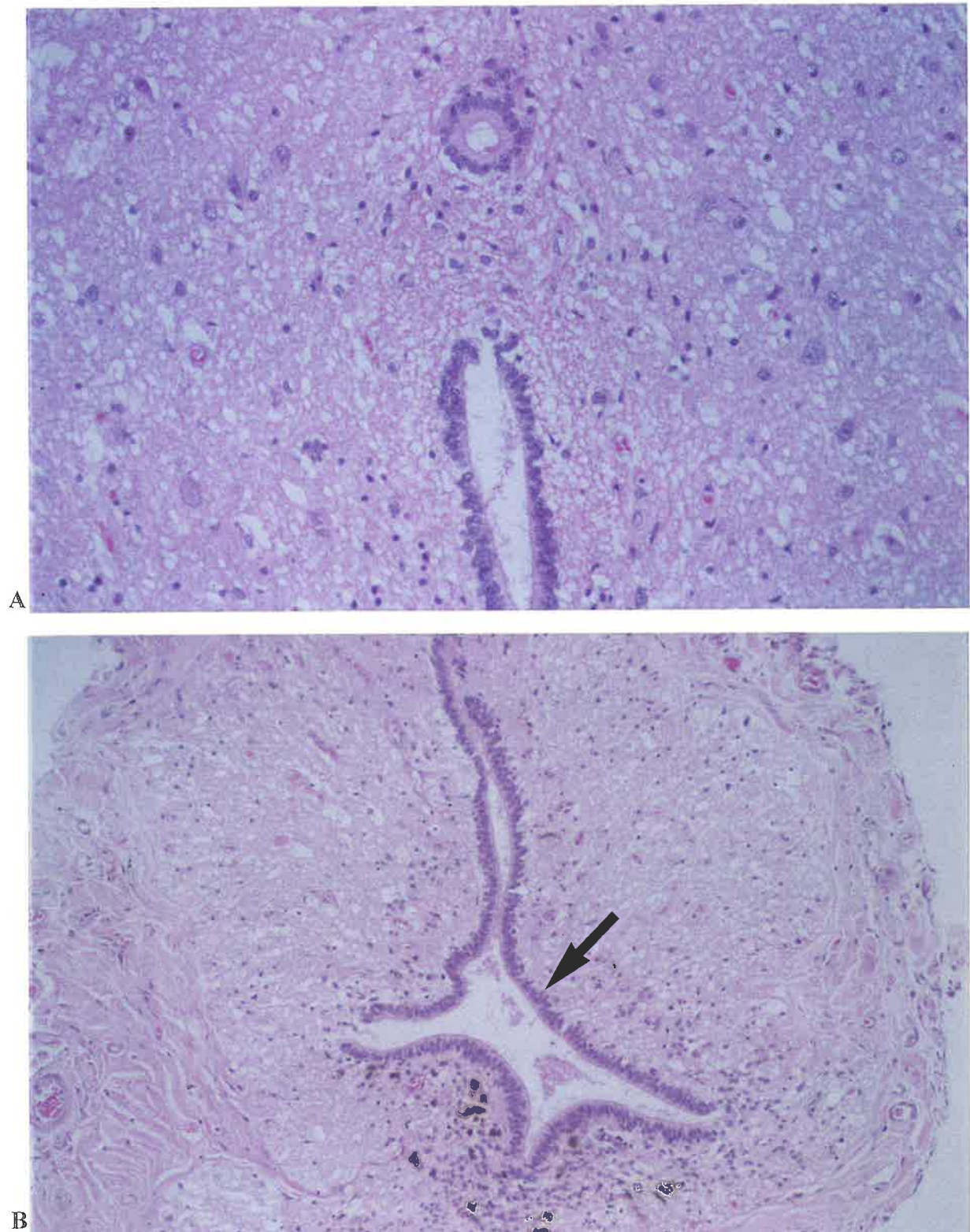


Figure 72. Photomicrographs of spinal cord sections from a five year old boy. *A: The central canal appears to be duplicated. H&E, $\times 100$. B: The central canal enlarged to form the terminal ventricle (arrow) in the caudal end of the conus medullaris. H&E, $\times 40$.*

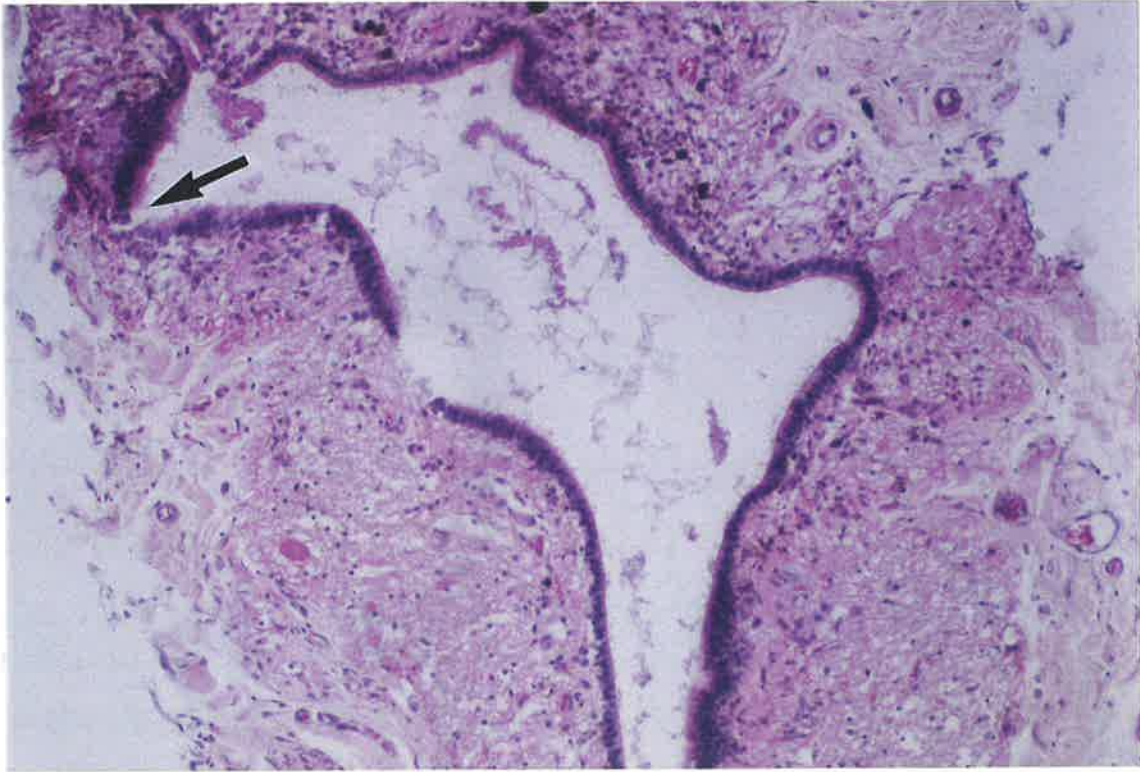


Figure 73. Photomicrograph of a section of filum terminale from a five year old boy. The central canal is large relative to the size of the filum, and is approaching the surface (arrow). H&E, $\times 40$.

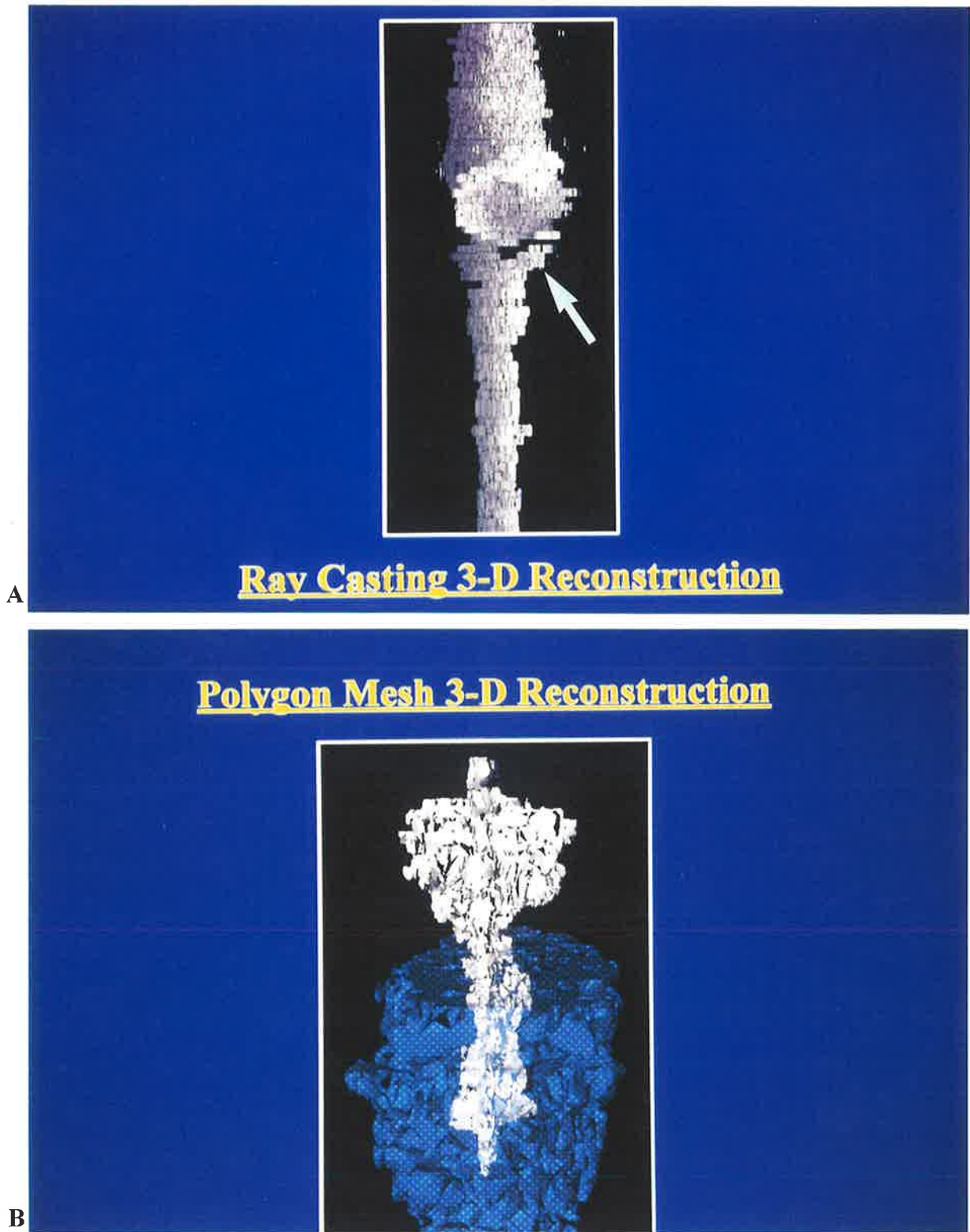


Figure 74. Three-dimensional reconstructions of the conus medullaris and filum terminale of a five year old boy. A: Ray casting reconstruction demonstrating forking of the canal (arrow) below which the canal enlarges to form the terminal ventricle. B: Polygon mesh reconstruction of the terminal ventricle (white) partly superimposed on the surface of the spinal cord (blue). Viewing the images on the computer screen with 3-D glasses confirmed that the central canal abutted the surface of the cord in this region.

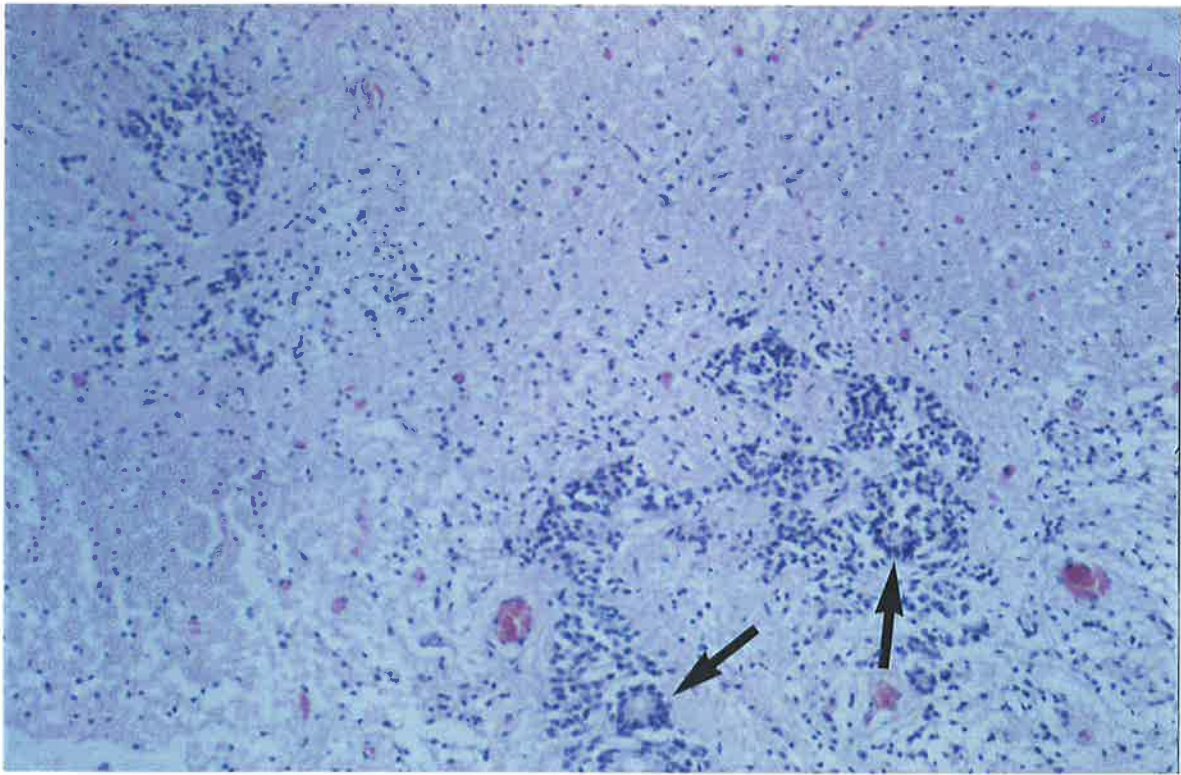


Figure 75. Photomicrograph of a section of conus medullaris from a 19 year old woman. There are clumps of ependymal cells with rosette formation (arrows). H&E, $\times 100$.

Ray Casting 3-D Reconstruction

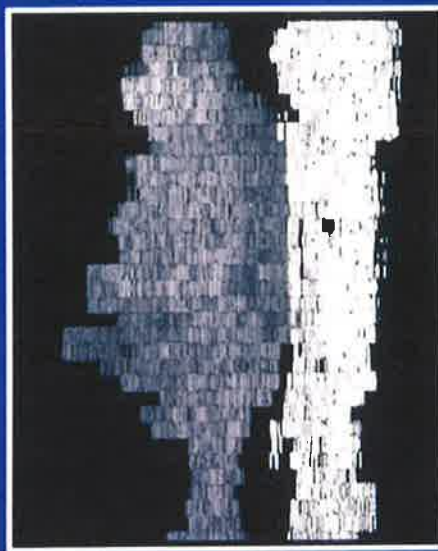


Figure 76. Three-dimensional ray casting reconstruction of the ependymal cells in the conus medullaris of a 19 year old woman. The cells are arranged in two discrete columns and no lumen is identifiable.

8.3 Discussion

A technique for studying the 3-D morphology of the central canal has been developed. The technique is time-consuming and requires expensive equipment. Rendering and reconstructing hundreds of images requires expensive and powerful computer hardware and software. The technique has produced detailed 3-D images that facilitate the study of central canal morphology. Much information can be gained from studying two-dimensional photographs of the 3-D reconstructions, but an even greater appreciation of the spinal cord and central canal structure can be gleaned from using special glasses to view the images in a true 3-D effect. These 3-D images can be manipulated on the screen to provide an even greater understanding of the morphology.

In the specimen from the 5 year old boy, there was forking of the central canal within the lower conus, closely coinciding with the location of the terminal ventricle. Forking began as a minor dorsal duplication of the main canal at the caudal end of the conus, which progressively increased in size in the ventricular region. This pattern is consistent with the findings of Lendon and Emery.²⁶³ Outpouchings of each fork of the central canal were also observed in the filum. There was also evidence of an opening into the subarachnoid space in the five year old child. Although the lumen of the central canal was not in continuity with the subarachnoid space, ependymal cells extending from the lumen did contact the pial surface. This arrangement may represent a functional communication between the central canal and the subarachnoid space similar to those described in other species.^{41,324}

The central canal in the specimen from the 19 year old female did not appear to have a lumen, but the ependymal cell clumps did come in contact with the pial surface within the filum terminale. Although this morphology is not suggestive of a functionally important fluid communication, it is conceivable that fluid does communicate between the subarachnoid

space and the ependymal extracellular space. Such a communication may have a 'lymphatic' function.

The information obtained using this 3-D technique could be theoretically achieved by studying each histological section, but it would be extremely difficult. The 3-D images allow rapid and clear appreciation of the central canal morphology and its relationship to the spinal cord surface. Using histological sections alone, it may be difficult to determine whether an apparent opening is real or artifactual. By using the 3-D image to assess the morphology of the central canal lumen, an overall impression of the shape can be appreciated - an artifactual opening would appear inconsistent with the morphology of the central canal near that point whereas a true opening would follow the overall shape of the canal.

Other potential uses for this technique include studying the 3-D morphology of syrinxes and their relationship to the spinal cord surface and central canal. The technique could also be used to study central canal and spinal cord morphology in congenital spinal cord abnormalities such as myelomeningocele and diastematomyelia. Study of a large number of human cords would allow a better understanding of age-related central canal changes and of the normal arrangement of the central canal in the conus medullaris and filum terminale.

9 Attempts to develop a sheep model of syringomyelia

The aim of this experiment was to develop a large animal model of syringomyelia that could be used in future studies of CSF dynamics in syrinx formation.

9.1 Methods

Sixteen merino wethers weighing 38 kg to 47 kg were used for this experiment (Table 5). Two methods were used in an attempt to develop syringes: intraparenchymal kaolin injection and mechanical occlusion of the central canal opening.

Eleven sheep were used in the intraparenchymal kaolin experiment. Seven of these were used to develop the techniques for kaolin injection and perfusion fixation and to determine the dose of kaolin and site of injection that could be tolerated without producing immediate neurological deficits. With the anaesthetised sheep in the right lateral position, a midline posterior cervical incision was made and the cervical spinous processes were exposed. In each of the seven preliminary sheep a laminectomy at a single level from C4 to T1 was performed. A dose of kaolin ranging from 50 μ L to 200 μ L was injected with a tuberculin syringe and a 30 gauge needle through the dura into the dorsal columns of the spinal cord at a depth of 2 mm to 3 mm. These sheep were sacrificed at various times up to 6 weeks after injection. Four sheep were studied with a dose of 200 μ L injected in the dorsal columns of the spinal cord at the C7/T1 vertebral level. One of these sheep was sacrificed at each of the following times after injection: 3 days, 1 week, 6 weeks and 11 weeks.

Three sheep were used in an attempt to occlude mechanically the rostral opening of the central canal. With the anaesthetised sheep in the right lateral position, a midline incision was made to expose the occipital bone, foramen magnum, posterior arch of C1 and the spinous process of C2. A suboccipital craniectomy was performed to expose the dura over the cerebellum and the posterior arch of C1 was also removed. The dura was opened in a 'Y' shape over the cerebellar hemispheres and the upper cervical cord. The cerebellar

hemispheres were separated inferiorly, without dividing the vermis, so that the opening of the central canal could be seen. A coronary balloon dilatation catheter (Advanced Cardiovascular Systems, Temecula, California) with a balloon outside diameter of 2.5 mm was inserted into the central canal. The catheter was inserted into the canal either directly through the gap between the cerebellar hemispheres and curving caudally at the fourth ventricle, or through the cerebellar vermis so that the curve required to reach the canal was less pronounced. The balloon was inflated with normal saline and the injection port was sealed. The dura was closed in a watertight fashion and the wound was closed in layers with absorbable sutures to muscle and nylon to the skin.

Two sheep were used as controls and did not have a kaolin injection or balloon occlusion of the central canal.

9.2 Results

A representative section of normal sheep spinal cord is shown in Figure 77. The central canal was patent at all levels examined and was lined by a single layer of ependymal cells.

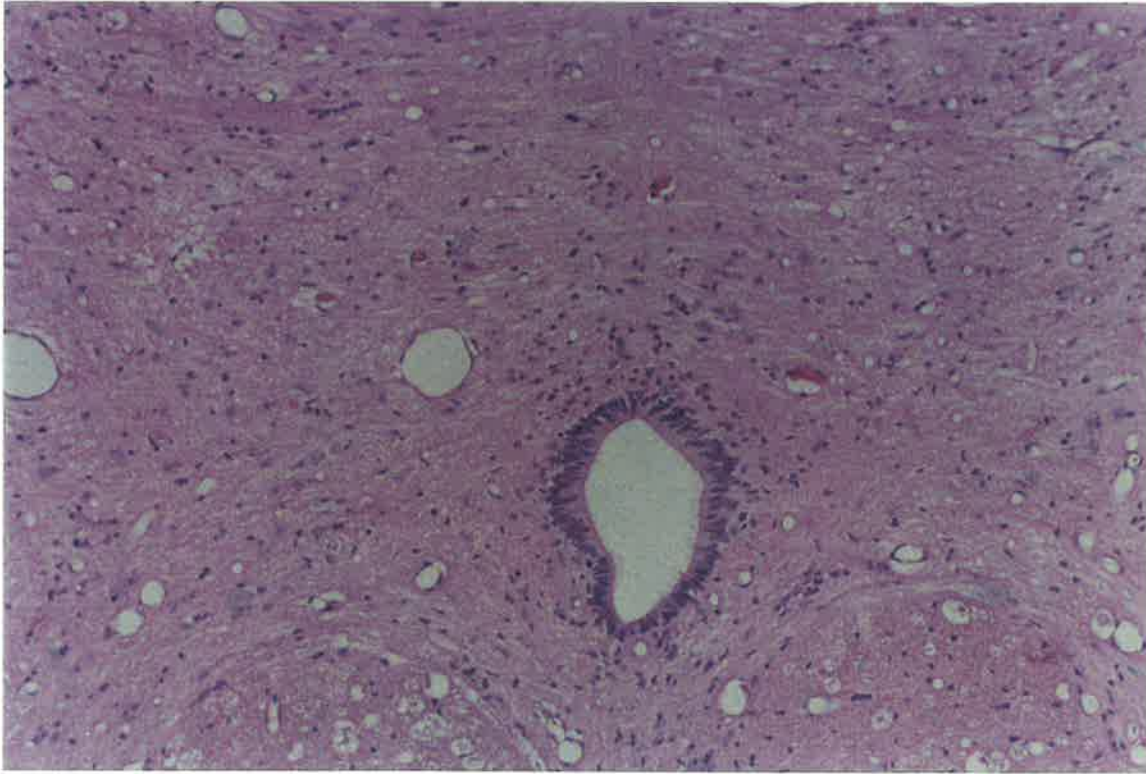
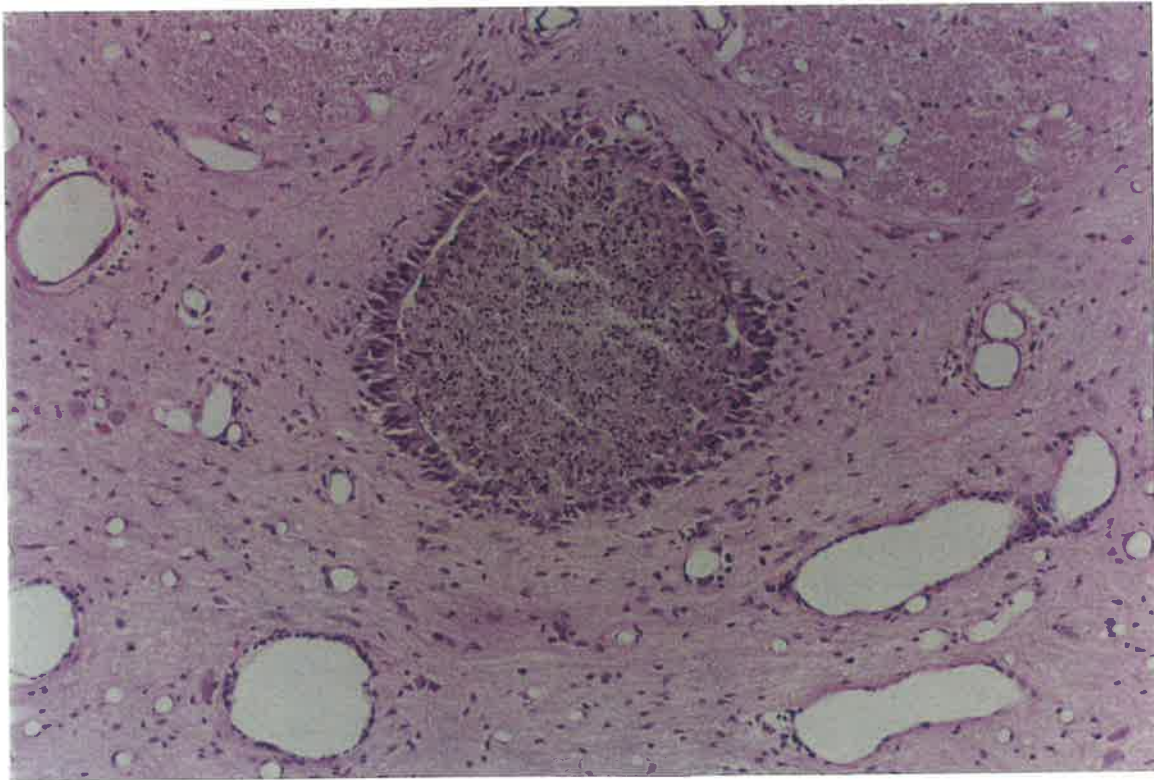


Figure 77. *Photomicrograph of a section from the cervical spinal cord of a normal sheep. The central canal is patent and the central grey matter perivascular spaces are not enlarged. H&E, × 100.*

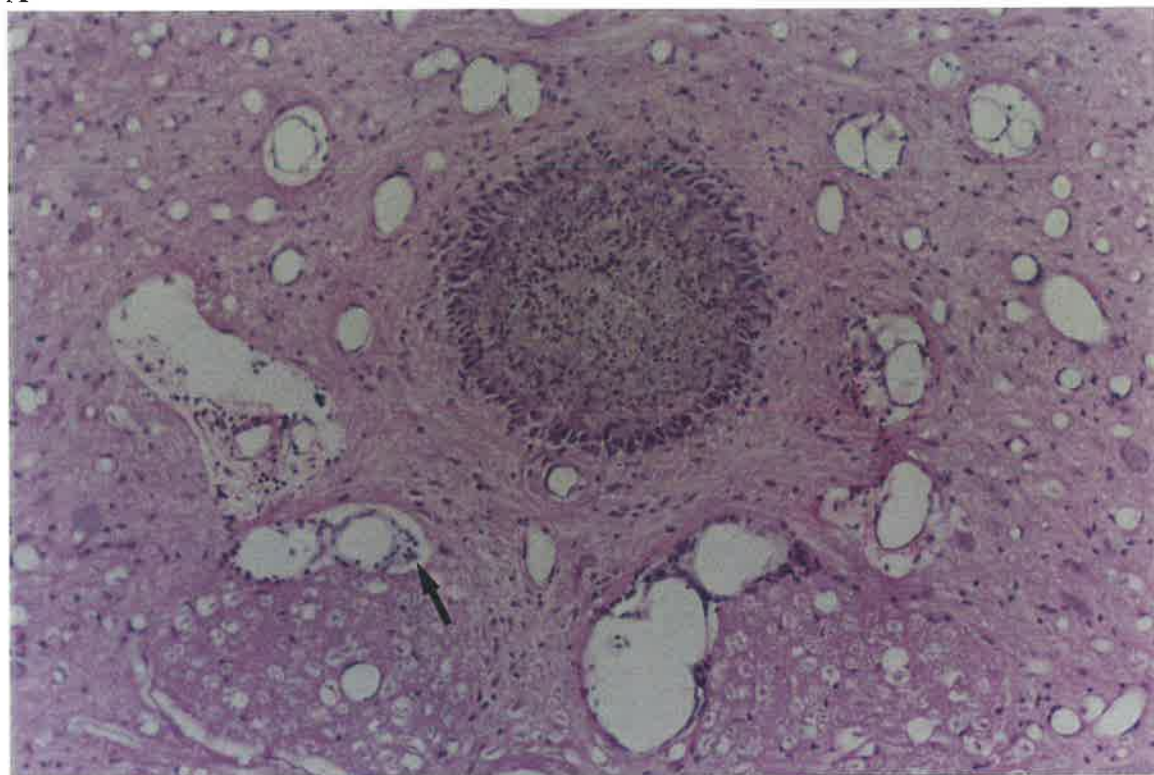
9.2.1 Kaolin injection

Of the sheep undergoing intraparenchymal kaolin injection, one had forelimb weakness and inadequate spontaneous respiration immediately after injection and was therefore sacrificed. No neurological deficits were detected in the other animals at any time after kaolin injection.

At 3 days after kaolin injection, the injection site was associated with an acute inflammatory response. The central canal at the level of injection and throughout the cervical cord was filled with kaolin crystals and neutrophils (Figure 78). The central canal was enlarged at all cervical levels and there was ependymal proliferation. Perivascular spaces in the central grey matter were dilated and in some cases contained neutrophils (Figure 78). The central canal in thoracic and lumbar sections also contained some kaolin crystals and neutrophils, but the central canal and perivascular spaces were not enlarged (Figure 79).



A



B

Figure 78. Photomicrographs of cervical cord sections from a sheep sacrificed 3 days after kaolin injection. A: C1, the central canal is enlarged and is packed with kaolin crystals, neutrophils and occasional erythrocytes. B: C5, perivascular spaces in the central grey matter are dilated and some spaces contain neutrophils (arrow). H&E, $\times 100$.

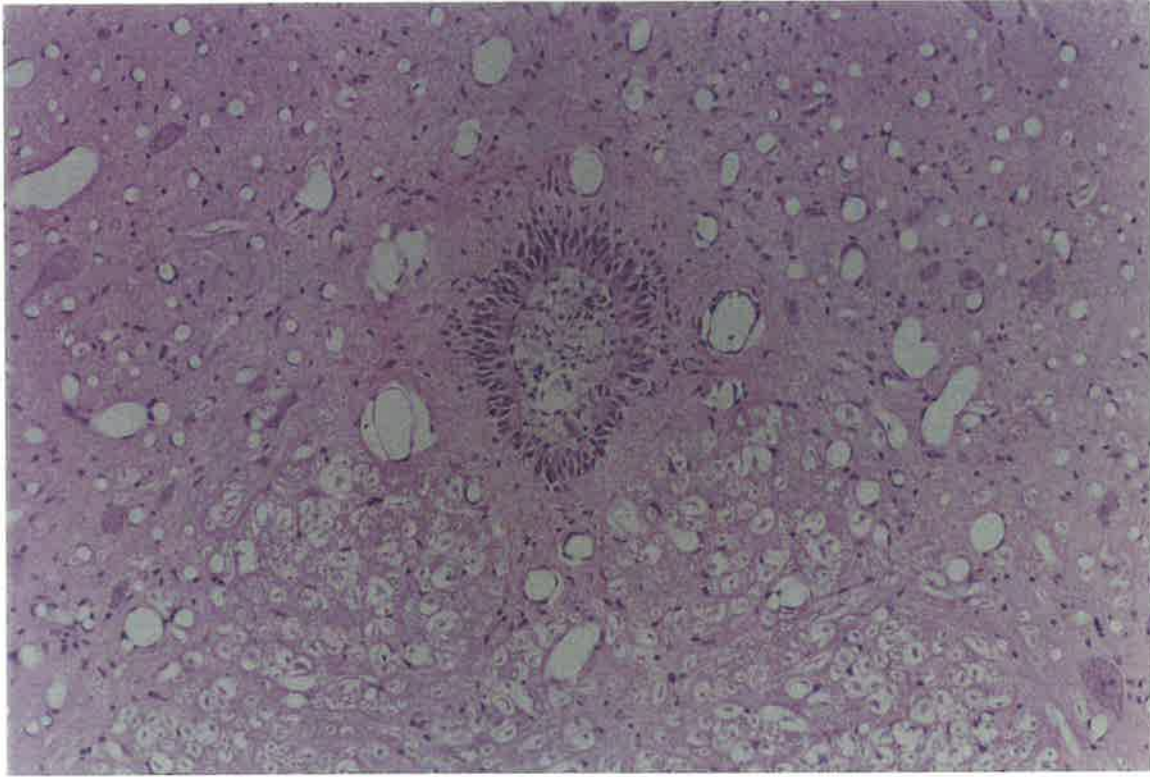


Figure 79. *Photomicrograph of a lumbar spinal cord section from a sheep sacrificed 3 days after kaolin injection. The central canal contains some neutrophils and kaolin crystals but is not completely packed with cells and is not enlarged. H&E, $\times 100$.*

The injection site at 1 week had become surrounded by macrophages and a fibroblastic reaction. The central canal did not contain kaolin crystals or inflammatory cells at any level. In several cervical cord sections, the central canal was stenotic and there were ependymal adhesions, however the canal always remained patent (Figure 80). Perivascular spaces were not enlarged.



Figure 80. *Photomicrograph of a cervical spinal cord section from a sheep sacrificed 1 week after kaolin injection. The central canal is stenosed and there is an ependymal adhesion (arrow), but the canal remains patent. H&E, × 100.*

A more mature fibrotic inflammatory response surrounded and invaded the injection site by 6 weeks after the kaolin injection. The fibrotic reaction also involved the subarachnoid space at the level of injection. The central canal was patent throughout the spinal cord and there was no evidence of stenosis or adhesions. Perivascular spaces in the central grey matter were not dilated but often contained an eosinophilic hyaline material (Figure 81).

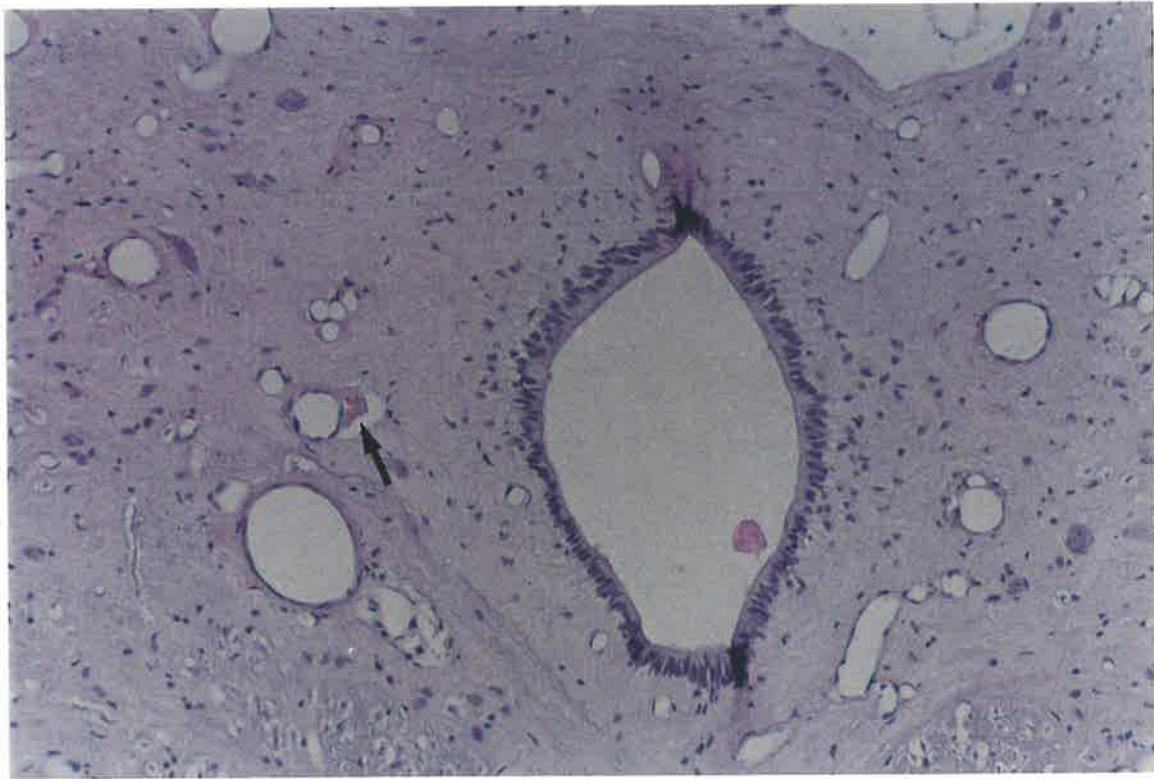


Figure 81. *Photomicrograph of a section through C7, taken from a sheep sacrificed 6 weeks after kaolin injection. The central canal is normal in size and shape. Some of the central grey matter perivascular spaces contain an eosinophilic hyaline material (arrow). H&E, × 100.*

By 11 weeks after the kaolin injection, there was a fibrotic arachnoiditis involving the entire cervical spinal cord. The central canal remained patent and of normal size. There were numerous hyalinised perivascular spaces in the central grey matter.

9.2.2 Balloon occlusion of the central canal

Insertion of a balloon catheter in the central canal proved to be a difficult technical exercise. Even when passed through the cerebellum to reduce the angle required for the catheter to enter the canal, it was difficult to pass the catheter more than a few millimetres down the canal. All of the animals had significant neurological deficits after the procedure, including quadriparesis and nystagmus. These deficits were severe enough in the first two animals to require that each of these animals be sacrificed immediately after the procedure. The third animal was unable to stand 48 hours after the procedure so it was sacrificed at that time.

Macroscopic examination of the brain stem of the first animal revealed that the catheter had kinked in the fourth ventricle and was pressing into the floor of the fourth ventricle. The catheter in the last animal had entered the canal but had passed through the ventral part of the cervical cord to reach the subarachnoid space (Figure 82).



Figure 82. *Low power photomicrograph of a sagittal section of medulla from a sheep after insertion of a coronary artery balloon dilatation catheter into the central canal. The catheter did not remain in the central canal; its point of departure from the canal is indicated by an arrow.*

9.3 Discussion

Neither of the techniques used in this experiment was successful in producing syringomyelia in sheep. Mechanical occlusion of the canal by an angiography balloon catheter would have had the advantage of having a catheter in the isolated canal that could have been used for pressure measurements, fluid sampling and injection of tracers. The catheters used in this study were of an appropriate size to cannulate the central canal and they were able to be inserted into the rostral opening of the canal. However, the catheters proved to be too stiff to be passed more than a few millimetres down the canal before they left the canal and penetrated the parenchyma, causing significant neurological deficits. It would have

been possible to occlude the canal with softer balloon catheters without a distal lumen, but there would not have been a catheter in the canal to allow measurements and sampling.

Nor did the attempted intraparenchymal model produce syringomyelia. There was passage of kaolin and inflammatory cells from the injection point into the central canal, reinforcing the concept of a fluid flow from the extracellular space into the central canal.^{294,301,312} The kaolin crystals and inflammatory cells were predominantly in the central canal rostral to the injection, but there were some crystals and cells in the caudal central canal. This did not occur in the rat model developed by Milhorat et al³¹³ but was seen in the rat model described earlier in this thesis (see *Intraparenchymal kaolin model of syringomyelia*, page 98). Milhorat et al³⁰¹ have argued that the central canal flow is in a caudal to rostral direction, while other authors^{56,88,245,324} have suggested that the flow is from the cerebral ventricles down the canal. The greater number of cells and kaolin crystals in the more rostral cord in this study supports the view of Milhorat et al.³⁰¹ The presence of cells and crystals in the caudal central canal does suggest that there may also be flow in the caudal direction, perhaps exiting from the caudal opening of the central canal. It may be that the inflammatory reaction resulted in an increased fluid flow from the lower cervical cord into the central canal and then both rostrally to the fourth ventricle and caudally to the caudal opening.

The central canal at three days after kaolin injection was enlarged and was packed with kaolin crystals and inflammatory cells. There was ependymal proliferation and the subsequent development of stenosis and adhesions. By 1 week the canal had cleared of crystals and cells. The reason the canal did not enlarge may be that the stenoses and adhesions did not completely occlude the canal at any level, allowing flow to continue. Sheep have a much larger central canal than rats and it may not be possible to create a model of syringomyelia in sheep using intraparenchymal kaolin for this reason. An alternative explanation is that a spinal subarachnoid block is necessary for syrinx formation. Injection into the sheep spinal

cord may result in less spillage of kaolin than in the rat with less arachnoiditis formation and consequently no spinal subarachnoid block.

Inflammatory cells were seen in the perivascular spaces at all levels in the cervical cord 3 days and 1 week after kaolin injection. After longer intervals, the perivascular spaces contained an eosinophilic hyaline material, which is presumably proteinaceous debris remaining from the inflammatory reaction. At three days after kaolin injection, when the central canal was packed with crystals and cells, the central grey matter perivascular spaces were dilated. This would be consistent with the normal flow from perivascular spaces to the central canal being prevented by the temporary occlusion of the canal with crystals and cells.

A large animal model of syringomyelia is desirable to facilitate the study of CSF dynamics during the development of syrinxes. The cisternal kaolin model is likely to produce an enlarged central canal but this would not be a model of non-communicating syringomyelia, which is more common and the type in which the pathophysiology is least understood. Other possible techniques include reproduction of a Chiari malformation by compression at the cervicomedullary junction, either with an intradural or extradural mass lesion.

10 Perivascular flow in the cerebellum and brainstem

Rapid perivascular fluid flow has been demonstrated in the cerebral hemispheres, brain stem and spinal cord,^{54,373,374} but not in the cerebellum. Brierley⁵⁹ injected India ink into the cisterna magna of rabbits and found the tracer in cerebellar perivascular spaces 12 to 24 hours after the tracer was injected, but did not examine flow at earlier time intervals or movement of tracer into the extracellular space. The purpose of this study was to determine whether there is a rapid flow of fluid from the subarachnoid space into cerebellar perivascular spaces and, if there is such a flow, to determine whether it continues into the surrounding extracellular space.

10.1 Methods

Ten adult male Sprague-Dawley rats weighing 280g to 340 g were used. Ethics approval was obtained from the Animal Ethics Committees of the University of Adelaide and the Institute of Medical and Veterinary Science, Adelaide.

The animals were anaesthetised as previously described (see *Anaesthesia of rats*, page 92). Ten microlitres of 4% HRP (Zymed Laboratories, San Francisco, EIA grade) in normal saline was injected at a rate of 2 μ L/min for 5 minutes. One control rat was injected with only normal saline. The needle was kept in place, to prevent leakage, until the animals were sacrificed at 0, 10, or 30 minutes after the completion of the injection. The animals were sacrificed by aldehyde perfusion (see *Perfusion-fixation of rats*, page 93). The rats were placed in the lateral position for aldehyde perfusion, which was always commenced within 2 minutes of terminating the experiment.

The skull and the spinal column were removed and postfixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer at room temperature for 2-3 hours. The brain and spinal cord were removed and sliced coronally to obtain blocks of the cerebrum, cerebellum, brainstem and cervical cord. Serial sections 50 μ m to 100 μ m thick were cut with a vibratome and kept

in Tris HCl buffer (pH 7.6) until they were mounted on slides and then left to air dry. HRP was localised using TMB (see *Vibratome sections and localisation of HRP with TMB*, page 96).

10.2 Results

The dark blue granular HRP reaction product was easily distinguished from the light blue staining of background endogenous peroxidase activity. In the cerebellum, the relatively acellular molecular layer was able to be distinguished from the densely cellular granular layer and the Purkinje cells were visible as large light blue cells. Residual erythrocytes were stained dark blue, but these were easily distinguishable from HRP reaction product.

10.2.1 Cerebellum

10.2.1.1 0 minutes

The pia was densely stained with HRP reaction product. Reaction product was present in the molecular layer, either uniformly or with greater density closer to the pial surface. This transpial diffusion stopped abruptly at the commencement of the granular layer. The granular layer did contain some reaction product, but this was mostly localised around blood vessels (Figure 83) and did not appear to have spread by transpial diffusion. The ependymal lining of the fourth ventricle roof contained reaction product. The white matter subjacent to this ependyma contained a diffuse, thin band of label limited to the subependymal white matter and not extending to deeper regions. Reaction product in the white matter throughout the cerebellum was otherwise almost exclusively localised around vessels. Some labelled perivascular spaces and surrounding focal areas of reaction product were observed in all layers of the cerebellum. In the arbor vitae and in the granular layer these focal areas of reaction product were in bold relief from the surrounding areas that were not labelled. In the molecular layer, areas around labelled perivascular spaces contained a greater density of reaction product

than surrounding regions. These appearances were suggestive of reaction product having spread from the perivascular spaces.

10.2.1.2 10 minutes

In animals sacrificed 10 minutes after HRP injection, the pia was densely labelled and reaction product extended across the molecular layer but not into the granular layer (Figure 84). Vessels were seen entering the molecular layer perpendicularly from the pial surface. The perivascular spaces of these vessels were often labelled for a short distance after entry and not for their entire course through the molecular layer. Many labelled perivascular spaces were present in all layers. There were more numerous labelled perivascular spaces than in the 0 minute group, particularly in the white matter. Labelled perivascular spaces in the centre of dense foci of reaction product were seen in all layers. The only label present in the granular layer and the white matter was around blood vessels (Figure 83). Reaction product was seen in the perivascular spaces of some vessels in the subependymal white matter close to the ependyma but no spread of reaction product towards the fourth ventricle was observed. The ependymal lining contained reaction product that was well localised with minimal spread into the subependymal white matter.

10.2.1.3 30 minutes

Transpial diffusion of reaction product extended throughout the molecular layer but not into the granular layer (Figure 84). There was less reaction product in the molecular layer compared with the 10 minute and 0 minute groups. Labelled perivascular spaces were present in all layers and were more numerous than in the previous two groups. Reaction product appeared to be spreading out from these labelled perivascular spaces. Reaction product in the granular layer and white matter was present only around labelled vessels. Diffusion across the ependymal lining was no greater than in the two previous groups.

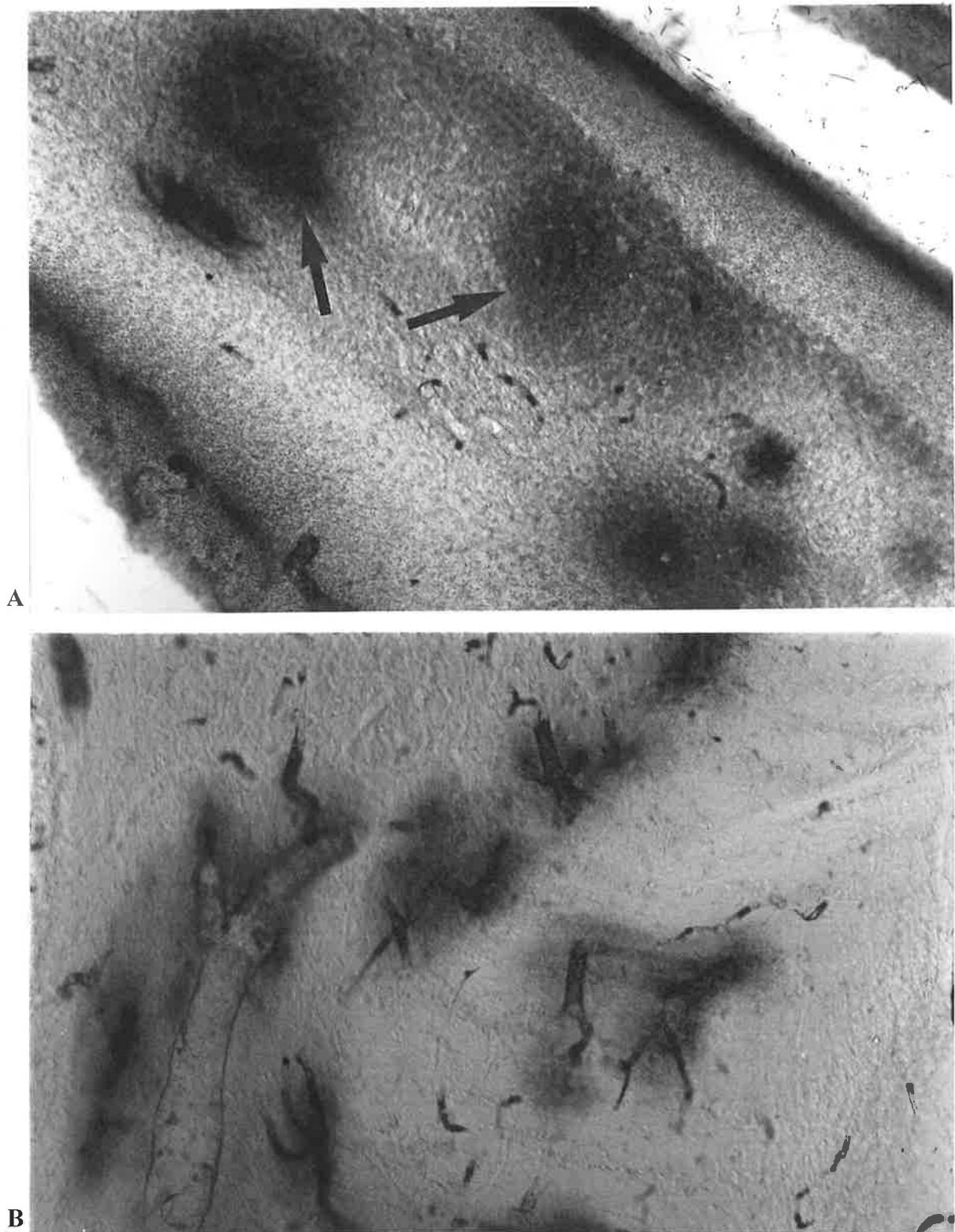


Figure 83. Photomicrographs of cerebellar sections from rats sacrificed after HRP injection into the cisterna magna. *A: 0 min. HRP reaction product has diffused through the pia but has not yet reached the molecular/granular layer interface. Perivascular spaces are labelled in the granular layer and reaction product is diffusing into the surrounding extracellular space (arrows). HRP/TMB, $\times 100$. B: 10 min. HRP reaction product is labelling perivascular spaces in the deep white matter and is spreading into the surrounding extracellular space. HRP/TMB, $\times 200$.*

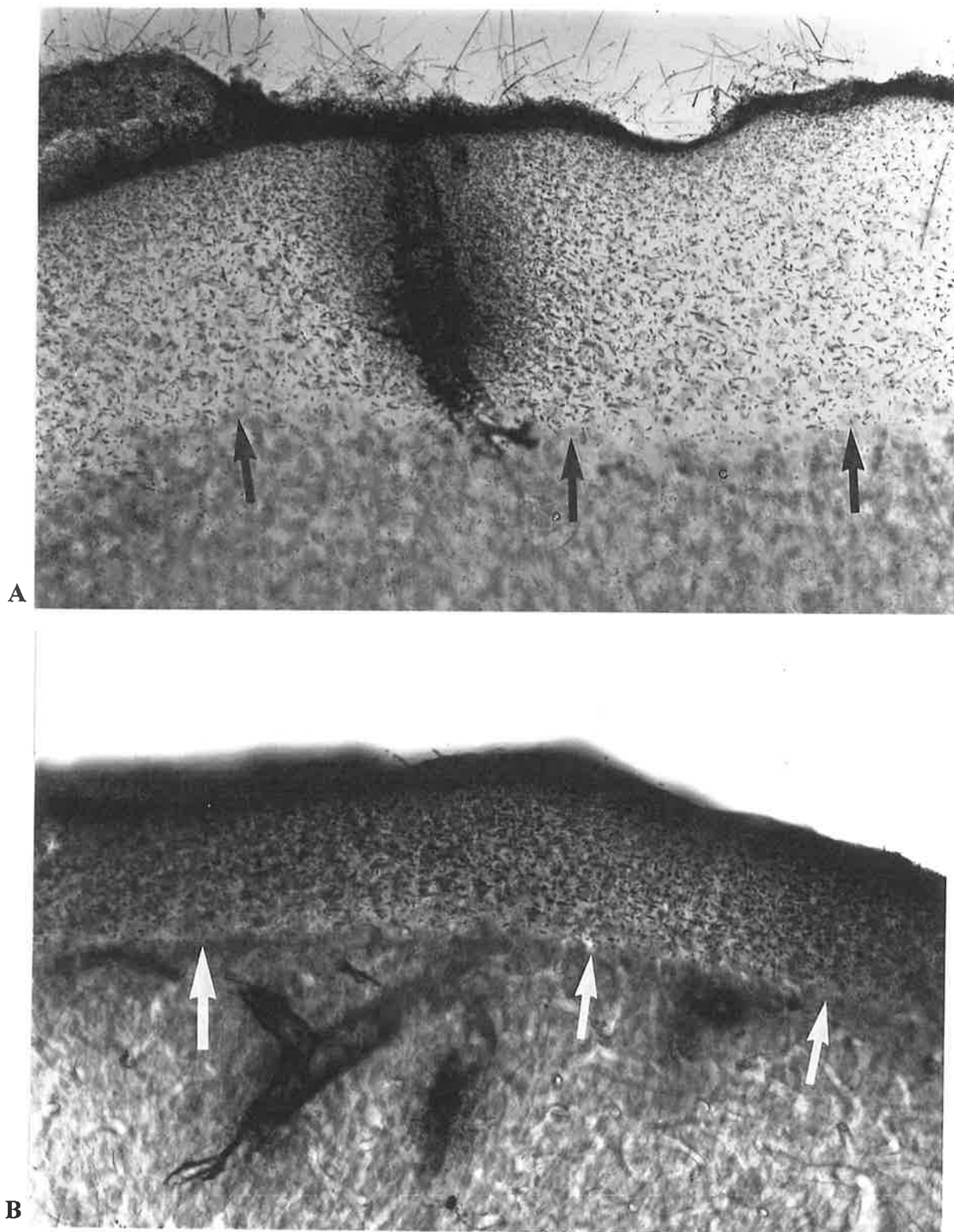


Figure 84. Photomicrographs of cerebellar sections from rats sacrificed after cisternal HRP injection. A: 10 min. HRP has diffused through the pia to reach the molecular/granular layer interface (arrows). A perivascular space in the molecular layer is densely labelled. HRP/TMB, $\times 200$. B: 30 min. HRP is densely labelling the molecular layer but stops abruptly at the granular layer (arrows). Perivascular spaces in the granular layer are labelled. HRP/TMB, $\times 200$.

10.2.2 Brainstem

The pia contained dense reaction product at all time intervals after HRP injection. Labelled perivascular spaces were present throughout the brainstem but were more numerous nearer the pial surface. The number of labelled perivascular spaces increased with each time interval. Reaction product was concentrated around labelled perivascular spaces and spreading diffusely into the interstitial space. Perivascular spaces were labelled for longer lengths than those in the cerebellum. The extent of transependymal penetration of HRP was always less than the transpial penetration.

10.2.3 Cerebral hemispheres

The extent of transpial diffusion in the cerebral hemispheres was less than that in the cerebellum at all time intervals. The perivascular spaces of vessels penetrating the cortex perpendicularly from the pial surface were labelled to greater depths than the transpial diffusion. Labelled perivascular spaces were present at all levels of the cortex and the white matter and were most numerous in the 30 minute group. Reaction product appeared to be spreading out from these perivascular spaces into the surrounding parenchyma. This interstitial reaction product was most dense in the 30 minute group. Compared with the cerebellar sections the labelling of perivascular spaces in the cerebral hemispheres was more numerous and more extensive, involving longer segments of vessels and their branches. The ventricular ependyma contained reaction product and there was a thin band of subependymal diffusion of reaction product.

10.2.4 Cervical spinal cord

Labelled perivascular spaces were present in both the white and grey matter of the spinal cord. Vessels entering the grey matter after passing through white matter were labelled with reaction product only in the white matter region. Vessels entering the grey matter from the ventral median fissure contained reaction product throughout their length. Reaction

product was seen spreading out from labelled perivascular spaces in both white and grey matter. More extensive and more numerous perivascular labelling was observed as one progressed from the 0 minute to the 30 minute group.

10.3 Discussion

In preliminary studies which used routine perfusion methods, the cerebellar sections contained numerous residual erythrocytes even when other parts of the brain and the spinal cord did not. These cells may obstruct fixative perfusion and prevent HRP fixation. Early anticoagulation by tail vein heparin injection and perfusing the animals in the lateral position minimised the number of residual erythrocytes.

10.3.1 Diffusion

Previous authors have suggested that the transport of molecules in the extracellular fluid is primarily by diffusion across the ependymal surface and by both diffusion and bulk flow across the pial surface.^{97,122,183,373} These different forms of fluid flow result in a more limited and uniform band of tracer in the subependymal region but a wider band of reaction product subjacent to the pial surface with radial spurs extending inwards along perivascular sheaths.^{54,373}

In this study, the transependymal movement of HRP was quite limited in its depth and did not increase with time. The pial surface however, had a much wider band of reaction product, which appeared to have diffused from the surface into the deeper regions. Borison et al⁵⁴ have suggested that the flow of the tracer along the perivascular spaces from the pial surface expands the diffusional area on that side, accounting for the wide pial bands of tracer. The subpial band of diffusion may also be deeper because of its proximity to the site of injection and the immediate exposure of the subpial cerebellar parenchyma to a high concentration of HRP. Unlike transpial diffusion in other parts of the brain, the density of HRP reaction product in the cerebellar cortex decreased abruptly at the Purkinje cell layer.

This may be due to an anatomical barrier posed by the Purkinje cells or to differences in the extracellular fluid spaces in the molecular and granular layers.

10.3.2 Evidence for perivascular flow in the cerebellum

In this study, there was rapid movement of HRP from the subarachnoid space into the cerebellar perivascular spaces. There were labelled spaces in all layers of the cerebellar grey and white matter even in the 0 minute group. However the cerebellar microvasculature was not completely outlined. Gregory et al¹⁸³ have suggested that the microvasculature in the cerebellum is completely outlined even after a five minute cisternal HRP infusion in cats and dogs. The small volume of HRP and the use of an injection rather than an infusion with concomitant drainage, probably explain the incomplete labelling in this study. Infusion with CSF drainage may increase the movement of tracer in the subarachnoid space and lead to more complete labelling of the perivascular spaces.

Labelling of perivascular spaces followed a temporal progression. Most numerous labelling, particularly in the cerebellar white matter, was observed in the 30 minute group. Rennels et al^{373,374} have demonstrated that solutes can enter the brain rapidly from the subarachnoid space into the perivascular spaces surrounding the vessels in the cerebral parenchyma. In as little as 4 minutes after the cisternal injection, HRP was distributed in the perivascular spaces at all levels of the neuraxis. In this study, some cerebellar perivascular spaces were labelled in the animals sacrificed immediately after injection, suggesting that perivascular flow is even more rapid.

In the spinal cord sections we observed the different types of blood vessels described by Cifuentes et al.⁸⁸ The 'Type A' vessels are labelled with the HRP reaction product only in the white matter and the 'Type B' vessels enter the central grey matter from the ventral median fissure or dorsal roots and are labelled throughout their course. The perivascular

spaces in the cerebellum were labelled throughout their course in grey and white matter, with no evidence of 'Type A' or 'Type B' vessels.

10.3.3 Movement of HRP from perivascular spaces into the extracellular fluid

The results of this study support the hypothesis that fluid from the perivascular spaces flows into the interstitial space. The evidence for this includes: 1) almost all the blood vessels at all levels of the cerebellar grey and white matter were associated with increased density of HRP reaction product in the surrounding extracellular space; 2) dense reaction product was seen around blood vessels in the granular layer while there was relatively little reaction product in other regions of the granular layer; and 3) the reaction product around blood vessels in the molecular layer was more dense than in areas of the molecular layer where there were no labelled vessels.

Rennels et al³⁷³ have suggested that there is a unidirectional movement of CSF in the perivascular spaces which is facilitated by pulsations of the penetrating arterioles with every cardiac contraction. According to these authors, CSF flows along the arterial perivascular spaces, continues along the capillary basal lamina and then possibly moves through the extracellular space to reach venous perivascular spaces. Metabolic end products incapable of traversing the blood-brain-barrier may be cleared by this bulk CSF flow.³⁷³ In this way the perivascular pathways may serve a 'lymphatic' role for the brain and spinal cord.^{282,292} Alternatively, bulk fluid flow in the extracellular space may play a role in non-synaptic neurotransmission.¹⁷ The rapid flow in cerebellar perivascular spaces and into the surrounding parenchyma demonstrated in this study is evidence for similar functions occurring in the cerebellum.

There was no evidence in this study for a preferential flow from perivascular spaces in the cerebellum towards the fourth ventricle. A few cross sections of blood vessels associated

with dense reaction product were observed in the subependymal white matter close to the fourth ventricle, but these were associated with diffuse surrounding reaction product with no evidence of a preferential flow.

This study has demonstrated that a perivascular pathway exists for solutes in the subarachnoid space to gain access to the cerebellar extracellular space. Tracers enter the perivascular space within minutes of their injection into the subarachnoid space. This rapid flow may act as a 'lymphatic' system or aid in non-synaptic neurotransmission. This pathway may also be involved in the formation of cysts associated with cerebellar tumours or with congenital malformations.

GENERAL DISCUSSION

1 *Choice of animal models*

Rats and sheep were used in this project. The rat was used for the majority of the work because it is a well-studied species that is easy to work with and because it was used in the model of non-communicating syringomyelia developed by Milhorat et al.³¹³ The major disadvantage of the rat is its small size that makes manipulation of CSF and arterial pressures difficult. The rabbit could have been used for this work but there is some evidence that central canal CSF physiology is unusual in rabbits.⁵⁶ The sheep was chosen as the large animal for study of CSF flow after manipulation of arterial and CSF pressures. The robust dura mater in this species allows cannulation of the subarachnoid space without CSF leakage. A disadvantage of using sheep for this experiment is that CSF physiology in this species is not well studied. Nevertheless, the results obtained in the normal sheep were similar to those seen in normal rats. A further disadvantage is that rapid perfusion-fixation of such a large animal is technically difficult, allowing several minutes for CSF tracers to diffuse before being bound to tissue proteins by fixative. A perfusion technique was developed for this project that produced satisfactory fixation of the spinal cord within 10 min of completion of each experiment.

Further experiments are planned in other species in an attempt to reproduce the results obtained in rats and in sheep and to develop an animal model that allows manipulation of pressures and rapid perfusion-fixation. Rabbits, cats and dogs are likely to be suitable species for this future study.

2 *Choice of CSF tracer*

The CSF and its pathways are extremely difficult to study,¹¹⁰ since none of the numerous tracer molecules that have been used is ideal. Of the available tracers, HRP has the advantage that its size (diameter 5 nm to 6 nm, molecular weight 43,000 daltons^{122,222,299})

limits simple diffusion yet allows it to pass readily between pial and ependymal cells, which have gap junctions.^{299,381,472} TMB was used as the localising chromogen because it is more sensitive than diaminobenzidine and provides better light microscopic visibility with less background noise.^{288,289,373} Fixation of HRP with perfused aldehyde occurs with sufficient rapidity to prevent post-fixation diffusion artifact,¹²² although there is a theoretical possibility that perfusion-fixation itself could artifactually move HRP in the perivascular spaces. Evidence against this possibility is that: 1) HRP injected into the subarachnoid space immediately after circulatory arrest and prior to fixation does not label the perivascular spaces;⁵⁴ 2) perfusion of fixative through the subarachnoid space does not translocate HRP;⁵⁴ and 3) that perivascular spaces are not labelled when arterial pulsations are damped.³⁷³ A disadvantage of HRP is that objective analysis is difficult. Although Cifuentes et al⁸⁸ measured the relative optic density of HRP reaction product, the influence of fixation variables³⁸⁴ would seem to make this an unreliable method except for analysis of very dense areas of reaction product.

3 Experimental techniques

3.1 Perfusion-fixation

Rapid tissue fixation with aldehydes was necessary in this project. Any delay in binding HRP to adjacent tissue proteins carries the theoretical risk of HRP continuing to diffuse through the tissue and give an erroneous impression of CSF flow.¹²² A combination of paraformaldehyde and glutaraldehyde was used for the rat experiments; this combination produces rapid fixation of HRP.¹²² Perfusion through the heart after thoraco-laparotomy is a standard technique in rats that allows rapid infusion of fixative.

Although many investigators^{58,208,235} have studied the sheep spinal cord without perfusion-fixation, it was necessary to use perfusion in this project for the reasons given above. Paraformaldehyde alone was used in sheep because the large volume of fixative

required for this animal made the use of glutaraldehyde impractical. Intracarotid perfusion of fixative was used because this gave the most rapid and reliable fixation of the brain and spinal cord.

3.2 CSF studies

Early studies of CSF physiology were largely concerned with the site and volume of CSF production and absorption. The technique of ventriculo-lumbar or cisternal-lumbar perfusion²²² was developed for this purpose. Other investigators have also used perfusion of tracers in the subarachnoid space to investigate patterns of CSF flow.¹¹⁰ This technique was not used in this project because a continuous perfusion of fluid into the subarachnoid space must alter the CSF dynamics, at least in the subarachnoid space if not in the perivascular spaces. Many investigators^{54,59,99,245,261,473,519,520} have studied CSF flow using a single injection of tracer into the subarachnoid space, but they have usually examined the location of tracer hours after the injection. Borison⁵⁴ and Rennels et al^{373,374} were the first to examine tracer location within minutes of the injection and similar methods were used in this project. A small volume of tracer was injected during these experiments so that the normal CSF physiology would be minimally disturbed. In each experiment in this study, the needle was left in place after injection to prevent CSF leak and an artifactual reduction of CSF flow.⁴⁰⁷

4 Experimental models of syringomyelia

The model of non-communicating syringomyelia developed by Milhorat et al³¹³ was reproduced in this project. The morphology of syrinxes in this model is similar to that of non-communicating syringomyelia seen in humans.²⁹⁶ The fact that the central canal dilates between occlusions is evidence in itself for a transparenchymal flow of fluid into the central canal. It is clear that at least in the animal model, the central canal does not enlarge by fluid flowing into it from the fourth ventricle. This is probably also the case in humans since there is no communication between the fourth ventricle and the syrinx.

The attempts in this project to produce a model of syringomyelia in sheep were not successful. Although it is possible that CSF physiology in the sheep is fundamentally different from other species, it is more likely that technical factors were responsible for the failure of syrinx production. For example, the dose of kaolin injected into the sheep spinal cord may not have been optimal to allow inflammatory cells to occlude the central canal. It may simply be that the sheep central canal is too large to be readily blocked by an inflammatory reaction. Further attempts at development of a large animal model of non-communicating syringomyelia will involve other species and different mechanisms of central canal occlusion, such as extradural compression of the spinal cord. Another possibility is to produce intraparenchymal cysts to mimic posttraumatic syringomyelia.

5 Normal CSF flow

Movement of CSF tracers from the subarachnoid space into the spinal cord perivascular spaces was first demonstrated by Brierley⁵⁹ in 1950. More recently, Rennels et al,^{373,374} Wagner et al⁴⁷³ and Borison et al⁵⁴ have shown that HRP injected into the subarachnoid space rapidly labels the perivascular spaces of the brain and spinal cord. In this project it has been demonstrated that this flow occurs in rats and in sheep. It has also been demonstrated in rats and in sheep that, after leaving the perivascular spaces in the spinal cord, CSF flows preferentially into the central canal (Figure 85). This preferential flow suggests that the source of the normal interstitial fluid flow into the central canal^{88,294,301,312} is CSF from the subarachnoid space flowing into the interstitial space via the perivascular spaces. It has not been clear what the driving force for such a unidirectional fluid flow is. Rennels et al³⁷⁴ have provided evidence that the flow of fluid from the subarachnoid space into the perivascular spaces is dependent on arterial pulsations; the flow was reduced when arterial pulsations were reduced by partially ligating the brachiocephalic artery in dogs. However, they did not measure spinal subarachnoid pulsations and did not specifically look for flow into

the central canal. In this project, reducing arterial pulsations also reduced flow into the perivascular spaces. In addition, reducing arterial pulsations abolished flow into the central canal. These results support the hypothesis that a unidirectional flow of fluid from perivascular spaces across the interstitial space and into the central canal is driven by arterial pulsations. Systolic expansion of arteries in the perivascular space may force fluid through the surrounding basement membrane, while in diastole fluid may enter the perivascular space from the subarachnoid space through gap junctions in the covering pia. Alternatively, it may be pulsations in the subarachnoid space that drive fluid into the perivascular spaces.

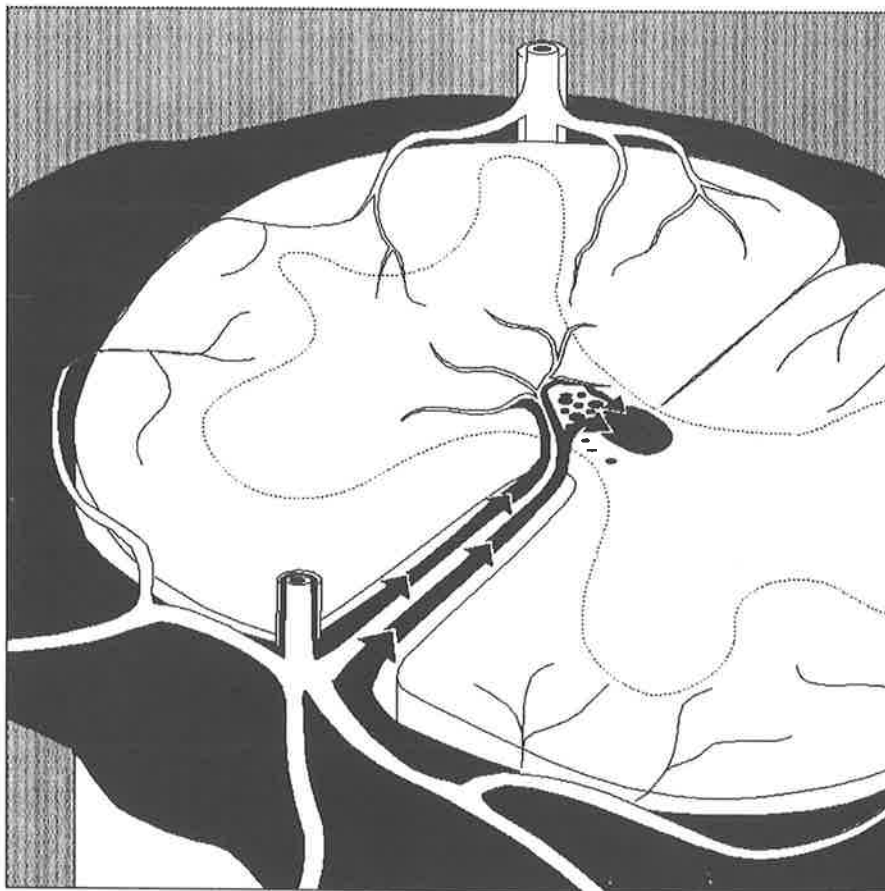


Figure 85. *Diagram illustrating the flow of CSF from the subarachnoid space to the perivascular spaces and into the central canal.*

The function of this flow may be a 'lymphatic' one, to clear the interstitial space of metabolites and neurotransmitters. Alternatively, it may have a role in non-synaptic neurotransmission.¹⁷ The existence of a one-way fluid flow into the central canal would add

considerable support to the theory that non-communicating syringomyelia develops in segments of central canal isolated by stenosis or occlusion.^{296,309,311,313} The continuation of an arterial-pulsation driven flow along perivascular spaces into the central canal would explain the accumulation of fluid and enlargement of cysts in such cases.

6 CSF flow in syringomyelia

The results from this project indicate that CSF flow into the central canal continues during enlargement of the central canal in an animal model of non-communicating syringomyelia. This does not prove that the fluid flow is responsible for canal enlargement. The results are consistent with the hypothesis that arterial pulsation-driven CSF flow in the perivascular spaces is enlarging the isolated segments of central canal (Figure 86). Other possible explanations include the alteration of subarachnoid CSF flow and compliance due to the partial subarachnoid block at the kaolin injection point. Hall et al^{195,197} have demonstrated a subarachnoid block in experimental communicating syringomyelia, but in the communicating model it is likely that the fluid entering the syrinx comes from the fourth ventricle. Subarachnoid block may cause non-communicating syringomyelia by forcing fluid along perivascular spaces or through the parenchyma to reach the central canal. These mechanisms seem unlikely given the pattern of HRP movement demonstrated in this study. An additional point in favour of the perivascular spaces being involved in syrinx formation is their enlargement at the same time that the central canal enlarges. Enlarged perivascular spaces are also seen in human syringomyelia.¹⁹ Whether perivascular flow is important in the development of human syringes, and indeed whether there is a normal flow of fluid from perivascular spaces into the central canal in the human, remains to be answered. Further work is aimed at determining whether altering arterial pulsations and subarachnoid space compliance affects syrinx development.

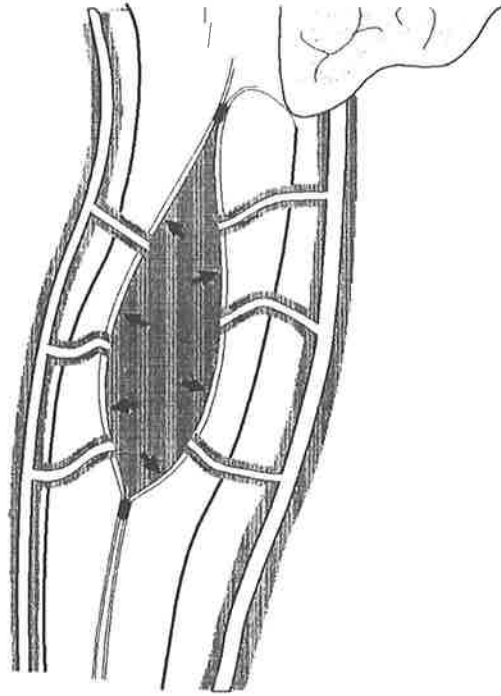


Figure 86. *Diagram illustrating the hypothesis that syrinxes form from an active perivascular flow of CSF into an isolated segment of central canal, even against a pressure gradient.*

7 Future work and unanswered questions

This work has provided good evidence that there is a normal flow of fluid from perivascular spaces into the central canal in some species and that this flow continues in an animal model of syringomyelia. Questions arising from these findings and from other parts of this work include:

1. Does this flow exist in humans?
2. Is the flow responsible for syrinx enlargement or is it an epiphenomenon?
3. Do arterial pulsations directly cause this flow, or is it due to transmitted pulsations from the subarachnoid space?
4. What is the normal morphology and function of the human central canal?
5. Is there normally a caudal opening of the human central canal?

Future directions of this work will be aimed at answering some of these questions. In particular, attempts will be made to develop an animal model that will allow pressure measurements of the central canal as it is enlarging. This would allow comparison of the pressures in the syrinx and the subarachnoid space to determine whether CSF is flowing from the subarachnoid space into the central canal against a pressure gradient. Avenues for exploration for syrinx models include cannulation and occlusion of the central canal, extradural compression of the spinal cord and cavity formation in the spinal cord parenchyma. Ideally a foetal model of the Chiari malformation would allow study of CSF physiology during this period.

More work also needs to be done on the normal physiology of perivascular flow and the effects on it of varying other physiological parameters such as venous pressure and respirations, performing Valsalva manoeuvres and performing surgical procedures such as CSF shunting and decompression. The effects of each of these on the development of syrinx models could then be examined.

The structure and function of the human central canal remain largely enigmatic. The 3-D morphological analysis technique developed during this project could be used to study normal and pathological human spinal cords to develop a better understanding of central canal structure. In particular, the relationship of syringes to the central canal and whether a caudal opening exists remain to be elucidated.

Finally, the most important aspect of this work is its application to the treatment and prevention of human syringomyelia. If the mechanisms underlying the development of syringes can be understood, a logical approach can be made to developing new treatments. For example, if subarachnoid space compliance is important, then dural decompression and insertion of soft gussets (using material such as expanded polytetrafluoroethylene) or opening alternative CSF pathways may alter the dynamics sufficiently to allow collapse of a syrinx. A

similar approach could be used in the prevention of posttraumatic syringomyelia. If cine-MRI, myelography or other tests of CSF flow are able to identify those at risk of developing syrinxes (for example those with a subarachnoid block) then intervention could be aimed at prevention rather than treatment.

CONCLUSIONS

The results of this project have provided evidence that CSF normally flows from the subarachnoid space, through perivascular spaces and into the central canal. This flow is dependent on arterial pulsations and continues during the development of an animal model of syringomyelia. These findings are consistent with the hypothesis that arterial pulsation-driven perivascular CSF flow is responsible for the development of non-communicating syringomyelia.

An additional finding was that rapid perivascular flow occurs in the cerebellum as it does in other parts of the brain and spinal cord. A technique for studying the 3-D morphology of the human central canal was also developed.

APPENDICES

1 Buffers and fixatives

Tris buffer 0.1 mol/L , pH 7.6²³

To make 4 L of buffer:

- 48.4 g TRIS in 4 L distilled water
- Add 5 mol/L HCl to adjust pH to 7.6

4% Paraformaldehyde/0.5% glutaraldehyde in phosphate buffer

To make 5 L of fixative:

Solution 1

- Dissolve 200 g paraformaldehyde in 2450 mL H₂O. Heat to 60° and stir
- add 1 mol/L NaOH by drops until clear
- Filter

Solution 2

- dissolve 0.19 mole of NaH₂PO₄ in 2450 mL H₂O
- add 0.807 mole of Na₂HPO₄

Combine Solution 1 and Solution 2.

Add 100 mL 25% glutaraldehyde.

4% Paraformaldehyde in phosphate buffer

To make 15 L fixative:

Solution 1

- Dissolve 600 g paraformaldehyde in 4 L H₂O. Heat to 60° and stir
- add 1 mol/L NaOH by drops until clear
- Filter

Solution 2

- dissolve 0.57 mole of NaH₂PO₄ in 4 L H₂O
- add 2.421 mole of Na₂HPO₄

Combine Solution 1 and Solution 2.

Make up to 15 L with distilled H₂O

2 TMB processing

Mounted section processing

1. Make ammonium molybdate solution (5%)

- 15 g ammonium molybdate in 300 mL 0.01 mol/L Na acetate buffer, pH 3.3
- Warm and stir until dissolved

2. Make nitroprusside solution
 - 289.5 mL distilled water
 - 300 mg Na nitroferricyanide (nitroprusside)
 - 3 mL 1mol/L Na Acetate buffer (pH 3.3)
3. Make TMB Solution
 - 15 mg tetramethylbenzidine
 - 7.5 mL absolute ethanol
 - Warm and mix until dissolved
4. Rinse sections for 15 seconds in distilled water
5. Add nitroprusside solution to TMB solution in staining jar
6. Immediately add slides; incubate for 20 minutes
7. Add 30 μ L hydrogen peroxide; incubate for 20 minutes
8. Wash in 2 changes $\times$ 15 seconds of 0.01 mol/L Na acetate buffer, pH 3.3
9. Transfer slides to ammonium molybdate solution for 15 minutes
10. Wash in 0.01 mol/L Na acetate buffer, pH 3.3 for 30 seconds
11. 95 % alcohol for 1 minute
12. 100 % alcohol for 1 minute
13. Transfer to xylene
14. Coverslip

Floating section processing

1. Make Ammonium Molybdate Solution (5%)
 - 1.5 g ammonium molybdate in 30 mL 0.01 M Na acetate buffer, pH 3.3
 - Warm and stir until dissolved
2. Make nitroprusside solution
 - 96.5 mL distilled water
 - 100 mg Na nitroferricyanide (nitroprusside)
 - 1 mL 1 mol/L Na acetate buffer (pH 3.3)
3. Make TMB Solution
 - 5 mg tetramethylbenzidine
 - 2.5 mL absolute ethanol
 - Warm and mix until dissolved
4. Rinse sections for 15 seconds in distilled water
5. Add nitroprusside solution to TMB solution
6. Immediately add sections; incubate for 20 minutes
7. Add 10 μ L 34 % hydrogen peroxide; incubate for 20 minutes
8. Wash in 2 changes $\times$ 15 seconds of 0.01 mol/L Na acetate buffer, pH 3.3
9. Transfer sections to ammonium molybdate solution for 15 minutes
10. Wash in 0.01 mol/L Na acetate buffer, pH 3.3 for 1 minute $\times$ 3
11. Mount on slides
12. 95 % alcohol for 1 minute
13. 100 % alcohol for 1 minute
14. Transfer to xylene
15. Coverslip

3 Publications

1. Stoodley MA, Jones NR, Brown CJ. Evidence for rapid fluid flow from the subarachnoid space into the spinal cord central canal in the rat. **Brain Research** 707:155-164, 1996.
2. Stoodley MA and Jones NR. Syringomyelia. In Clark C. (Ed), *The Cervical Spine* 3rd Edition. The Cervical Spine Research Society. Lippincott. 1996 (In press).

Papers submitted for publication

1. Stoodley MA, Brown SA, Brown CJ, Jones NR. Arterial-pulsation dependent perivascular CSF flow into the central canal in the sheep spinal cord. **J.Neurosurg.**

4 Presentations at scientific meetings

1. Stoodley MA, Jones NR and Brown C. Evidence for a rapid, transparenchymal flow of CSF into the spinal cord central canal. Presented at the **Royal Australasian College of Surgeons Annual Scientific Congress**, Perth, May 1995.
2. Stoodley MA and Jones NR. Rapid fluid flow from the subarachnoid space into the spinal cord central canal. Presented at the **Annual Scientific Meeting, SA State Committee, Royal Australasian College of Surgeons**, Adelaide, September, 1995.
3. Storer K, Toh J, Stoodley MA and Jones NR. The human central canal: a computerised three-dimensional study. Presented at the **Annual Scientific Meeting, SA State Committee, Royal Australasian College of Surgeons**, Adelaide, September, 1995.
(*Winner of the Jepson Medal*)
4. Stoodley MA and Jones NR. CSF flows rapidly from the subarachnoid space into the central canal via perivascular spaces. Presented at the **Spine Society of Australia Annual Scientific Meeting**, Broome, September 1995. (*Winner of the Rob Johnson Award*)

5. Stoodley MA and Jones NR. CSF flow into the central canal in a rat model of syringomyelia. Presented at the **Neurosurgical Society of Australasia Annual Scientific Meeting**, Broome, September 1995.
6. Stoodley MA, Jones NR and Brown SA. Influence of spinal subarachnoid compliance on CSF flow into the central canal. Presented at the **Neurosurgical Society of Australasia Annual Scientific Meeting**, Broome, September 1995.
7. Stoodley MA, Storer K, Toh J and Jones NR. Computerised three-dimensional morphological analysis of the human conus medullaris central canal. Presented at the **Neurosurgical Society of Australasia Annual Scientific Meeting**, Broome, September 1995.

5 Prizes

1. **Nimmo Prize** (Full-Time Research Category) 1995
For the best presentation by a medical researcher working in the University of Adelaide, Royal Adelaide Hospital, or Institute of Medical and Veterinary Science
2. **Jepson Medal** 1995
For the best presentation by a surgical trainee
(As co-author of paper presented by K. Storer)
Royal Australasian College of Surgeons (SA Branch)
Annual Scientific Meeting
3. **Rob Johnson Award** 1995
For the best presentation by a surgical trainee
Spine Society of Australia
Annual Scientific Meeting

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