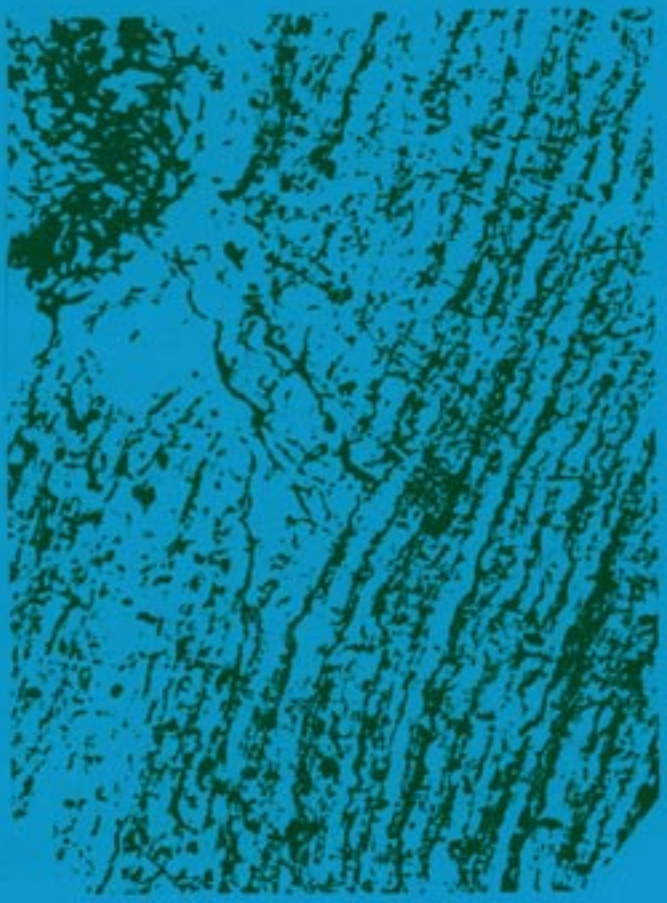
Treatment of Traumatic Spinal Cord Lesions During the Past 5000 Years in Animal and Man

Including a Future Perspective with Fetal Transplants

by

Benedicte Dahlerup



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The body can give the most beautiful pleasures to the soul, but it can also be its prison.

Benedicte Dahlerup

Preface

The aim of this book is to give a detailed history of all the attempts that have been made throughout the years - since Imhoteb in the 13 century BC till now-on treating spinal cord lesions and their repair.

Scientists have spent numezous hours in the laboratory doing experimental work on spinal cord. lesions to discover the mechanism behind the lack of functional recovery of the spinal cord. I have tried to give a summary of the work performed until now.

The original thought behind my own experimental work was to do fetal spinal cord transplants in rats to try to gain functional synapsis between the host and the graft, but in this I did not succeed with the spinal cord. However it has been shown in cerebral tissue by other scientists.

The final goal is to give my view on how to treat spinal cord lesions today based on history, earlier experience and my own experiments.

The book is dedicated to all para- and tetraplegics and to the central ethical scientific committee for whom it is to decide the extent of our efforts for new approaches to spinal cord lesions in human beings.

This work is produced at the Institute of Neurobiology, Arhus Univerity, Department of Neurosurgery, Aarhus University Hospital and at the Department of Neuropathology, Arhus University Hospital.

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Spinal Cord Lesions in Man 3000 Bc-1895

Ancient Egypt

The oldest descriptions of surgical treatises were bought in 1862 by an American, Edwin Smith, in Luxor (Thebes) in Egypt. These descriptions contain the earliest history of cervical lesions.

Edwin Smith studied Egyptology in London and Paris and started to translate this papyrus which was in hieroglyphic writing. The document dating from the seventeenth century BC is a copy of a original manuscript produced perhaps a thousand years earlier, from the 6 dynasty (about 3000 to 2500 BC). Since the beginning and the end of the manuscript are missing, the name of the author is unknown. It is possible though, that a surgical treatise of this importance appearing in the Pyramid age might have been written by the first known physician, Imhoteb. He lived in the seventeenth century BC.

In 1930, Professor James Henry Breasted from Chicago published a magnificent edition of the Edwin Smith papyrus in facsimile with a hieroglyphic transcript and with an English translation and comments.

Because of its historical interest and value,! will include the part which concerns the cervical lesions both in the hieratic writing, the hieroglyphic translation and the English translation made by Professor Breasted. The paper used as a textbook for medical students or doctors, teaching how to deal with different cases. Each case has a number. Thus, case 29 to 33 are cases dealing with the back of the neck and injuries of the cervical vertebra.

Case 29

Instructions concerning a gaping wound in a vertebra of his neck.

If thou examinest a man having a gaping wound in a vertebra of his neck penetrating to a bone and perforating a vertebra of his neck; if thou examinest that wound and he shudders exceedingly and he is unable to look at his two shoulders and his breast thou shouldst say concerning him: “One having a wound in his neck penetrating to the bone, perforating a vertebra of his neck and he suffers with stiffness in his neck. An ailment with which I will contempt”. Thou shouldst bind it with fresh meat the first day. Now afterwards moor him at his mooring stakes until the period of his injury passes by.

Case 30

Instructions concerning a sprain in a vertebra of his neck.

If thou examinest a man having a sprain in a vertebra of his neck them shouldst say to him: "Look at thy two shoulders and thy breast". When he does so the seeing possible to find him is painful thou shouldst say concerning him: "One having a sprain in a vertebra of his neck. An ailment which I will treat". Thou shouldst bind it with fresh meat the first day, afterwards thou shouldst treat with ymrv* and honey every day until he recovers.

Case 31

Instructions concerning a dislocation in a vertebra of his neck.

If thou examinest a man having a dislocation in a vertebra of his neck shouldst thou find him unconscious of his two arms and his two legs on account of it while his phallus is erected on account of it and urine drops from his member without his knowing it; his flesh has received wind, his two eyes are bloodshot; it is a dislocation of a vertebra of his neck extending to his backbone which courses him to be unconscious of his two arms and his two legs. If however, the middle vertebra of his neck is dislocated it is an emissio seminis which befalls his phallus. Thou shouldst say concerning him: "One having a dislocation in a vertebra of his neck while he is unconscious of this two legs and his two arms and his urine dribbles. An ailment not to be treated".

Case 32

Instructions concerning a displacement in a vertebra of his neck.

If thou examinest a man having a displacement in a vertebra of his neck whose face is fixed, whose neck cannot turn for him thou shouldst say to him: "Look at the breast and thy two shoulders", and he is unable to turn his face that he may look at his breast and his two shoulders. Thou shouldst say concerning him: "One having a displacement in a vertebra of his neck. An ailment which I will treat". Thou shouldst bind it with fresh meat the first day. Thou shouldst loose his bandages and apply grease to his head as far as his neck and thou shouldst bound it with ymrv*. Thou shouldst treat it afterwards with honey every day and his relief is sitting until he recovers.

ymrv*: we have no exact translation of this word.

Case 33

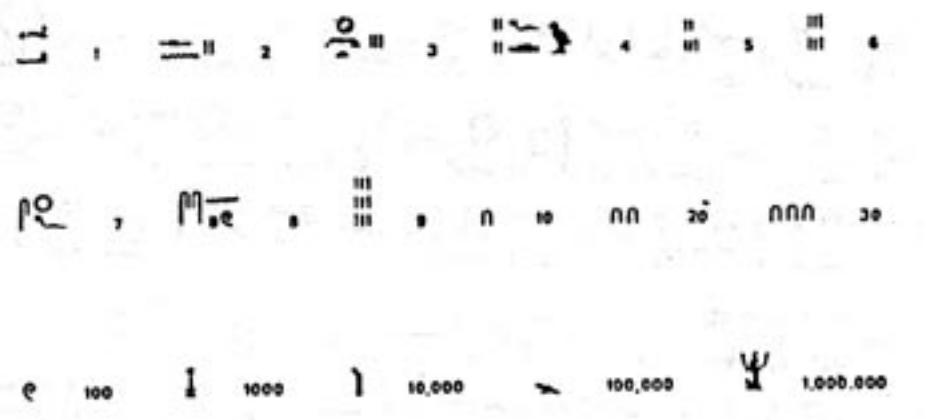
Instructions concerning a crushed vertebra in his neck.

If thou examines! a man having a crushed vertebra in his neck and thou findest that one vertebra has fallen into the next one while he is voiceless and cannot speak; his falling head downwards has coursed that one vertebra crush into the next one; and thou shouldst find that he is unconscious of his two arms and his two legs because of it. Thou shouldst say concerning him: "One having a crushed vertebra in his neck; he is unconscious of his two arms and his two legs and he is speechless. An ailment not to be treated"."

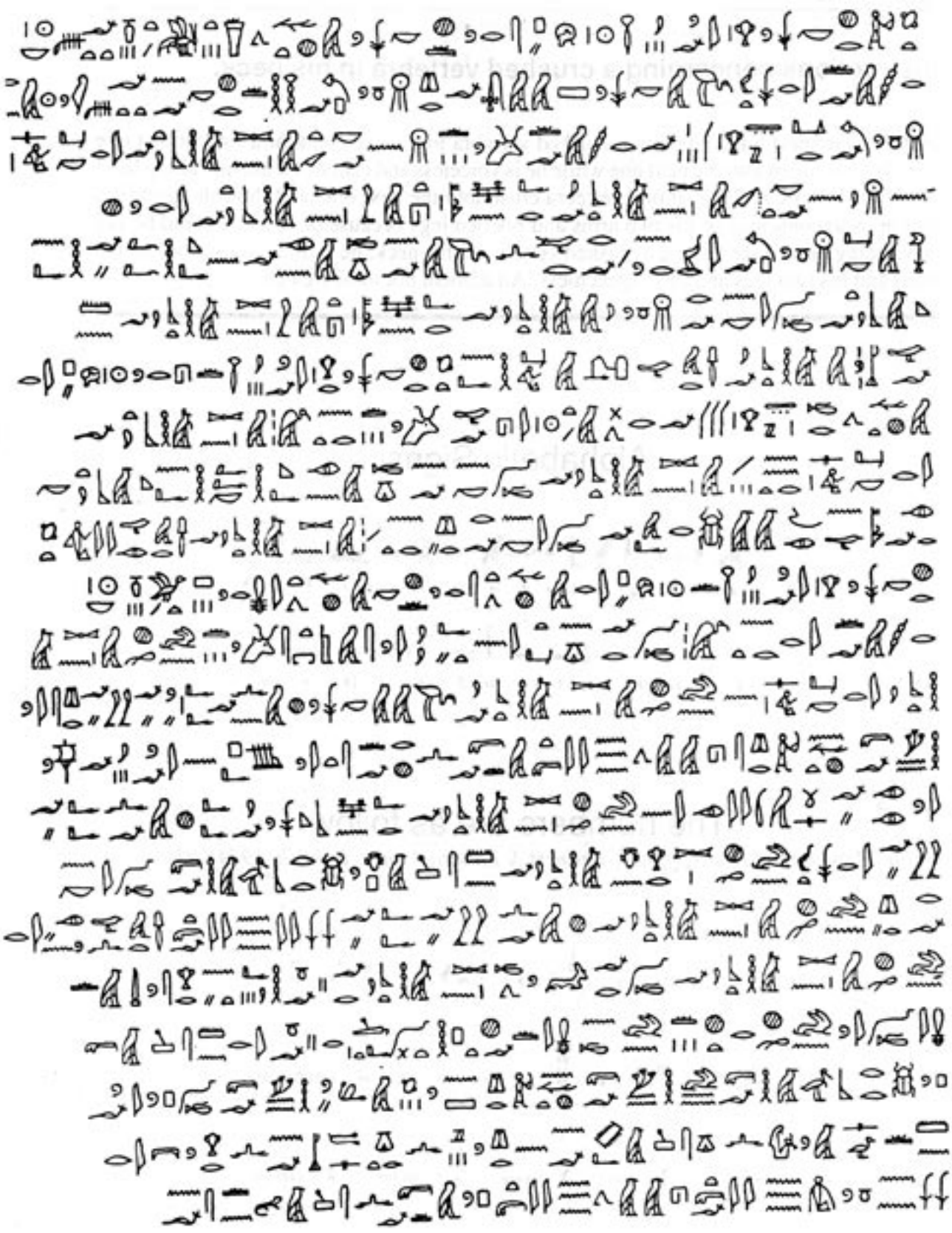
Alphabetic Signs

The numbers are as follow

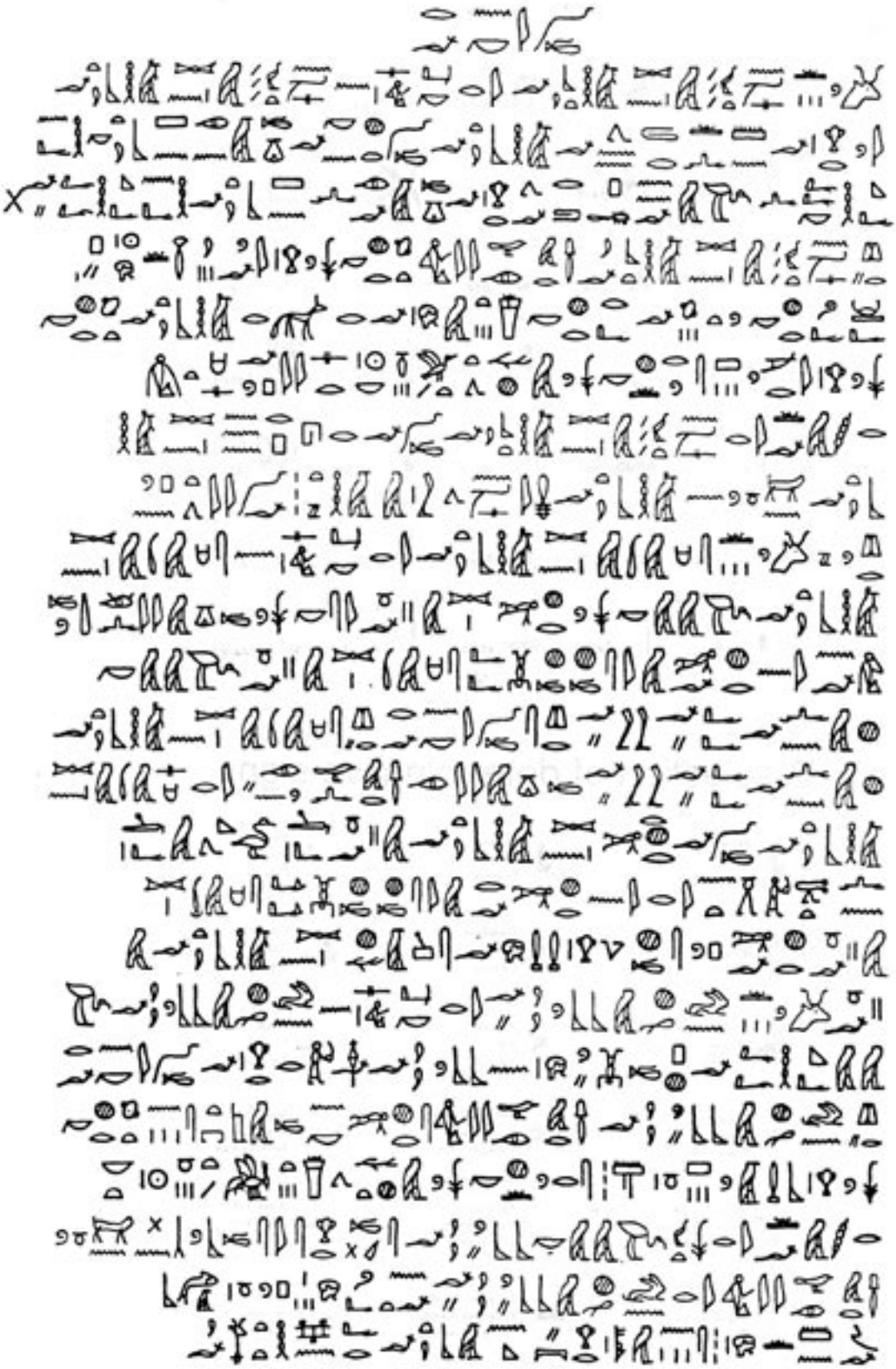
From "How to read Hieroglyphs". Lelinert & Landrock succ. Publish. 1974 Cairo



The surgical treatise. column X



The surgical treatise. column XI



Some Syllabic Signs

A selection of determinative Signs

Hieroglyphic Transliteration of column X

1 Case 32
2
3
4
5
6
7
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9 Case 33
10
11
12
13
14
15
16
17 Case 34
18
19
20
21
22
23

Hieroglyphic Transliteration of column XI

1
2
3 Case 35
4
5
6
7
8 Case 36
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10
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12 Case 37
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The Greeks - Hippocrates

The Greeks who founded the Alexandrian Library assembled all the knowledge of ancient medicine. They did that approximately 200 years BC. They found several writings of Hippocrates who was born approximately 460 BC., although one is not sure, that all the documents were really written by him [116]. About back injuries he writes:

> *"Each of the posterior spinal processes is so constructed that it would sooner be broken than undergo any great inclinationforwardfrom a force which would have to overcome the ligaments and the articulations mutually connecting them. The spinal marrow would suffer if, from the displacement of the vertebra it were to bend even to a small extent; for the displaced vertebra would compress the spinal marrow if it did not break it; if compressed and strangled it would induce insensibility of many great and important parts, so that the physician need not give himself any concern about rectifying the displacement of the vertebra, accompanied as it is by many other ill-consequences of a serious nature. It is evident then that such a case could not be reduced either by succussion or by any other method unless one were to cut open the patient and then, having introduced the hand into one of the great cavities were to push outward from within, which one might do on the dead body but not at all on the living. Why then, do I write all this? Because certain persons fancy that they have cured patients in whom had undergone complete dislocation forward, some indeed suppose that this is the easiest of all these dislocations to be recovered from, and that such cases do not stand in need of reduction but get well spontaneously. Many are ignorant and profit by their ignorance for they obtain credit from those about them".*

Hippocrates even writes about callus:

"Callus readily forms in all such bones as are porous".

About gibbositas or projections backward from falls on the verify this word or on the shoulders he writes that in this case some of the vertebra must necessarily appear higher than natural and those on either side to a less degree; but yet no one generally has started out for the line of the others but everyone has yielded a little so that a considerable extent of them is curved. On this account the spinal marrow easily bears such a distorsion as they are of circular shape and not angular. The apparatus for the reduction of this case must be managed in the following manner:

> *"A strong and broad board having an oblong furrow in it is to be fasten in the ground or in the place of the board we may scoop out an oblong furrow in the wall about a cubit above the floor or at any suitable height, and then something like an open bench of a quadrangular shape is to be laid along the wall at a distance from the wall which will admit of the persons to pass around if necessary, and then the bench is to be covered with ropes or anything else which is soft but does not yield much, and the patient is to be stowed with vapour if necessary or bathed with much hot water, and then he is to be stretched along the board on his face with his arms laid along and bound to his body; the middle then of the thong which is soft, sufficiently broad and long and composed of two cross-straps of leather is to be twice carried along the middle of the*

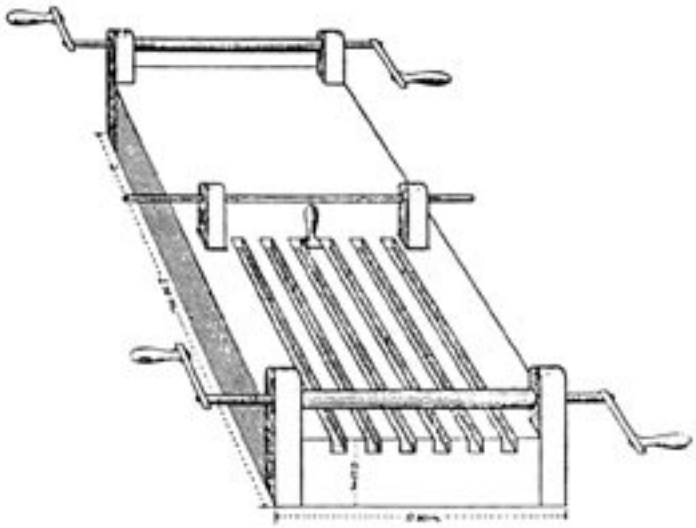
patient's breast as near the armpits as possible. Then what is over of the thongs at the armpits is to be carried around the shoulders and afterwards the ends of the thong are to be fasten to apiece of wood resembling a pestle; they are to be adapted to the length of the bench laid below the patient and so that the pestle-like piece of wood resting against this bench may make extension. Another such band is to be applied above the knees and the ankles, and the ends of the thongs fastened to a similar piece of wood, and another thong brought soft and strong in the form of a swathe, having breadth and length sufficient, is to be bound tightly round the loin as near the hips as possible, and then what remains of this swathe-like thong with the ends of the thongs must be fastened to the piece of wood placed at the patient's feet, and extension in this fashion is to be made upward and downward equally and at the same time in a straight line. For extension thus made could do no harm if properly performed unless one sought to do a mischief purposely. But the physician or some person who is strong and not uninstructed should apply the
palm of one hand to the hump, and then having laid the other hand upon the former he should make pressure attending whether the force should be applied directly downward or toward the head or toward the hips. This method of applying force is particularly safe and it is also safe for a person to sit upon the hump while extension is made and raising himself up to let him fall again upon the patient. And there is nothing to prevent a person from placing afoot on the hump and supporting his weight on it making gentle pressure".

Fig. 1.1
The bench of Hippocrates from Guido Guidi's latin translation from the 16th century of Ancient Greek surgeons.

From "Lægekunsten gennem tiderne". Kaj Birket-Smith (ed.) (1946), vol. 1[149]"

Fig. 1.2

Reconstruction of the bench of Hippocrates to rectify broken limbs.[149]



From the Byzantines to the Twentieth Century

Fig. 1.3

Two settings (reduction) of spinal vertebrae when the patient is put on a ladder with the head downward or the head upward. (Apollonius ofCitium, c.81-58 EC)[15].

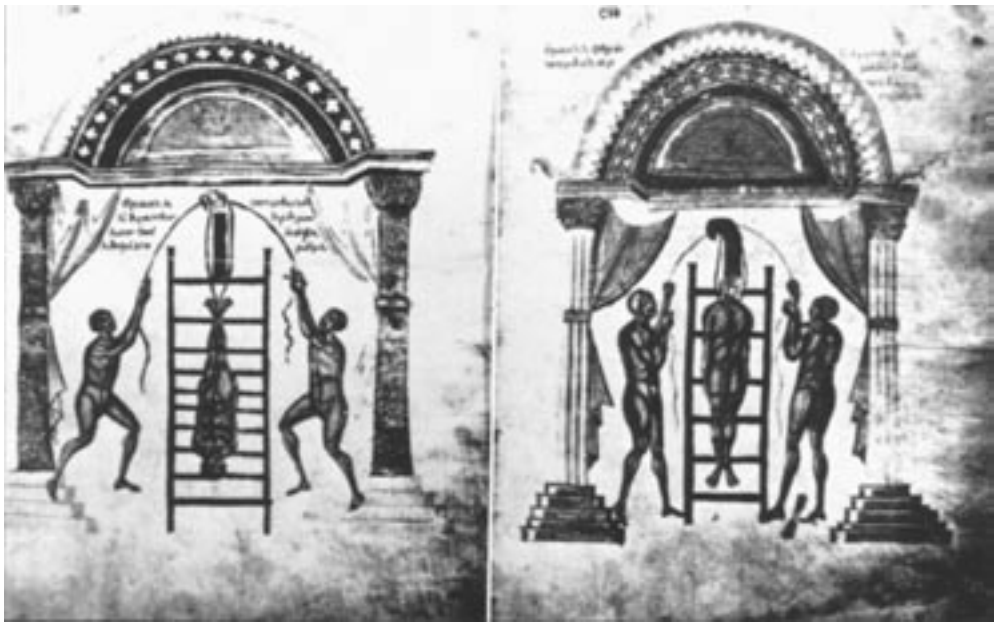


Fig. 1.4

Reduction of a dislocation of the spine. The physician is seen standing upon the gibbosity. (Avicenna, 980(?)-1037[15.]

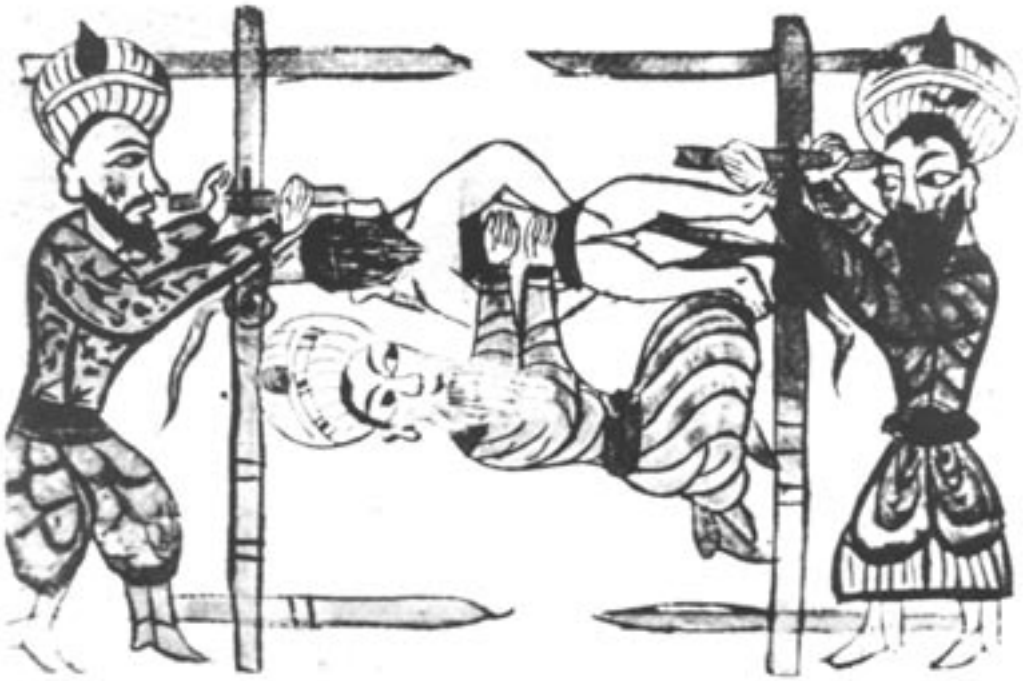
These byzantine illustrations are believed to be direct copies from the original Greek drawings in Hippocrates's treatise "On articulations". It is found in one of the comments made on the work of Hippocrates written by Apollonius of Citium, a surgeon who lived in the middle of the first century BC. This manuscript was copied in the ninth or tenth century, and it is part of the Niketas collection of Greek manuscripts which the emperor Constantine requested to be gathered in the eleventh century [192.]

After the fall of the Roman Empire the civilization, culture and medicine started to declined. However, the Eastern Empire developed their own medicine based upon the works of Hippocrates, Paul of Aegina, a surgeon living in Alexandria in the 7 century and Galen. Thus, a combination of Greek and Hindu medicine was practised in the Eastern Empire. The most famous of these works are written by Avicenna. He was a Persian living from 980 to 1037. He followed Paul of Aegina's treatment for dislocations of the spine where the physician stands upon the gibbosity with his heels to reduce a dislocation of the spine. Avicenna does not mention Paul of Aegina's surgical treatment for removal of compressing bone in fractures. Actually, Paul of Aegina was the first to recommend a laminectomy with removal of all pieces of bone compressing the spinal cord. Paul of Aegina was very cautious about this operation and stressed the danger of the operation before performing it. Avicenna and his Arab colleagues do not mention this operation because they believed that jractures of the bodies of the vertebra were fatal if they were accompanied by paralysis 15.

The Turk Serefeddin Sabuncuoglu was inspired by Paul of Aegina to write a book about surgery. Some of his work has been translated and published with of the reproduction illustrations which are believed to have been drawn by him. In this illustration extension and

Fig. 1.5

Treatment of dislocation of the spinal column. (Serefeddin Sabuncuoglu, 1465) [15.]



counterextension are applied to the patient to reduce a dislocation of the dorsal vertebra. Two bands are passed around the patient above and below the luxation and fastened to windlasses. In the meantime the surgeon is exerting direct pressure upon the dorsal vertebra with the dislocation [15].

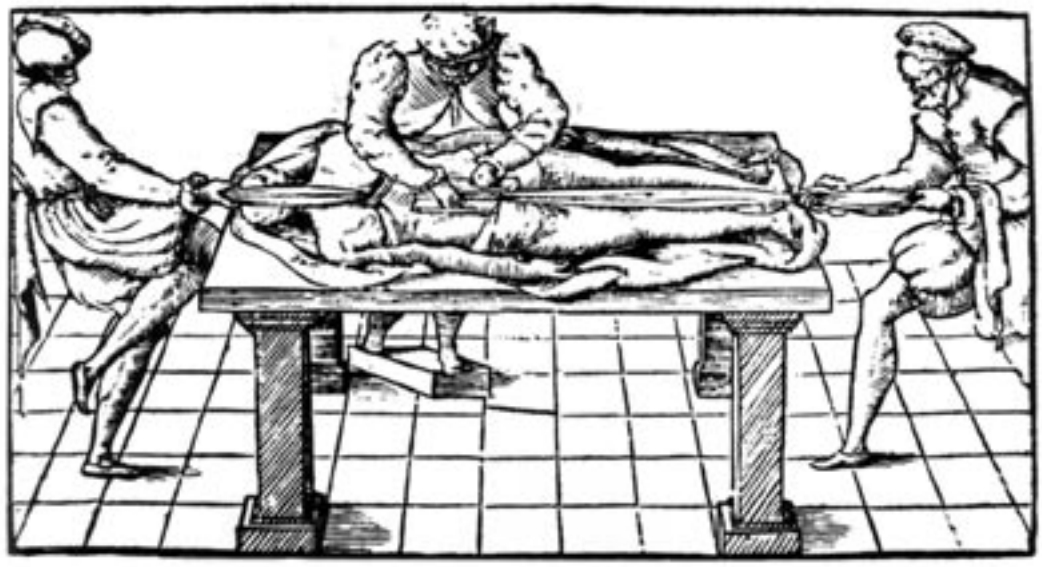
Among others who wrote about spinal cord lesions was Ambroise Pare 1510-1590, who was a Parisian surgeon in the sixteenth century. He wrote from the battle of Saint Denis in 1567: [173.]

> *"And for this battle of Saint Denis there were diverse slain as well on one side as on the other: ours being hurt went back to Paris to be dressed together with the prisoners who were taken whereof I dressed a great part. The King commanded me to be the request of the Lady high Constable to go to her house to dress my Lord who had received a pistol shot in the middle of the spondills of his back, whereby he presently lost all senses and motion of thighs and legs with retention of excrements, not being able to cast out his urine nor anything by the fundament, because that the spinal marrow (from whence proceed the sinews to give sense and motion to the interiour parts) was bruised, broken and torn by the vehemence of the bullet. He likewise lost his reason and understanding and in a few days he died. The surgeons of Paris were a long time troubled to dress the sad wounded people, I believe that you saw some of them. I beseech the great God of victories that we may never be employed in such evil encounters and diasters".*

In Ambroise Fare's writings upon surgery, he writes about the spinal marrow:

Fig. 1.6

Reduction of a dislocation of the spine by traction and direct manual and mechanical pressure. (Ambroise Pare, 1517-1590) [15.]



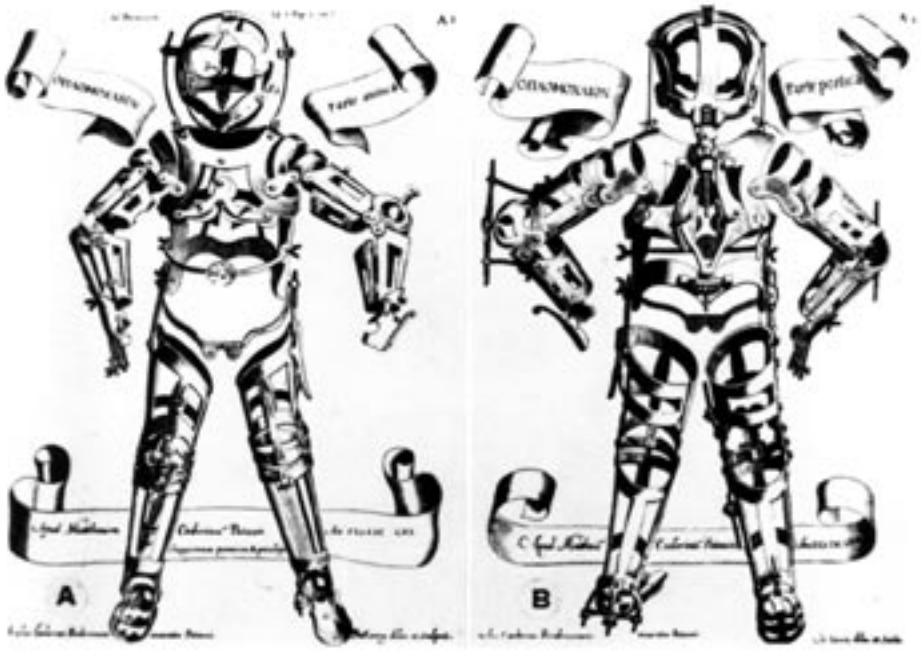
"The marrow of the backbone being pierced the patient is assaulted with a palsy or convulsion very suddainely and since the motion fails in the parts beneath it, the excrements of the bladder are either evacuated against the patient's will or else are altogether stopped ... Now because by hurting the spinal marrow men become lame sometimes of a legge and it is fit, you know, that the spinale marrow descends from the braine like a rivulet from the distribution of the nerves who might distribute sense and motion to all the parts under the head; wherefore if by hurting the spinale marrow the patient's arms or hands are resolved or nwnme or wholly without sense, it is a sign these nerves are hurt which come forth of the fifth, sixth, seventh vertebra of the necke. But if the same accident happens to the thigh, leg or foot with refrigeration so that the excrements flow unvoluntarily without the patient's knowledge or else are totally suppressed it is a sign that the sinewes which proceed from the vertebra of the loins and the holy-bone are hurt or infault; so that the animal faculty bestowing sense and motion upon the whole body and the benefit of opening and shutting to the sphinctermuscle of the bladder and fundament can not shew itself in these parts by which means suddaine death happens especially if there be difficulty of breathing therewith".

In some cases, with open wounds Pare did even perform an operation to remove all the splintered parts of the bone from the spinal marrow.

Ambroise Pare was inspired by Paul of Aegina's idea about surgery of the spine. He reviewed Paul's work of laminectomy for fractures producing compression of the spinal cord. He agreed with Paul in doing laminectomy and removing parts of bone that injured and the spinal cord.

Fig. 1.7

Surgical armor (front - left, and back - right). (Hieronymus Fabricius ab Aquapendente, c.1533-1619) [71.]

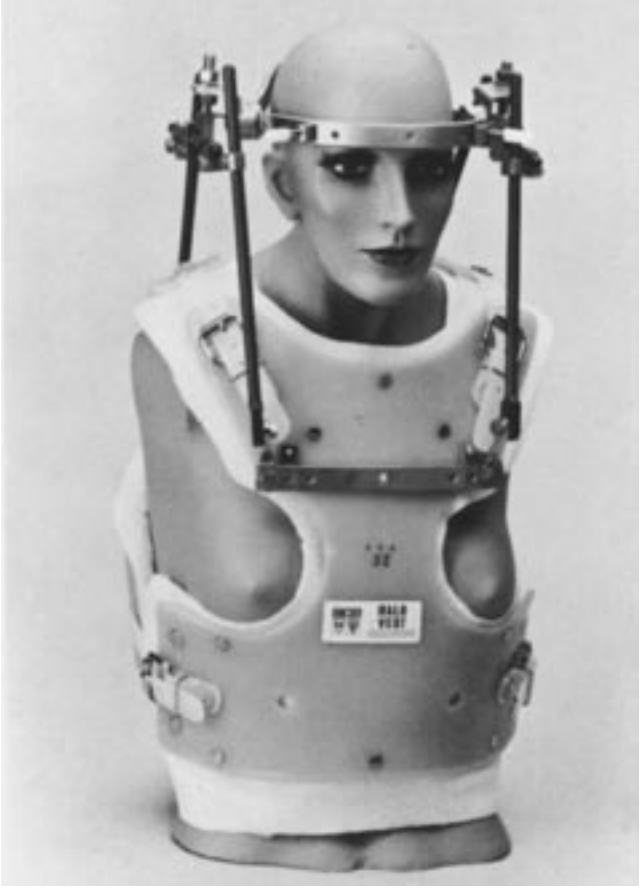


He did, howerer, make one modificattion to he operation: if the process was attached to the periosteum then the pieces should be set in the original position, it should be splinted and allowed to reunite. He also thought that if the spinous process was fractured, the fractured part should be removed because it would cause pain. Pare also gave his recommendations on how to reduce dislocation of the cervical spine as many others throughout the centuries had done, but he emphasized especially the great care to be taken when handling these cases. The picture shows how Pare uses the method of Hippocrates to reduce a dorsal and lumbar dislocation of the spine. The patient is placed in prone position on a table and has traction applied for extension of the dislocated part. At the same time the surgeon applies pressure with his hands an should this fail, he applies two small sticks of wood wrapped in cloths to either side of the vertebra being cautious not to press on the spinous processes. Afterwards the patient is placed on the back and kept in this position for a long period.

The surgical armor is fabricated by an Italian physiologist and anatomist. Fabricius ab Aquapendente. He was also a surgeon and interested in all kinds of braces.This illustration appears In his work Opera Chirurgica . In the description of the reduction of spinal gibbositas he writes [15]:

> *"One comes to the surgical operation which is done particularly with iron instruments and plates. They are made in such a way as to push the spine toward the part contrary to that toward which it has been projected, seizing this not violently but gently and gradually. So one puts under a rather long and round iron hollow all along in the shape of a screw which is inserted in a similar cavity and which can with a plate iron push the ribs and the spine toward the side contrary to the situation which they have acquired".*

Fig. 1.8
ACE ortopedic Halo-vest first presented by Perry and Nickel in 1959.



The equipment used today for cervical immobilisation, stabilization and slight traction, the Halo-vest. This is used when the patient is mobilized with no severe lesion of the spinal cord. Thus one can compare the evolution in stabilization equipment to be worn by the patient from the 16th century till now.

The Development Of Laminectomy

Hippocrates did not and could not distinguish between fractures of the vertebra and dislocations. Until the beginning of the nineteenth century fractures of the vertebra were confused with dislocations. In the beginning of the nineteenth century the surgeons eventually realized the mistake and wrote that in trauma of the spinal column, fractures almost always were present, and some even stressed that a dislocation without a fracture was impossible because of the strength of the muscles and the ligaments of the vertebra.

Paul of Aegina was the first to describe the treatment of a spinal injury with a laminectomy. The first surgeon who did a real laminectomy was Henry Cline in 1814 in London [218]. The patient died nine days later but since then it has been discussed whether laminectomy should be performed in treatment of fractures of the vertebra with or without spinal cord lesion. Each new case of fracture and lesion of the spine and spinal cord is still a subject of discussion whether or not one should operate to remove bone parts in order to relieve the pressure of the spinal cord with the risk of reducing the stabilization of the spine.

The first person to report on cervical spine fractures with traction and weight was the French neurosurgeon Bontecou in 1887. He put adhesive tape on the patient's face and fastened a cord over the top of the bed and put on 12 to 20 pounds weight. The patient lay with this for two weeks and after six months he had recovered from the fracture [26].

A breakthrough to guide the treatment of lesions of the spine appeared in 1895, when the German physicist Conrad Röntgen developed the röntgenography making it possible to take X-rays pictures of the injured part of the body [15].

References: 15, 26, 71, 116, 120, 149, 173, 192, 218.

2
Spinal Cord Regeneration in Animals and in Man. Human Operations 1889 -1967

Evidence of Spinal Cord Regeneration in Pigeons, Guinea Pigs and Rabbits

The French neurologist Charles Edouard Brown-Sequard (1817-1897) was the first to describe the regeneration of the spinal cord in pigeons where the spinal cord was completely transected. Three months after the transection, he found traces of voluntary movements and very brisk reflexes. The animal was killed and the spinal cord was examined under microscope. Together with Follin, Brown-Sequard found cells in the grey substance and nerve fibers apparently at the site of transection, but they also found that the quantity of nerve cells and fibers were much fewer than in the normal parts of the spinal cord.

Brown-Sequard continued his experiments, and in 1849 he published a great number of experiments made with guinea pigs, rabbit and pigeons [40,41]. He found that the pigeons were doing much better than the mammals which were all dead after six months. He found that the pigeons had sensation and voluntary movements of the legs, although to a much lesser degree than the control pigeons which were not operated upon. At the autopsy he found in two of the pigeons with returned functions that the ends of the spinal cord had reunited by a grey-reddish substance which was more consistent than the spinal cord, and he found it was transversed by white fibers.

In 1851, Brown-Sequard had another publication in the Gazette . He wrote that one pigeon, after complete transection of the spinal cord, had after 15 months returned to normal and was capable of running. At the autopsy he found that the spinal cord was covered with fibrous tissue. When this was removed the spinal cord was narrowed in an odd way. At the place of coaptation he found a trace of white tissue. In this he found cells and fibers which he described minutely.
His conclusion was:

1. The spinal cord is capable of producing scar tissue.

2. The function of the spinal cord can return to normal even after complete transverse section.

Since the first guinea pigs died a few months after transection of the spinal cord, he decided to make only a partial transection finding what we today would call the Brown-Sequard's syndrome, which is a paralysis of the motorneurons of the same side of the lesion and loss of pain and temperature on the opposite side of the lesion. Tactile sensibility is normal or slightly impaired on the same side. In 1892 Brown-Sequard wrote that the human spinal cord was able to regenerate too. He supported this theory with many minutely documented case stories of patients with spinal cord injury but he lacked histological evidence.

In 1899, Nageotte demonstrated fine nerve fibers going through the scar of a hemisected spinal cord in non-mammalians (in William F. Windle, Walther & Lockhart (eds.) (1955): "Regeneration in the Central Nervous System", p. 260). He believed that these nerve fibers represented an attempt to regenerate and his opinion was that the nerve fibers derived fromthe ventral roots [157,220].

Evidence of Spinal Cord Regeneration in Human Beings

After Myelorrhaphy

The first report I have been able to find about myelorrhaphy of the spinal cord in man is written in 1906 by Estes:

> *"I have done two complete and one partial excision of the spinal cord. As a matter of interest, though it is not important at all, I should like to state that my first case of complete resection -1 mean the resection of a section of the whole thickness of the cord - was done in October 1889.*
>
> *I made a complete resection of a disintegrated cord at the first lumbar vertebra, removed about three quarters of an inch of the cord, squared off the ends and brought them together with catgut sutures and sutured the dura mater over the united cord. The operation was done on the tenth day after an injury from indirect violence (the man fell from a high telegraph pole). The cord was lacerated entirely through and many fragments of the bone had been driven into the spinal canal. The man was considerably improved as regards the sensory and trophic disturbances but never regained the use of his lower limbs. I again resected about half the thickness of the cord in the lower dorsal region (ninth and tenth dorsal) which was lacerated and entirely disintegrated by sharp spiculae of bone. I left the anterior columns only intact, then I drew the ends of the lateral and posterior columns together by catgut sutures, and sutured the dura over the united ends. Sensory and trophic paralysis improved almost immediately. The man finally recovered the use of his lower extremity and the use of the flexors of the right extremity, so that with the aid of a steel and leather brace under the weak side he was able to walk with comparative ease. His sphincteres entirely recovered". (From Haynes' article 1906)* [113].

Another case from 1898 where the operation was carried out by W.D.Briggs in St. Louis and reported by T.F. Prewitt is as follows:

> *"A boy aged 17 had his spine crushed by a falling tree and symptoms of complete division of the cord followed. Laminectomy was performed on January 5th, 1898, three weeks after the injury; removal of the posterior arches of the ninth, tenth and eleventh dorsal vertebra. The cord had been completely severed at the level of the tenth dorsal vertebra. "I had once conceived the idea", writes Dr. Briggs, "of cutting off the ends of the cord and attempting to reunite them". Four sutures of fine catgut were passed transversally through the ends of the cord and membranes tied bringing the severed ends of the cord into apposition. There was gradual improvement in the boy's condition, partial control of the bladder, motion of the muscles to the knee in one limb and nearly the whole of the other limb"* [178].

This makes Prewitt ask in 1898 about the cases with severed spinal cord:

> *"Shall we leave them to survive or perish without attempting to improve their chances of recovery? Would it not be better to do a laminectomy, remove all foreign bodies, spiculae of bone, the bullet if possible and the devitalized tissue of the cord, thereby diminishing the dangers of meningitis and myelitis and promoting more prompt hea-ing of the wound? [178]".*

One of the most famous publications concerning the regeneration of the spinal cord in human beings is written by Richard Harte and Francis Stewart from Philadelphia in 1902. They were among the first to do myelorrhaphy (suturing of the spinal cord) which they described in the article called "A case of severed spinal cord in which myelorrhaphy was followed by partial return of function". They described a waitress Clara Nicholas of 26 years of age, who was admitted to Pennsylvania Hospital on January 21st in 1901 after having been shot twice with a 32 calibre revolver 111:"

> *One ball entered about one inch to the right of the seventh dorsal spine and passed directly into the spinal canal. There was immediate complete abolition of motion and sensation below a line transecting the lower part of the tenth dor sal spine at a point 3 inches above the umbilicus, the distance between the ensiform and the umbilicus being 5 inches. This line of demarcation was sharply defined and was superimposed by a belt ofhyperaesthesia reaching as high as the ensiform cartilage. Just before operation this layer of hyepraesthesia also became anaesthetic. The superficial and deep reflexes of the lower limbs could not be elicited ... Three hours after the accident the patient was etherized and an incision about 5 inches long made over the dorsal spines with the eighth dorsal spine for its centre. After dissecting back the*
> *muscles on either side the right lamina of the seventh dorsal vertebra was found to be crushed in, and the left lamina of the same vertebra fractured at its base. With the aid of forceps the spines and laminas of the seventh and the eighth dorsal vertebra were removed and the enter in the membranes, through which could be seen the bullet and a number of small fragments of bone lying between the ends of the severed spinal cord was exposed. After removing the bullet, the fragments of bone and dilacerated nervous tissue the distance between the segments of the cord was 3/4 of an inch ... The wound was flushed with salt solution and the ends of the cord approximated with three chromicized catgut sutures passed by means of a small staphylorrhaphy needle, one suture being passed antero-posteriorly through the entire thick ness of the cord and the other two being passed transversally. This part of the .operation was attended with unusual difficulties because of the narrow space in which suturing was conducted because of the consistency of the cord, and because of the wide interval between the fragments the catgut frequently tearing out before the ends were finally brought together. The dura mater could not be approximated. A small gauze drain which was allowed to remain 24 hours was carried down to the cord because of the oozing, the muscles were united with deep sutures of catgut and the skin closed with silkworm gut. The patient was in better condition after than before the operation. During the first 24 hours after operation there was a sharp pain over the front of the chest and in the vicinity of he wound".*

After this follows a very elaborate day by day description of the improvement of the patient. On the 42nd day it says:

> *"Pinpoint felt several inches below the iliac crest on each thigh; massage distinctly felt over the legs. Can voluntarily extend the right big toe and also the left leg slightly. Can tell when the bowels are going to move but has no control over them".*

The 60th day:

> *"Is out of bed in a wheeling chair; moves the right big toe quite readily and with great effort can feebly flex the knees".*

The 14 month:

> *"The patient takes a tub bath; can feel the water on the lower extremities and can distinguish between the water from the hot and cold spigots".*

The 16 month:

> *"The general health is as it always has been excellent. The patient voluntarily flexes the toes, flexes and extends the legs, flexes and extends the thighs and rotates the thighs. While sitting the extended leg can be raised from the floor, flexion is more powerful than extension and any movement is increased by strongly contracting the muscles of the upper extremities at the time of making effort to move the lower extremities. The patient slides out of bed into a chair by her own effort and is able to stand with either hand on the back of a chair thus supporting much of the weight of the body. The bowels move every second day and are under perfect control except for the presence of diarrhoea. About one pint of urine is passed three times during the 24 hours. There is sometimes incontinence during sleep. The menses are regular, preceded by sharp pains in the lower limbs and accompanied by cramps in the lower abdomen. The patient has the sense of touch, temperature, pain and position all over. The difference between heat and cold is not satisfactory elicited when small testtubes filled with hot and cold water are used... A pinprick can be localized as low as a line running transversally through a point 2 inches below the umbilicus. A pinprick can be differentiated from several pinpricks and the pinpricks from a sharp blow from a pencil as far as the knee but the localization of these sensations is not accurate. The muscles are moderately rigid, and there is present on both sides marked, but easily exhausted ankle and patella clonus. The deep reflexes elicited by tapping the tendo-achilles, the ligamentum patellae and the hamstring and may be reinforced by muscular exertion on the face and arms and by painful sensations such as a sharp pinch in the arms. On tickling the sole of the foot of the big toe flexes, the little toe abducts and there is a feeble contraction of the tibialis anticus, the hamstring muscles and the tensor vaginae femoris. The rectus abdominis reflex is seen on both sides of the abdomen. There are no reactions of degeneration and no trophic changes in the skin or nails".*

Harte and Stewart believed that this report was the first one given on myelorrhaphy performed in man. They write that in the cases in which every indication points to acomplete crush of the cord and in which recovery ensues, these cases are regarded by some neurologists as only partial lesions

because it is frequently impossible to diagnose a total transverse destruction of the cord unless the paralysis et cetera persists. If sensation and motion return, the question of regeneration has never even been thought of, but the return of function is considered as conclusive evidence that the axons were not cut or crushed. When the cord is divided or crushed the maxim of spinal surgery was "no interference", but who can say that the cord is divided without inspecting it? The axiom of spinal surgery was at that time "that compression and compression only without injury to the cord can be benefitted by operation", but Harte and Stewart concluded that abolition of motion, sensation and reflexes is no safe guide diagnosing severe destruction of the cord and if the cord is exposed and found severed why not suture it, no harm can be done and much good may be the results.

This article is of great value because it is one of the first evidences of myelorrhaphy in man. Many doctors have found it interesting and have tried to look up upon the patient to see if any further examination was made, or if any attending the operation or knowing Dr. Hart and Stewart could give some further information and evidence of what happened with the patient afterwards. Dr. Walther S. Lockhart and Dr. William F. Windle who both are considered to be among the founders of research of spinal cord regeneration, mentioned this patient at "the Bethesda-meeting of regeneration in the central nervous system" in 1954. In the biographical sketch of the book "Spinal Cord Reconstruction" edited by Kao, Bunge and Reier, published in 1983, Raleigh K. Pettegrew writes that perhaps no other person than William Frederic Windle has done so much to stimulate the research in the area of spinal cord injury and repair and to inspire students and colleagues to the dream that one day, research would provide the answers to what he called "The enigma of spinal cord regeneration" [128].

When Dr. William F. Windle in the fall of 1953 visited Philadelphia he found the opportunity to examine the original hospital records of the (previously mentioned) patient, Clara Nicholas, who was shot in a restaurant where she was a waitress. The restaurant was located two blocks away from the Pennsylvania Hospital where she was seen after the accident by the resident Dr. Charles Mitchel, who immediately summoned Dr. Stewart from his home not far away. Dr. Windle had the opportunity to talk to Dr. Charles Mitchel, who remembered the operation and recalled the observation of the complete transection of the spinal cord. The last entry of the patient on September 30th, 1903, says:

> *"Stands about 30 minutes with only knee-braces. Can feel in feet that she is standing. Has infected toe recently that was exquisitely tender".*

It is known that between 1906 and 1920 Clara Nicholas lost her ability to perform voluntary movements, and sensory perception disappeared below the level of the transection. In 1906, Stewart wrote a letter to Haynes where he indicated that the patient's condition had not changed since she was dismissed from the hospital in 1903. Dr. Cadwalader examined the patient in 1920, and his examination showed that she was completely paraplegic, so therefore he concluded that there must have been errors of observation and that:

> *"It can therefore be assumed that regeneration of the spinal cord does not take place after complete section and end to end suture"*

Many neurologists have of course been considering the mystery with this patient.

Especially one neurologist, Professor Spiller, suggested the presence of a spinal cord and two dural sheaths where only the one component had been injured. When Clara Nicholas died in the Pennsylvania Hospital on January 21st, 1924, Dr. Charles Mitchel gave permission for a partial autopsy, and he found a single spinal cord constricted by a dense scar tissue at the site of the end to end suture. This is exactly what Dr. Windle found in the chronic spinal cats where the spinal cord had been transected. The histological section showed no regenerative phenomena which is in accordance with the clinical findings. This did not prove though, that there had been no regeneration of spinal cord neurons in the early months and years after the accident. Windle writes that the current opinion is that the human spinal cord can not regenerate, and when the patient later recovers some function, this cannot be due to regeneration but must be a failure to have recognized a subtotal transection. This is still the current opinion in 1988, although it has been shown that the spinal cord can regenerate in mammals, which means that it probably also does regenerate in man, but this regeneration, to what extent it might be, is not a functional one.

Lockhart who in 1954 recalls the paper of Stewart and Hart summarizes that surgical apposition of spinal cords has been reported by Fowler in 1905, Lortat-Jacob, Girou and Ferrand in 1915, Collica in 1917 and Claude and L'hermitte in 1918, but there has been no acceptable method of spinal cord approximation [49, 78, 220].

Spinal Cord Transplantations from Dog to Man

In 1905 Dr. Shirres gives this following very interesting report in the service of Dr. Armstrong in the Montreal General Hospital. There is no case history of the patient or the trauma19 [196]:

It is a case of complete severance of the spinal cord with a gap of one half inch between the ends. No attempt was made to suture the divided ends at the time of the first operation as there was no hope that the patient would survive. After many months of treatment there was no return of the reflexes or changes in the flaccid state of the limbs. A few trophic or vasomotordistur bances appeared in the extremities eleven months after the injury. It was then decided to make an attempt to secure a regeneration between the ends of a severed cord by a second operation. A segment 3 inches long of the spinal cord of a large dog was placed along side of the severed ends of the patient's cord, the interval now between the ends having increased to 1 and 1 1/2 inches. No attempt was made to diminsh this gap. A few fine sutures united the pia arachnoid of the one to the other, the dura was closed, wound was sutured, and a plasterjacket applied.

The patient made a perfect recovery from this unique operation. A month passed without change but the electrical and massage treatment was carried out by Dr. Shirres and a corps of assistants as before. Dr. Shirres writes:

> *"By the fifth week the patient was conscious of flatus in the lower quadrant of the adomen. This he had never experienced before. Six days later he was conscious of the passage of the catheter and two days later he informed the orderly bowels were about to move and could tell when faeces passed the rectum (anus?). On this date he complained of sensations of pins and needles in the right foot and a week later of the same symptoms in the left. Two months after the operation he had subjective disturbances in both feet as high as theknees. Little other changes were noted until the 80th day after the opera-*

tion when for the first time with a pleximeter a certain amount of tone was noted in the muscles of the flexor surface,of the thigh and leg".

A month after this description the patient died from a large abscess in the right kidney. A post mortem examination of the spinal cord showed the following conditions:

"Above the site of the lesion there was a typical ascending degeneration of the columns of Goll and Burdach, the direct cerebellar and Gower's tracts. Section below the lesion showed definite degeneration in the direct and crossed pyramidal tracts. The dura and tissue at the site of the grafting experiment showed a mass of minute myelin sheaths of nerve fibers which laid closely adherent to the dura and one traces upward and downward united with the segment of the cord above and below, demonstrating the fact that regeneration of the axons of the spinal neurons had taken place to a limited extent. At the time of the operation the dura between the two segments was perfectly clear of nerve fibers to the naked eye. Sections of the lower segment showed to be in fairly healthy condition. In the cuada equina 90% of the nerve fibers were to all intents and purposes normal by the Pal-Weigert stain".

This is the only report of a spinal cord transplant from a dog to a human being, and as far as the results are concerned apparently there was an improvement in the neuorological status of the patient. So it seems that the first myelorrhaphy took place in 1889 and the first xeno-transplantation of spinal cord in 1902 - at the same time when Stewart and Harte [111] believed to be the first ones to do the myelorrhaphy of the spinal cord of human beings.

Operation and Treatment of the Patient With a Spinal Cord Lesion

In 1906 IS. Haynes published an article called: "Gunshot wounds of the spinal cord. A plea for early myelorrhaphy with report of a case of a bullet wound through the liver, spinal column and cord. Laparotomy, laminectomy, recovery" . Haynes refers especially to and quotes the works of Prewitt, Estes, Briggs, Stewart and Hart, and Shirres and Fowler. Haynes writes that it is primarily to advocate active interference in the worst cases of spinal injury and that he writes mainly of gunshots. He does, however, stress that all patients who survived the initial shock of the injury of the cord and its complications should be operated upon. He feels that the damaged region of the spinal canal should be laid open in order to examine the extent of the injury and be certain that the proper treatment is instituted. He refers to the earlier surgeons who have done myelorrhaphy, and states that in the instances of complete severance of the cord by a bullet, it has been shown, beyond any doubt, that the spinal cord does regenerate. The operation should take place at the earliest possible moment and in every case in order to avoid the bad effects of pressure degeneration.

Heynes describes carefully the operating procedure.This is very interesting since it was written 80 years ago and the operation is still performed in exactly the same way, if one chooses to operate upon a fractured spine with or without lesion of the spinal cord. What one does not do today is the myelorrhaphy. Haynes also describes the after treatment of the patients and even this is done in the same way today.

"A free median incision through the skin to the spinous processes, a clean severance of the tendons of the spinal muscles and a rapid stripping up of the muscle mass so as to bare completely the laminae up from 3 to 5 vertebrae. Pack the wound with hot gauze, compress for a few moments to check thefi'ee mostly venous hemorrhage; then with the muscles well retracted you will have a dry field. With a rongeur forceps bite off the spinous process of one vertebra to its base and open into the spinal cord. Gnaw away the lamina laterally with rongeur or cutting forceps, severe one lamina after the other until the spinal canal is open for the desired extent. Leave no sharp bony point. Osteoplastic methods take time and are not necessary as the spine is not appreciably weakened by this procedure. Deal with the conditions as you find them, with the minor details I will not delay you; open the dura for the full extent of the wound, place 2-4 traction sutures in its cut-edge; they work much better than clamps or tenaculae and are not in the way. Remove the bullet if present, spiculae of bone, foreign bodies as clothing and blood-cloths, the latter preferably with a stream of saline solution. By drawing on the traction sutures and lifting up (backward) the dura, the cord is brought into better view. If it is completely severed, treat it in a surgical way by carefully trimming of the bruised and lacerated ends (myelorrectomy), and then bringing them into coaptation by fine chromic catgut sutures (myelorrhaphy) introduced well away from the severed ends, so that in tieing them they will not cut their way through the soft cord. One suture is passed anteposteriorly through the centre of the cord, the other two from side to side; one near the ventral, the other near the dorsal surface of the cord. To aid in the coaptation of the ends use the expedient of extension of the head, thighs and trunk, which I have found in work on the cadaver assists this object very materially. In the upper dorsal and cervical region, greater morbility of the ends of the cord may be obtained by tearing off with a blunt hook the attachment to the dura of two pairs of the serrations of the dental ligaments above and below the site of the lesion.

Tie the sutures in the cord, smooth out the mushroomed ends of the cord (in a severed cord the ends form "mushroom" contraction ofthepia arachnoid; after suture this tendency still exists but can be counteracted in a measure by close application of the dura as suggested) and over them adjust the dura closely by three interrupted sutures. Close the rest of the dural opening loosely so as to allow an escape of any excess ofexsudate or cerebrospinal fluid. Drain from the dura outward with strands of silkworm gut or strips of rubber tissue for 24 hours. Suture the muscular mass with interrupted and the skin with continuous catgut. Apply a moderate dressing. Maintain the trunk fully extended until the operation has been completed and a plasterjacket has been applied".

Human Cadaver Studies

Haynes then refers to his work on cadavers. On cadavers that have been frozen in the morgue and injected with arsenic and formaline solution, the spinal cord was exposed and a block of the spinal cord was removed. Haynes found that the ends of the cord could be retracted over an inch with a trunk in flexion. Three sutures were then passed through the spinal cord as recommended by Stewart and Hart and Fowler and as described in theoperation-procedure above. With the trunk in the flexed position coaptation could not be obtained of less than half an inch, but by placing blocks to extend the head, thighs and trunk the severed ends of the cord came together nicely. The

effects were then noted of a change of position. The thighs were dropped to the table in a semi-flexion, this made no change; the head was allowed to drop in a flexed position and there was still apposition of the ends; but as soon as the body was placed flat on the table the ends of the cord began to pull away until contact was preserved only over the central part of the fragments. Fixation of the spine in a fully extended position Haynes considered a very essential factor in maintaining coaptation until healing took place. This is exactly what Alf Breig, Michel Renard, Stanislaw Stefanko and Catherine Renard state in their article "Healing of the severed spinal cord by biochemical relaxation and surgical immobilization" in Anatomia Clinica in 1982 [35], a little less than 80 years after Haynes' experiments on cadavers. Breig et al. used dog cadavers for their experiments.

Patient Care

The healing in Haynes' patient was thought to take place in 2-3 weeks after which the stabilizing jacket could be removed. The patient should be kept flat on a waterbed if available, and active electrical stimulation with massage and passive motion of the trunk and lower extremities should be undertaken. Retention apperators should be employed to prevent deformities in the limbs and the bladder should be regularly catherized and irrigated under strict aseptic precautions to prevent a surgical kidney. Everything else for the preservation of strength, nutrition and comfort should be utilized. As soon as possible the patient should get out of bed and be coached to attempt to use his muscles. The stimulation to regeneration from voluntary efforts is very considerable.

Eighty years ago Haynes recommended to use a waterbed, and this is still today the best way to prevent bedsores. Thus, the postoperative treatment is the same, the mortality rate, however, is not. The mortality rate for Haynes was 42.5% for the patients who had undergone operation; for those who had not been operated upon 69.25% died. Today a patient with a spinal cord injury does not die from the lesion of the spinal cord or the neurological defects that follow the lesion. The overall technique is so much better today and the patients do not die from the wound, visceral complications, hemorrhage, septic infections, bedsores, abscesses or what is called a surgical kidney. Such complications were the most frequent causes of death. Today also nursing has vigorously improved.

A Rise Against Myelorrhaphy

In a report from 1918, Henri Claude and Jean L'hermitte [49] described a case in which the spinal cord was completely severed and a myelorrhaphy was carried out. The patient survived for eight months and a post mortem autopsy of the spinal cord was performed. The patient was a 22-year-old soldier, Fernand Gant, who was hit by shrapnel in the dorsal region at the tenth thoracical vertebra. The day after the accident a laminectomy was performed and a piece of shrapnel was removed from the spinal cord and a meningo-myelorrhaphy carried out. Four months later it is described how the patient was paraplegic with complete anaesthesia of the right side from Th.6 and of the left side from Th.10 and distally. There were no tendon reflexes in the legs and there was complete retention of urine. Six months later spontaneous automatic movements of the legs were seen. After eight months the patient died from diarrhoe, loss of weigh and hypothermia.

A post mortem autopsy was performed and this showed that the laminectomy had been carried out at the level of TH.8 and at that site, the dorsal part of the spinal cord was totally adherent to

the vertebral column. The meninges were narrowed, and between the two ends of the normal looking spinal cord a block of hard, fibrous tissue having the consistence of cartilage was found. The spinal cord next to the fibrous mass was soft and changed, the grey substance was totally replaced by scar tissue and cysts. Between the two ends of the spinal cord there was absolutely no evidence of nervous tissue, but only homogenous fibrous tissue with small narrow tracts of collagene tissues in which many vessels were seen. Distal to the scar, the central canal was found surrounded by proliferating nervous tissue. After the twelfth dorsal segment the spinal cord began to appear normal and so did the anterior and posterior roots. In short, the most pronounced changes were in the ninth dorsal segment, the tenth segment was replaced by a fibrous scar where the sutures were placed. The eleventh segment was soft and without any functional value; around the twelfth segment the architecture of the spinal cord began to look more normal.

They conclude that the reappearance of knee-jerk reflexes after six months does not mean that it is a sign of an incomplete spinal cord lesion, and the voluntary movements are assumed to be automatic reflexes from the distal part of the intact spinal cord and roots beneath the lesion. The sensation and paresthesia which patients sometimes can feel are considered to be hallucinations of the same kind as those experienced by persons who have had a leg and an arm amputated.

Henri Claude and Jean L'hermitte do not advise to use myelorrhaphy concluding that it will only leave the patients with a disillusion.

Spinal Cord Transplant in Man

For a long period nothing is heard of myelorrhaphy in human beings. In the following report from 1944 in Experimental Medicine and Sugery, there is a paper on human spinal cord transplant, written by Dean Woolsey et al [228]. This paper is inspired by Le Gross Clark and Towers' work on the regenerative capacity of mammalian central neurons. At the time it was already known that regrowth of peripheral nerve fibers could take place, and the supply of a bridge in a nerve defect of the peripheral nerve would encourage the regrowing of nerve fibers. With this in mind, Woolsey et al. report on the following:

A 16-year-old male was shot in the right shoulder. He fell to the ground immediately and was unable to get up. He did not lose consciousness. He was admitted to the St. Louis County Hospital on the 14th of June, 1942, shortly after the accident. At the physical examination, there was no action of the intercostal muscles and he was unable to move this trunk and lower extremities. At X-ray examination, a bullet embedded in the spine was found just to the left of the midline of the upper border of the fourth thoracic vertebra. After the injury the patient's condition deteriorated and the course was progressively downhill with tremendous decubitus, ulcers and high fever. On the 31st of June, 1942, the patient had a spinal cord transplant operation.

The operation was performed as follows, quoted directly from the original paper:

"Under drop ether anaesthesia with iodine and alcohol preparation a midline incision was made over the spines of the first three thoracic vertebra. Laminectomy was carried out in a classical fashion with subperiostal dissection of muscle and removal of spines and lamina. Exposure revealed a gross, ragged defect in the right pedicles of the third and fourth thoracic vertebra. The anterior 2/3 of the dural cylinder was severed at this level. The bullet was embedded at the base of the left pedicle of the third vertebra and projected downward into the intervertebral space. The spinal

cord was completely transected with superior and inferior segments retracted and separated except for ragged connective tissue bridge incorporating disorganized softened nervous tissue. The ends of the cord were freed and cut with a sharp knife at which time they retracted further leaving a gap of approximately 3 inches. Abed of cellophane was prepared in the vertebral canal and fastened with Cushing's clips. A piece of spinal cord previously prepared and sterilized was fitted to the gap and placed in apposition to the cut-ends with acacia glue (after Rezende). The transplanted piece had been removed at the autopsy two weeks previously and had been prepared as follows: fixation by immersion in 10% formaline for twelve days; running tap water was overnight; repeated immersion, washes in distilled water for eight hours; immersion in 70% alcohol for sterilization and keeping. The morning of the operation the piece was washed in distilled water and delivered to the operating room in sterile saline. The cellophane cylinder was closed over the posterior aspect of the transplant with the clips; muscles were closed in midline with three layers of interrupted silk sutures: silver foil; gauze-dressing.

> *Postoperative course. - The operative wound healedperfectly without reaction. Laboratory work is non-contributory. The patient's condition remained unchanged. An autopsy was obtained at death of the patient on the 20th of November, 1942, 5 months and 6 days after admission and 3 months and 21 days after operation".*

An autopsy was performed and about the transplanted area it says:

> *"The operative defect is palpable but firm and clean. The vertebral column and operative site are exposed by posterior incision. The defect is limited posteriorly by skin and subjacent nonmuscular tissue of approximately 1 cm thickness. Superiorly the end of the transplanted piece lies free and the space is limited by a transverse septum of false dura which closes the defect at the extremity except for an ostium of approximately 0.5 cm diameter in the centre. The cellophane bed has remained in place and there is no gross evidence of tissue reaction to its presence. The inferior end of the transplanted piece is fused to the superior end of the distal parent segment of spinal cord. A dural or false dural expansion covers the fusion incorporating the lower part of the cellophane bed and fixed both parent and transplanted piece firmly to the dura anteriorly. The superior segment of the spinal cord is fixed firmly to the septum, limiting the upper end of the defect and as well to the sides and anterior walls of the vertebral canal. The dura of this segment is thickened and separates with difficulty down to the defect."*

The microscopic examination was carefully made and figures with Swank-Davenport and Protragold stains are shown.

> *"In the transverse sections through the spinal cord above and below the transplant showed complete degeneration of ascending and descending fiber tracks. In the longitudinal section of the transplanted piece there was demonstrated unusual preservation of both grey and white matter, even though the piece had lain as a foreign body within the host vertebral canal for 3 months and 21 days. The only dissolution sight of this tissue was at the junction with the lower segment of the viable spinal cord. At this place the grafted piece was torn down by macro/ages at the junction with encroaching host tissues. The histology at the union is characterized by a jumble of disorganized tissue in*

which a definite process of oriented regrowth can be recognized. There was growth cones and neurofibrils oriented toward the graft and a viable nerve cell body with processes. These processes continued up to and within the transplant. Fat granule cell reaction was evident at the margins of the invaded graft and vascularization of the growth channel was also apparent. There was peripheral nerve tissue presumably deriving from the spinal roots and incorporated at the junction between host and transplant".

In the discussion, the authors write that in the matter of choice of graft material there seems to be a general agreement that predegenerated peripheral nerve tissue is best suited experimentally, but in human surgery a substitute must be sought for. In the future there will unquestionably be prepared graft material available for immediate use. The authors do not draw any conclusions from their description of the microscopic findings. The interpretation of the findings was difficult and they hesitate to draw any conclusions, however they state that the following conclusions are justified:

"1. The capability of central neurons to regenerate is confirmed in human material; 2. Regrowth in the human spinal cord may be oriented to transplanted formalinized spinal cord; 3. Formalinized spinal cord will maintain good preservation as a transplant for a period of months; 4. Regrowing central nervous tissue appears to follow a vascularized connective tissue tract; and 5. Dissolution of transplant material occurs at its junction with regenerating tissue".

In my opinion the reason why the transplanted piece is so correctly preserved is because it was fixed by immersion in 10% formaline for twelve days, before it was transplanted into the spinal cord. The outgrowth of the processes and neurofibrils oriented towards the graft will always be seen in a cut spinal cord because of the attempt to regenerate. The question is, why does the regeneration stop at a certain point in the central nervous tissue?

There has been no further reports on transplantation of the spinal cord in which the word transplantation has been used correctly. There are papers like O. R. Hyndmann's on: "Transplantation of the spinal cord; the problem of cyphoscoliosis with cord signs" [122]. J. G. Love and H. R. Erb's paper: "Transplantation of the spinal cord for paraplegia secondary to Pott's disease of the spinal column" [148], and J. G. Love's paper from 1956: "Transplantation of the spinal cord for the relief of paraplegia" [147]. What they have been doing, however, is an anterior decompression of the spinal cord in the management of Pott's paraplegia. In cases of paraplegia secondary to scoliosis it was found that compression of the cord was caused by a tight dura mater. The compression was relieved by opening the dura on the convex side of the curve, and occassionally rhizotomy with or without severance of teeth of the dentate ligament was performed in order to provide maximum freedom of the cord. What the authors called a transplantation is actually a decompression and a anterior transposition of the spinal cord. This is not a transplantation.

Intercostal Spinal Nerve Anastomosis to the Spinal Cord

L.W. Freeman [80,81,82,83] was the first to do an intercostal spinal nerve anastomosis to the spinal cord. The operation was carried out in 1951 when a 33-year-old male was admitted to the Robert Long Hospital of the Indiana University Medical Center. The patient suffered from a gunshot wound inflicted by a police officer, with immediate paralysis of both legs. Two days after the gun-

shot, the missile was removed and a laminectomy of the tenth and eleventh thoracic vertebra was performed. The missile had completely transected the spinal cord. After five months there was no change in the neurological status, and the patient volunteered for an experimental procedure that might restore the neurological function of his legs. He stated that since he had wronged society he wanted to make retribution. He was granted amnesty from his prison sentence by the proper officials and admission to the Robert Long Hospital was arranged. At the time of operation he was paraplegic and all sensory modalities were lost below the umbilicus. He had withdrawal reflexes in the lower limbs.

An operation was performed on August 27th, 1951:

> *"The old laminectomy wound was reopened and extended upward through the eight thoracic vertebra and downward through the second lumbar vertebra. At the thoracic eighth and ninth vertebral levels there was dense scar replacing the spinal cord. Oblique skin incisions were made over the ninth ribs posterior laterally. Through these incisions the eighth and tenth intercostal nerves were freed laterally and were sectioned at the axillary line. These nerves with their central connections intact were brought back paravertebrally and into the spinal canal. Two of the four nerves were anastomosed with autologous plasma clot to the distal stumps of the severed sacral nerve roots. In diameters of the proximal and distal neural elements were about the same. The other two nerves were implanted into the cephalad end of the conus medullaris through small midline stab wounds and were held in place by loosely tied 6-0 black silk sutures passed through them and through the thickenedpia mater".*

The postoperative result was poor. There was no obvious improvement of his neurological status, and when his wife decided to divorce him he refused to leave his bed for therapy or to participate in the self care program. He died on January 6th, 1952. Since it was Sunday morning and a coroner's case, the dissection was restricted to reopening of the laminectomy wound through which most of the cord from Th.8 to L 4 was obtained. Pathological report:

"Gross decryption of the specimen recorded at that time reads as follows: The specimen consists of 14 cm of spinal cord with the upper most spinal nerve roots being the eighth thoracic. The cross sectional area of the spinal cord at the upper most portion measures 8 x 12 mm. Several mm below the tenth thoracic nerve root the cord narrows to a hard scar which measures 4 x6 mm.

> *This area extends over a length of 18 mm of cord. The second, third and fourth sacral spinal nerve roots are absent from the conns. One of the intercostal-spinal nerve roots anastomosis has poor continuity of the sacral roots but the other appears to have a fine branched continuation. One of the intercostal nerves is seen to enter the conus medullaris and the other cannot be found in the specimen. Microscopically the most successful nerve root anastomosis revealed many axons traversing the area of suture. Despite the distorsion secondary to fixation, most of the serial sections show continuity of axons from the proximal intercostal nerve to the sacral roots. Indeed, it was necessary to make constant reference to sections where the anastomosis was clearly evident to maintain orientation. The sections through the conus medullaris showed liberal growth axons from the intercostal nerve into the substance of the spinal cord "* [30].

In experimental animal studies, fibers of root origin grow into the divided distal stump of spinal cord and establish functional connections. The assumption is that implanted fibers may act as secondary interneurons which then could be utilized to control distal function. It has been shown that function can be demonstrated by neurophysiologic means and that sections of the implants will return the animal to the paraplegic state. Freeman has several articles about functional regeneration of spinal nerve roots, the first from 1949 [80], later in1952 [82] and 1958, where Turbes and Freeman [210] showed that histologically nerve fibers and Schwann-like cells extended from the distal end of the inserted peripheral nerve into the isolated distal segment of the spinal cord. Schwann cells can myelinate axons within the central nervous system, especially following spinal cord trauma, but it has also been seen in other conditions. Raine showed in 1975 [182] that Schwann cells even occurred in the normal central nervous system. In his work, he says that Adelman and Aronson in 1972 found that 40% of otherwise normal human spinal cord from a large series of autopsies contained Schwann cells (Schwannosis) which might imply this phenomenon as being more prevalent than previously suspected. Later on, Kao [127, 130] transplanted the sciatic nerve to the transected spinal cord to obtain structural continuity of spinal cord, and he concludes that nerve grafting is a feasible therapeutic approach to a treatment of spinal cord trauma in which loss of tissue has occurred. The experiments still go on, and lately Fernandez, Pallini, Maira and Rossi (in 1985) [76] made experiments of peripheral nerve autografts into the injured spinal cord of a rat. They found that the glial and connective scarring at the site of insertion of the nerve into the spinal cord and the degree of cystic cavitation cannot be avoided but it can be reduced. They found regenerated axons in the nerve grafts by passing the transversed spinal cord lesion, and some of the axons could be
followed for a short distance into the white matter of the spinal cord both rostrally and caudally to the site of grafting. They found no improvement in the neurological deficit due to the spinal cord lesion.

To my knowledge peripheral nerve transplants into the spinal cord have been done in man since Freemann did the first in 1951, however they have not been published.

In 1967, Dana M. Street [202] gave a paper at the Annual
Clinical Spinal Cord Injury
Conference on traumatic paraplegia treated by vertebral resection, excision of spinal cord lesion, suture of the spinal and interbody fusion. She found that the problem in suturing the spinal cord was that of tension of the sutureline. To avoid this problem, Dr. Street shortened the spinal column by excising a vertebra in order to provide enough slack in the cord for excision of the scarred portion and resuture without tension. The first attempt was made on dogs but the three dogs operated upon all died. Four cases of traumatic spinal cord injury with complete paraplegia were presented. One of the patients was a 20-year-old male who had been involved in a motorbike accident in which he sustained a compression fracture of the ninth thoracic vertebra. He was totally paraplegic with a sensory level at Th.8 and loss of bowel and bladder function. Three years after the accident, he underwent surgery. He was found to have an anterior dislocation of Th.8 on Th.9 with a compression of Th.9.

The operation was performed as follows:

"This area was exposed through a midline incision from Th.6 to Th.ll spinous processes, a distance of 6 inches. The transverse processes of Th.8, Th.9 and Th. 10 were exposed. The transverse process of Th.9 on the left was resected along with the proximal inch of the ninth rib. The left lateral 113 of the body of Th.9 was exposed and resected with an osteotom. The same was then accomplished on the right, after which the central portion of the body was excised, and the anterior portion finally removed with a Kerrision rongeur. What remained of the intervertebral discs were removed with the end plates of Th.8andTh.10.

An anterior downward projecting break of Th.8 was excised, after which the back was hyperextended, thereby producing slack in the spinal cord. Sutures of no. 18 stainless steel wire were passed around the transversed processes of Th.8 and Th.10 bilaterally. An inch ofthelamina of Th.8 was then excised. The scarred area of the cord was found to measure 3/8 of an inch in length. The dura was adherent in this area and the cord was constricted. The cord above and below measured 7/16 of an inch in width by an inch in thickness, while at the scarred area, it was 3/8 of an inch wide and 3/16 of an inch thick. This scarred area for a distance of 3/8 of an inch was excised by cutting transversally above and below. It was found to cut with a gritty feel. Both ends of the cord were observed to bleed and the circulation appeared to be intact. The cut surfaces were reapproximated with 5-0 cardiac silk sutures, one placed at each side and one madras suture in the sagittal plane in the midline. After placing the silk sutures, the wire sutures were tightened and silk sutures in the cord were then tight. A third wire suture was then placed encircling the spinous process of Th.8 and Th.10. This suture passed through the lamina of Th.8 on each side of the spinous process close to the spinous process. A slight step appeared in the approximation of the tenth vertebra being slightly anterior; therefore 114 inch up with the lamina of Th.10 was excised. Gelfoam was placed posterior to the cord and the excision closed. A 114 inchpenrose wick was left extending to the depth of the exposure. The anaesthesia time for this procedure was nine hours and the patient received six units of whole blood".

The excised specimen of cord as described by the pathologist measured 2x2x1 cm. It was firm, rubbery and of bluish colour. It appeared to be dense fibrous collagenous granualation tissue enclosing some nerve bundles and a small fragment of degenerated nerve tissue resembling the spinal cord. In the postoperative care a well-padded plastershell or a Striker frame was recommended as a supplementary fixation. In the discussion, Dr. Street emphasizes that it is difficult to suture the spinal cord. Ideally, one would use no sutures at all or perhaps a fibrine dot; this would require total absence of tension in order to keep the cut surfaces in apposition. Approximation of vessels with microtechnique is also suggested and the anastomosis of the cord should be shielded from the invaison of fibroblasts by wrapping with some kind of membrane, perhaps the use of millipore membrane as used by Bassett in his experiments upon cats.

In summary, Estes in 1889 was the first to perform a myelorrhaphy. In 1902 Shirres did a spinal cord transplant from dog to man, and Hart and Stewart made what they thought was the first myelorrhaphy in 1902. Claude and L'hermitte in 1918 advised against myelorrhaphy and since then, Dana Street is the first to recommend it again in 1967. Woolsey did the first spinal cord transplant in man in 1942, and Freeman did the first intercostal nerve transplant in the spinal cord in 1951.

To operate or not to operate that is the question! It has always been and will always be as far as fractures of the vertebral column with or without a spinal cord lesion are concerned. Today the tendency is not to operate if there is a complete spinal cord lesion. The main point is that if one performs a laminectomy and removes the pieces of bone, the site of fracture will not become stable. If there is a partial lesion of the spinal cord and an X-ray or CT has shown that pieces of bone are affecting the spinal cord, operation is generally recommended. With our present knowledge, myelorrhaphy in these patients is now obsolete. If the fracture is unstable orthopedic procedures will take place to stabilize the site of the fracture.

The overall view is mainly to be as conservative as possible, but this view is now changing again. What is treated today is the position of the bones and not really the spinal cord. One thing, however, is certain: if an axonal outgrowth over the lesion in the spinal cord is desired, traction should definitely not be applied - on the contrary, the opposite procedure should be done as suggested by Alf Breig. A close approximation of the severed stumps should be attempted to promote axonal regeneration induced by a fetal spinal cord transplant or an axonal growth factor.

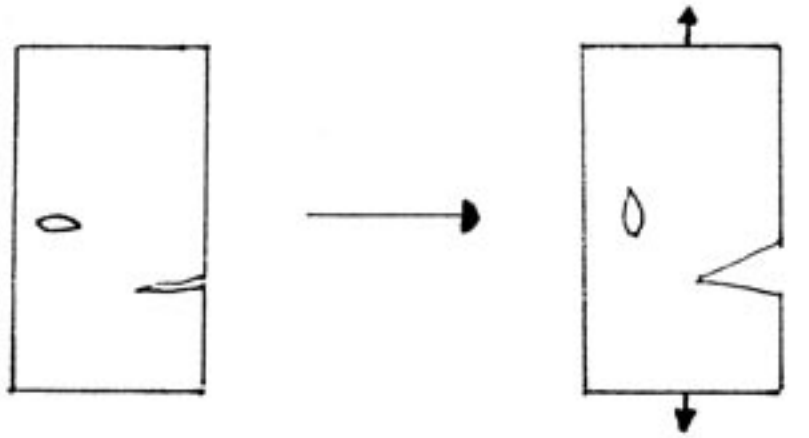
References: 35, 39,40,41,49, 69, 70,75, 76, 78, 80, 81, 82, 83, 111, 113, 118, 122, 127, 128, 130, 144, 147, 148, 157, 182, 196, 202, 210, 220, 228.

3
Orthopedic Procedures within Spinal Cord Surgery

Orthopedic Procedures within Spinal Cord Surgery

Alf Breig et al. (1982) [35] have suggested to abandon the treatment of skull traction on patients with fresh spinal cord injuries to avoid the wound surfaces from drawing apart, forming an open gap. He suggests that spinal cord lesions are treated like lesions of peripheral nerves, where the cut surfaces are brought together in a long-lasting close and tensionless contact. The rationale is that axonal sprouting and regeneration can more easily take place if the cutends are brought into close contact, and that the autonomy described by Ramon Y Cajal (1928) [123] will not occur, thereby avoiding the development of scar tissue and necrotic cavities. Alf Breig et al. do not believe that the cystlike cavities found in the spinal cord after spinal cord lesions contain lysosomal enzymes. They state, but they have not proved, that living tissue in any part of the body is normally protected from damage by the response of protective biological mechanisms. Lysosomal enzymes do not become active before the tissue pH has fallen substantially, which does not until there is a considerable rate of necrosis. Instead, they tend to presume that the cavities are produced by elastic stretching of the cord as shown in figure 3.1. By measuring the spinal cord of fresh human adult cadavers, they have found that the difference in the length of the spinal cord between maximum extension and full flexion is 5 to 7 cm.

Furthermore, Alf Breig et al. [35], studied how the intramedullary wound surfaces can be brought into a close, long-lasting tensionless contact which would promote the primary healing and outgrowth of fibers without formation of cavities or scar tissue. To study this, they made hemitransections of the spinal cord in dogs and used surgical orthopedic procedures to immobilize the spinal cord.



from Alf BREIG et al.

They conclude that the many sprouts in the histological section at the site of transection in the immobilized dogs were the result of the close contact of the wound surfaces and early capillary outgrowth in the well-oxygenized tissue with possible activation of neurotrophic substances. As for grafting they have no confidence in the ideas of positive effects of delayed grafting.

Even though the theory may be correct, it has not been possible to apply this to rats, as shown in my own experiments figures 3.2 to 3.5. In figure 3.2 a spinal cord lesion of a rat without immobilization is shown. Gliar scar tissue is seen to occupy most of the lesion site. Figure 3.3 to 3.5 show the spinal cord of a rat in which a complete spinal transection was made and the spine subsequently was immobilized by a steel-wire introduced into the spinal processes of the adjacent laminas to the laminectomy. In this case, there is no evidence of axonal sprouting but only invasion of scar tissue from the periphery, probably Schwann cells.

In conclusion, close contact and immobilization are essential in spinal cord lesions when a transplantion is carried out.

Immobilization of the spine in human beings can be carried out in numerous ways depending on the type of fractures and the equipment available in the different departments.

For the stabilization of the cervical spine one can perform a spondylodesis ad modum Cloward with removal of the fractured vertebral body and bony stumps affecting the spinal cord. A Bob plug in completed or autograft mixed with tissue. The operation with the Roy-Camille silver plate fixed on staples or the Casper plates with screws. See fig. 3.6. for the posterior fixation in the cervical spine. The Halifax inter-laminar clamps can be used with autograft bony fusion between the laminas. (See fig. 3.7.) Normally the anterior spondylodesis and fixation is more than sufficient and no external fixation is needed in the postoperative course.

For thoracical and lumbar fractures, the vertebral body is corrected in shape and place as well as possible. Bony stumps are removed and the dura sutured with or without dura replacement for example a fascia autograft. A dura glue should always be used to avoid cerebrospinal fluid leak, especially when the needle has passed through the dura and a flattened piece of muscle should be applied over the sutures and the glue to keep it all waterproof and tight. For fractures over just one or two vertebral levels, Dick's internal fixator for the spine can be used, see fig. 3.8.

If a longer fixation is needed, the Harrington rods are used at times combined with the Luque wire technique.

By using these fixation techniques it gives the patient the possibility of almost immediate maximum capacity of physical training and rehabilitation, and no external fixation is needed postoperatively, at least in the case of cervical fractures. Thus the Halo-vest is about to become obsolete.

References: 17, 23, 35, 50, 87, 100, 120, 126, 151, 155, 181, 206.

Own Experiments

Fig. 3.2

Transverse spinal cord lesion in a rat. Survival time 10 days. The rat spine was nonimmobilized. There is an intense growth of glial tissue scar formation within the spinal cord. (Thionin x 20).

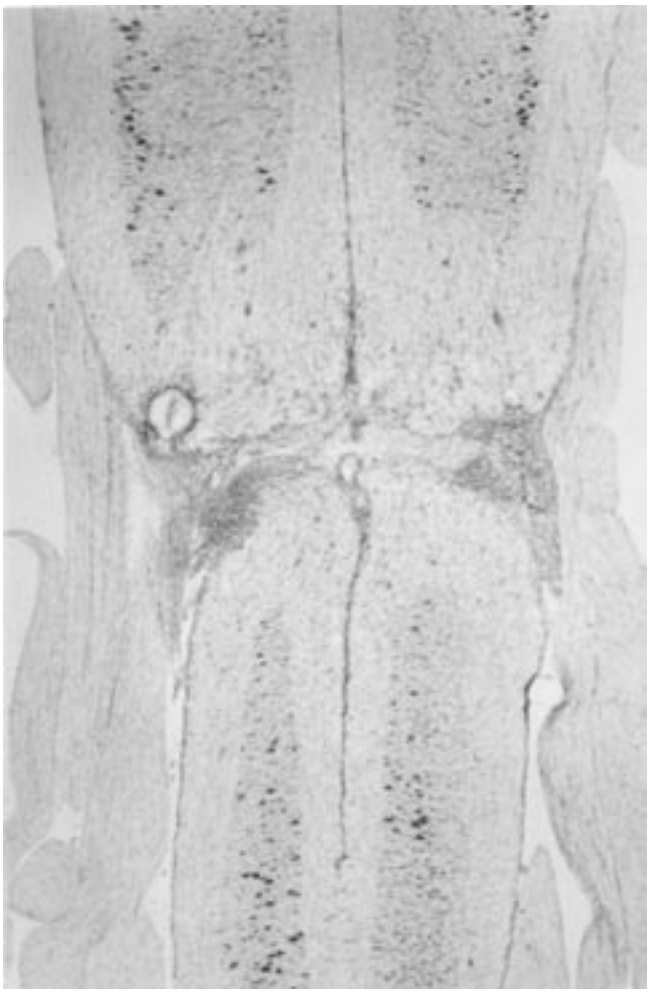


Fig. 3.3

Transverse spinal cord lesion in a rat. Survival time 10 days. The rat spine was immobilized with steel-wires that had been forced through the spinal processes of the lamina on each side of the laminectomy. It seems as if the scar tissue comes from the periphery and grows into the spinal cord. There is no evidence of axonal outgrowth passing the site of transection. (Thionin x 20).

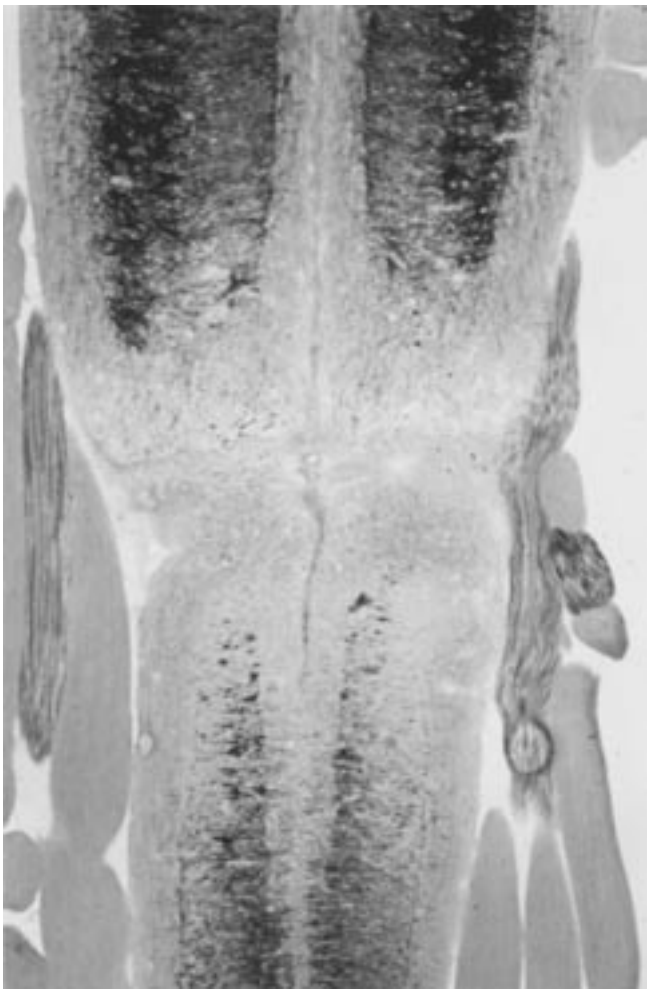


Fig. 3.4

The same as shown in fig. 3.3, but in an ACHE stained section. No ACHE-containing axons transverse the transection. (This can be compared to the ACHE containing axons in the roots (R) lying along the spinal cord) (x 20).

Fig. 3.5

The same as shown in fig. 3.3 and fig. 3.4 in a Fink-Heimer stained section. There are no degenerated fibers at the site of transection, but old degeneration of fibers are seen in the caudal part of the cord which is also diminished. (Cranical upwards - caudal downwards), (x 20).

Fig. 3.6 *Roy-Camille*

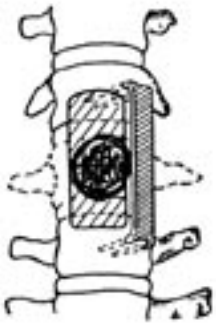


Fig. 3.7 *Halifax*

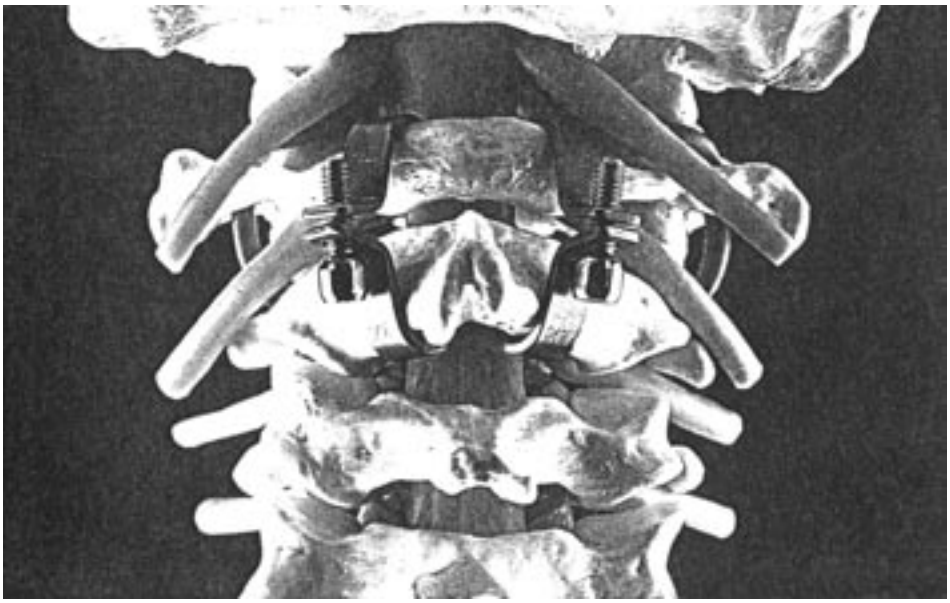
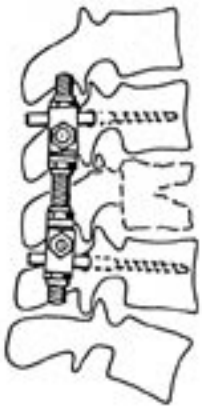


Fig 3.8 *Dick*



4
Anatomy

A Comparative Study of the Spinal Cord in Rat and in Man with Some Corrections to the Anatomy of the Meninges

The goal of this chapter which deals with the gross anatomy and histology of the spinal cord of the rat and man is to clarify some anatomical details and differences causing confusion when textbooks are compared to reality. When the results of research are compared, it is important that the structures discussed by scientists be the same.

Finally, a comparison between the motor functions of the spinal cord of the rat and in man is described based upon literature.

When a laminectomy is performed in man, the epidural space containing epidural fat and veins are directly exposed. Underneath is the dural sac. The dura is a one mm thick white tough, non-translucent membrane containing vessels. It consists of dense connective tissue built up by collagen and elastic fibers. When the dura is cut open longitudinally (figs. 4.1, 4.2 and 4.4) the arachnoid membrane is seen. The arachnoid is a thin, glossy, translucent avascular membrane consisting of a few layers of cells. The cells are flat with a large pale oval nuclei. The arachnoid in man is especially well demonstrated in figure 4.2. When the arachnoid is perforated and opened carefully to avoid hemorrhage from the vessel-containing pia, cerebrospinal fluid pours out from the subarachnoidal space. The space is not built up by trabeculae (not along the spinal cord according to Leeson and Leeson (1967) [143]. The cord is suspended in the dentate ligaments passing from the pia to the dura, and is kept in place by the roots emerging from the dorsal and ventral horns of the cord. The subarachnoidal space is thus a continuous cerebrospinal fluid-containing space without trabeculae. This is how the space appears when it is looked upon with the naked eye during open surgery. The only place in the arachnoid membrane where vessels appear is where the arachnoid cross the fissurian sylvii of the brain. Here the vessels run along the arachnoid and a meshwork of trabeculae is seen between the temporal and frontal lobe.

When a spinal laminectomy is performed in the rat, the arachnoid is directly exposed (fig. 4.3). There is no obvious free dura. The dura is adherent to and removed with the bone when the laminectomy is done. Fig. 4.3, which is from a rat, should be compared with figs.4.2 and 4.4 which show the human arachnoid. The non-vascular, translucent, glossymembrane is seen clearly in all the figures. When this membrane is perforated carefully in the rat, to avoid hemorrhage from the underlying vessel-containing pia mater, cerebrospinal fluid pours out from the subarachnoidal space. Again this space is not built up by trabeculae, but is a continuous cerebrospinal fluid-containing space, where the spinal cord is suspended and kept in place by the spinal roots. When the rat arachnoid is removed, the pia mater remains as the last membrane. This membrane is, like in man, a thin, translucent, glossy, vessel-containing membrane. The dorsal spinal vein is seen in both the rat and in man (figs. 4.3 and 4.4).

To investigate these anatomical differences of man and the rat, several experiments were performed on rats.

After a laminectomy had been performed and the spinal cord exposed as in fig. 4.3, the rat was transcardially perfused with a 4% paraformaldehyde in a 0.15 M S0rensen buffer (pH = 7.4) and hereafter the meninges easily could be removed. Only two membranes could be removed: the outer membrane (the arachnoid) which is seen in fig. 4.8 and the inner membrane (the pia mater), seen in fig. 4.7. The stain is a thionin cell body stain. It is seen that the density of the cells in the pia is not as marked as in the arachnoid. No dura was found.

In order to recognize the subarachnoidal space in later preparations, 0.5 ml Evans Blue was injected with a little plastic tube into the subarachnoidal space and spread around with the CSF. It is seen in fig. 4.3 how the arachnoid is lifted with a subcutaneous needle with a bent point to introduce the plastic catheter.

The result is seen in fig. 4.6. The rat was killed after three minutes, the spinal cord was removed and put in a fixative. What is seen is the arachnoid (2), the pia mater (1) and the subarachnoidal space (A).

It is interesting to see that the Evans Blue apparently by pinocytose (in the living anaesthetized rat) or by diffusion (in the dead rat) passes over the pia mater and the external glia limiting membrane to the white and grey matter of the spinal cord. The grey matter in particular is heavily stained by Evans Blue.

To further clarify this assertion, a block of the vertebral column with the laminas and muscles was prepared in a decalcification process in RDO and stained with van Giesson (fig. 4.5). It is seen that the dura is a layer of fibroconnective tissue and that it is built up in two layers (like in man) by the periost and endost. These two layers are removed because of its close attachment to the bone when a laminectomy is performed in the rat.

The same preparation is seen in fig. 4.9, but stained with toluidin. The close connection of the dura (periost) is clearly seen at the arrow 3.

Underneath is the arachnoid at arrow 2 and the pia mater at arrow 1.

This section (fig. 4.9) is compared with a human fetus (fig. 4.10). The fetus is 14 weeks old, the stain is toluidin. The two figures 4.9 and 4.10 are very much alike, especially with regard to the meninges. In fig. 4.10 it is seen that the dura (3) derives from the bone and not from the meninges primitiva of the neural crest.

In 1934, Hochtstetter [117] investigated the development of the spinal meninges in human beings. He believed that the dura cells arose from a perichondrium of the vertebra and intervertebral discs rather than from the meninges primitiva. According to Hochstetter the dural layer becomes separated by the peridural space from the vertebra and thus, close to the spinal cord, the subarachnoid space appears. The arachnoid becomes identifiable much later and the dura completely lines the internal wall of the vertebral canal by the eighth week of gestation. This assumption is supported by my own investigation of the vertebral column of human fetuses (fig. 4.10).

According to Harvey and Burr (1926) an artefact is frequently mistaken for the arachnoid. They state that the arachnoid does not appear until the third trimester orpostnatally. This assumption cannot be supported by my investigations.

In 1951, Sensenig [194] concludes that all three spinal meninges are derived from the meninges primitiva and that they are mesodermal with the exception of a contribution from the neural crest to the pia mater.

In 1986, O'Rahilly and Müller studied 61 brain and cranial meninges in human embryos from the eighteenth post-ovulatory day to the eighth post-ovulatory week. They do not have any further contributions to the development of the meninges in the spinal cord. They write:

> *"The cells of the neural crest clearly contribute to the pia mater of the occipital part of the hind brain and perhaps to the pia of the spinal cord ... Although most workers now regard the origin of some meningeal cells from the neural tube as of merely historical interest, it would be premature to exclude this possibility".*

My investigations give reason to believe that the dura mater arises from the mesodermal layer and the arachnoid and pia mater from the neural crest. This is demonstrated in fig. 4.10.
The conclusion is thus:

1. The periost and the dura in the rat is one and the same and that the periost is removed during laminectomy exposing the arachnoid membrane directly.

2. The subarachnoid space in the spinal cord does not contain a network of trabeculae. The subarachnodial space is filled with cerebrospinal fluid and the only structures passing through are the denticulate ligament and the spinal nerve roots.

3. No villi are present only granulations arachnoidialis going into the intracranial sinusis.

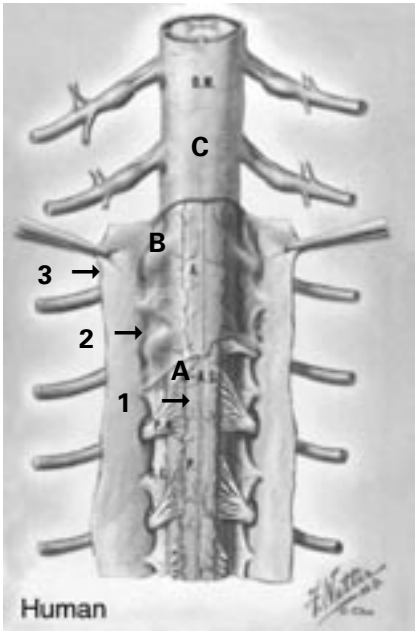
4. No nerves nor vessels are present in the arachnoid membrane. (George Winckler also found this in 1960, when in his article "Remarques sur la structure histologique de la leptomeninge chez rhomme" he writes: "The arachnoid is characterized by the absence of vessels and nerves"). The only exception is where the vessels cross the fissurian sylvii on the cortex of the brain. See fig. 4.15.

5. The appearance of trabeculae is due to the loosely adherent cells in the pial membrane. This is illustrated in figs. 4.14 and 4.16. Fig. 4.14 is my own drawing, and fig. 4.16 is an illustration from Wheather, Burkitt and Daniels (1987) [216]. The only exception is where the vessels cross the Sylvian fissure in the brain.

Even the latest histology textbooks rely upon the old drawings from Weed (1923) [215]. These should be revised according to the most recent knowledge that brings the actual findings in accordance with the appearance of the arachnoid and the subarachnoidal space during operations. In the future, scientists dealing with spinal cord transplants ought to be aware of the immediate appearance during a laminectomy in the rat of the arachnoid membrane and not the dura mater [75].

Fig. 4.1

A drawing by F. Netter in the CIBA collection showing the human spinal cord and the meninges ' Shown with the permission ofR.R. Donally and Sons Company.



1: Pia Mater

2: Arachnoid

3. Dura Mater (periost)

4: Periost 5: Endost

A: Subarachnoid Space

B: SubduralSpace

C: Epidural or Artifical Space between Periost and Endost

Fig. 4.2

The spinal cord exposed in an adult human. The dura is cut open and so is the arachnoid. It is seen how the arachnoid is a non-vascular, glossy, translucent membrane. A tumor appears to lie in the subdural space compressing the spinal cord. Shown with the permission of Steen Midholm, M.D.

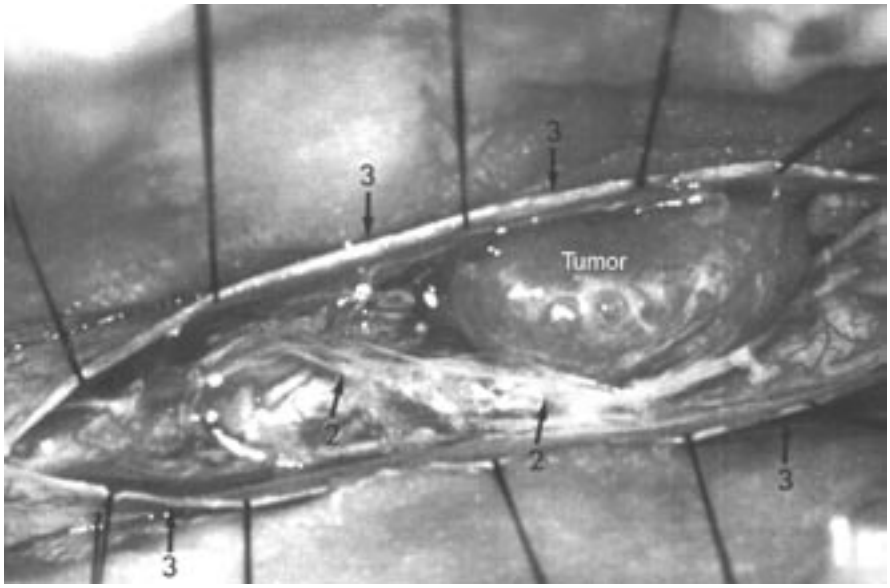


Fig. 4.3

A laminectomy is performed at Th7-9 of a 5 week old rat. It is seen that when the laminectomy is performed, the arachnoid is exposed directly. The arachnoid is the non-vascular, glossy, translucent membrane as in man. The dura which is identical with the periost is removed with the lamina during laminectomy. The vessels seen are located in thepia mater underneath the arachnoid membrane. The vessel is the dorsal spinal vein. Between the two layers is the spinal fluid. This figure should be compared with the figure below, 4.4.

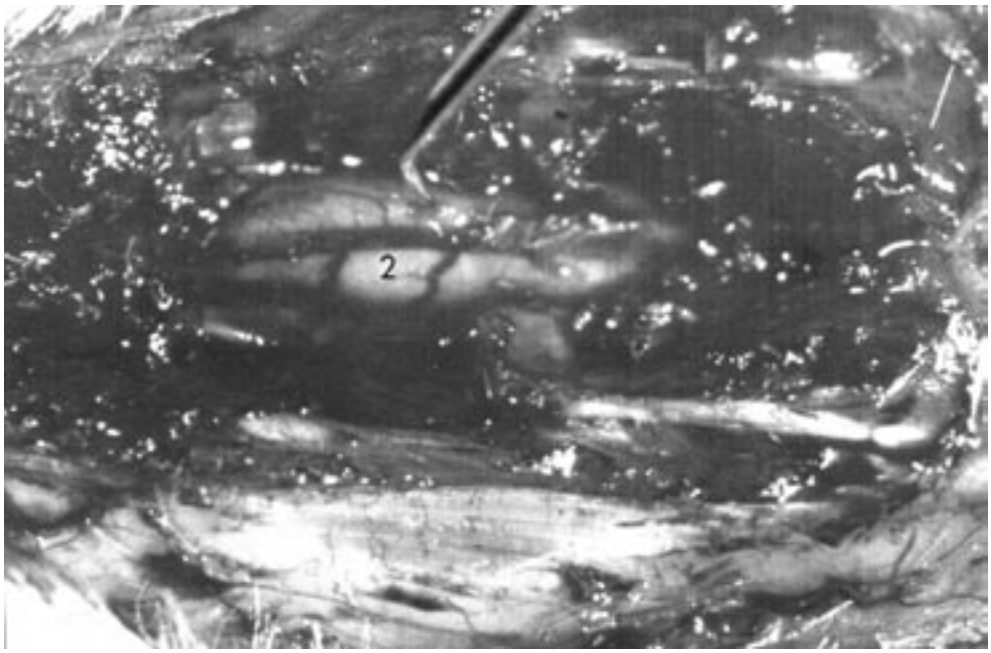


Fig. 4.4

This shows, as in figure 4.2, the spinal cord exposed in an adult man. The dura is cut open and held aside with sutures. The arachnoid is exposed as in the rat in figure 4.3.

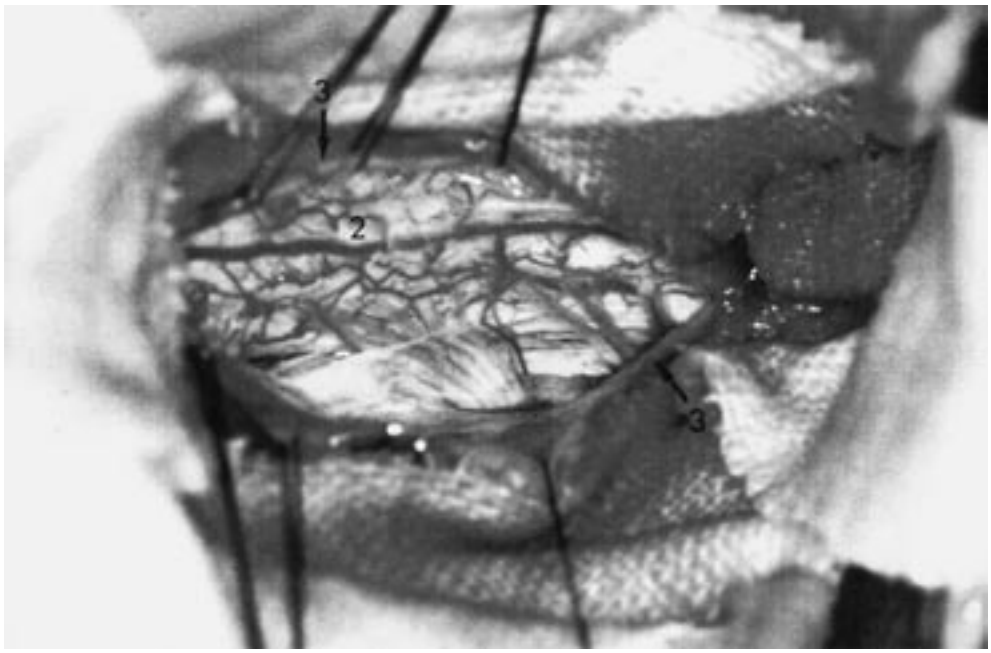


Fig. 4.5

The spinal cord of the rat in the vertebral bony column. It is seen how the dura (3) is identical to theperiost that is detached from the bone (endost (5)). The periost is seen on both sides of the bony lamina (4). The arachnoid (2) is seen and so is the pia mater (1).(RDO decalcification process and a van Giesson stain).

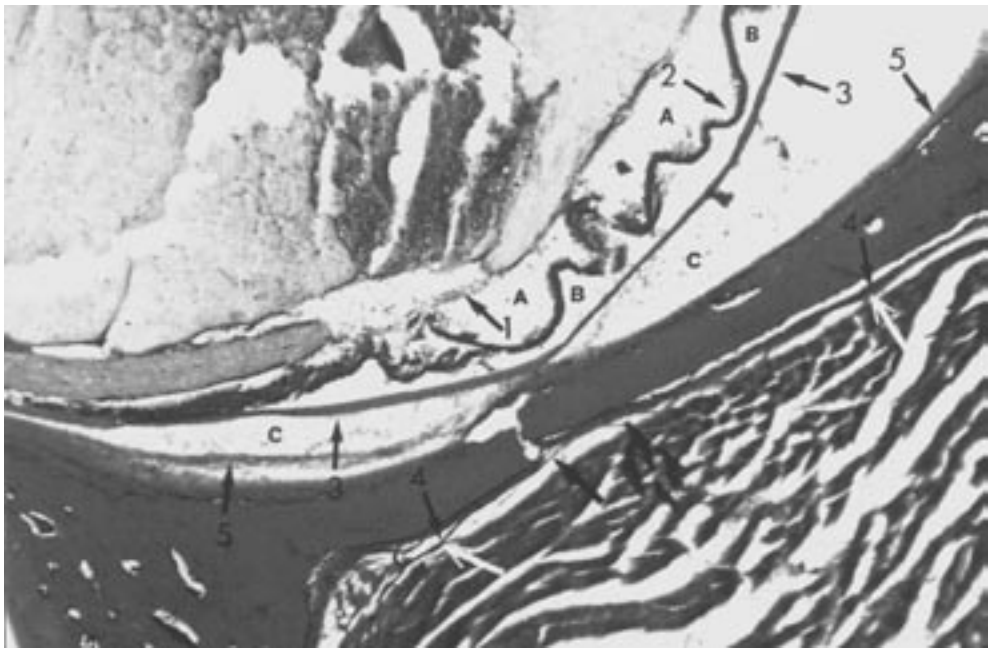


Fig. 4.6

The spinal cord of a rat. Evans Blue has been injected into the subarachnoidal space after a laminectomy has been performed. The spinal cord has then been removed with the meninges. No dura is seen, only the pia mater and the arachnoid mater.

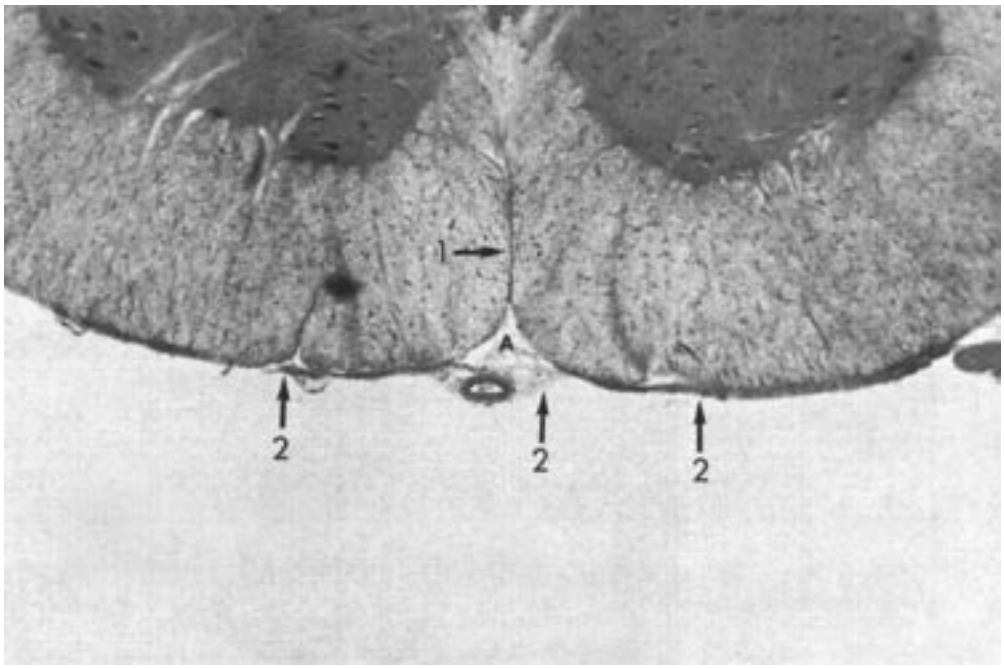


Fig. 4.7

The inner membrane, the pia mater. Thionin stain. (Rat)

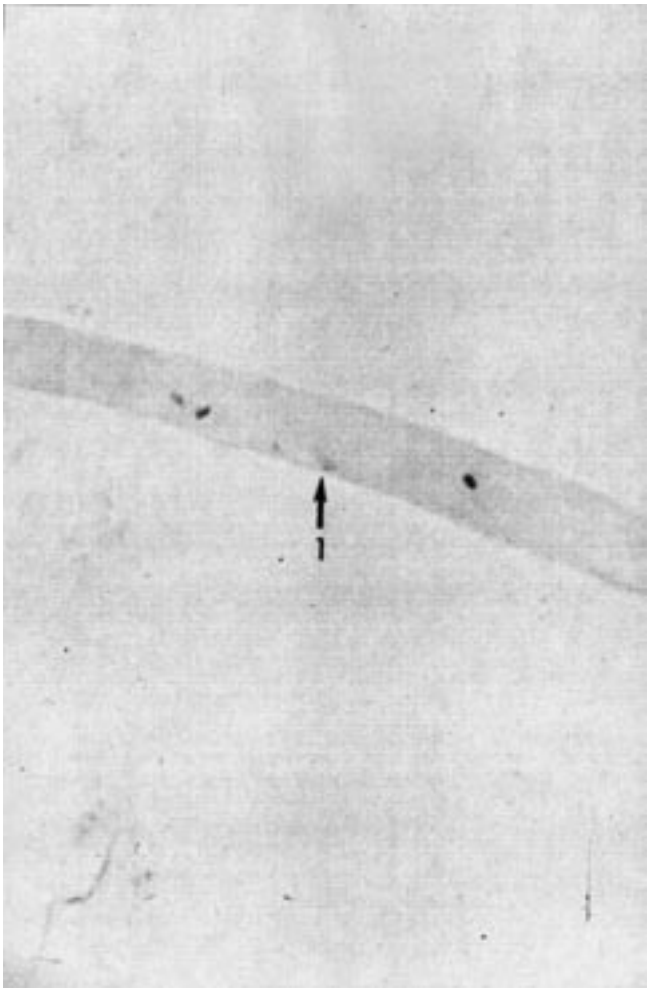


Fig. 4.8

The outer membrane, the arachnoid. The glossy, translucent, avascular membrane as it is seen in fig. 4.3. It is a layer of flat cells with a large pale oval nuclei. This is not the dura. Thionin stain. (Rat)

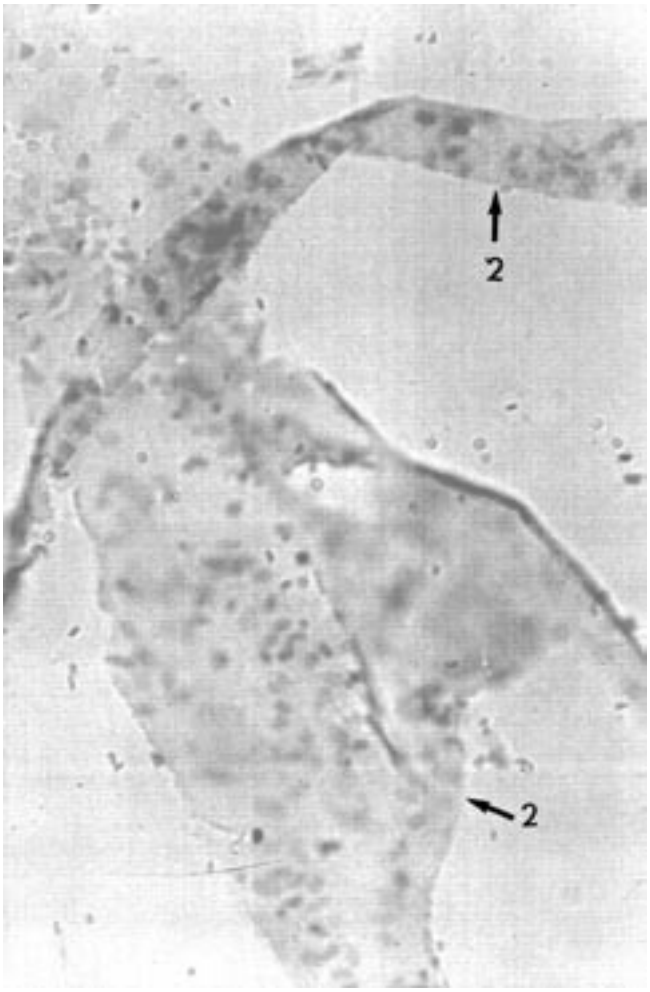


Fig. 4.9

The same as fig. 4.5, but in a tohddin stain. The spinal cord of the rat in the vertebral bony column demonstrate the relations between the bone and the dura mater (periost), the arachnoid and the pia mater. (RDO decalcification process and a tohddin stain)

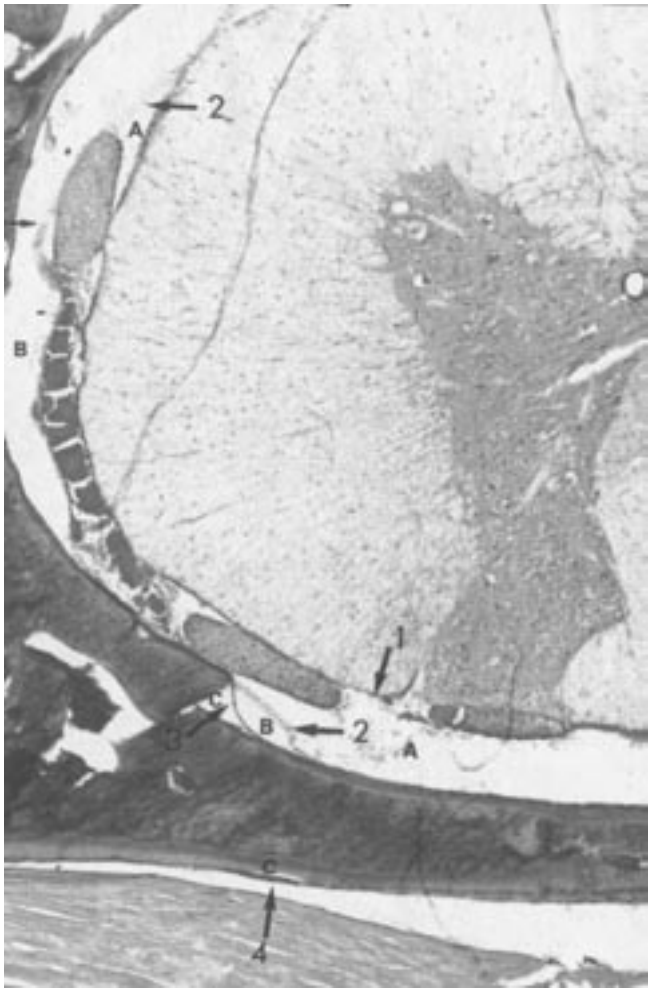


Fig. 4.10

The spinal cord of a 14 week old human fetus in a tohddin stain. These two figures are very much alike, fig. 4.9 from the rat and fig. 4.10 from a human being. In the preparation it is seen in a microscope how the dura mater is separating from the periost and how the pia mater and arachnoid membrane is separating from the neural crest. The arachnoid is closely attached to the pia that contains the vessel.

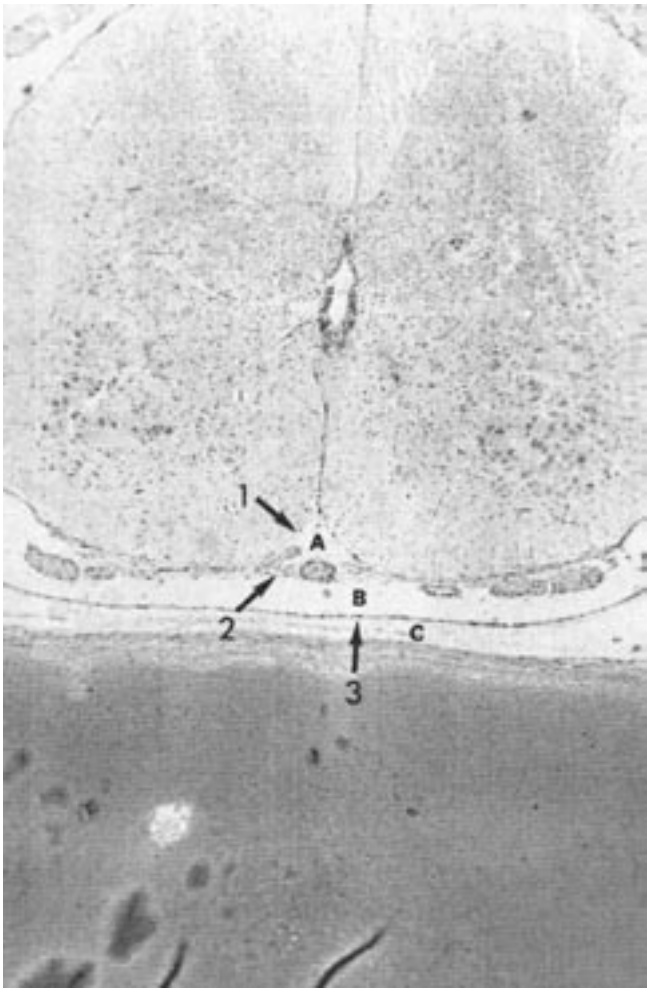


Fig. 4.11

Diagram to illustrate the relationship oftheperivascular space, art.sp. artefact space; bs., brain substance; b.v., blood vessel; d.mat., dura mater; epi.sp., epispinal space of His; pn.sp.,perineuronal space;pr. sp.,perivascular space, p.mat., plater mater;r.p.s., reticular perivascular sheath, subarachn.sp., subarachnoid space.

Woollam and Miller's original drawing from RATS. 1955 [227].

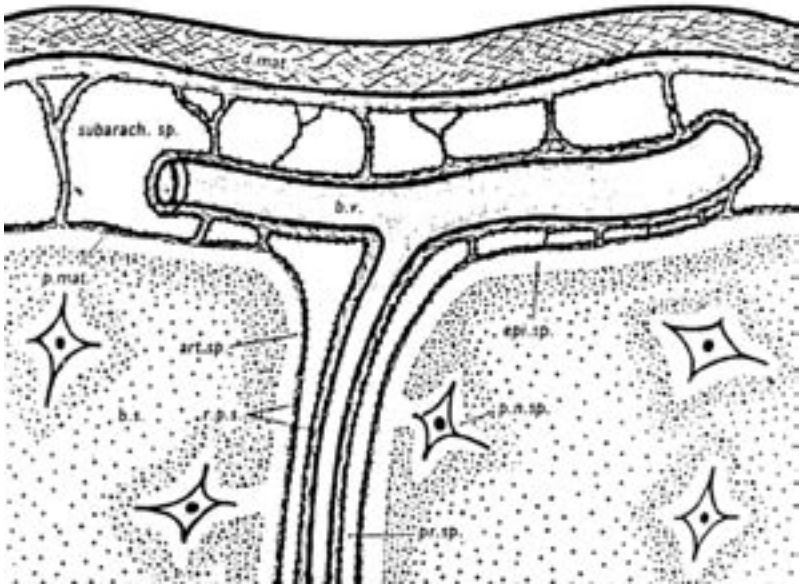


Fig. 4.12

The subarachnoidal space according to Ham's histology 1979 (redrawn from Weed and Miller from 1954). A net\vork oftrabeculae is seen [109].

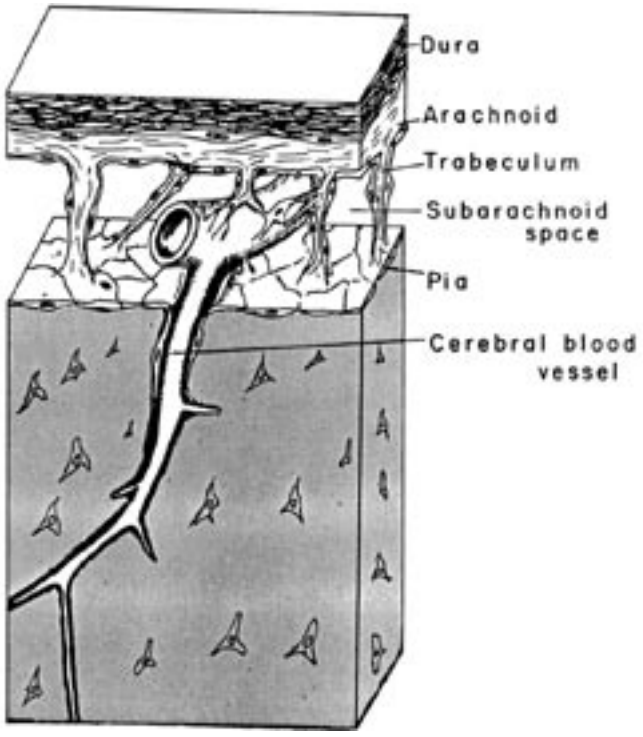


Fig. 4.13

After Weed 1923. From dogs and cats [215].

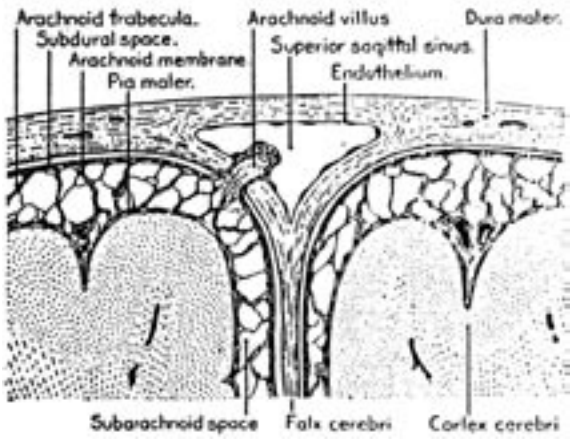


Fig. 4.14

In the spinal cord of the rat and in man there is no network of trabeculae in the subarachnoidal space. The spinal cord is kept in place by denticulate ligament. The dura contains vessels. The arachnoid is a glossy, avascular, translucent membrane consisting of a few layers of flat cells that are only loosely attached to each other. The pia is also a glossy, translucent membrane but the pia contains vessels that are embedded in the layer of the cells (drawing made by author).

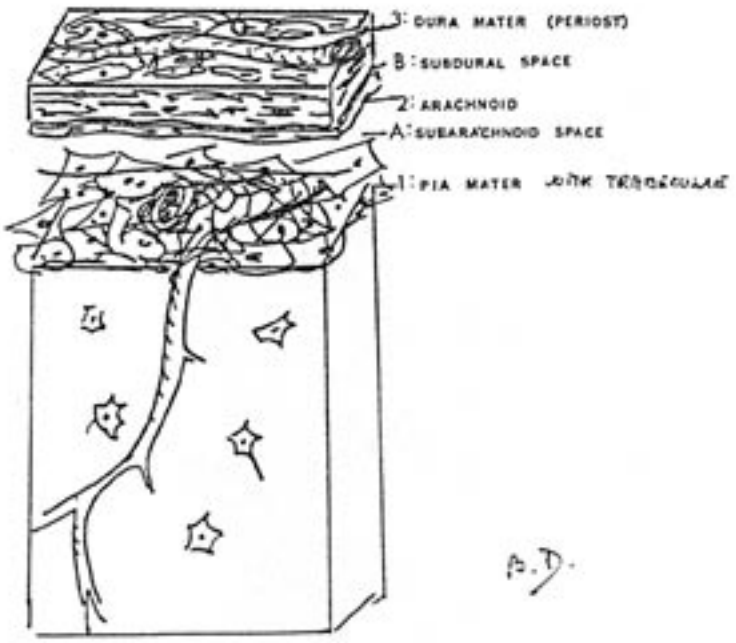


Fig. 4.15

The arachnoid membrane from a human being. It is translucent and avascular (H & E stain). Compare to the membrane from the rat fig. 4.8.

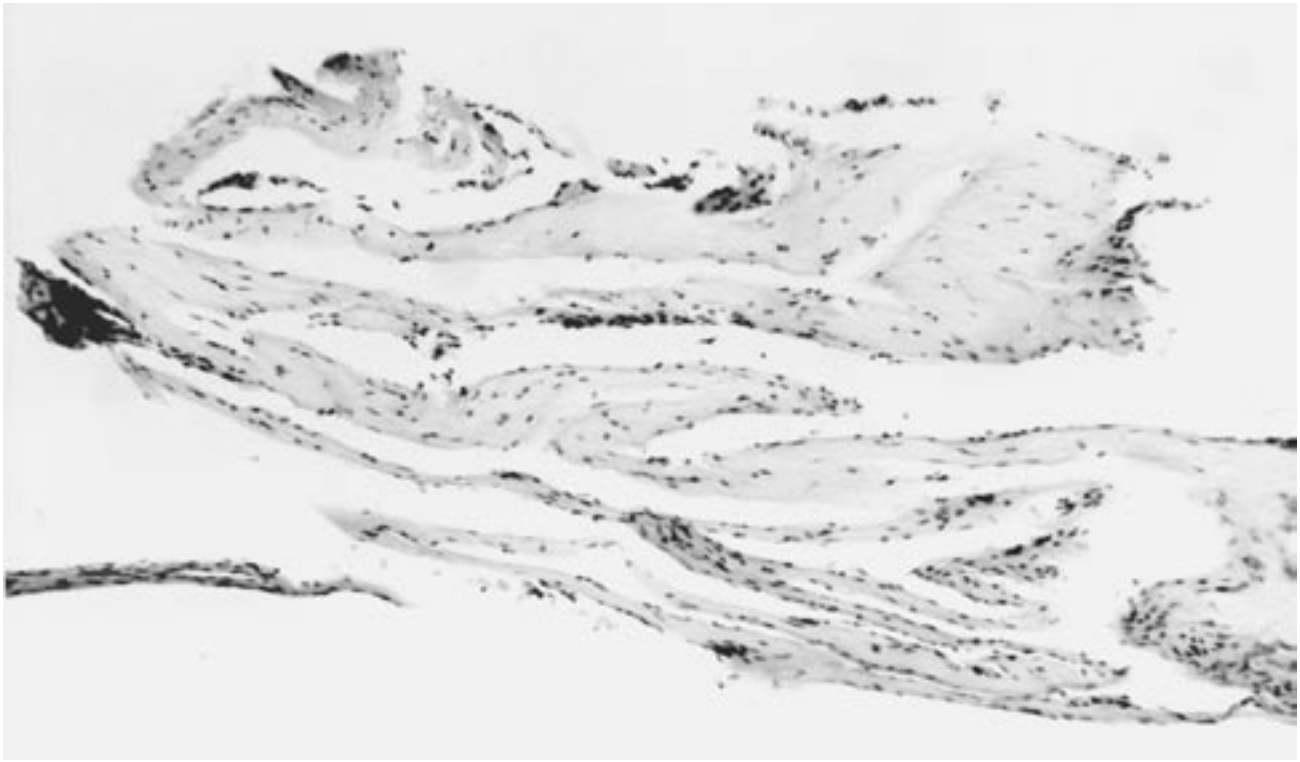


Fig. 4.16A

Meninges (H & E x 198)

"The pia and arachnoid layers of the brain meninges are illustrated in the micrographs , the dura mater remaining adherent to the skull when the brain is removed from the cranial cavity.

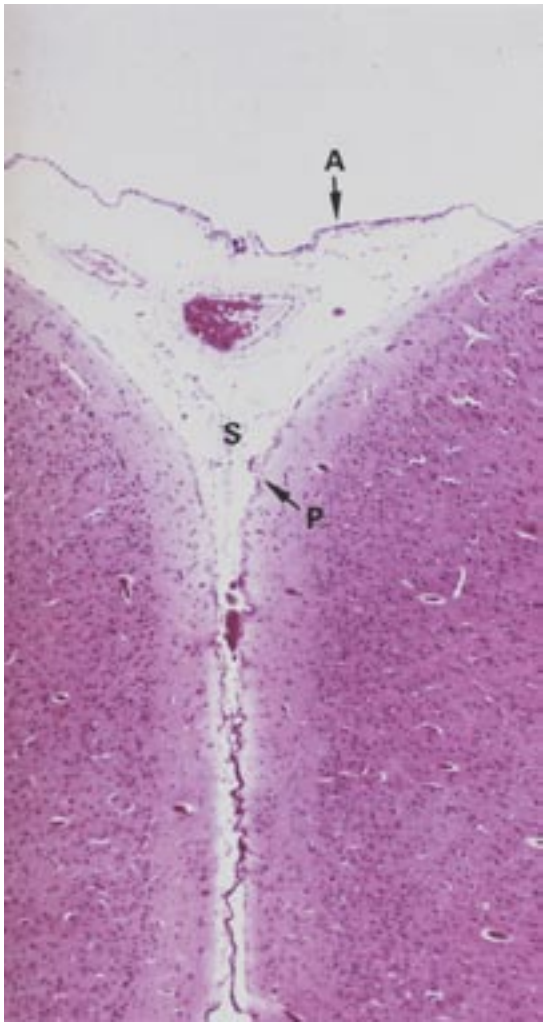
*The pia mater **P** is intimately attached to the surface of the brain and continues into the sulci **S** and around the penetrating vessels. The arachnoid mater **A** appears to be a completely separate layer and bridges the sulci. Delicate fibrous strands can be seen transversing the subarachnoid space to connect the pia and arachnoid layers. Both these layers consist of delicate connective tissue the surface of which is lined by flattened mesothelium.*

The subarachnoid space contains arteries and veins, their branches extending Into the brain substance surrounded by a perivascular space which is continuous with the subarachnoid space and which is thus filled with CFS. The CNS contains no lymphatics and interstitial fluid is thought to drain outwards from the brain substance to join the subarachnoid CSF via the perivascular spaces."

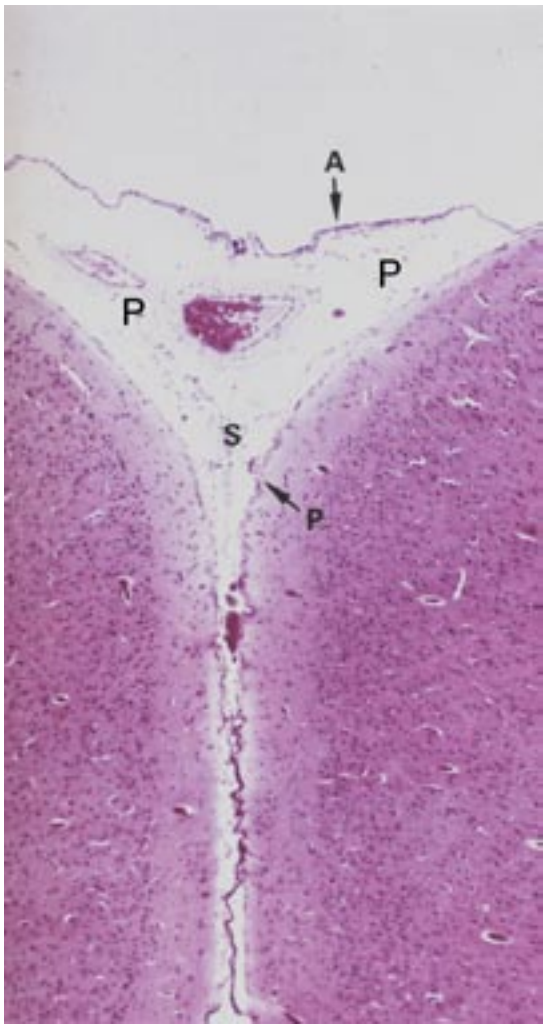
Fig.4.16B

Section of the brain showing the arachnoid and the pia and the underlying brain. The authors have described how the dura was closely attached to the skull and removed with the skull. The underlying layer is the arachnoid and it is seen how the cells are loosely attached to each other, and therefore have become a part giving rise to the belief that the subarachnoidal space is a network oftrabeculae. In my opinion, this is not true, except in fissures between the lobes of the brain. The pia is shown attached to the external glial limiting membraneg. Between the arachnoid and the pia is a subarachnoidal space and it is seen that it is empty as it only contains the cerebro spinal fluid. It is the pia that contains the bloodvessels not the arachnoid. The picture is from Functional Histology by Wheater, Burkitt and Daniels (1987). The authors are of the belief that the subarachnoidal space is a network oftrabeculae.

The assumptions made by Weed (1923) [215] and'Woollan and Miller (1955) [227] are still carried on in the new histology books (Ham 1979, Geneser 1986, Wheater, Burkitt and Daniels 1987) [216].



my own conclusion:



(Wheater, Burkitt and Daniels) [216].

Shown with the publishers permission. Churchill Livingston.

It is also important to know the localization of the ascending and descending tracts of the spinal cord in the rat when one is dealing with spinal cord transplants. In 1934, Barron [12] investigated the pyramidal tracts in the rat, since these tracts are of specific interest with regard to the motor function of the rat; in particular concerning paraplegia.

In Hebel and Strombergs book "Anatomy and Embryology of the Laboratory Rat [114], a detailed study of the tracts in the rat spinal cord is presented.

It is known that the corticospinal tract in the rat descends from the cortex and appears as the pyramid on the ventral surface of the spinal cord; the decussation of the fibers is completed in the pyramid. The tract then descends in the posterior columns of the cord lying beneath the ascending sensory tracts on either side of the posterior longitudinal sulcus (fig. 4.17).

Barron (1934) [12] considered it important to determine the function of the corticospinal fasciculus in rats. For this purpose, he made unilateral pyramidal sections at the beginning of the spinal cord. Other workers had made observations upon decorticated animals, but they found that after purely cortical destruction in adult rats no motor disturbances would persist more than 18 hours following the operation. If the motor cortex and the caudate were destroyed, it was followed by a permanent hemiplegia, and therefore it was suggested that the function of the corticospinal tract was concerned with the regulation of posture and tone.

After complete section of a single pyramidal tract, there was an obvious paresis in the toes of the fore and hind limbs on the side affected, coupled with a decrease in the flexor tone. The paresis disappeared in parts during the first two or three weeks, although a definite residual deficiency in the limbs was always present. Following the complete section of the pyramid it has not been possible in any of the rats tested to elicit movements of either the ipsilateral or the contralateral limbs by bipolar faradization of the hemisphere on the site of the lesion. Movements, however, were always to be elicited from the opposite hemisphere. The stimulation of the motor-area resulted in flexion of the opposite hind leg and extension of the hind leg of the same side. It is quite unlikely that the extension of the ipsilateral hind leg is due directly to the impulses from the cortex because apparently there are no uncrossed fibers in the pyramidal tracts. The movements thus appeared to be a typical extensor thrust of spinal origin, and as atest that the dorsal roots of the lumbar and sacral nerves of the contralateral limb were cut thus removing the proprioceptive sensory input from its muscles. Excitation of the hemisphere several days later still produced flexion of the contralateral hind leg, but extension of the ipsilateral leg was never observed to occur after the dorsal root section.

Starlinger (1985) [198] found that dogs after both pyramids had been sectioned could run,climb, jump and rise themselves on their hind legs with such ease and success that only with diffulty could they be distinguished from normal dogs. This was in particular true after three weeks. These facts are equally true as regards the rats. The deficiency in the rats, however, in the digits has no parallel in the dogs as they do not use their digits to the same extent as the rats do. Therefore, a comparison of spinal cord motor function in rats after a fetal spinal cord transplant cannot be made to man. After section of the corticospinal tract, the rat shows hardly any deficits after two weeks. This has also been shown in my own experiments, and consequently, it is not possible to make any neurophysiological experiments with electrostimulations of neurons or muscles to determine whether a transplant is functioning.

The localization of the cortisospinal tract in man is also different from that in the rat. The corticospinal pyramids in man are located in the lateral part of the spinal cord and not in the dorsal part as in the rats. To demonstrate the differences, figs. 4.17 and 4.18 can be compared. Fig. 4.21 shows a list of ascending and descending columns in the human spinal cord [115]. For demonstration of the tracts and the connections of the neurons in the spinal cord, fig. 4.22 can be studied (J.V. Priestly: Neuroanatomy of the Spinal Cord. Paraplegia 1987; 25:199) [179]. The spinal cord has the size of a little finger in man and through that, all the motor and sensory functions distal to the foramen magnum takes place in a complicated neuro-physiological and biochemical teamwork.

It seems to be a rather hopeless task for a transplant to be capable of a proper combination of these connections so that they will regain the original functions prior to a trauma. As a step towards the main goal of regaining motor function of the spinal cord, the primary task will be a) to avoid the formation of scar tissue, and b) to promote the axonal regeneration either with a transplant or with a neuronal growth-factor. Two chapters will be dealing with the prospects of the research within these areas.

Fig. 4.17

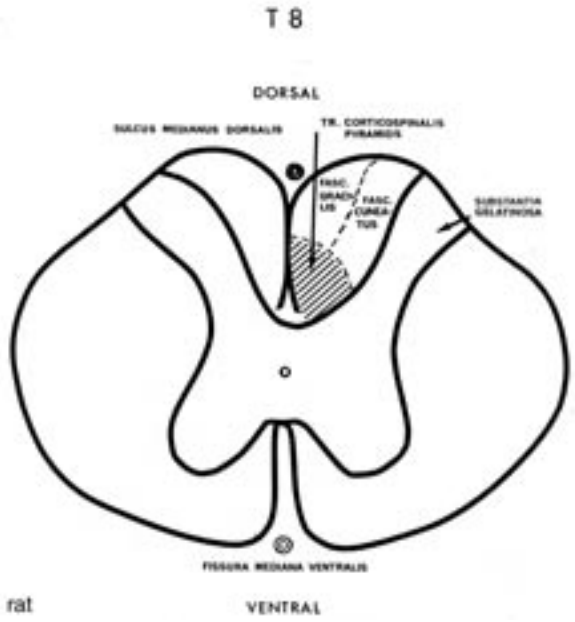


Fig. 4.18

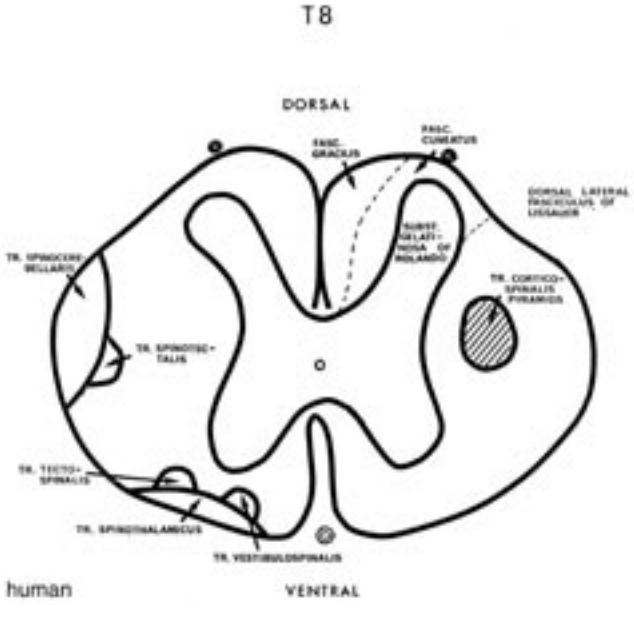
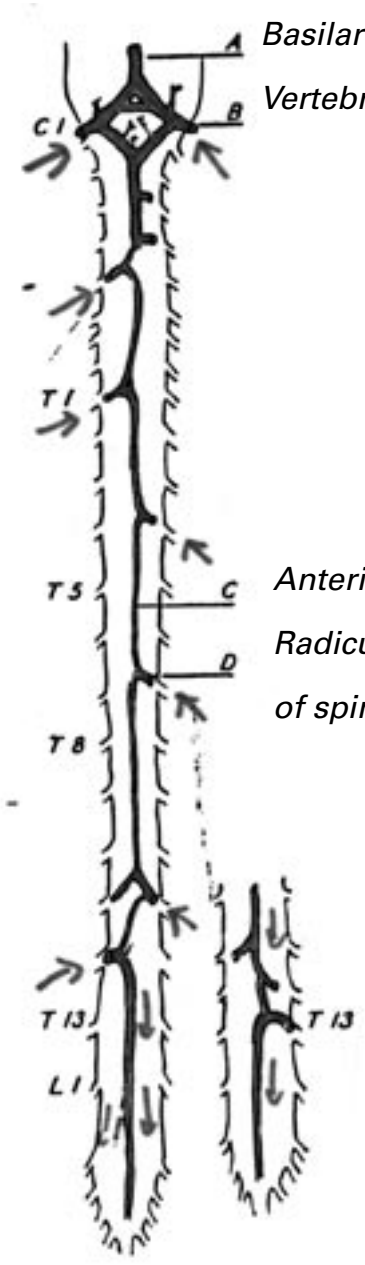


Fig. 4.19

The anterior spinal artery in the rat is formed by the confluence of branches of a number of vessels ascending along the dorsal roots. This is true from Cl to Thl2. At Thl2 to LI a large entering vessel anastomoses through a fine artery and then cur-ves caudally to become the lumbo-sacral portion that receives no other bloodsupply.

This distribution of arteries explains why animals with the spinal cord severed above Thl2 can become "spinal animals" with spinal reflexes, and animals with a cord lesion below Thl2 have no spinal reflexes and no automatic bladder. The caudal stump of the spinal cord simply degenerates [204].



Basilar artery

Vertebral arter

Anterior spinal artery

Radicular branch

of spinal ramus of intercostal artery

After Sugar and Gerard 1940.

Fig. 4.20

71 mm.2 of the ventral horn tissue from the cervical spine contains about:

0,85 mm3 of neuropil
0,04 mm3 of blood vessels
0,02 mm3 of neuroglia cells
0,08 mm3 of motorneuronperikarya
0,02 mm3 of small neuronperikarya
45.000 neuroglia
11.650 neurons

(Hebel and Stromberg) [114]

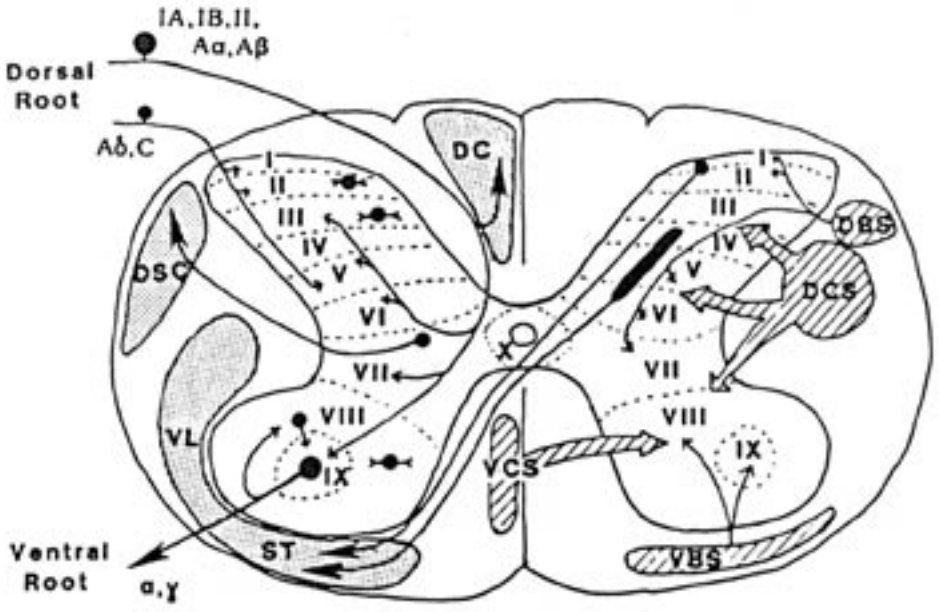
Fig. 4.21

Ascending and descending columns in the human spinal cord [115]

ASCENDING SENSORY TRACTS	FUNCTION
fasciculus gracilis and cuneatus	propriception and touch
tr. spinothalamus (lateral)	pain, temperature
tr. spinocerebelli (anterior and posterior)	proproception
tr. spinothalamicus anterior	touch
tr. spinoreticularis	visceral information
tr. spinovestibularis	posture
tr. spinotectalis	sight and sound rereflex
tr. spinoolivaris	proprioception
DESCENDING MOTOR TRACTS	
tr. corticospinalis, crossed and uncrossed	arbitrary
anterior and lateral	fine movements
tr. reticulospinalis tr. rubrospinalis tr. tectospinalis	sight and sound reflex
tr. vestibulospinalis	position and posture

Fig. 4.22

J. B. Priestley. Neuroanatomy of the spinal cord. In Paraplegia 1987; 25: 199. (Both drawing and text) [179].



"Diagram showing some of the basic cell types and connections of the spinal cord. This scheme is based on the results of classic neuroanatomical studies supplemented by data from the tract tracing and intracellular HRP techniques. For more details, see Brown (1981, 1982), Kemplay and Webster (1986), Kuypers and Huisman (1982), Perl (1984). All cells and connections occur bilaterally in the spinal cord but for simplicity 1° afferents, interneurones, motoneurones and ascending tracts (stippled) are represented on the left half of the diagram while descending tracts (hatched) and cell bodies of spinothalamic projection neurones are represented on the right half. Visceral and autonomic afferents and efferents are not shown, nor has any attempt been made to take account ofsegmental differences. Roman numerals indicate the laminae of Rexed and as far as possible the location of cells and the site of termination of projections in the different laminae have been shown accurately. For clarity certain details have been omitted from the diagram but are described in the legend below.

1° afferent neurones comprise two broad classes. Small neurones convey information mainly about cutaneous pain and temperature sensibility (A, C conduction velocity) and terminate mainly in laminae I (A), II (C),V (A) and X (A, not shown). Large neurones convey information form cutaneous touch and hair receptors (A, A□), muscle spindles (IA, II) and tendon organs (IB) and generally have axons which ascend in the dorsal columns (DC) to terminate in the dorsal column nuclei. However, these neurones also have collaterals which enter the spinal grey matter to terminate in laminae HI (A, Aft hair follicle afferents), HI-VI (A, AJ3 low treshold mechanoreceptors), VI, VII, IX (IA spindle), IV-VII, IX (II spindle) and V-VII (IB tendon).

Spinothalamic (ST) projection neurones are located in laminae I, IV-VI, VII (not shown) and VIII (not shown) and send axons across the midline to ascend in the ventrolateral (VL) funiculus. A few cells (mainly in lamina VIII) have ipsilateral projections (not shown). Also contained within the VL funiculus although not shown are the axons of ventral spinocerebellar neurones and various spinobulbar pathways including spinotectal, spinoreticular, spinoolivary, spinovestibular and spinopontine projections. The cells of origin of many of these pathways are located in similar laminae to those of the spinothalamic pathway and some cells have projections both to the thalamus and to more caudal bulbar locations. In the dorsal funiculus run axons of the dorsal spinocerebellar tract (DSC) derivedfrom cells of Clarke's column which is located in laminae VI, VII between the third lumbar (L3) and eight cervical (C8) segments. The spino cervical tract in the dorsolateral funiculus and the post-synaptic dorsal column system are not shown on the diagram. These pathways have been best characterised in the cat but are thought also to occur in humans.

Descending pathways shown on the diagram are the dorsolateral (D) and ventral (V) bulbospinal (BS) and corticospinal (CS) tracts. Bulbospinal pathways include rubrospinal (terminating in laminae VII, VIII), raphespinal (I, II, V, VII-X), hypothalamospinal (7-7/7, V77, X) and tectospinal (VI, VII) tracts. Inter neurones are located in all areas of the spinal cord but are shown only in laminae II, HI and VIII. Some of those in lamina VIII (Renshaw cells) are innervated by recurrent motoneurone collaterals. Not shown are the numerous short axon neurones which innervate adjoining laminae or which project for only a few spinal cord segments (propriospinal cells). Motoneurones are shown as either (innervating extrafusal muscle fibres) or (innervating intrafusal fibres)."

References: *12, 36, 37,48, 54, 75, 86, 109, 112, 114, 115, 117, 124,134, 143, 144, 171, 179, 194, 198, 204, 215, 216, 227.*

5
Spinal Cord Transplants in Animals Especially Rats 1890 -1986

Early History of Transplantation

The first report on grafting of central nervous tissue dates back as far as 1890, when W. Gilmann Thompson, MD, in The New York Medical Journal, wrote about successful brain grafting [207].

This primary Original Communication describes how attempts have been made to graft nearly every different tissue of the body such as skin, bone, teeth, muscle, nerves, glands, eyes and mucous membranes. Grafting or transplantation of tissues is by no means a new or recent highly developed technique but was tried as early as the nineteenth century had been with more or less success.

The very first report of brain-grafting is 100 years old and is indeed interesting. I present the whole article because of the historical value and the author's enthusiasm. He finishes his article by writing:

> *"I have no doubt that other experimenters will be rewarded by investigating it".*

This has proven to be very true.

THE NEW YORK MEDICAL JOURNAL
June 28, 1890

Original Communications

"SUCCESSFUL BRAIN GRAFTING By
W. Gilman Thompson, MD

Professor of Physiology in the New York University Medical College;
Visiting Physician to the Presbyterian and the New York Hospitals.

Attempts have been made to graft nearly all the different tissues of the body. Skin, bone, teeth, muscle, nerves, glands, eyes, mucous membrane, etc., have all been grafted with more or less success, but successful brain grafting has not heretofore been performed. With the exception of skin grafting, andpossibly mucous membrane grafting, the results of such experiments have been of little

practical value. They are, however, of great scientific interest in demonstrating the relative vitality of different tissues and the histological changes which accompany degenerative processes. The laws of atrophy and final disappearance of disused organs, so ably advocated by Darwin, are equally striking with *regard to individual tissues and cells, and it is a well-recognized fact that the higher the original development of a tissue or cell has been - i.e., the more it has been differentiated or specialized from the amceba type - the more profoundly is it affected by alterations in the environment or nutrition, so as to degenerate completely, or be replaced by some form of tissue like the connective, which is of lower development but stronger vitality. The result of nerve grafting and of nerve suture after complete section have varied greatly in the hands of different operators, but despite many discouraging failures, there is no doubt that in man, as well as in the lower animals, nerve fibers may reunite when sutured even aftersecondary degeneration has occurred, and they exhibit restoration of function. For this to occur, however, the nerves must be in communication with some trophic center. Nerve grafting does not succeed as well as nerve sutures in favorable cases. It occurred to me recently, while studying cerebral localization in the lower animals, that it would be interesting to graft a piece of brain tissue from one side of a dog's brain to the other, or from one animal's brain to another's, and study its vitality. Of course, I had no expectation of being able to restore abolished function by the operation, but the question of vitality of the brain tissue and the course of its degeneration is a subject which is of very wide interest. The first experiments were preliminary, made in order to ascertain whether the transplanted brain would be immediately absorbed or would slough away.*

Experiment I. *- Two large dogs, A andB, were simultaneously trephined over the right occipital region; 8 c.c. of brain tissue were excised in one piece and exchanged; the piece from dog A was put into the opening in the brain of dog B, and vice versa. On the third day both dogs were killed, and the transplanted pieces of brain tissue looked normal, and in each case they were so adherent and firmly covered with fibrous exudation that it was impossible to pull them off with forceps without laceration. Total blindness of the eye opposite the lesion resulted in each dog, as was excepted.*

Experiment II. *- A cat and dog were simultaneously trephined, and 3.5 c.c. of brain tissue were removed from the dog's left occipital region and transplanted into an opening of the same size in the cat's left occipital region. Three days later the cat was killed. The transplanted dog's brain was found where it had been placed, firmly adherent to the cat's brain by a layer of fibrin, which varied from one fourth to half an inch in thickness. The cat was, of course, totally blind in the right eye.*

Experiment III. *- Another cat and dog were simultaneously trephined; 4 c.c. of brain tissue were excised from the dog's right occipital region and replaced by an equal quantity of cat's brain from the same region. On the fourth day the cat's brain was found adherent to the dog's by a layer of fibrin.*

Photographs relative to this paragraph are not presented because of the poor quality.

Micro-photograph of a brain graft. The upper half of the picture reproduces a section through the cat's brain; the lower half, a section through the dog's brain; the connective tissue uniting the two crosses the median line. Three large bloodvessels are seen in transverse section, and one (in the center) in longitudinal section. On the right center the line of union is very perfect, there being a minimum of connective tissue here, and the two varieties of brain seem fused together. On the left center, just above the line of union, a slight tear was made in the cat's cortex inpreparing the section; this was unavoidable, in order to get the section thin enough for photographing. In all the other sections no such tear exists, and the line of union of the two brains is as perfect as it is seen to be on the right of this photograph. The picture is not magnified to show detail; it is merely intended to present the relative thickness of the line of connective tissue and some of the larger nuclei and blood-vessels. (For this photograph I am indebted to the kindness of Dr. H.S. Stearne, instructor in the Pathological Department, and director of the Photographic Department of the Loomis Laboratory).

No microscopic examination was made in connection with these experiments, as it was intended only to determine the possibility of the transplanted tissue adhering. Being satisfied in regard to this matter, I secured a large dog and performed.

***Experiment IV.** - A half-inch trephine was used and a button of bone was cut nearly through over the left occipital region, leaving a small attached margin so that the button could be elevated and then depressed like a little trap-door. Through the opening 2 c.c. of brain tissue were removed. A cat was simultaneously trephined and 1.5 c.c. of brain from her left occipital region were transferred in eight seconds to the opening in the dog's brain. The trephine opening was closed by the button, and the wound, which had been opened under careful antisepsis, was closed and dressed with layers of antiseptic gauze wet with thick collodion, which is the only practical dressing for brain operations on dogs, because they can not tear it off. The dog was totally blind in the eye opposite the lesion, and so remained until his death. He was, unfortunately, not tested as carefully in regard to the other eye. He was very dull - a street mongrel - and it did not occur to me to do more than test the vision of the eye opposite the lesion (which I have invariably found absent in such cases, with normal vision on the side of the lesion) .As the autopsy showed, however, there was secondary degeneration of the opposite occipital region, which must have progressed far enough to have greatly impaired the vision of the left eye. The dog made a good recovery from the operation, although he was very feeble for a few days and had to be fed by nutrient enemata. Subsequently he appeared normal in every way excepting the loss of vision. He was killed at the end of seven weeks, when the piece of transplanted cat's brain was found firmly adherent to the dog's brain, with the pia mater intact. The brain was hardened in Muller's fluid for some weeks, and the following report of its examination has ben kindly furnished me by Mr. Warren Coleman, assistant in the Physiological Department of the Loomis Laboratory, who prepared specimens of the brain for microscopic demonstration:*

Gross Examination. *- The cerebral hemispheres measure 5.2 ctm. in breadth, 5.5 ctm.in length, and 3.5 dm. in depth. The cerebellum was 3.5 ctm. broad and 2.9 ctm. deep through the medulla. The portion of brain transplanted now measures 1.9 x 0.5 ctm. It was grafted into the middle of the second occipital gyrus, its long axis extending outward and backward and involving a small part of the third occipital gyrus. The surface of the brain over the transplanted portion was somewhat shrunken. At a corresponding point in the opposite (right) hemisphere there appeared to be degeneration extending somewhat farther forward. Microscopical Examination. - The whole of the transplanted tissue was removed, together with a surrounding zone of dog's brain. This tissue was embedded in celloidin, and vertical sections were cut in various parts. The vitality of the transplanted tissue has been maintained throughout, except at its inner extremity; here degenerative changes are well marked. The cells in the region are shrunken and irregular in outline, the protoplasm is granular, and the nuclei either stain very badly or refuse to take up the stain at all. In other parts the cells are intact, but their outline is somewhat irregular. From the surface of union of the two brains, lines of degeneration extend down into the dog's brain, along the margin of which are seen cells larger than those found elsewehere. Many of the cells observed show beginning atrophic changes; these might be due, however, to the hardening process. Their nuclei are situated eccentrically. Between the two kinds of brain tissue a narrow band of connective tissue has formed, which firmly unites the grafted cat's brain to the dog's throughout their entire contiguous surfaces. This is most marked at the middle and inner extremity, where it reaches the width of a dozen fibers. Along its line numerous bloodvessels have developed, some of which are of considerable size. The transplanted cat's brain is covered with pia mater perfectly continuous with that over the dog's brain. This pia was transplanted together with the cat's brain. The vessels of the cat's pia are large and numerous; the pia itself is somewhat thickened. The examination of the corresponding portion of the opposite hemisphere of the dog's brain gives the following results: Degenerative changes here are very marked. A portion of the brain tissue and the pia covering it are entirely gone. The cells in the neighborhood are granular and their nuclei do not appropriate the stain. A little removed from the point of greatest degeneration, the brain shows a reticulated structure from the absence of cells. Here there is extensive diapedesis of the red blood cells. In other parts of the section no red blood cells are to be seen, but the brain cells contain a deposit of brown granules, showing that the red blood cells had broken down with the formation of hcematoidin. The vessels of this region are enlarged and actively congested.At no point am I able to trace any communicating nerve fibers or axis cylinders between the two varieties of brain tissue, although here and there, the line of union of connective tissue appears so narrow as to make the two brains almost continuous in structure. While some of the cells of the cat's brain are completely degenerated, the majority of them are still quite distinct and in some the walls are yet visible. Many of them do not look at all different from any piece of cat brain tissue kept for some weeks in hardening solutions, as this one was.*

The features of interest of this experiment are the facts that

1. *There is complete union, through organized connective tissue, of the contiguous portions of the two brains.*

2. *After seven weeks the cat's brain still maintained enough vitality to bedistinctly recognized as brain tissue.*

3. *Brains of animals of two very different species were thus made to unite.*

4. *The cat and dog pias present perfect union as well.*

5. *There is a sympathetic degeneration of the corresponding convolutions upon the opposite side of the dog's brain. For this curious fact I can not account. I have never noticed it before, in as many as fifty operations upon this region of the brain of cats and dogs (W. Oilman Thompson and Sanger Brown: Experiments upon the Labor-aty, No. 1,1980, p. 13), although I have sometimes seen removal of apart of the occipital region result in extensive softening of the entire hemisphere of the same side. The opposite degeneration in this case may possibly be a mere coincidence; if so, it is a very unusual and remarkable one. There was no meningitis to favor it.*

6. *There was descending secondary degeneration of the dog's brain on the side of the graft, as is usual in the cases of simple excision of brain cortex; hence the cat's cortex had not succeeded in acting as a nutrient center for the dog's brain. (The microscopic specimens showing the line ofmicroscopists, who indorsed their appearance as herein described, so that there can be no question of the accuracy of the observation).*

I think the main fact of this experiment - namely, that brain tissue has sufficient vitality to survive for seven weeks after the operation of transplantation without wholly losing its identity as brain substance - suggests an interesting field for further research, and I have no doubt that other experimenters will be rewarded by investigating it.

Thompson used grown-up dogs and cats as donors and recipients of the occipital lobe from one dog to another and from a dog to a cat. He made four experiments using eight animals. He killed the dogs and cats after three or four days. His work was followed by Saltykovs in 1905[190], by Del Conte in 1907 and by Elisabeth Hopkins Dunn in 1917 [67]. In 1905 Saltykov describes in "Versuche iiber Gehirntransplantation" how he made excisio and reimplantation of a part of the parietal lobe in 41 six weeks old rabbits. The implanted piecewas 0,5 cm x 0,5 cm, the period of survival was eight hours to 233 days. The neurons in the transplanted piece degenerated and died whereas the glial cells had mitotic activity and survived. In 1917 Elisabeth Hopkins Dunn wrote an article on "Primary and secondary findings in a series of attempts to transplant cerebral cortex in the Albino Rat" in the Journal of Comparative Neurology.
She carefully explained her method and many of the difficulties she had in grafting the central

nervous tissue are the same as those today. She operated upon 46 rats; two of the rats died soon after the operation and nine other brains of the rats showed nothing of interest on microscopical examinations. 35 brains were sectioned and studied. The donor-tissue was taken from rats at the age of nine or ten days because she found that the cortical tissue at this time was not mature and the rats were fairly simple to operate upon because of the thin cartilage skull that was easy to cut with sterile scissors. The recipients were of the same age. The cartilage flap was made in the parietal region of the skull and a little piece of cortex was then cut out with a warm knife maintaining a warm body temperature as well pre- and postoperatively, which was found to be very essential. The transplanted material was handed rapidly and carefully with sterile instruments and no antiseptic drug was used. The anaesthesia given was ether on a small piece of cotton. She found, as it is found today, especially in spinal cord transplantations:

> *"... that an almost insuperable difficulty appeared to be that of retaining the transferred material in the desired places".*

Finally she found that if a thin blood clot was put over the transferred piece of tissue the transplant could be kept in place.The animals were killed from 7 to 211 days after the operation and thus were examined at every different time range. The brains were removed and fixed in 10% formalin and then mordanted in toto in Miiller's fluid. The blocks were cut in 30 pm and 45 pm series microsections and stained by the Weigert-pal method and some sections were
counterstained with Upsons Carmine. In four of the rats she found successfully surviving transplants. There was continuity of vitality in the transplanted bits and growth had continued in the neurons transplanted. Furthermore, she found that the neurons which had survived had retained their morphological relations to other neurons within the transplanted bit and that growth-changes within the transplants were very similar to those of normal material about the same age. Medullation was fully accomplished. In none ofthe four successfully transplants brain had the transplanted bits attached themselves so that fibers could cross the line of attachment to unite with adjacent host neuron masses. Elisabeth Dunn further found that the transplanted cortex which was placed near the choroid plexus of the lateral ventricle apparently had received its blood supply from that source. The same was found by S. Walther Ranson who in 1914 wrote an article in the Journal of Comparative Neurology about transplantation of the spinal ganglion with observations on the significance of the complex types of spinal ganglion cells. He found nearly normal conditions in those ganglias which were within or adjacent to the choroid plexus, thereby receiving sufficient bloodsupply. The chief problem seemed to be the nourishment of the transplanted tissue. The problem of nourishment to the transplant is probably the main reason why it is still today so difficult to make successful spinal cord transplantations and to prove functional restoration of the donor-host theory.

In 1917 Elisabeth Hopkins Dunn demonstrated living and maturing neurons in the transplants of the cortex with no proven axonal connectivity to the host. That is the point where we - to be quite honest - find ourselves today with regard to spinal cord transplants, where immature spinal cord tissue can grow, mature and differentiate, without **proven** functional connectivity to the host-donor donor-host tissue. However there are **heavy** indications that there are functional synapsis between host and grafts in the spinal cord.

Regeneration Of The Injured Spinal Cord

Since 1901, it has been known (Brown-Séguard, Vanlair, Masisu, Müller) that the spinal cord could regenerate within amphibians. In 1921 Lorente De Nó [221]wrote that a spinal cord in a 20 to 35 mm long larva could regenerate. He made an incomplete section at the level of entrance of the eighth and ninth pairs. He then made histological examination from the fifth to the twentieth day after the operation. According to Lorente, the axons of the lesioned white matter became crowned with clubs and many of them crossed the peripheral portion of the wound and reestablished the spinal continuity. Before this repair, the ependyma appeared dilated and full of liquid; slowly these cysts became resorbed and the grey matter of the two stumps distally and proximally came into contact. The neurons did not divide, but adapted themselves in form and position to the new state of things. This is possible in larvae and this is exactly what we want to achieve, when the spinal cord in mammals has been lesioned either with contusion or transection of the axons.

In 1928 Ramon Y Cajal [183] in his book wrote about degeneration and regeneration of nerve centers, spinal contusion and laceration. He used young animals, cats, dogs and rabbits from 15 days to two months old in the experiments, in which the spinal cord was crushed and split into longitudinal pieces. Sometimes he would even cut the dorsal roots and then transplant a ganglion with its roots into the spinal cord. He is the first to report of an attempt to graft a peripheral nerve into the spinal cord suffering from grave contusion and laceration.

Cajal describes all the characteristics of necrotic phenomenas of the white matter: vast cysts, the preserved axons, the free and torn axons, the fibrillar masses, the necrotic and degenerated phenomena of the grey matter with exudates of cysts and torn edges of irregular wounds, ulcerations brought about by the death, and autolysis of the elements. The particular signs of compression to the spinal cord are the formation of residual neuro-fibrillar masses and superficial vacuolisation.

Of the regeneration of the spinal cord he describes the axonic sprouts which are fine, short, nodose collaterals that spring from triangular swellings in the axons. They end in butts, reticulated grumes or lanceolated eminences. In those animals where a graft of a nerve was inserted, he found that at short distance from the necrotic zone the neurons appeared deeply stained, furnished with expansions, thick or fine but clearly striated and displaying signs of a new formation. One could see sprouting in the dendrites as well as in the axons. He therefore concluded that in certain conditions the axons as well as the dendrites, and the soma of spinal neurons that have been shaken by traumatic violence, are able to emit ramified sprouts. “We can say nothing concerning their fate”, Cajal wrote. In summary, as Cajal stated un till 1928 the following is known about regeneration of the spinal cord:

" 1) The sprouting of the dendrites of the spinal cord;

2) the excitatory action of the mesodermal scar on the white matter;

3) the partial reconstruction of the posterior bundles following destruction of the proximal stump of the sensory root;

4) the migration of the bundle-fibers across the degenerated motor roots which liberate the trophic or attractive substances;

5) the capacity of the recurrent axons, sensory or motor, to innervate the roots and penetrate into the spinal cord;

6) and to sum up the demonstration that the irregenerability of the cord is not an eminent and fatal property of the neuronal architecture but the result of the absence in the neuroglial scar of a trophic and orienting environment similar to that produced in the peripheral stump of the nerves by the prolifer ated cells of Schwann".

In 1990, this is still true!

From 1927 until 1940 only a few articles about regeneration in young or adult mammals appeared. One was written by Marburg in 1936 in The Journal of Psychiatric Neurology in "Experimentelle Untersuchen über Pyramiden-Lasionen beim Hund zugleich ein Beitrag zur centralen Regeneration", where he found no regeneration in dogs with one or both lateral columns cut. So what is known until 1936 about regeneration in the central nervous system is that in non-mammalian vertebrates it is generally accepted that the central nervous system can regenerate, in mammals regeneration does not occur or is at least abortive.

In 1940, Sugar and Gérard [204] wrote about spinal cord regeneration in the adult rat. They used rats although Ssamarin in 1926 [221] abandoned the use of adult rats in his spinal cord studies, because he found that rats could not tolerate spinal cord transections. They died a short time time after the transection. Sugar and Gerard found four important missing factors in the non-regeneration of mammalians:

1. The absence of embryonic cells to help restore the grey matter.

2. The lack of Schwann cells.

3. The lack of neurotropism and

4. The lack of growth pathways.

To investigate the embryonic factor in mammalian central nervous system regeneration, Gérard and Koppanyi in 1926, and Gérard and Grinker in 1931 transected fetal rats in utero or just after birth (neonatals). They found a considerable return of function but could present only little evidence of an anatomical restitution. Complete negative anatomical findings were reported by Hooker and Nicholas in 1927 and in 1930, and Nicholas and Hooker in 1928; they found return

of function of the hind limbs, but because of the lack of the anatomical restitution they meant that the transmission of impulses were attributed by collateral pathways and to mechanical pulling of tissues to initiate reflexes past the cut. Miglia Vacca in 1930 reported of two cases of anatomical and physiological regeneration in fetal rats in utero and neonatal rats who had had the spinal cord transected. In 1935 Rossi and Gastaldi wrote that the attribution of anatomical regeneration was a regrowth of spinal roots and a posterior incomplete section of the cord, leaving the return of physiological function still to be explained by the collateral transmission as stated earlier [204, 220, 221].

Thus, my own findings are similar to Ssamarin. If the spinal cord is completely transected in rats, the animals will die soon after the transection, if they are not very carefully attended. They die of bladder disturbances, edema and loss of motor and sensory function allowing big necrotic wounds to develop. It seems that if the section of the spinal cord is incomplete, leaving just a few intact axons on the ventral side of the spinal cord, and leaving the anterior spinal artery intact, there is a very good chance of regaining some function of the hind limbs after two to three weeks. This might be by the spontaneous regeneration in the injured part of the dorsal cord and restoration of the intact fasicles in the pyramidal tract and reflexes from the intact spinal cord distal to the lesion. Whate-ver it is, the rats will do well after some weeks developing a spinal bladder, reflexes of the hind limbs and a good functional use of the limbs. Sugar and Gérard (1940) [204] found that the bloodsupply was especially of extreme importance after the transection of the spinal cord.

They found that the anterior spinal artery in the rat did not arise from the vertebral arteries entirely, but was rather formed by the confluence of branches and a number of vessels ascending along the dorsal roots. From C I to T XII they found that the artery consisted a lot of small branches (see fig. 4.19). At T XII and below to the lumbar-sacral region they found one large entering vessel to supply this part of the cord. The small confluencing branches and the large lumbar-sacral vessel made a fine anastomosis with the small confluencing branches around T XII. Therefore, the cord was always cut above T XII to avoid the large bloodvessel at the section below. If they cut below T XII, this led to degeneration of the caudal stump of the cord with atrophy of the hind limbs and failure to regain bladdercontrol. Sections above T V interfere with forelimbs and respiratory movements, for which reason they made their sections of the spinal cord between Th 6 and T h 9, so that the rats stayed alive and got an automatic bladder after eight to twelve days.

Thus, in the spinal animal, there are spinal reflexes and a spinal bladder because of a functional spinal cord distal to the lesion.

In the animals where the section has been made below Th 12 there is a degenerated caudal stump of the spinal cord, and no automatic bladder or spinal reflexes, and the animals soon die.

After two weeks the reflexes return in the spinal animal. The reflexes are walking movements of the hind limbs which are mainly alternate flexion of the hips which appear irregularly, usually after the legs have been dragged passively for some distance. There are scratch reflexes and stretch reflexes; this is stretching out the toes of the hind limbs when the rat is lifted by the tail. Apparently there is no return of sensation. Sugar and Gérard found that there was scarring and degeneration changes in the white matter (as described by Cajal in 1928) with retraction balls, autonomy of fiber ends, axon varicosities and infiltration of leucocytes and connective tissue bundles and cysts. They were the first to use embryonic tissue from the brain and the spinal cord, because of

the theory that a lack of growth in spinal cord lesions might be due to the lack of "embryonic factors". When they used transplanted embryonic material, as Elisabeth Hopkins Dunn had found in adult CNS transplants in the brain, they found that in most cases the transplant did not stay-between the cut-ends of the cord. Today it is still a problem to keep the transplant situated at the transplanted site. Sugar and Gerard found no nerve cell mitoses nor any other sign of grey matter regeneration, but many new fibers in the scar area ineffectually crossing from one side to the other in the scar tissue. Occasionally, however, the thin bundle of nerve fibers could be traced across the whole scar area from one stump to the other. In those animals who had a complete spinal cord transection it was seen that with either a piece of muscle or nerve interposed between the cord-ends, the growing nerve fibers were affected resulting in the orientation of the muscle fibers were parallel to the direction of the cord axons growth across the gap. The nerve implants from adult rats served likewise as a growth bridge and new nerve fibers followed faithfully the path laid down by the degenerated fibers even when the nerve was curved. Sugar and Gerard also found a fine correlation between the anatomical and physiological picture in those rats who showed functional recovery - new fiber bundles connecting the cord stumps across the scar, and also the amount of restitution of structural connections and functional capacities were alike. In the rats with incomplete cord sections, they found functional recovery far outdistanced the anatomical findings of regeneration. The scarred area contained many new nerve fibers, some from regenerating posterior roots and some arising from posterior columns of the spinal cord.

In their conclusion the authors write that

> *"It seems inescapable that nerve fibers can grow from spinal elements across a region of complete discontinuity and can then conduct nerve impulses which restore transmission across the lesion and some degree of normal function"* [204].

This is the first report on restoration of normal transmission of nerve impulses across a lesion of the spinal cord. This might be true, but even today it has not been proven that there is synaptic connectivity between the regenerated axons and its connections in the spinal cord of mammalians. Many have since then tried to reproduce the results of Sugar and Gerard. The first was Le Gros Clark144 who in 1942 and 1943 found growth of spinal ganglia and nerve fragments grafted into the brain. In 1947, Brown and McCouch found only scar tissue in transected spinal cords of cats and dogs with no trace of functional regeneration (described in their article "Abortive regeneration of the transected spinal cord" in the Journal of Comparative Neurology). They had used prothrombin, nerve stumps and sheath cell mixture to bridge the gap in the spinal cord. Their work was followed up in 1949 by Bernard and Carpenter in "Lack of regeneration in spinal cord of rat",in which they made spinal cord transections in 159 rats, using also sciatic nerve, muscle or tantalum to conduct the axons over the transected lesion. They even conclude that:

> *"It is our impression that regeneration would not occur even if the connective tissue were absent",*

and they continue:

> *"Regeneration in the central nervous system of mammals will probably have to wait until a more complete understanding is obtained of the more subtle aspects of the chemical background of growth"* [9] .

In 1951, Feigen, Geller and Wolf wrote "Absence of regeneration in the spinal cord of the young rat" [73]. In their summary they conclude:

> *"102 immature rats in which the spinal cord had been transected were studied functionally and anatomically to observe evidence of regeneration of axons. In most of these animals grafts of fresh or pre-degenerated autogenous sciatic nerve were interposed in the spinal cord gap. No evidence of regeneration was observed. Previous observations suggesting regeneration may have been misinterpreted" [73].*

In 1957, a report appears in the medical journal Science of "regeneration of adult mammalian spinal cord" by Campbell, Bassett, Husby and Noback [46]. This work was inspired by the works of Windle and Chambers who had shown, in 1950, that axons can regenerate across gaps in completely transected spinal cords of animals treated with Piromen. The authors made a complete spinal transection in the third thoracic level of the spinal cord in cats. The proximal and distal end of the severed portion of the spinal cord was then encased by a nylon tube impregnated with cellulose acetate (millipore). A sling stitch was used between the severed ends to maintain the ends within the tube. 30 days after transection the proximal and distal ends of the spinal cord were found to be anatomically connected by a firm bridge of tissue. A microscopic examination of the histological section of the material from the cords 30 days after transection showed an orderly linear regeneration of axons in the gap without overproliferation of glial tissue or of the pia arachnoid complex. In this paper, however, there was no report on functional recovery, only the anatomical signs of axonal regeneration.

Grafting To The Spinal Cord

The immense problem of the formation of intraspinal astroglial and collagenous scar in the gap between the proximal and distal stumps of the transected spinal cord making a definite barrier to the axonal regeneration and elongation across the lesion, provoked many scientists to use transplants over the lesion such as muscle, peripheral nerves and micropore. Such materials turned out to be very good in guidance of the regenerating axons over the lesions. After Ramon Y Cajal [183], the next scientist to use the graft of a peripheral nerve was Le Gros Clark in 1943 [144]. He inserted a piece of peripheral nerve-stump into the brain and found regeneration of brain-axons growing into the peripheral nerve, but found only penetrating axons sprouts in the new-born brain. Turbes and Freeman [210]. found some histological evidence of synapse-information in the mammalian's spinal cord from axons penetrating through a peripheral nerve grafted into a spinal cord lesion. Many other scientists, especially C. C. Kao [127, 128, 130, 132], have carried out peripheral nerve graft transplants but there has been no sign of physiological restoration.

Because of this lack of finding functional synapses and host transplant host connection of a functional transplanted bridge or relay, scientists tried to implant embryonic CNS-tissue into the spinal cord. The second attempt of an embryonic transplant to the spinal cord was made in 1972 by Thuline and Bunge [218], who used 15 to 16 days fetuses from which they removed the entire spinal cord and cultured a hemicord for 6 to 13 days in a collagen substrate. They then made a laminectomy in an adult rat, and the cultured fetal cord was placed between the dura and cord surface or in a longitudinal grove of the dorsal column white matter. They found that:

a) Implants may survive and be identified for as long as eight weeks,

b) maturation (myelination)

c) implants become vascularised

d) neurons and synapses are demonstrable within the implant.

There is no report of astroglial scar tissue or connective tissue. The culture system for hemisections of embryonic spinal cord is most carefully described by Bunge and Wood (1973) [44]. Olson and Bunge continued the work and in 1974 reported of 27 adult rats subjected to transection of which 14 received cultured embryonic rat spinal cord. Ten of the animals survived for long term observation. Histological examinations showed that in no case did any substantial portion of the spinal cord implants survive to provide a bridge between the cut cord ends. They concluded that this method of using spinal cord transplantation is ineffective in achieving functional recovery. They write that the substantial tissue reaction to the trauma of the spinal cord transection apparently precludes the survival of the implanted tissue so that future experimenters should make an effort to control the acute edema and host tissue reaction to injury.

In 1977, Nygren, Olson and Seiger [167] took fetal brain stem tissue within the locus coeruleus, containing noradrenalin cells as well as the raphe nuclei containing 5-hydroxytryptamin cells and implanted these with a glass pipet into the lumbar intumescence of adult rats. The rats had previously been lesioned with a neuro-toxic drug at the cervical level to disturb the noradrenalin and 5-hydroxytryptamin axons. After the transplant was inserted, the cord was thereafter under ether anaesthesia transected two vertebrae above the transplant. Thus, the transplanted monoamine neurons which have a high growth capacity were exposed to a completely denervated spinal cord. They found about 50% recovery of transplants for locus coeruleus and 35% for the raphe nuclei. The low survival rate was probably partially due to the expelling of the grafts and necrosis of the graft. However, they found five locus coeruleus transplants and four raphe transplants that were fully viable as evidence by normal looking monoamine containing cell-bodies and other non-fluorescent, normal looking nerve-cell-bodies after both short and long postoperative terms. They used the Falck-Hillarp fluorescence histochemical technique at different time intervals after transplantation. This technique visualizes especially the catacolamines and the 5-hydroxytryptamine containing neurons and axons. Their conclusion was that grafted immature noradrenalin and 5-hydroxytryptamin neurons can produce fibersystems in the white and grey matter of adult spinal cord that is devoid of its normal monoaminergic fiber-supply. In their study they used a neurotoxic (6-hydroxydopamin (6 OHDA) drug to deprive the spinal cord of its descending monoamin system at cervical level. It was shown by histochemical studies and uptake of a labelled transmitter that both noradrenalin and 5-hydroxytryptamin nerve terminals can regenerate in distal cord sequence leading to a recovery of normal hind limb reflex responses. Also they found a normal varicose appearance of the nerve terminals in the transplant indicating the possibility of a functional transmitter release. 14 rats were given locus coeruleus grafts with a survival of 50%, and 15 rats were given raphe nuclei grafts with a survival of 25%. Both were transplanted into the spinal cord.

In 1981, Das [59] transplanted embryonic neural tissue from neocortex, tectum, cerebellum and spinal cord obtained from embryo of 16 or 18 gestational day to the adult spinal cord. He found

that neocortex had grown and become integrated with the spinal cord but the other neural tissues did not survive the transplantation very well. He found that they were isolated and necrotic. The neural tissue from embryonic neo-cortical regions survived, grew and became anatomically integrated with the host spinal cord. He found normal looking and well integrated pyramidal and stellate cells. Furthermore the transplant was anatomically continuous with the grey matter of the spinal cord, and a number of fibers could be seen going through the interface from the transplant to the spinal cord. He thus concluded that it is possible to transplant neural tissue into the adult rat spinal cord and that the transplant receive afferents from the immediately available fiber tracks of the spinal cord. After this preliminary study, Das in 1983 published that he had transplanted embryonic tissue to the spinal cord of 208 adult rats. He divided the hosts into four groups depending on the lesion of the spinal cord. In the first group the cord was intact, in the second a complete transection of the spinal cord was performed, in the third a partial transection was made and a single injection of embryonic tissue was injected in the host; in the fourth group a partial transection was made, but a double injection of embryonic tissue was given; i.e. neocortical tissue, diencephalic tissue, tectal tissue, brain stem tissue, spinal cord tissue and cerebellar tissue. The embryonic tissue was obtained from embryos 15, 16, 18 and 20 days. The survival time of the host was four to six months after the transplantation.

The general findings in the transplanted rats were that those which had a complete transection were generally emaciated, they had a fluctuation in bodytemperature, irregularities in breathing, they were dehydrated and lacked control of urination and defecation. Thus, these findings are exactly the same as found by Ssamarin in 1926 , which caused him to abandon the use of rats in further studies of complete transection of the spinal cord. The rats that survived after the complete transection of the spinal cord showed some degree of recovery of autonomic function in two to three weeks after the surgery, but there was no evidence of recovery of locomotor function of the hind limbs. This autonomic function and the spinal reflexes are probably due to the vascularisation of the spinal cord described by Sugar and Gérard in 1940 [204]. See figure 4.19. Animals with only partial transection of the spinal cord or with the transplant directly injected into the spinal cord do not show any of these symptoms, probably due to the blood-supply of the spinal cord in the rat.

The problems of having the transplant stay in the spinal cord at the injection site in the lesion of the cavity were also described most carefully by Das in the book “Spinal cord reconstruction” edited by Kao, Bunge and Reier 1983 [128]. These difficulties still remain to be solved in the methods of transplantation but are discussed in a later chapter. In his study Das showed that the transplant of the diencephalic, the tectal, the brain stem, the spinal cord and the cerebellar tissue did not survive at all. He found that the site of the lesion was filled with meningeal membranes and dense connective tissue. The transplants that had survived were the neo-cortical transplant primarily from the neo-cortical tissue from embryos of 15, 16 and 18 days, especially those of day 16 which showed a transplant survival rate of 93% (14 out of 15), in which the parenchyma from the transplant was well integrated with the parenchym of the host spinal cord. No transplants from 20 days embryos survived. From histological observations of the parenchymally integrated neo-cortical transplants he found pyramidal and stellate cells in which neurons were not organized in layers but in clusters. Between the clusters, the space was occupied by bundles of axons.

The transplants were parenchymally opposed to the spinal cord but seemed to be separated from it by an intervening glial scar-formation. Most of the transplants were found to beopposed to the

grey matter of the spinal cord and had established an anatomical integration through the neuropil. The myelinated axons from the spinal cord were seen to course around the contour of the transplant when this was placed intraparenchymally in the white matter. The axons were neither interrupted nor did they penetrate the transplant, being pushed away by the growing transplant. He found a single cellular band of interfasicular glia between the transplant and the myelinated axons of the transplant. When the transplant was placed in the grey matter of the spinal cord, Das found that it appeared to have established an intimate anatomical integration with the host tissue. In the grey matter he found a true interface. Through the neuropil interface a number of thin and thick axons were seen to course between the transplant and the spinal cord. When the axons had entered the transplant, they formed thin bundles that appeared to run through the transplant for some distance. In the Golgi-Cox preparation he found typical pyramidal cells but they had a few secondary branches emerging from the apical dendrites and also a fewer, basal dendrites than the normal pyramidal cells. In some of the pyramidal cells, he found abnormally oriented apical dendrites. The stellate neurons seemed to be deficient in the secondary and tertiary dendritic branches. In those transplants that had become extraparenchymally and not integrated with the spinal cord of the host, Das found the transplant completely isolated from the host by intervening meningeal membranes or glial scar-formations or both. In the transplant he found the neuronal elements densely packed, poorly differentiated and necrotic, and the transplants were gradually atrophied and degenerated. Das found furthermore that the glial scar always appeared to be present when the white matter of the host spinal cord was damaged and when the transplant and the spinal cord were closely opposed. The white matter of the host spinal cord and of the transplant was always separated by an intervening glial scar. This was not nearly as clear as when the transplant was placed in the grey matter of the spinal cord host.

The idea of transplanting neocortex and spinal cord embryonic tissue to the spinal cord of adult rats was taken up by Hallas who reported of his primary results in 1982 [106] and later on in 1983 [107, 108]. In a series of adult rats, he had made a hemisection of the spinal cord between C V and C VI and then transplanted 16 days old embryonic neocortex at the site of the hemisection or 13 days old embryonic spinal cord to the hemisected cervical host spinal cord. The survival time was five months. He investigated the transplants with cresyl violet Golgi-Cox or Bodian stain horseradish peroxidase (HRP) injections or Fink-Heimer staining the lesioned fibers. In those animals stained with the Fink-Heimer method an electrolytic lesion was made directly into the transplant, one week before a transcardial perfusion with 10% formalin. Unfortunately, Hallas does not mention how many animals he operated upon, but he does tell that in all host animals the 15 days old embryonic neocortex and the 13 days old embryonic spinal cord survived, grew, differentiated and established connections with the host spinal cord or brain. The transplants of the neocortex were large and completely filled in the lesioned site and grew both rostral and caudal to the initial transplantation site. Hallas describes that the cresyl violet and the Bodian stained material revealed numerous fibers crossing the interface between transplant and host **brain** which I do not understand, because according to the description, no transplants had been put into the brain.

In transplants injected with HRP, labelled neurons were found in the thoracic and cervical grey matter of the spinal cord, the lateral reticular nucleus, contra-lateral red nucleus and motor cortex. The embryonic **spinal cord transplants** were markedly smaller than theneocortical transplants just as Das described it. Das also described that numerous fibers and fiber bundles were crossing the interface between the transplant and the host brain. Hallas concludes that both embryonic neocortex and spinal cord transplants into the spinal cord of adult rats could survive, grow, differenti-

ate and establish connectivity with the host central nervous system. In 1982 [106], he stated that an embryonic neural transplant receives afferents from the host central nervous system which normally has axons coursing through the hemisected area and also provides afferents to the intact spinal cord.

Hallas finds that the homotypical transplant may have an advantage over heterotypical transplants being in a more natural environment and therefore are able to establish better connection with the lesion's ends of the spinal cord. This however remains to be proven. In all the control animals a neuroglial scar was observed in the spinal cord within the lesioned area. Like Das, Hallas has the assumption that the transplant might prevent neuroglial scar formation in addition to re-establishing some of the severed connections of the host spinal cord or it may function as a bridge.

Brain Stem Transplants And Synaptic Connections

In 1982, Bregman and Reier 34 studied whether implants of fetal spinal cord tissue would survive and form axonal connection with host spinal cord in rats with spinal cord lesions as neonates and as adults. They used neonates because the tissue reaction to injury is less severe in the immature spinal cord, and the late developing axons, which are the cortical spinal axons, do not become damaged directly in this way. They found that immature cortico-spinal fibers took an apparent route through intact CNS, but did not regenerate across a lesion site in the absence of a suitable substrate. In the recipients they made both complete and incomplete lesions sparing some of the most ventral fibers. Bregman and Reier used prelabelled 3H-thymidin donor tissue and the donor tissue itself was from the fourteenth gestational day. Spinal cord implants were identified in 71% of the adults and in 73% of the neonatal operates. In total, 37 rats were operated upon. Furthermore they found that the implants contained mature neurons surrounded by a neuropil of myelinated and non-myelinated fibers. Extensive areas without intervening glial barrier and cellular bridges containing axons extending between the implants and the host tissues was found. With HRP-injections Bregman and Reier found retrograde and anterograde labelled fibers within the implant. In addition, there was retrograde labeling of cortico-spinal tract neurons in the neonatal recipient. Anterograde tracing experiments indicated that some of the cortico-spinal tract fibers grew through the implant. The implant of the fetal spinal cord survived and formed anatomical connections with the host. Neurons from the graft projected axons for a long distance within the host spinal cord. In the article, it is suggested that the implants served as a relay for supraspinal input or bridge for growing fibers. In 1983, Bregman and Reier [186] continued their work using immunohistochemical techniques to investigate the development and the distribution of chemically defined neuronal elements within the transplants. The reason for the evolution within spinal cord transplants in rats was not whether the transplant survives and differentiates and matures, because it does, but to examine the evolution within the transplant, its histochemistry and synaptic connections with the spinal cord of the host.

The donor tissue was obtained from the 14th and 15th gestational day of the fetal rat and placed into the dorsal columns of the funiculies or hemisections of the spinal cord of adult rats. The time of survival was one to four months and the tissues were prepared forimmunocytochemical reactivity for antisera to metencephalin, neurotensin or serotonin. Also the distribution of myelinated axons were demonstrated with an antimyelinic basic protein. The transplant in the spinal cord lesions of the adult rats showed that the implants were partially confluent with the host neural pa-

renchyma containing a sparse population of serotonin positive fibers, in contrast to those transplants completely isolated from the host spinal cord, which failed to exhibit any immuno-reactive elements. Thus Reier and Bregman conclude that the distinct unmyelinated regions which develop embryonic spinal cord transplants share morphological and immunohistochemical characteristics with the substantia gelatinosa. Just like immuno-reactive fibers, serotonin indicate invasion of the transplants by at least one population of chemically defined neurons in the host CMS [186].

In 1983. Nornes, Björklund and Stennevi [164, 165] continued the work of Nygren and Olson in 1977 [167] by replacing the loss of brain stem inputs to the severed spinal cord in adult rats by grafting embryonic brain stem tissue containing noradrenergic neurons. In the spinal cord, locomotion is generated by neuronal networks intrinsic to the cord. The intraspinal locomotion generators are controlled by supraspinal systems, and a disconnection of the cord from such supraspinal control systems results in profound loss of motor function. To restore this function and to regain motor functions CA (catecholamine) containing motor neurons were transplanted from the locus coeruleus complex to lesions into the spinal cord. The supraspinal CA system was removed by injecting a neurotoxin to cause the CA fibres in the spinal cord degenerate. Three types of cavities were made in the recipient spinal cord. A central cavity, by removing the central grey matter, a small subpial cavity and a large subpial cavity. The donor tissue was from embryos 11 to 12 days and 16 to 17 days. The survival time was 4 to 6 months before the animals were injected with fluorescent tracers 13 to 17 mm caudal to the transplant. The animals were perfused five to twelve days after the injection. For histochemistry the survival time was two to six months and CA-histofluorescens was performed.

Exactly like Elisabeth Dunn [5] and Ranson [184], the authors found that the placing of the transplants in direct contact with the vessel-rich pia is essential for a good survival. The survival of the LC (locus coeruleus) transplants in the centrally placed cavities of the grey matter was poor, only six out of 16 transplants survived. In the surviving grafts, only a few scattered CA containing neurons were found and the amount of CA containing fibers was quite limited. In the host, small pieces of cervical ganglia had been transplanted into the central cavities of the spinal cord in twelve animals; only three transplants survived. In the transplants were found CA containing ganglionic neurons. Some CA containing fibers was found within and around the transplant, forming a capsule-like structure. However there was no growth of CA fibers into the host spinal cord. The grafts placed in the small subpial cavities showed the best survival and the grafts had successfully fused with both the white and the grey matter of the host without any obvious intervening scar. In this group 14 out of 17 grafts survived and all of the grafts contained CA neurons at the range of 10 to 50% of the normal locus coeroleus. In the three animals with the large subpial cavity all the grafts survived, and the grafts contained large numbers of flurescent CA containing cell-bodies and an extensive network of fluorescent CA axons. Of the transplants with at least 50 to 60 surviving CA cell-bodies CA fiber outgrowth was seen. The outgrowth of fibers occurred especially at the sites of close fusion between the transplant and the host cord. The fibers extended within both white matter and grey matter but the route within, grey matter was generally the most prominent. Fluorescent retrograde tracing was performed in six rats with LC-transplants into the small subpial cavities. In four of the rats the graft had survived. It had fused well with the host cord and in three of the transplants CA axons coursed into the host cord and could be traced for six to twelve mm caudal to the graft.

This work shows that both noradrenergic and non-monoaminergic neurons in LC-grafts can rein-

nervate the host and that both kinds of neurons and their fibers can grow to a distance of more than one cm away from the transplant. The authors suggest that such transplants can be used as a substitute for activating command driving systems in the severed cord, and that embryonic donor tissue can restore continuity across the spinal cord transection. This may provide the possibilities for a tissue bridge that could serve to relay influences from the supraspinal centers across the site of the lesion. In the same year, 1983, Jones, Buchanan and Nornes [125], together with a group from the Colorado State University, tried to graft adrenal medulla into the adult rat spinal cord to determine whether chromaffin cells from the adrenal medulla would survive such an implantation and thus be able to reinnervate the transected cord with catecholamin containing cells. It was found that eleven of twelve dorsolateral cavity implants and thirteen of fifteen central cavity implants contained surviving chromaffin cells.

However, only very few of the cells formed processes, and regeneration of host CA containing fibers into the implant was rarely observed. There was a good tissue continuity between the host spinal cord and the implant although the interface contained a layer of collagenous tissue.

In 1983, Commissiong [51] also used fetal locus coeruleus transplants in the transected spinal cord of adult rats in an attempt to reinnervate the transected cord by a bridging technique to examine the theory that neuronal substrates for coordinated locomotion may exist entirely within the spinal cord. The so-called spinal generator for locomotion remains depressed after the spinal cord transection because of loss of their operative descending control projections. This generator can be reactivated pharmacologically. Commissiong also tried to establish a local endogenous source of neurotrophic producing cells within the spinal cord by transplanting the locus coeruleus from 16 days old embryos into the transected spinal cord of adult rats. The survival time was six to twelve weeks and the spinal cord was removed and processed for monoamin fluorescens histochemistry. In eight out of twenty animals the grafts survived. In five out of eight animals axons from the host locus coeroleus were seen to grow from the cut rostral catecholaminergic axons in the ventral horn of the cord into the transplant, but those catecholaminergic axons that were not derived from the host locus coeruleus did not proliferate in this way. Thus this proliferation is possibly a result of neurotrophic substances produced by the implanted fetal locus coeruleus. This neurotrophic substance apparently does not affect catecholaminergic axons not deriving from the host locus coeruleus. In two out of eight cases the axons of the fetal noradrenergic locus coeruleus neurons remained completely confined to the fetal nuclear tissue itself. It appeared as “a fluorescent island in a completely non-fluorescent sea”.

Peripheral sympathetic fibers grew from the spinal ganglions into the spinal cord in those cords where the transplant survived. Bernstein and his group (Patel, Kelemen, Jefferson, Turtil and Hoovler) from Washington DC have in 1983,1984 and 1985 done several worksupon spinal cord transplants in rats [18, 19, 20, 21, 22]. In 1983 [21], 70 adult rats were operated upon with neocortical and spinal cord transplants from gestation day 11, 12 and 15 (E-ll, E-12 and E-15, E stands for embryo) placed subpialy by pressure injection between the dorsal horn and the dorsal column at the Th5 to Th7 level. The survival time was 2 weeksand 3 weeks and 1,2 and 3 months. A total of 68 animals survived and of these 47 appeared to have had successful transplants (69%). The authors found that the transplants in the host grew in a proper temporal sequence in such a way that the transplant at the different stages of survival reached the same size, as it would have done if it had not been transplanted. They also found that the most successful implants were the young E-ll fetal spinal cord into the adult host spinal cord, and are thus the first to succeed in having surviving fe-

tal spinal cord transplants in adult rat spinal cord, what Das was unable to demonstrate in 1981 and 1983. Das found that only the neo-cortical tissue survived in the spinal cord, and that transplants from 15 days old embryoes grew far more extensively than those obtained from 21 days embryoes. Bernstein found the transplants to grow, maturate and differentiate. The neurons became unipolar, bipolar or multipolar. Occasionally he found some very large neurons in the fetal spinal cord implants with the characteristics of a motorneuron, but occurring in the host dorsal horn where the transplant was placed. Bundles of myelinated nerve fibers were observed and appeared to have penetrated the host spinal cord. This work was continued in 1984, and Bernstein[20] then writes about the ultrastructure of fetal spinal cord and cortex implants into the adult rat spinal cord. The donor tissue is cortex and spinal cord from 11,12 and 15 days old fetuses, implanted subpialy between the left dorsal column and the dorsal horn in 70 adult rats at the Th . 6 level. The survival time was 14 and 21 days and 1, 2 and 3 months.

At different stages of postimplantation interval, Bernstein studied the ultrastructural characteristics of fetal CNS-implants into the spinal cord of adult rats. He implanted the grafts in three zones: centre, middle and peripheral zone and found that at 30 days after the implantation, the central zone contained basal lamina and lined channels communicating with the surface of the spinal cord. The middle zone contained differentiating neurons and the outer zone was covered with the host grey matter. Furthermore, there were many projections of neurons and neuroglia from the implant to the host. In the cortical transplant he found that the neurons had large extracellular spaces in the neuropil. The neurons observed had at 30 days postimplantation a decreased stack of granular endoplastmatic reticulum, but there was no axosomatic synapses although many mature axodendritic synapses. The number of myelinated nerve fibers were increasing with the postimplantation interval. In the region between the host and the implant there was an overlapping neuropil, and in this it was impossible to distinguish between the host and implant in the electron micrographs. Bernstein found no differentiation of the fetal cortical or spinal implant into the recognizable ultrastructural cytoarchitecture. The anatomical overlap and the possible integration was demonstrated and the basal lamina, which is the interface between the central and peripheral nervous system, indicated that there was a continuity between the implant and the periphery with the formation of a syringomyelocele similar to the cavitation formation in post traumatic syringomyelia in paraplegics.

This work was continued in 1985 when Bernstein [19] transplanted E-ll fetal cortex or spinal cord implants into 36 adult spinal cords in rats. The observation time was from 1 to 10 days and during this period he found the neuroepithelia to contain cytoplasmatic organels. Later, the transplants would grow to fill up the traumatic cavity and had an overlapping neuropil in the spinal cord.

In 1985 Bernstein [19] continued his work and wrote about initial growth of transplanted E-llfetal cortex and spinal cord in the adult rat spinal cord. This continues the work just mentioned in which he transplanted E-ll fetal cortex and spinal cord into T VI spinal cordlevel of 50 adult rats and examined these 1, 3, 7 and 10 days postimplantation by means of light and electron microscope. He found the cells in the transplant to be neuroepithelia that had started to differentiate at the seventh to the tenth day after implantation. Thus, the E-ll fetal tissue formed neuroepithelia which differentiated into neurons typical for cortex or spinal cord. How these neuroepithelia are formed is not quite clear. Neuroepithelia might be either growth-centers for the fetal CNS-tissue transplant or cell surface factors, or release various trophic factors from the transplant or from the

injured host influencing the neuroepithelia cells and the differentation of them. The hypothesis was that fetal cortical transplants in the adult spinal cord from early donors E-11 might develop into spinal cord specific cell types.
The E-ll cortical and spinal transplants did not contain any cells recognizable as neurons or neuroglia, the cells being undifferentiated neuro- and spongioblasts. The E-ll cortex however develops differentiated neurons recognizable as cortial neurons and the E-ll spinal cord transplant develops neurons recognizable as spinal neurons.

In 1983 Patel and Wells [175] showed that spinal cord implants can survive in injured and uninjured host spinal cords for up to one year, whereafter they seem to degenerate. Like Bernstein, the authors found that younger embryos E-ll to E-12 seemed to best survive. The transplants were labelled with tritiated-thymidine. In 1984, Williams, Hymes, Winkler and Tesslar [217] from Philadephia, Pennsylvania, found that dorsal root ganglion neurons from E-14 or neonatals would survive in the adult spinal cord. The dorsal root ganglion develops specific characteristics in the spinal cord throughout the survival period of the host.

Electron Microscopic and Immunohistochemical Studies

Earlier the group from the University of Maryland School of Medicine, Baltimore, with Reier, Bregman, Perlow, Guth and Wujek [30, 31, 32, 33, 34, 97,185, 186, 187] contributed to the attempts to obtain functional recovery of traumatized spinal cords in rats by using fetal spinal cord transplants. In 1983, a baseline of information was obtained of the development of spinal cord transplants by inserting the transplants intraventricularly and intracerebrally in adult rats. 72 adult rats were operated upon using embryoes of various ages. The investigators found the most successful survival rate in the group where they had used fetal transplants from 12 to 15 days old embryoes. In this group 90% survived in contrast to those transplants where 16 to 17 days old embryos were used and in which only 22% of the transplants survived. To study the graft they used light and electron microscopy, autoradiographic identification of transplants and wheat germ agglutinin-horseradish peroxidase (WGA-HRP) [187].

From five to seven days after the transplantation they found distinct masses of immature neutral tissue extensively vascularized with a large population of neuroblasts. The nuclei were surrounded by a thin rim of cytoplasm and the neuronal elements had a granular cytoplasmatic containing polyribosomes, small metochondria and immature Golgi-complexes. Some neurons had a greater number of organels ineluding microtubuls neurofilarnents and rough endoplasmic reticulum. Some immature presynaptic profiles were also seen. Furthermore, glioblasts were present. By three to four weeks the transplants were up to 15 times larger than those of five days. The transplant now contained mature neurons and heavily myelinated axons and the implants were highly vascularized without any necroses. From 50 days to 8 months after transplantation the transplant increased further in size and degree of myelination although there was little qualitative changes. It frequently was found that bundles of neuropil contained neuronal pericarya, and glia extended through the trabecular lattice of the surrounding gelfoam. Numerous dendrites and myelinated axons were distributed throughout the parenchyma and many axosomatic and dendritic synapses were established as well. The tritiated-thymidine revealed that regions of the transplant had become confluent with the surface of the host brain, and studies in the electron microscope revealed an intermingling of axonal and dendritic processes without the presence of any glial or connective

tissue. In those animals where the anti-GFAP was made (a total of five animals), the anti-GFAP showed a considerable astrocytic reaction in the connective tissue partitions between host and donor tissue. A dense capsule of a GFAP-like immunoreactive process was present at the surface of the transplant, but not in the region where the grafts were directly fused with the host neural parenchyma. In two animals the silver stained section showed axons crossing over the border zone from the host to the donor, and tissue fusion was demonstrated. In one of the animals injected with WGA-HRP it was shown that the adjacent tissue contained many intensely labelled neurons, but because of diffusion it was not possible to determine if this was a sign of axonal interaction. In view of the presence of numerous astrocytes in the transplants, the lack of intervening glial scar is striking. Some degree of topographical differentiation like clusters of large neurons resembling the substantia gelatinosa was found, but the absence of a normal configuration of the grey matter was probably due to the lack of surrounding longitudinal tracks and to tissue distorsion during preparation.

In 1984, Wujek and Reier [229] transplanted E-14 rat fetuses into the spinal cord to examine the glial composition of the border zone between host and implant by means of immunocytochemistry. They found very little gliosis in those areas where an intimate fusion between the host and the donor tissue could be observed, although there was no obvious intervening cellular interface. When the implants were separated from the host by a cyst or mesodermal tissue, they found glial incapsulation to the same degree as when the spinal cord does not contain an implant.

In 1983 Bregman [30] showed that neural tissue transplants could rescue.rubrospinal neurons after neonatal axotomy. After the axotomy, the new-born rats received a fetal spinal cord transplant from E-12 to E-14; survival time was from one month to one year. The volume and the number of rubrospinal neurons was determined. A series of unoperated new-borns and a series of axotomized new-borns without transplant served as control groups. Furthermore, HRP-WGA was injected bilaterally into the lumbar spinal cord caudal to the lesion or caudal to the lesion and implant. The results indicated that implants of fetal spinal cord could rescue immature axotomized rubrospinal neurons. The cell loss in the animals with the axotomy was 45% compared to those not operated upon and to those operated upon with a transplant. The animals with the lesion had only a 25% decrease of the volume of the rubrospinal nucleus whereas in those having the implants no decrease was found. The HRP labelling indicated a retrograd transport through the transplant to the contra-lateral localized rubrospinal neurons. These studies were continued in 1986 [33], when 48 new-born rats had a laminectomy performed at Th.5 to Th.6 and a transplant from E-12 to E-14 placed at the site of transection of both dorsal columns plus one lateral funiculus. The survival period was one month to one year postimplantation. When the laminectomies are made at an early stage even a complete transection of the spinal cord of the new-born does not damage the cortico-spinal tract because the tracts do not reach the mid-thoracic region until several days after birth. When the tract is cut, however, there is an extensive retrograd death of red nucleus neurons. In those aminals where a transplant was performed at the site of the lesion, most of the rubrospinal neurons were rescued and there was no significant difference from the control animals.

HRP injected 10 to 15 mm caudal to the transplant showed bilateral labelling in the animals with the transplant of the rubrospinal neurons, but in the animals with spinal cord lesion and no transplant only the un-axotomized rubrospinal neurons were labelled. The reason why the transplant can prevent retrograd cell-death of immature axotomized rubrospinal neurons is not known, but

the authors speculate that the transplants provide a trophic support for the young axotomized neurons, and thereby allow them to survive the immediate effects of the lesion. The immature neurons are then able to use the favourable terrain provided by the transplant to support axonal elongation and thus reestablish appropriate connections. The primary work of the Reier group in 1983 wascontinued in 1986 [187] by further investigations of intraspinal transplantation of embryonic spinal cord tissue in neonatal and adult rats. In this experiment 42 adult rats and 48 newborn rats were used, the gestation day of the transplant tissue was E-14, and the survival time of the host from one to six months.

The main purpose of their study was to examine:

1. The survival and cytological features of homotropic tissue implants.
2. Organotypic differentation and development of specific neuronal populations.
3. Glial responses to intraspinal transplantation.
4. Axonal interactions between host and donor tissues."

The methods used for investigations are light and electron microscopy, autoradiographic demonstration of tritiated-thymidine labelling, immunocytochemical localization of glial fibrilary acidic protein (GFAP) and wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP). Finally immunocytochemistry was used to determine whether supraspinal serotoninergic (5-hydroxytryptamin referred to as 5-HT) axons would extend into H-thymidine-labelled donor tissue.

The overall survival rate in both adult and neonatal hosts was 83%. The volumetric measurement of a representative four month old implant was an approximate 230% increase from the initial volume of the donor tissue. Reier also finds that it is very important for the graft survival and growth that the transplant is in close contact with the adjacent meningeal blood-vessels or the central canal of the host spinal cord. In all the grafts the vascularization was extensive and he found no zones of necrosis, not even in those transplants that had survived for 16 months. The neurons contained rough endoplasmic reticulum, Golgi-complexes and mitochondria. No degeneration could be seen and the size of the neurons was the same as the size of mature neurons in the intact spinal cord. In the transplants there was no typical cytoarchitecture looking like the characteristic butterfly configuration of the grey matter, but there was distinct myelin-free areas resembling the substantia gelatinosa. In all the transplants there was extensive myelination and with the exception of the substantia gelatinosa-like regions the white matter was distributed uniformly throughout the grafts. There were numerous mature axodendritic andaxosomatic synaptic arrangements with some axosomatic synapses circumscribing the surface of the neuronal perikarya. Fiber-degeneration was not seen. The axon terminals in the grafts contained many clear vesicles. In the anti-GFAP sections the astrocytes showed moderate hypertrophy and hyperplasia and the amount of astrocytes seemed to be more numerous than seen normally in the grey matter of the spinal cord. A dense gliosis was only seen rarely in the well-integrated transplants, but in those cases where they did not fuse very well with the host spinal cord, there was a substantially greater degree of glial reactivity in the grafts. Concerning the fusion between the host and the donor tissue it was found that the greatest degree of integration was at the transition zone between the transplant and the host ventral grey matter in both the adult rats and in the neonatal group.

There was an intimate fusion which could especially be demonstrated in the tritiated-thymidine labelled graft and unlabelled host tissue. In a few transplants there was an intervening zone of gliosis or connective tissue infiltration, probably originating from injured roots and their Schwann cells. A substantial gliosis was also observed between transplants and degenerating white matter of the host. The axonal tracing was done by means of WGA-HRP which was injected into the adult and neonatal recipients 5 to 7 mm and 10 to 20 mm rostrally or caudally to the transplant to follow the retrograd transport in axons extending from the transplant into the spinal cord of the host or to demonstrate a penetration of the graft by intraspinal axons. In a total of 29 adults and neonatals, 16 grafts were found with WGA-HRP filled neurons and a few WGA-HRP filled axons in the grafts, thus showing that the transplant is projecting axons into the host and that a few axons from the host will grow into the transplant. In three animals the graft was injected by WGA-HRP; two of the grafts were heavily labelled and fibers from the graft were seen growing into the host for at least one mm to three to five mm from the graft. A few were present in the white matter of the host, but the majority of the neurons seemed to grow into the grey matter. Some retrogradely labelled cells were seen approximately three to five mm above or below the transplant. Cell labelling was seen. At the sites of host-donor-tissue approximation the cystic cavities and transplants were seen surrounded by necrosis, macrofages and myelindebris, but still outgrowth of the graft parenchyma extended toward the host spinal cord. The most prominent integration was seen at the transition zone between the transplant and the host ventral grey matter. The most prominent examples were seen in neonatal recipients in which the implants also extended and fused with the dorsal horns of the host. Dense connective scar tissue was found only rarely in the transplant recipients but substantial gliosis was observed between the transplant and degenerating white matter in the host.

The results of Reier, Bregman and Wujek's [187] show that the graft from an embryonic spinal cord has potential for replacing damaged neuronal tissue and for restoring anatomical continuity between the separated stumps of the injured spinal cord although many technical problems remain to be solved. The intraspinal transplant survival rate was 80% in both neonatal and adult recipients for periods ranging from 1 to 16 months. Most neurons originate between day 11 and day 16 of gestation and the most successful survival rates were found when fetal donor tissue was used from E-14 donors.

Many transplants of CNS-tissue have demonstrated that the cytoarchitecture of the transplants closely resembles the corresponding regions of the normal brain. This is also found in fetal spinal cord grafts. It is especially found in Reier et al. 's study that the neuropil of the graft has features resembling the substantia gelatinosa. This shows that transplantscan replace specific neuronal populations in the injured cord. The fetal spinal cord grafts can restore the anatomical continuity and at the host graft integration some axonal projections between the two tissues have been found. A synaptic functional connectivity, and thus recovery of function, has yet to be demonstrated, but more and more influential factors are still being revealed.

In 1986, Privat, Mansour, Geffard and Sandillon [180] showed that transplanted 5-HT neurons can be integrated morphologically and functionally into the spinal cord of a paraplegic rat. The rats underwent complete transection of the spinal cord at the lower thoracic level. After one week half of the rats were injected with a cell suspension from the raphe nuclei of 14 days old fetuses just below the site of transection at the lower thoracic level. After 10 days to 6 months they were perfused intracardially and Vitratone sections were made. The sections were prepared with anti 5-HT

antiserum. In grafted animals many 5-HT neurons were detected at the level of the graft. They were shown to innervate the anterior horn and the intermedio-lateral column over more than 15 mm. In the non- grafted animals no 5-HT neurons could be detected. Using electron microscopy synapses were seen at the two locations. After intraperitoneal injection of 5-HT a re-uptake inhibitor Zimelidine sexual reflexes of erection and ejaculation were restored in the grafted animals but were absent in the control paraplegic rats. Consequently the authors claim that 5-HT neurons can be integrated morphologically and functionally into the spinal cord of paraplegic rats.

In 1987, Yakovleff, Mansour, Bussel, Roby-Brami and Privat [230] showed evoked motor activity after the injection of a suspension of fetal locus coeruleus cells (containing NA neurons) into the spinal cord of paraplegic rats. The NA containing neurons were injected below the lower thoracic transection of adult rats. The effect of the graft on polysynaptic reflexes and locomotor activity was tested. It was shown that DOPA transformed into NA, depressed the short latency flexor reflexes and released a late flexor reflex. Thus grafts of NA cells in spinal rats did not modify polysynaptic reflexes in rats, but NA containing neurons grafted into the spinal cord seemed to have a slight improving effect on locomotion.

Up to the state of demonstrating functionally effective synapsis between host and graft and graft and host all the results are merely indications. The only way to actually prove the function of a synapse is to have a well integrated transplant and then perform electrophysical examinations by an electrode inserted in a neuron from the graft and a neuron from the host and to obtain an electrical activity over the synapse by electrical stimulation in one of the electrodes conducted over the synapse into the other electrode. The difficulty is to know exactly whether the electrode is inserted into the graft and if the neuron is actually making a synapse with the host. This can only be done by making simultaneous electromicroscopic sections of the areas in question to ensure the electrodes are in the correct position. Such kind of investigation is carried out, although complicated by the fact that the grafted neurons make synapses with both other grafted neurons and its own neurons by its dendrites. Electrophysiological examinations of this kind were performed in our Institute in Arhus where fetal cortical tissue was transplanted into the cortex of an adult rat. Electrophysiological examinations showed that the stimulation of the transplant gave rise to electric recordings in the host cortex [233].

The first to use fetal tissue from the brain and spinal cord resulting in the survival of transplanted tissue were Sugar and Gerard in 1940 [204].

The first to use cultural spinal cord as a graft to the spinal cord were Thulin and Bunge in 1972 [208].

In 1981 Das [59, 60] transplanted neocortex to the spinal cord with good Bernstein [21] was the first scientist who actually transplanted pieces of lesioned spinal cord with good survival results.

Reference: 5, 9, 13, 18, 19, 20, 21, 22, 30, 31, 32, 33, 34, 38, 44, 45, 56, 57, 58, 59, 60, 63, 64, 65, 67, 73, 89, 90, 93, 94, 96, 97, 98, 99, 121, 123,125,127, 128, 129,130, 131, 132, 135,141, 142, 144, 150, 164, 165,166,167,170,174,175,176, 180, 183, 184,185,186,187, 197, 199, 204, 205, 207, 208, 209, 210, 211, 214, 217,219, 220, 221, 232, 233.

Summary

Types of grafting in mammalians		CNS → CNS
		PNS → PNS
		PNS → CNS
	Other tissue (muscle)	→ CNS
		CNS → outside CNS (eye)
1890 Thompson		CNS → CNS (brain transplant between diff. species)[207]
1914 Ranson		PNS → CNS (ganglion to brain)[184]
1928 Cajal		PNS → CNS (ganglion to spinal cord)[183]
1940 Sugar and Gérard	muscle, PNS,	CNS → CNS (spinal cord)[204]
1942 Le Gros-Clark		PNS → CNS (spinal ganglia, PNS → brain)[144]
1957 Campbell	nylon millipore	→ CNS (spinal cord)[45,46]
1972 Thulin and Bunge		CNS → CNS (spinal cord → spinal cord)[208]
1977 Nygren, Olson, Seiger		CNS → CNS (brain stem → spinal cord)[167]
1981 Das		CNS → CNS (neocortex to spinal cord)[59,60]
1983 Bernstein		CNS → CNS (spinal cord to spinal cord)[21]

6
Material and Methods. Personal Experiments

The aim of this study was to see how transplanted fetal neuronal tissue from the spinal cord would grow and react in the adult brain and how different types of cerebral tissue would grow in the adult spinal cord. Furthermore the goal was to see if the transplant and the host in the spinal cord would establish functional synapses as it has been shown it does in the brain [233].

The Transplants

To have a baseline for a comparison for the grafts at differents sites in the central nervous system, fetal rat spinal cord was transplanted into the anterior eye chamber, the hippocampal area of the brain and the spinal cord of adult rats. The hippocampal area was chosen as a recipient site because this structure is well-known from other experiments performed with fetal transplants from various parts of the fetal brain of the rat.

To examine the organotypic specificities of the neurons, fetal hippocampus was transplanted into the spinal cord of the adult rat and ganglionic cells and cerebellar tissue was transplanted from the fetus into the spinal cord of the adult rat.

The amount of surviving neurons and axons with possible synapses has not been counted. One of the problems with transplanted tissue is that not many cells survive.

Two types of transplant were used: a) cell suspension and b) blocks of tissue. In one serie a cell suspension of fetal spinal cord was transplanted into the anterior chamber of the eye, the hippocampus and the spinal cord of the adult rat, and in another serie blocks of fetal spinal cord were transplanted into the anterior chamber of the eye, the hippocampal area and the spinal cord of the adult rat.

The donors and the recipients

The present study comprised 200 rats in which various transplants were performed. The transplants were not necessarily performed in series and some of the transplants shown are from the pilot study.

The recipients were adult female inbred albino wistar rats. Female rats were continuously chosen

because of their minor bladder problems compared to the problems of male rats when the transplants disturb the innervation patterns to the bladder. The rats always had the same age of five weeks, and approximately the same weight - about 140 grams.

The donors were also inbred albino wistar rats. At first, the age of the donor tissue varied from embryo-day 13 to embryo-day 17. However the best survival was reached when the transplant was taken as early as possible, either day 14 or 15. Fig. 6.1 shows two fetuses,one of day 14 and one of day 16, the latter having been dissected to show the brain, the cerebellum, the brainstem and the spinal cord with its meninges.

The operations

The animals were anaesthetized with mebumal 0.5% 0.1 ml./100 mg rat.

The operations were always performed in the same way. Five female rats were anaesthetized simultaneously and a laminectomy was performed at the level around Th.8 just below the interscapular fat and at the curve of the back where the lamina and the spinal cord is easy to expose to the surface. One lamina was removed. Thus, the arachnoid, the subarachnoid space and the pia containing the spinal cord were exposed. After all five female rats had quickly had a laminectomy performed, one fetus was taken from an anaesthesized pregnant albino wistar rat through a laparotomy and the spinal cord was dissected promptly out of the fetus. The fetus in its amnion sack was placed on a little plastic cover with the result that the spinal cord throughout the whole period was only embedded in its own fluid. No other solution was used. The spinal cord was stripped of its meninges and the cord cut into small pieces. One of those small pieces or a suspension was used as a transplant. A little piece of approximately 1 mm by 1-2 mm was aspirated into a 25 cm long tiny plastic tube and placed either in the subarachnoidal space or intramedullary into the five rats in which the spinal cord was exposed. A subcutaneous needle with a bent end as shown in fig. 4.3 was used to lift up the arachnoid membrane. A little incision was made so the plastic tube could be inserted as shown in fig. 6.2 in order to place the transplant. The transplants were easily introduced into the subarachnoid space where they had excellent growth conditions because of the cerebrospinal fluid being their natural environment. Furthermore they were also in close contact with the pia containing the vessels. When the transplant was introduced into the subarachnoidal space it was always attempted to make a little incision in the pia enabling the transplant to get into close contact with the white matter of the spinal cord. When the transplant was inserted intramedullary, the transplant was inserted cranially underneath the adjacent lamina of the thoracic vertebral canal in order to avoid extrusion from the spinal cord because of the mass effect, the pressure and the edema developing immediately when the lesion in the cord had been made. The rat was always placed with the head toward the left so that the plastic tube could be easily handled with the right hand, and the transplant, whether it was placed in the subarachnodial space or intramedullary was always placed in the right column of the spinal cord making it easier to find again. Thus the interval in which the fetal cord transplant was outside its natural environment was very short. When the fetus was taken out of the animal there was a maximum period of 15 minutes before it was inserted into the recipient rat. During this period the transplant was constantly kept in its own amnion fluid.

The lesion in the arachnoid membrane was rather small about 2-3 mm, and no attempt was made

to suture the membrane. At first I tried to cover the small lesion with either a foil or a little plastic cap. Also a small piece of dried human dura (Lyodura, L0vens Kemiske Fabrikker, Braun, Germany) was sometimes used. Whatever was used to cover the lesion, it did not seem to have the slightest effect on either expelling or the growth of the transplant. The cover was always completely embedded in scar tissue and sometimes actually removed from the lesion in the arachnoid. For this reason the skin was closed in one layer with interrupted silk sutures and nothing else. This procedure was used in thewhole serie and considered to be in accordance with general surgical principles of human surgery: to do the least possible and to leave as little foreign material as possible. After five weeks or more the rats were sacrificed. When a period of five weeks had passed, the transplant had a certain size and if the rats were kept alive for a longer period of time, the transplant did not seem to change.

A complete transection of the spinal cord in the rat was not used because from the pilot studies it was soon realized that what Saltykov found in 1905 appeared to be quite true. The rats became very weak, they had nutritional problems, bladder problems and hypothermia. Of course they could not use their hind limbs, as they were paraplegic, and furthermore developed wound problems. They were as a whole terribly weakened and would often die. To avoid this condition a smaller lesion of the cord was made. However it turned out that whether transplanted or not the rat would start to move its hind limb after approximately one to two weeks. Thus neurophysiological examinations would not reveal whether a piece of transplant had made functional synapses from the spinal cord to the transplant to the spinal cord or if it was used as a bridge over the lesion. Consequently the way of transplantation was an insertion of the transplant into the intact spinal cord without any efforts to first make a lesion. In this way the least harm was done to the rats who also had no neurological deficits postoperatively. In case a lesion was made and it was intended to place a transplant, this was extremely difficult because of the reactive edema of the tissue.

As a consequence the lesion should be made close to the wound and then be left alone for three or four weeks. After this period an insertion should again be made into the spinal cord at the lesioned site and all scar tissue and possible cysts should be removed, and the transplant should be placed. Following this procedure the transplant will not be expelled and this method should thus be the proper approach in human beings.

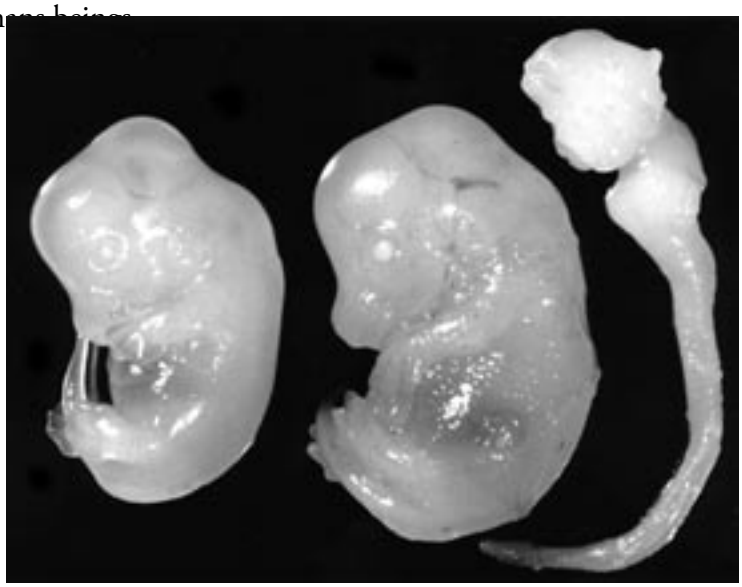


Fig. 6.1
Rat embryos

SpE-14 *SpE-16*

SpE = Spinal Embryo

Fig. 6.2

A plastic catheter is inserted intermedullary to place the transplant.

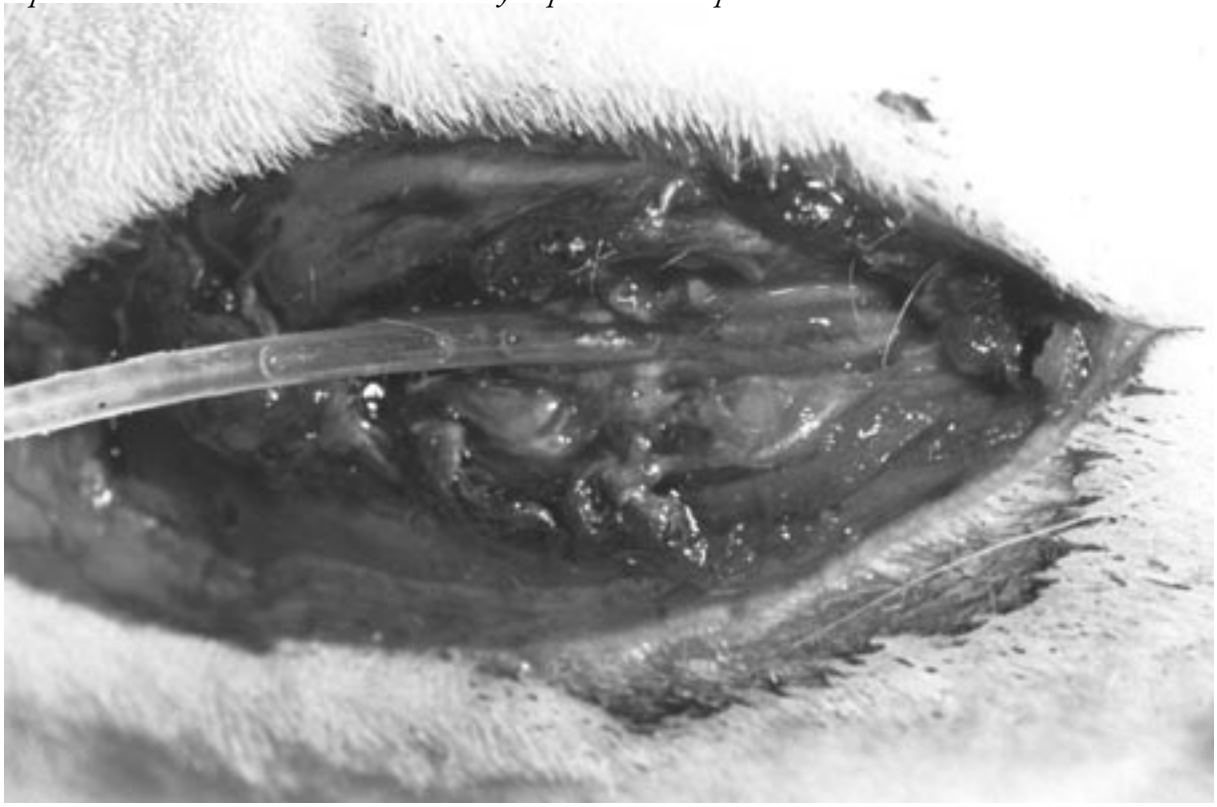


Fig. 6.3

Fetal anterior horn cells Thionin.

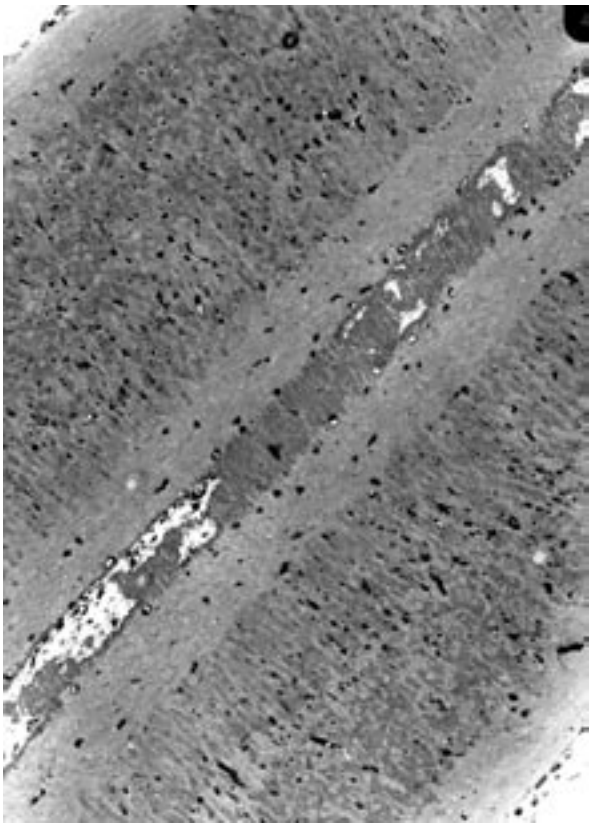


Fig. 6.4

Large magnification.

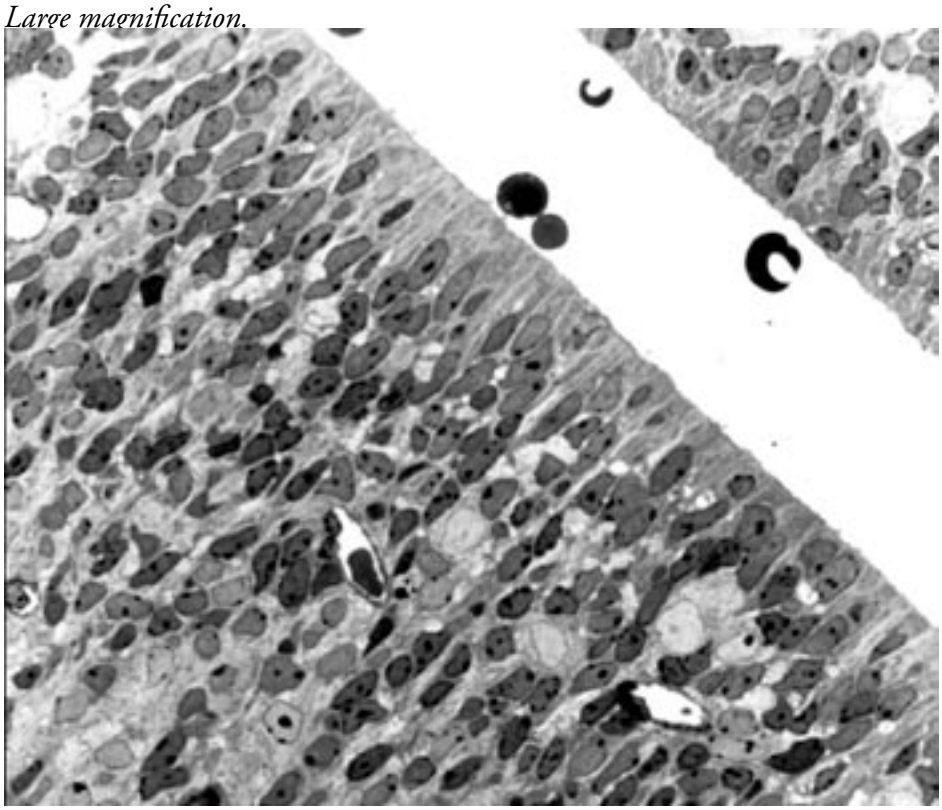


Fig. 6.5

Larger magnification

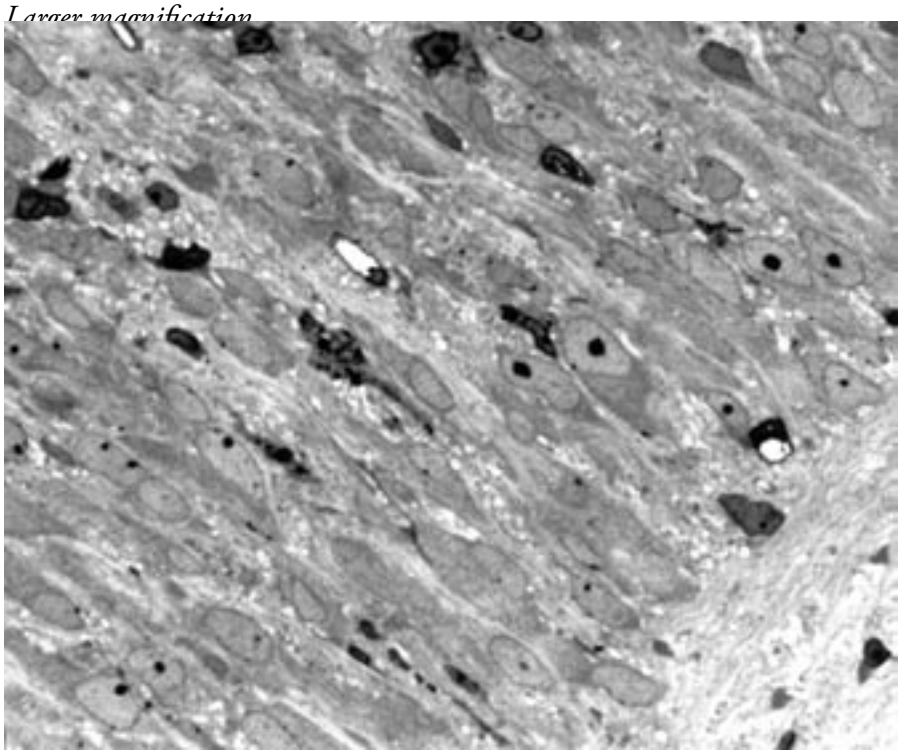
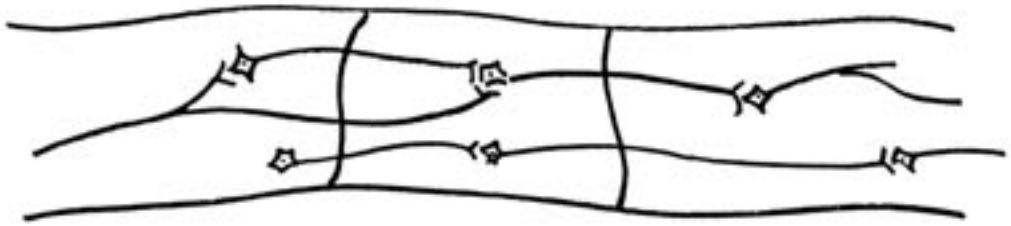
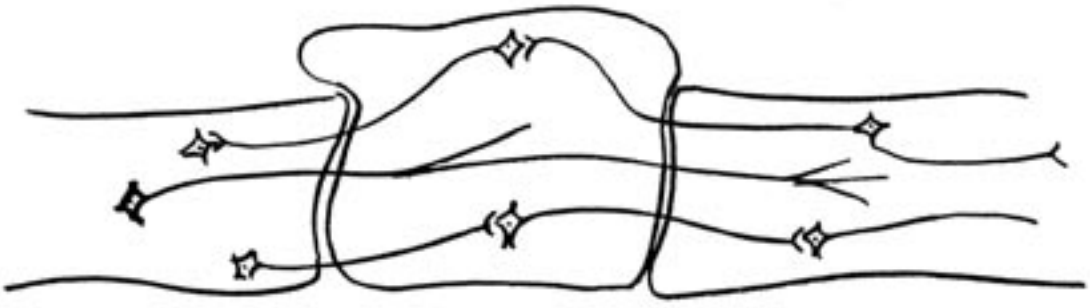


Fig. 6.6
Bridge versus relay of transplants

TP BRIDGE



TP RELAY
After P. J. Reier



COMBINATION
Benedicte Dahlerup

Laboratory Techniques

Suspension of spinal cord tissue

The vital spinal cord tissue was placed in 0.6% glucose + 0.9 sodium chloride. To remove the spinal cord and place it in the solution would take about five minutes. It was then kept in the glucose-sodium chloride solution for one hour, and then with a pipette put into a trypsin 0.025% solution and placed in a heater for 20 minutes. The trypsin was removed by a pipette and replaced with Dulbeccos PBS (phosphate buffered NaCl solution and NaHCOs without glucose). The spinal cord was filtered in a sterile way six times to make it into a suspension, and it was washed six times. With a 0.6 mm. glass pipette the suspension was made by using the pipette eight to ten times to achieve a milky fluid. After this the suspension was placed in a Hamilton syringe. Before the suspension was put into the rat as a transplant, a viability-test was made with FD-PI (fluorescein diacetate propidium iodide). The living cells becoming green and the dead cells red in the fluorescent microscope. About 20-30% of the cells in the viability-test were dead. Then the suspension was ready for grafting into the adult rat.

Perfusion methods

After five weeks the rats were perfused transcardially with either 1) sulphide perfusion 2) paraformaldehyde perfusion 3) fluochrome perfusion (fluofix) or 4) EM perfusion.

1.Sulphide perfusion

0.5% sulphide perfusion is made by ll.7g.Na2S, 11.9 g NaHiPCUx IHzOand 1000 ml distilled H2O. The rats were perfused for one minute with full flow and thereafter for four minutes with the sulphide just dripping. Then the brain and the spinal cord were removed and dried with carbondioxide ice.

From this five series 30m thick were made. Each series was stained with a thionin cell body stain, a Timm light + dark stain for the heavy metals and Nauta-stain for normal fibers and acetylcholinesterase to visualize the cholinergic system.

2.Paraformaldehyde perfusion

A 4% paraformaldehyde solution was mixed with Sørensen's buffer 0.15 mol (pH = 7.3). 375 ml H20 was heated to 60°. Then 20 g PFA was dissolved in NaOH until the solution was clear. It was then chilled and filtered in a waterfilter. 125 ml 0.6 mol buffer was added (1 drip of NaOH per 2 g PFA makes pH = 7.4). The rat was then perfused transcardially for one minute with full flow and hereafter for three to four minutes with a slowly dripping flow.

The brain and the spinal cord were removed and put into a 30% sucrose solution overnight until they dropped to the bottom of the solution. They were thereafter frozen in carbondioxide ice and cut into 30 m thick slices. This was done in three series and it was coloured with PAP immune stain.

Thionin

1% thionin in H_2O. 0.1 g in 100 ml Diff in 70% alcohol.

Histochemically acetylcholinesterase staining

The cut tissue was put on object glass and dried by room temperature. The slices were then fixed in acetone by 4° centrigrade for 15 minutes and thereafter dried by a ventilation for 15 minutes. They were incubated for two hours by 37°. The main contents of the incubation solution was 499.4 mg CuSO4 ml and 750.7 mg glycin which was diluted with 750 ml H_2O. To this was added 74.0 ml of 0.2 mol acetid acid and 176.0 ml 0.2 mol natriumacetate. The pH had to be 5.0.

This solution was kept cool. The incubation solution was made by adding 115.8 mg acetylthiocholin and 6.2 mg etopropazin to 100 ml of the original solution. The object glasses were then rinsed shortly in five cups of 0.05 mol acetic acid-Na-acetate buffer, and then put into a 1% AgNOs for one minute and rinsed in four cups of 0.05 mol acetic acid-Na-acetate buffer. Thereafter they were fixed from one hour up to several days in a neutral buffered 10% formaline in the refrigerator. It was dehydrated by ethanol xylol and finally mounted with Dammar resin.

Nauta silverstaining

The tissue was cut and put on glasses and dried by room temperature. If the tissue was fixed in formaline, the slices might be kept for just one night in neutral buffered formaline. If the tissue was fresh, the slices had to remain for at least 14 days in neutral buffered formaline. Thereafter the slides were kept for one to three days in 50% ethanol containing 6.00 ml of 25% ammonia per 1000 ml 50% ethanol. The slices were then most carefully put through distilled water until the ethanol had diffused. They were then kept overnight in 1.25% $AgNO_3$ with 5% pyridin on top of a heater to maintain a temperature somewhat higher than the room temperature. Then the slices were placed directly into two cups of ammonia-$AgNO_3$, 30 seconds in the first cup and 60 seconds in the second cup. The solutions were completely fresh not to vapourize. The first solution was made of 132 ml of 1.4% $AgNO_3$, 60 ml of absolute ethanol, 8 ml of 25% ammonia and 5 ml of 4% NaOH. The slices were then put into two cups of reduction baths, five seconds in the first and one minute and 55 seconds in the second one made of 90 ml distilled water, 10 ml absolute ethanol, 3 ml 10% formaline and 3 ml 1% citrus acid. The slices were then rinsed three times for three minutes in distilled water. For half an hour the ethanol was dehydrated to xylol-phenol-creosol consisting of 80 ml xylol, 10 g phenol and 10 ml creosol. Finally followed three baths of xylol. The slides were mounted with Dammar resin.

Slice cultures of nervous tissue

A few experiments were made with tissue cultures, but only cultures of cerebellar tissue were grafted. The cultures were made the following way: the cerebellar tissue was cut into thin slices and cultured by means of the roller-tube technique.

All the sections were prepared under lamina flow, using sterile techniques. The tissue for this spe-

cific experiment was from grown-up five weeks old, 140 grams female wistar rats.
The tissue to be cultured was removed and washed in GEYS balance salt solution (BSS), enriched with glucose to a final concentration of 6.5 mg/ml. The tissue block was placed on an Aclar foil and cut into 400 m thick slices using a Mcllwain tissue sectioner. The sliced tissue block was then transferred into a plastic dish containing glucose enriched with GEYS BSS and quickly separated into individual slices using spatulas. The slices were kept covered in the solution in the petri dishes at 4° centigrade until they were embedded a few hours later. The slices were placed on glass coverslips that had been thoroughly washed, sterilized and coated with 451 reconstituted thick enplasma (DIFCO, lyophilized). Then the coverslips bearing the slices were placed in plastic cultured tubes (16 x 100 mm) which contain 1 ml of medium. The test tubes were fixed in a roller drum which was housed in an oven at 33-36° centrigrade. The drum rotated at 10 revolutions/hour to ensure proper feeding and aeration of the cultures. The roller drum was tilted 5° with respects to the horizonal axes so that the slices were immersed in the medium only during half a cycle. Concentrations of oxygen and carbon dioxide and humidity of the air in the oven did not need to be regulated. The monocultures were only fed once per week with a sterile medium consisting of 25% horse serum, 50% of the Eagle base medium and 25% of Hans BBS, supplemented with glucose and glutamine to a final concentration 5 mg/ml and 0.3 mg/ml respectively. To reduce the population of non-neural cells the cultures were exposed during 48 hours 4 days after the culture had started to a medium containing antimitotics.

The avidin-biothin method

Immuncomponent staining of the surface antigens (for light microscopy).

The rat was perfused in 4% paraformaldehyde in 0.15 M S0rensen's buffer, pH 7.3, + 1% glutaraldehyde in 0.15 M Sørensen's buffer. The brain and/or spinal cord were kept overnight in 20% sucrose in a 0.15 M Sørensen's buffer. Frozen sections were cut in 5-8 m and put in a glass of gelatine, two sections in each glass and kept in a freezer.

It was kept in TBS + 10% PCS for 30 minutes, incubated with primary antibodies for 60 minutes and TBS +1 % triton for 3 x 15 minutes. Thereafter it was incubated with secondary antibodies 1:2000 and biotinyleret antimus - immunoglobulin for 60 minutes. Then it was blocked with endogen peroxidases with 0.2% H_2O_2 methanol for 30 minutes. Again TBS + 1% triton for 3 x 15 minutes incubated with avidin-peroxidase for 60 minutes. TBS + 1% triton for 3x15 minutes and it was exposed with diaminobenzidin for 30 minutes and rinsed in TBS twice, dehydrated with xylol and thereafter mounted in permount.

The PAP-staining for a light microscopy

The animal was perfused with 4% paraformaldehyde in 0.15 M S0rensen's buffer, pH 7.3 + 1% glutaraldehyde in 0.15 M Sørensen's buffer. The specimen was cut in 30 frozen sections and put in a gelatine glass, two sections in each glass and kept in a freezer. Thereafter it was put in a 1% triton, TBS for 15 minutes, incubated with 1% pig serum in TBS by room temperature for 30 minutes. Thereafter it was incubated with primary antibodies at 4° overnight and put in TBS (1% Triton) for 3 x 10 minutes. Kept for 30 minutes with unmarked antirabbit 1:30 and again TBS (1 % Triton) for 3x10 minutes. PAP 1:175 for 30 minutes and TBS (1% Triton) for 2x10 minutes, again TBS, this time without triton, for 10 minutes and exposed with diaminobenzidin for 30 minutes. Rinsed twice in distilled water, dehydrated with xylol and mounted in permount.

Reference: 3, 24, 61, 168, 169, 191, 195, 212, 231, 232.

7
Results

Results

No surviving grafted neurons could be found in any of the animals in which the suspension had been used (N = 20).

Fig. 7.1 shows a total spinal cord in which a suspension was inserted. The suspension was made from E- 13 and the survival time was six weeks, the staining was thionin. Only massive masses of glia tissue were seen and in the close-up in fig. 7.2 , possibly some cartilage has also developed.

In fig. 7.3 part of the suspension of the spinal cord was injected into the hippocampal area and exactly the same picture appeared with lots of dense glia tissue and no neurons. As a consequence the experiments with suspension were temporarily given up.

In the following I will let the photographs of the grafts speak for themselves.

Fig. 7.0

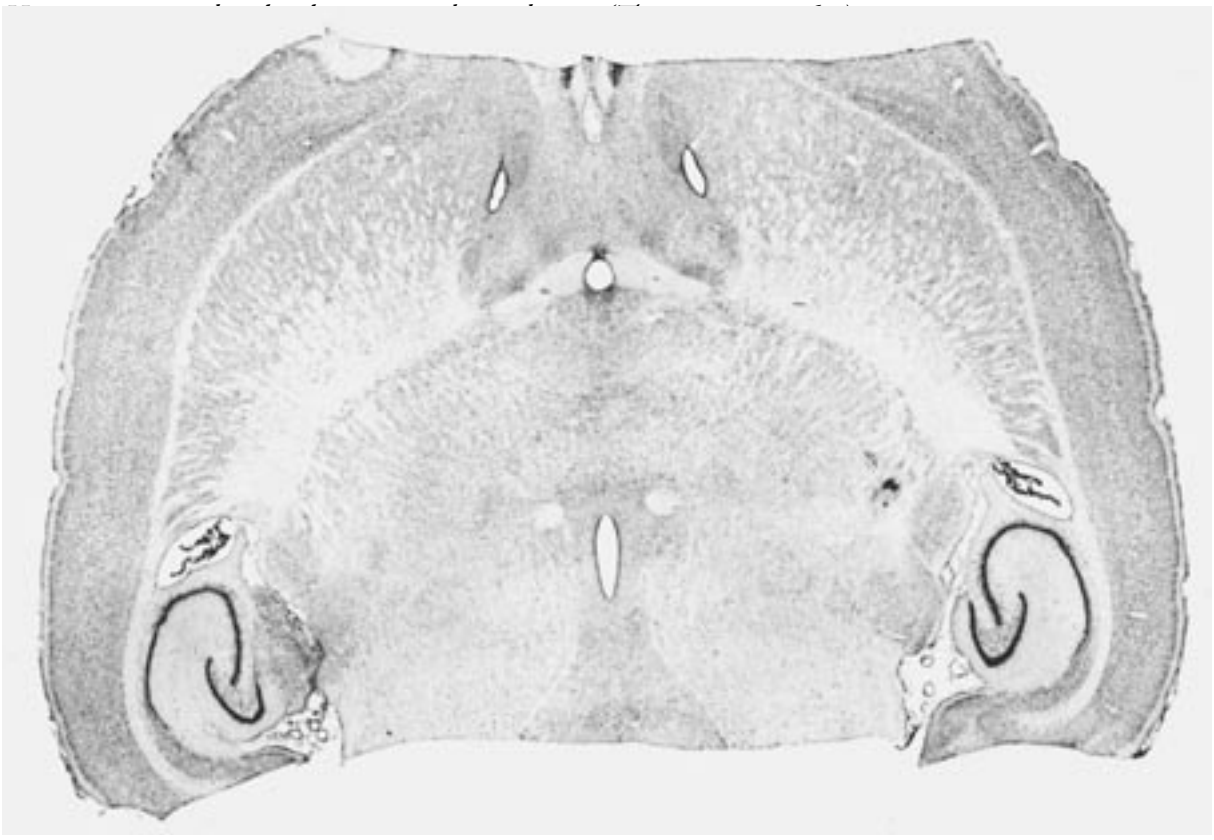


Fig. 7.1

Suspension of the fetal spinal cord transplanted into the adult spinal cord (x 6,5) Gliatissue and cartilage

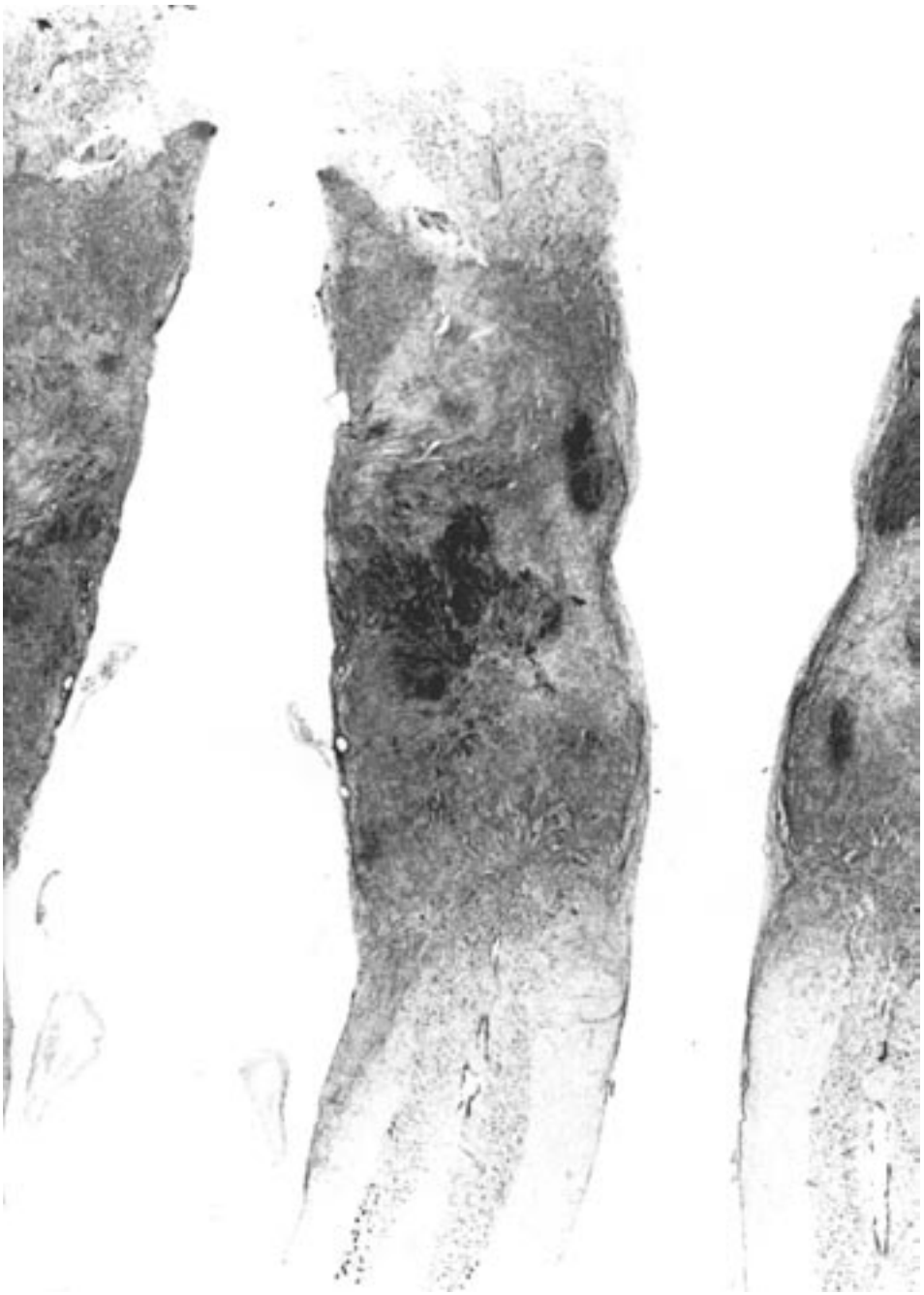


Fig. 7.2
Close-up of fig. 7.1 (Thionin x49) Cartilage

Fig. 7.3
Suspension in the anterior thalamus near the hippocampus (Thionin x31)

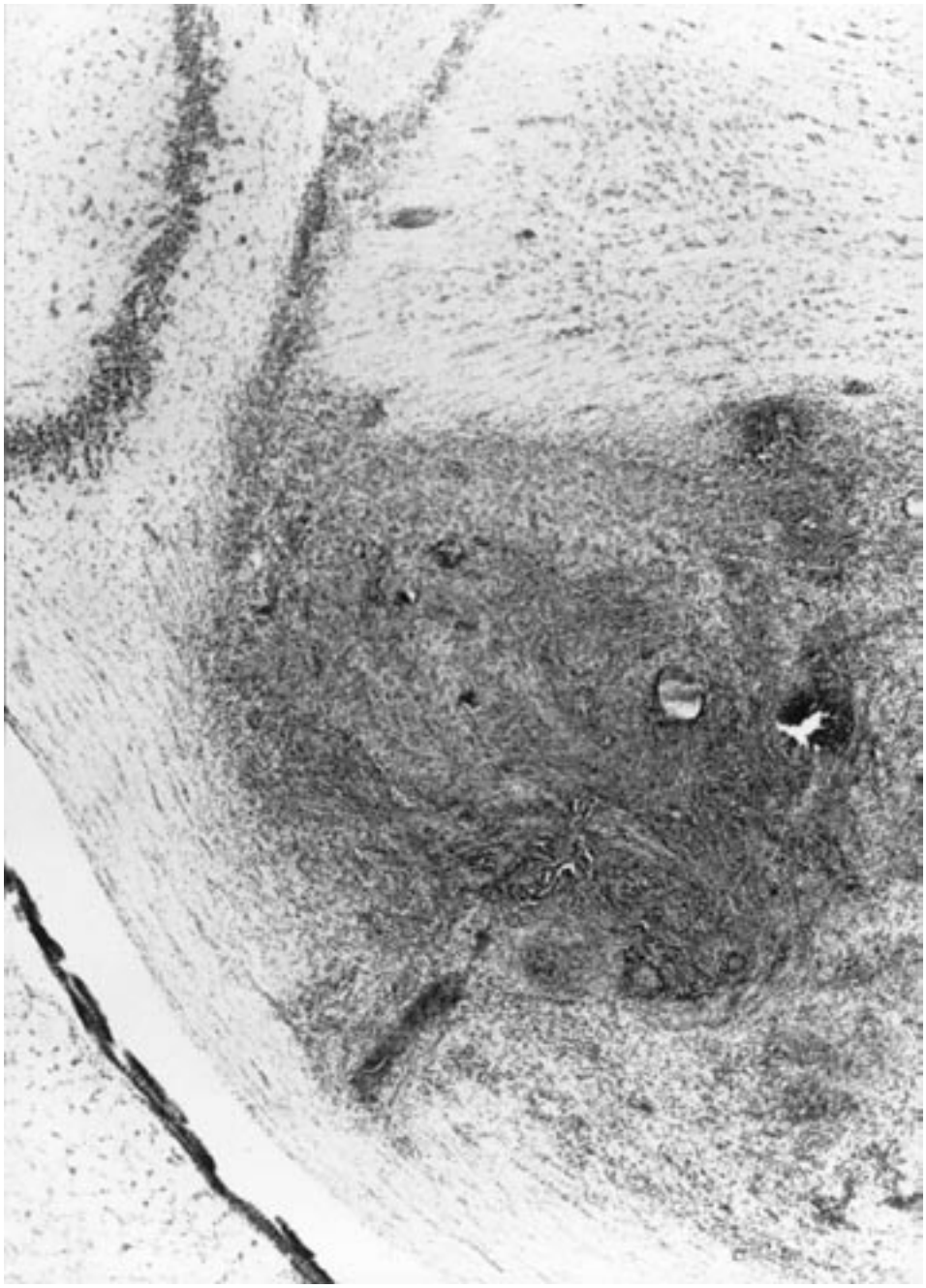


Fig. 7.4

Figs. 7.4 and 7.5 show blocks of E 14 transplanted into the anterior chamber of the eye of an adult rat. The survival time was six weeks, thionin staining. The cornea is well defined and clusters of neurons are seen between astrocytes and glia cells. In fig. 7.5 a cluster of small neurons has been magnified, (x 31)

TP = transplant C= cornea L- Lens

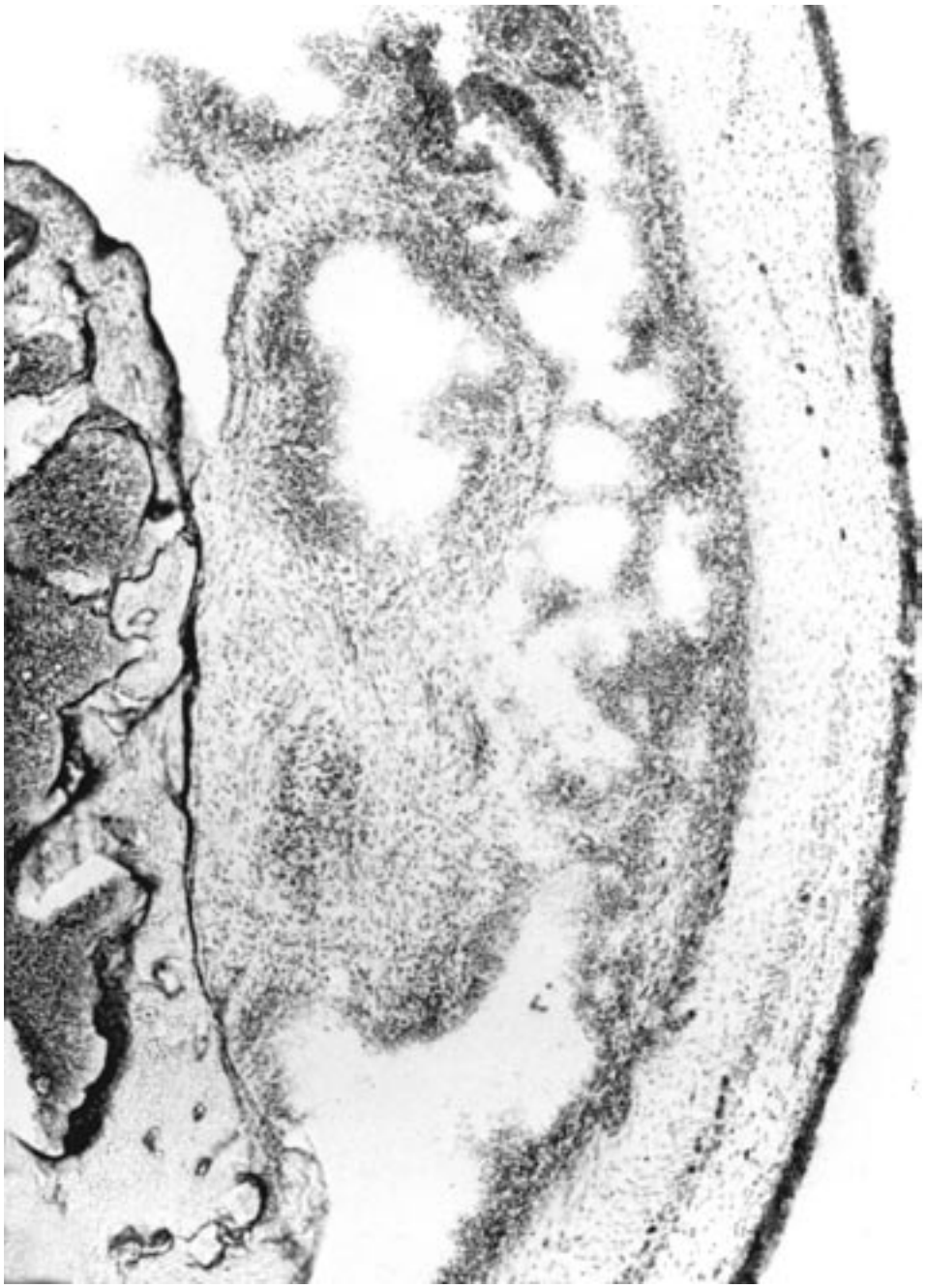


Fig. 7.5
Thionin (x 275) Cluster of neurons in the anterior eye chamber of a rat.

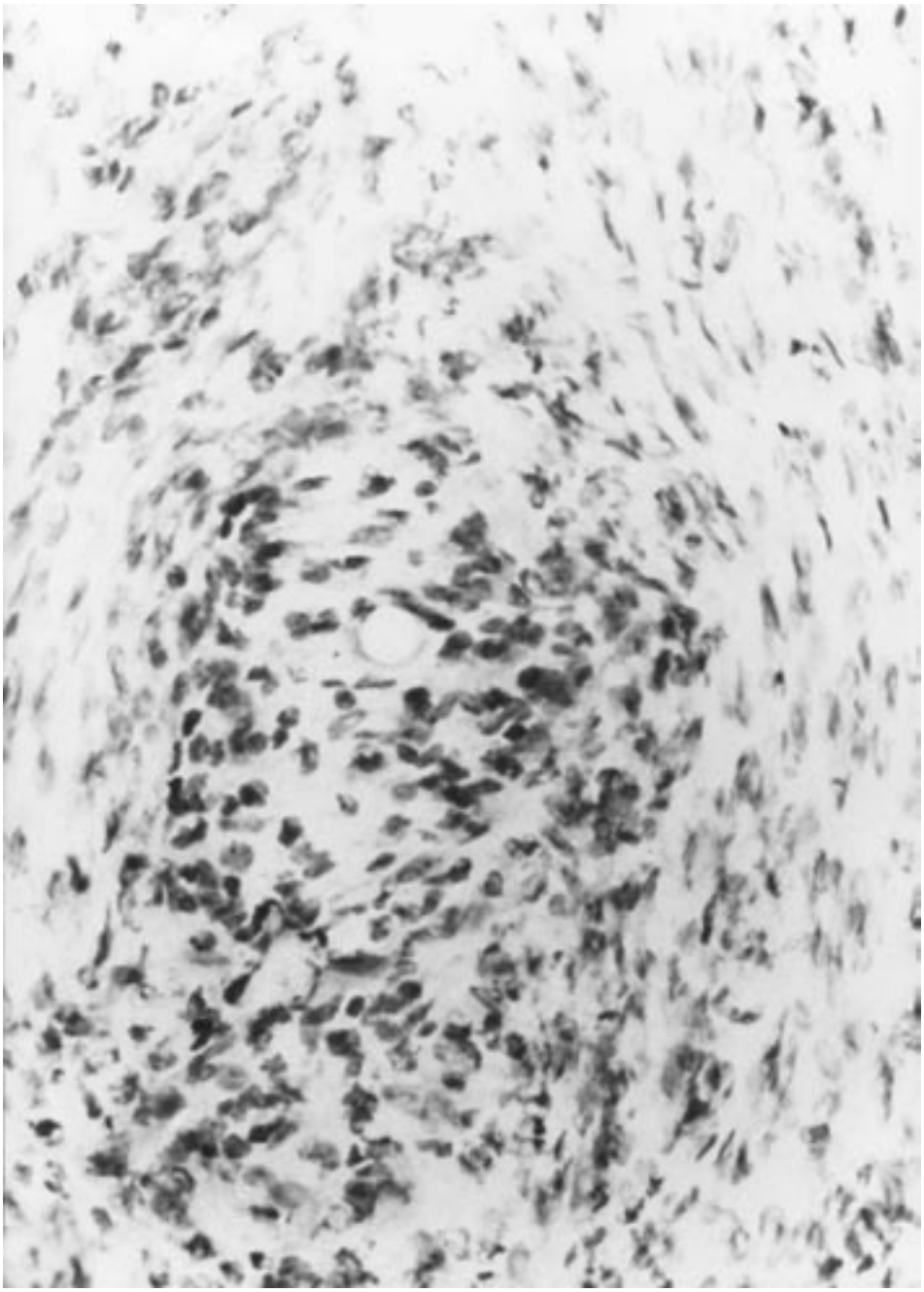


Fig. 7.6

In fig. 7.6 the hippocampus has been grafted into the spinal cord and can be recognized as the dentate area. Thionin staining. (Sunde and Zimmer). (x 44)

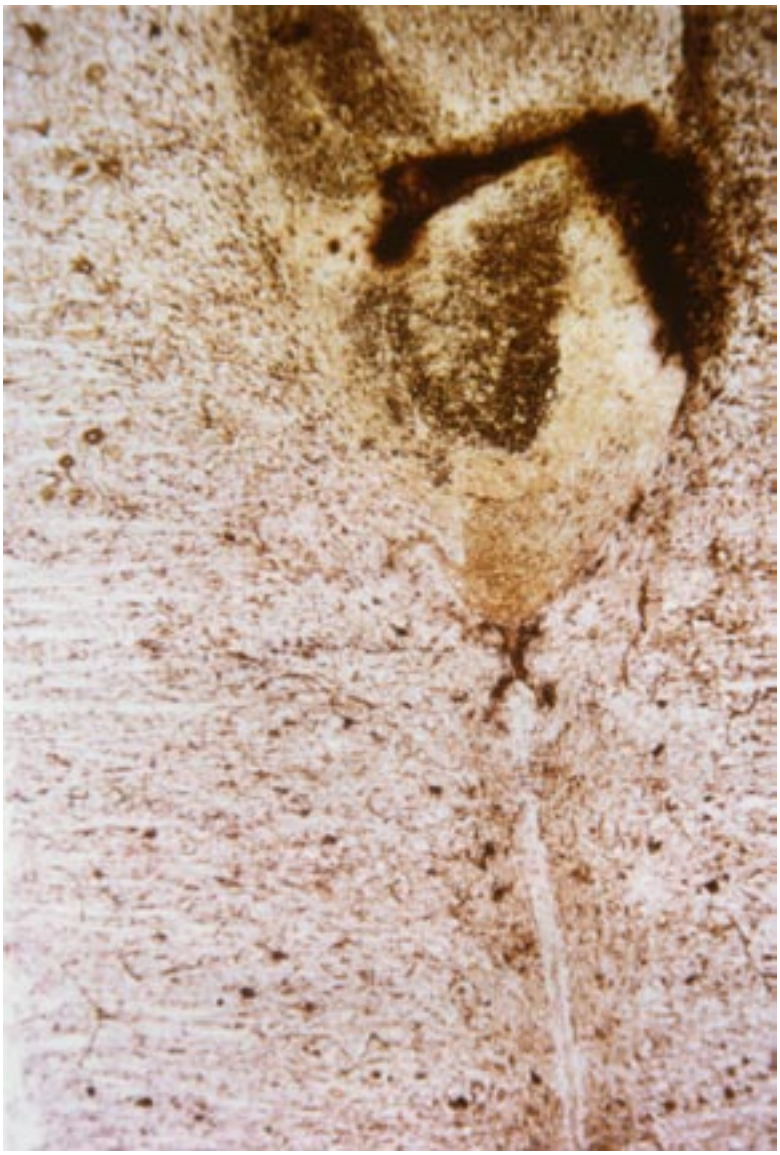


Fig. 7.7

Fig. 7.7 shows an E15 cerebellar tissue graft into the spinal cord. The staining is thionin. The cytoarchitecture is typical for cerebellar tissue with a molecular layer, a Purkinje cell layer and a granulation cell layer, (x 25)

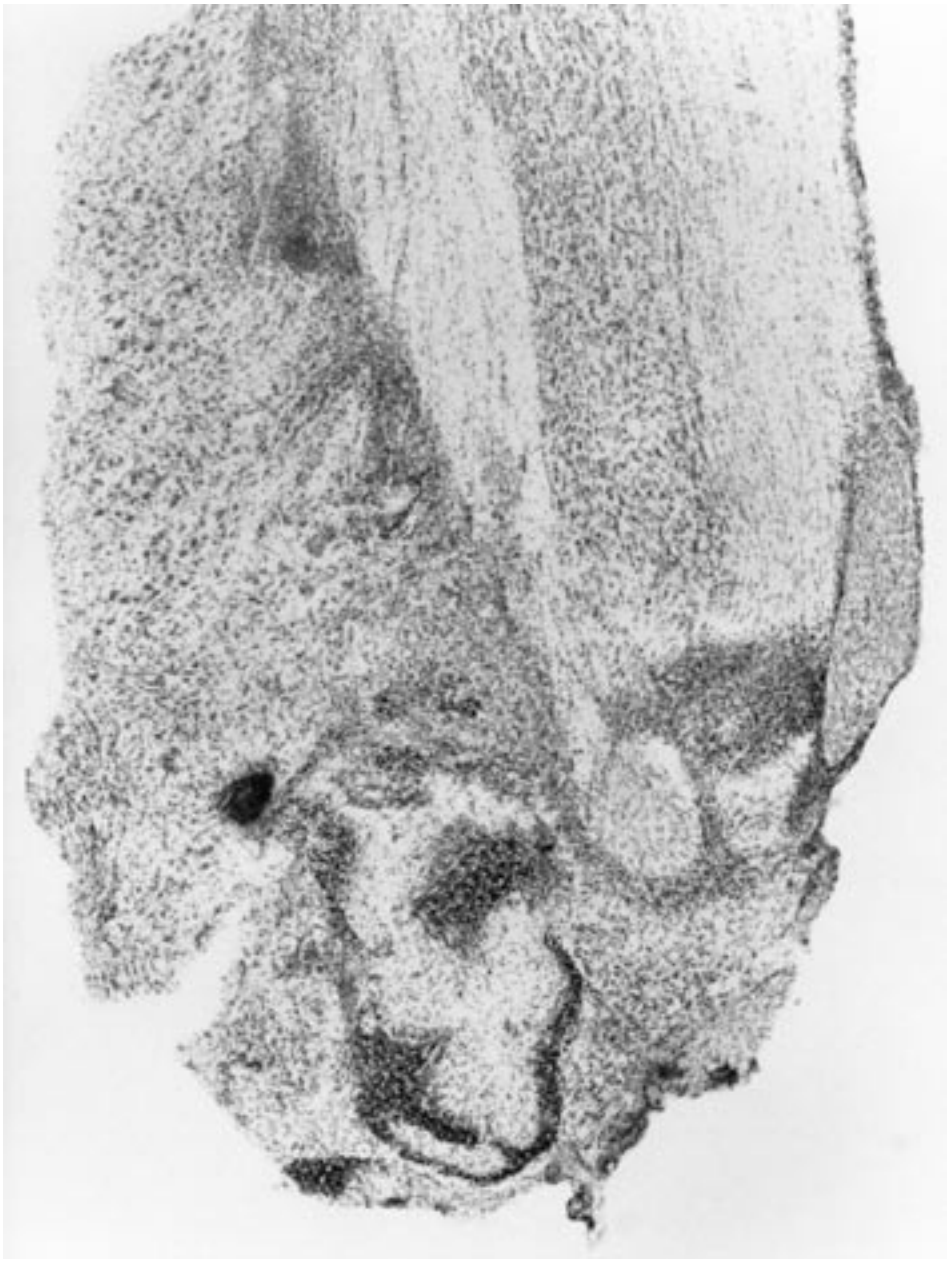


Fig. 7.8

Fig. 7.8 shows the same picture as fig. 7.7 in a close-up and could as well have been taken from the cerebellum of an adult rat, each demonstrating how well defined and organotypic the tissue is, although it has been transplanted into a different area of the central nervous system. (Thioninx 108)

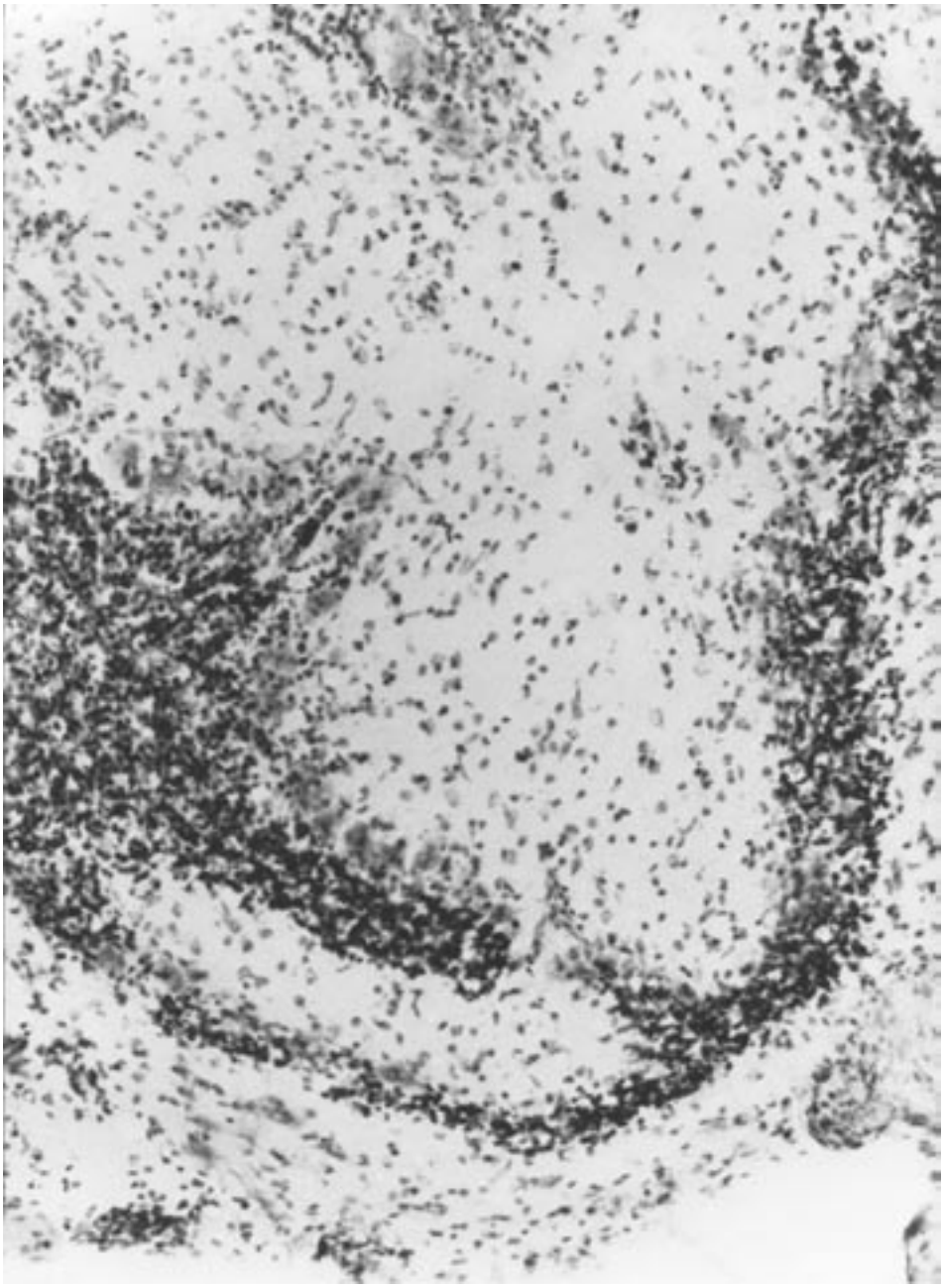


Fig. 7.9

Fig. 7.9 shows E 14 dorsal root ganglion cells grafted into the hippocampal area in an ACHE-staining. Survival time is six weeks. The first to show that the ganglionic cells could survive as a transplant in the brain was Ransohof, 1906. (x275)

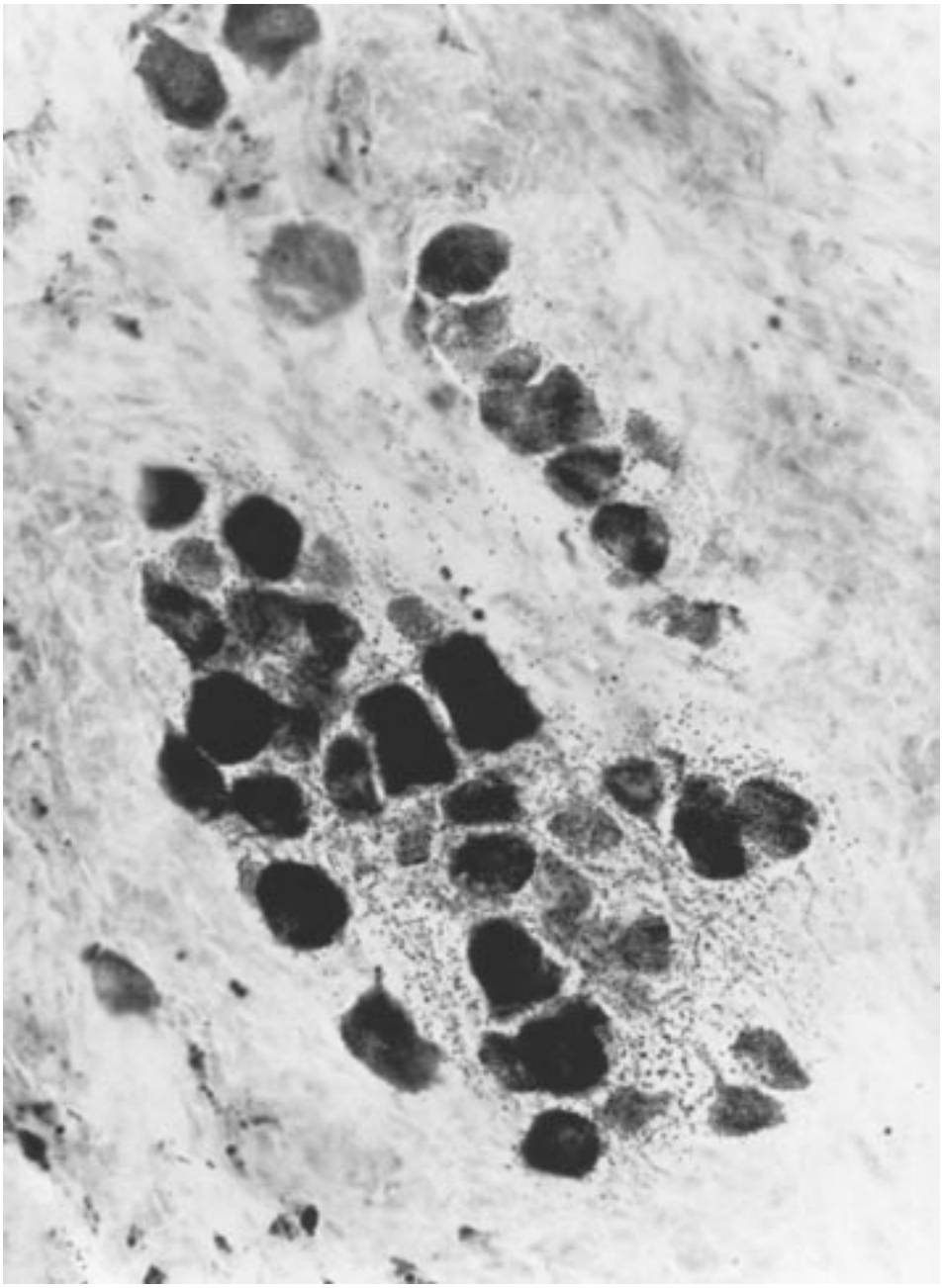


Fig. 7.10

This figure shows an anatomically well integrated spinal cord transplant in the hippocampal area. Thionin staining (x 44)

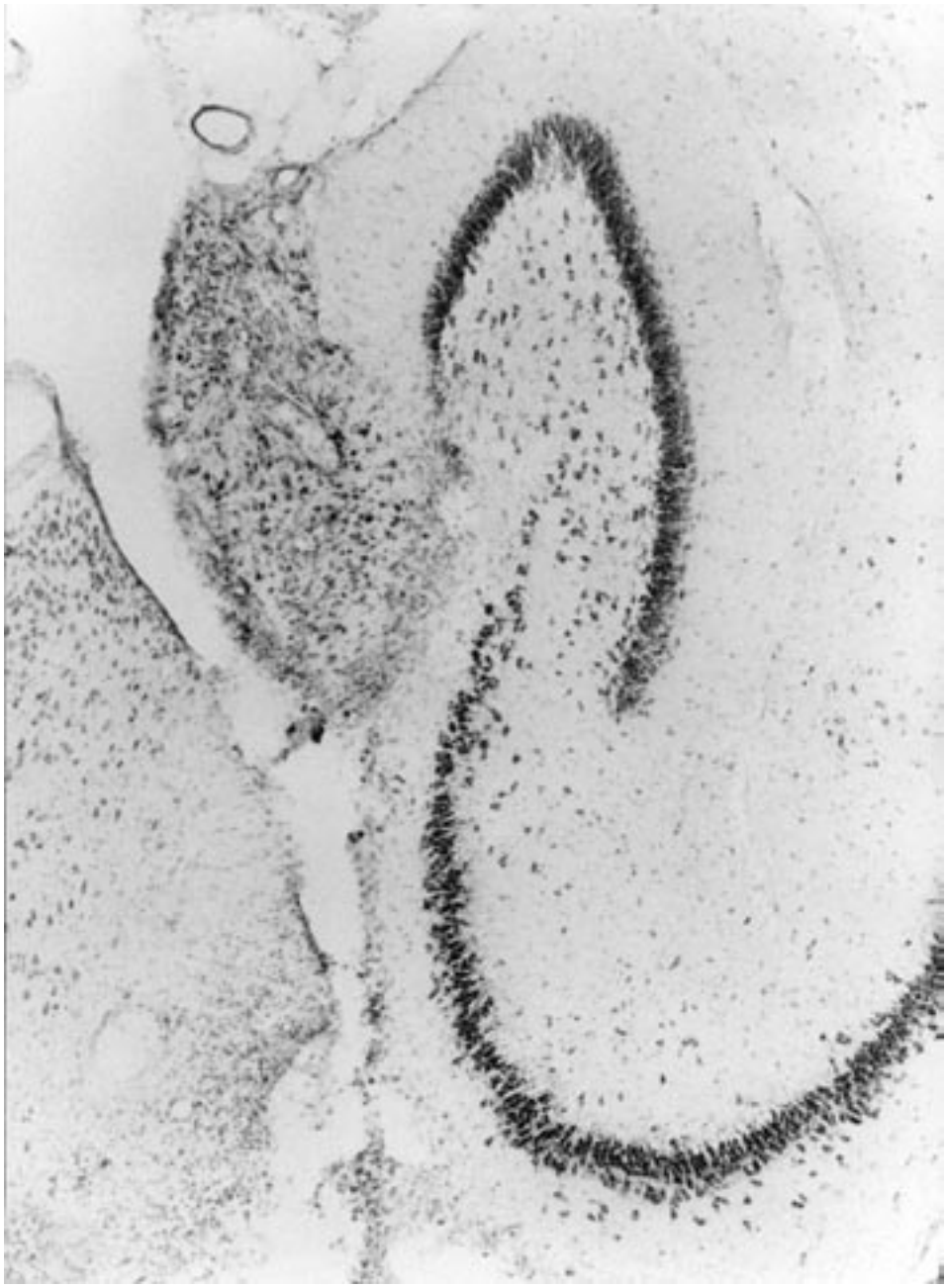


Fig-7.11

Another spinal cord graft in the hippocampal area showing a large transplant, extremely well-integrated into they area. Clusters of neurons are seen in between dense glia tissue. The overlapping zones are well defined, (x 108)

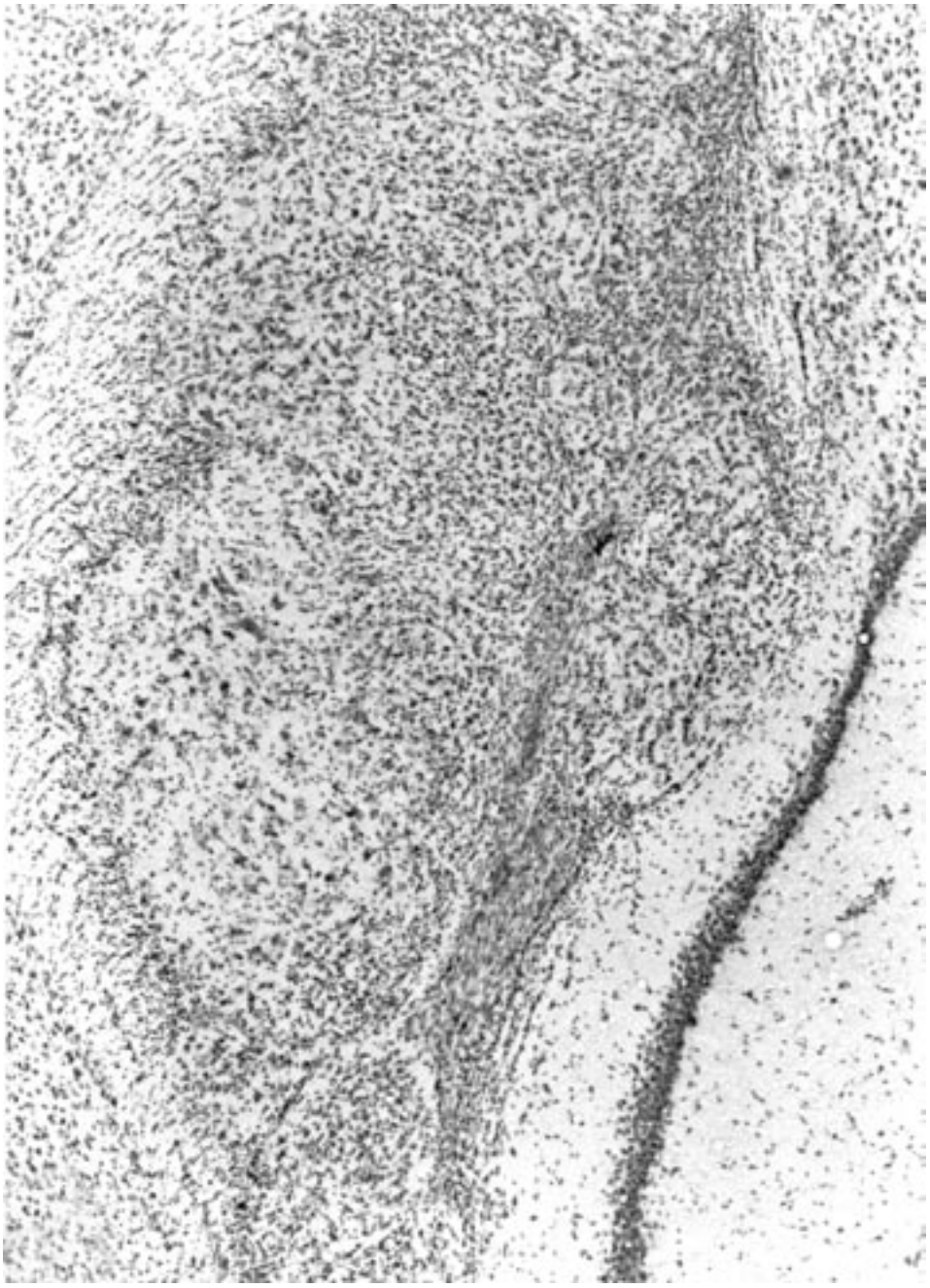


Fig. 7.12

A transitional zone in the hippocampal area of an E15 spinal transplant in an ACHE-stain. It seems as if fibers from the transplant are trying to integrate and penetrate the host, (x 275)

H = host TP - transplant

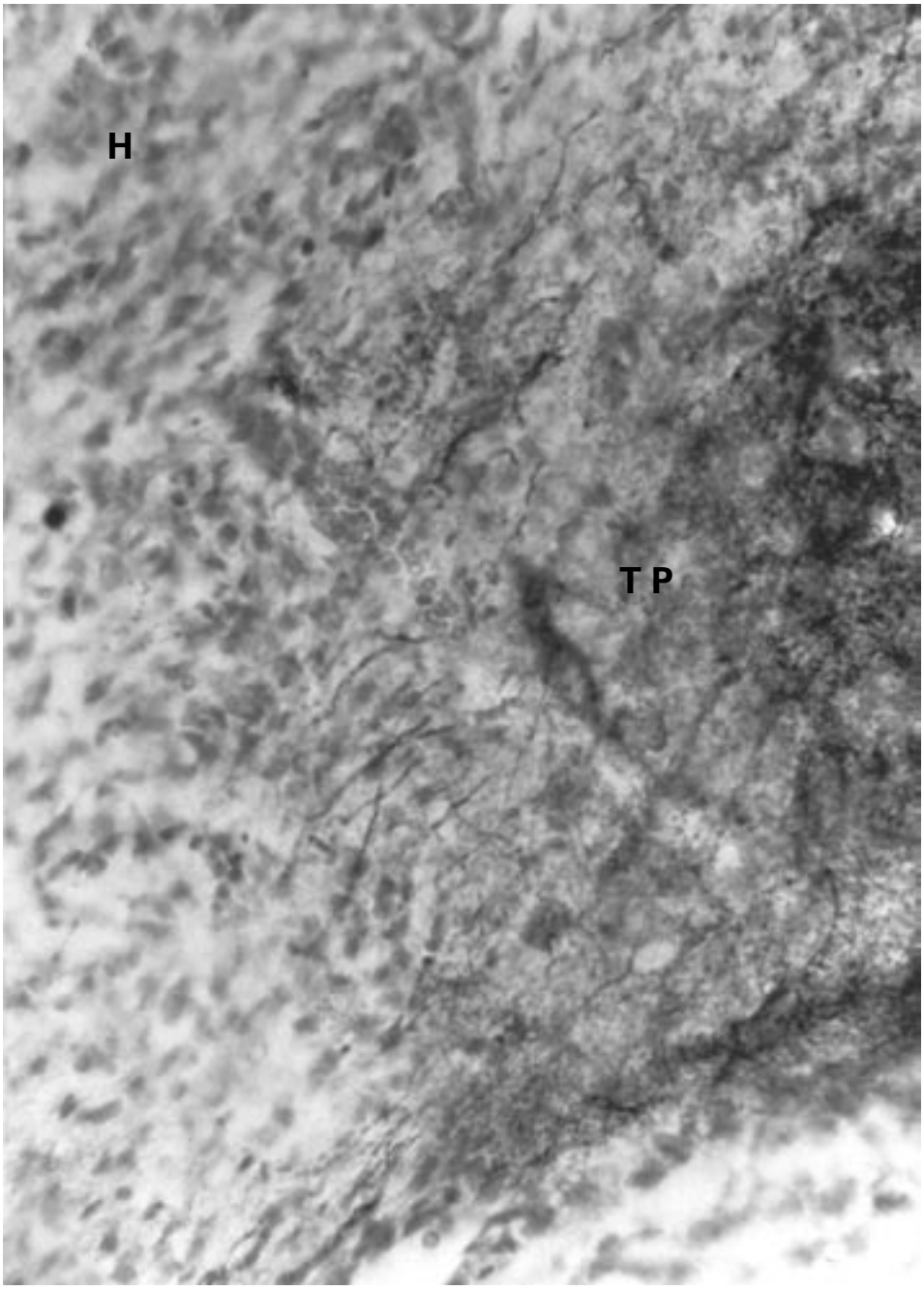


Fig. 7.13

A large neuron of a spinal transplant in the hippocampal area with a long axon and dendrites. The survival time was five weeks. ACHE stain (x 275)

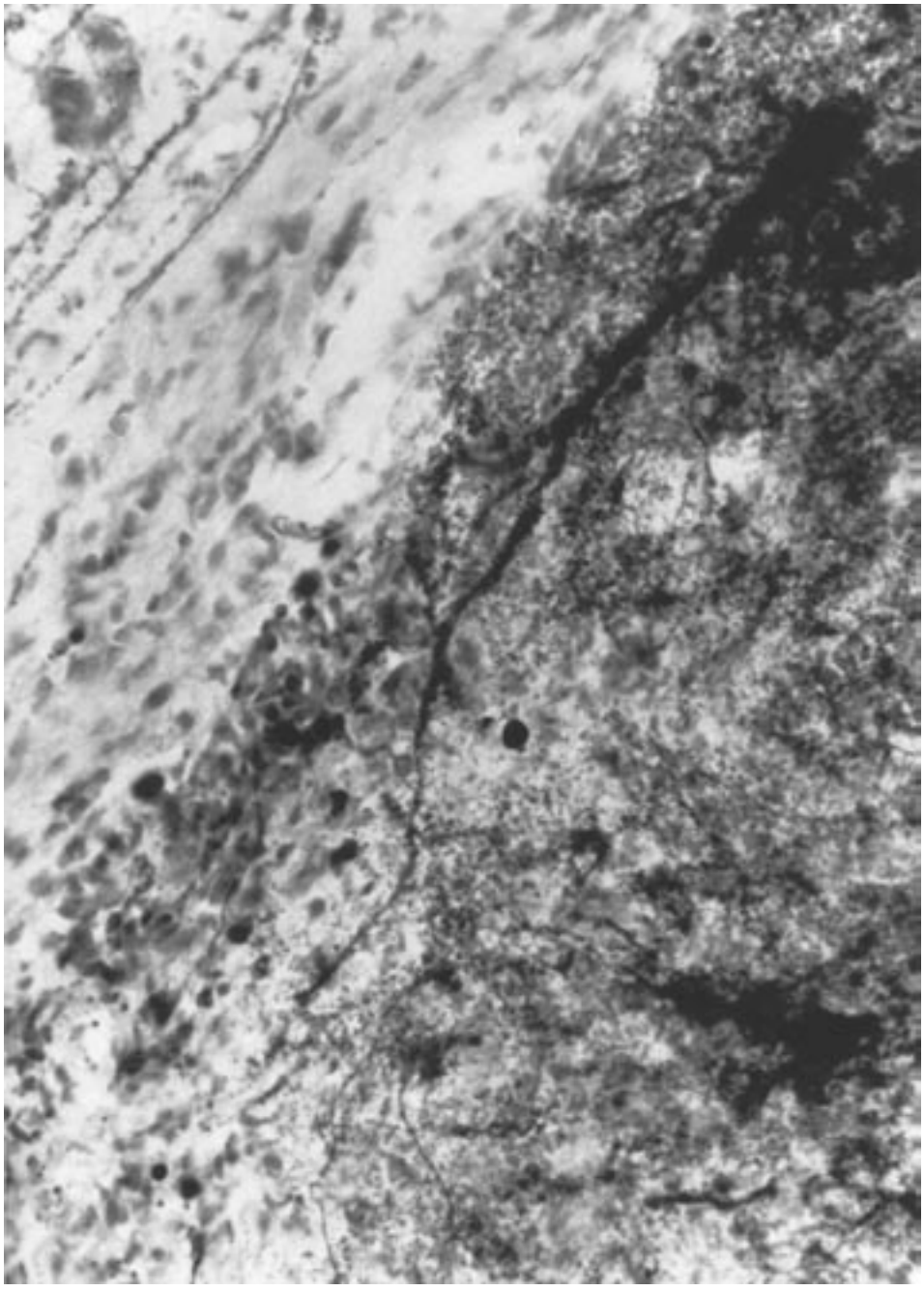


Fig. 7.14

A large spinal graft in the hippocampal area is seen to be lobulated and isolated with a well defined border zone with no apparent interaction with the host - or possibly embedded into white tissue from the brain. The survival time was six weeks. The stain is ACHE, (x 108)

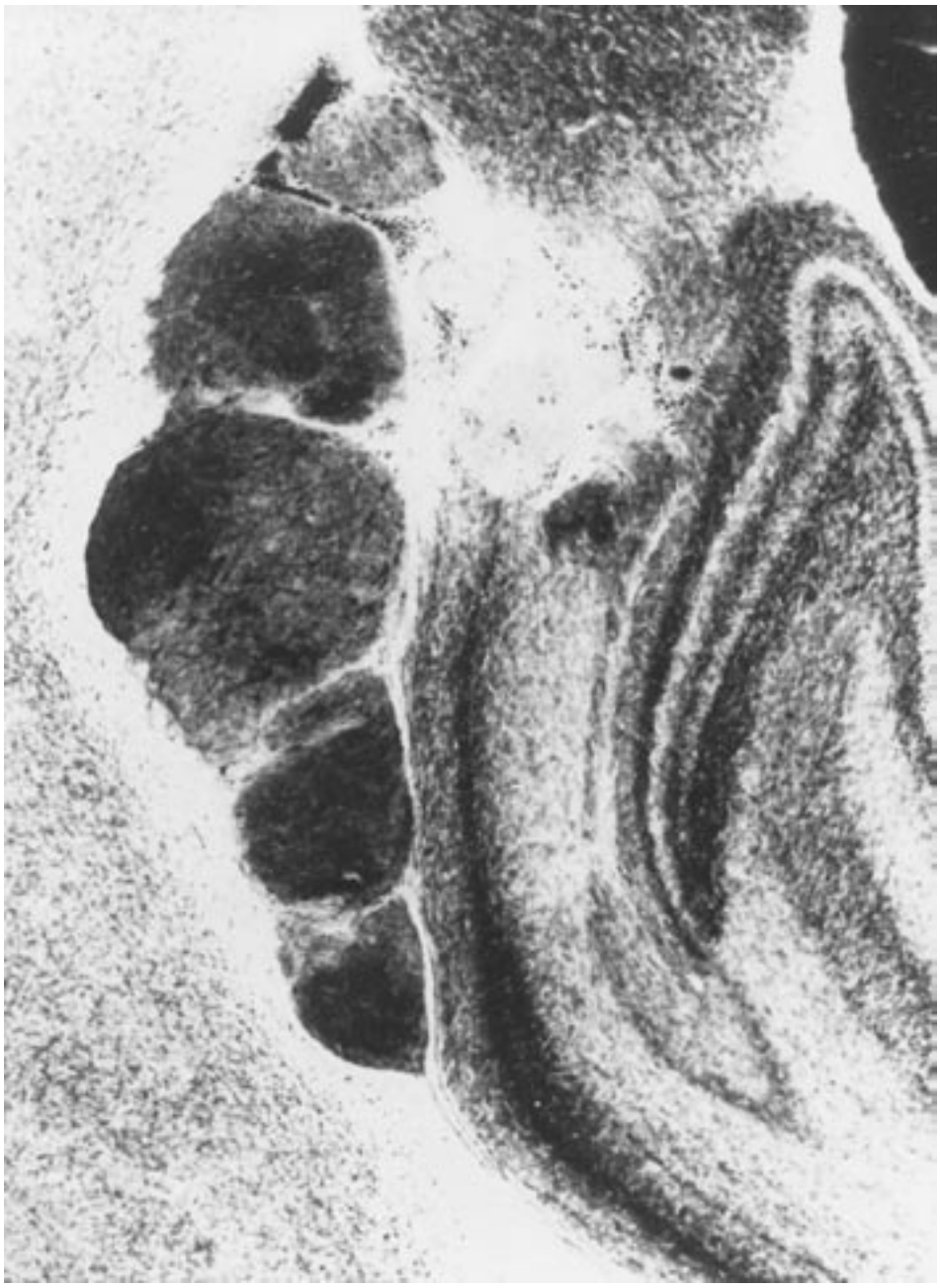


Fig. 7.15

Another spinal graft in an ACHE stain. Lots of neurons are seen in the transplanted tissue. SpE14 = Spinal Embroy (x 108)

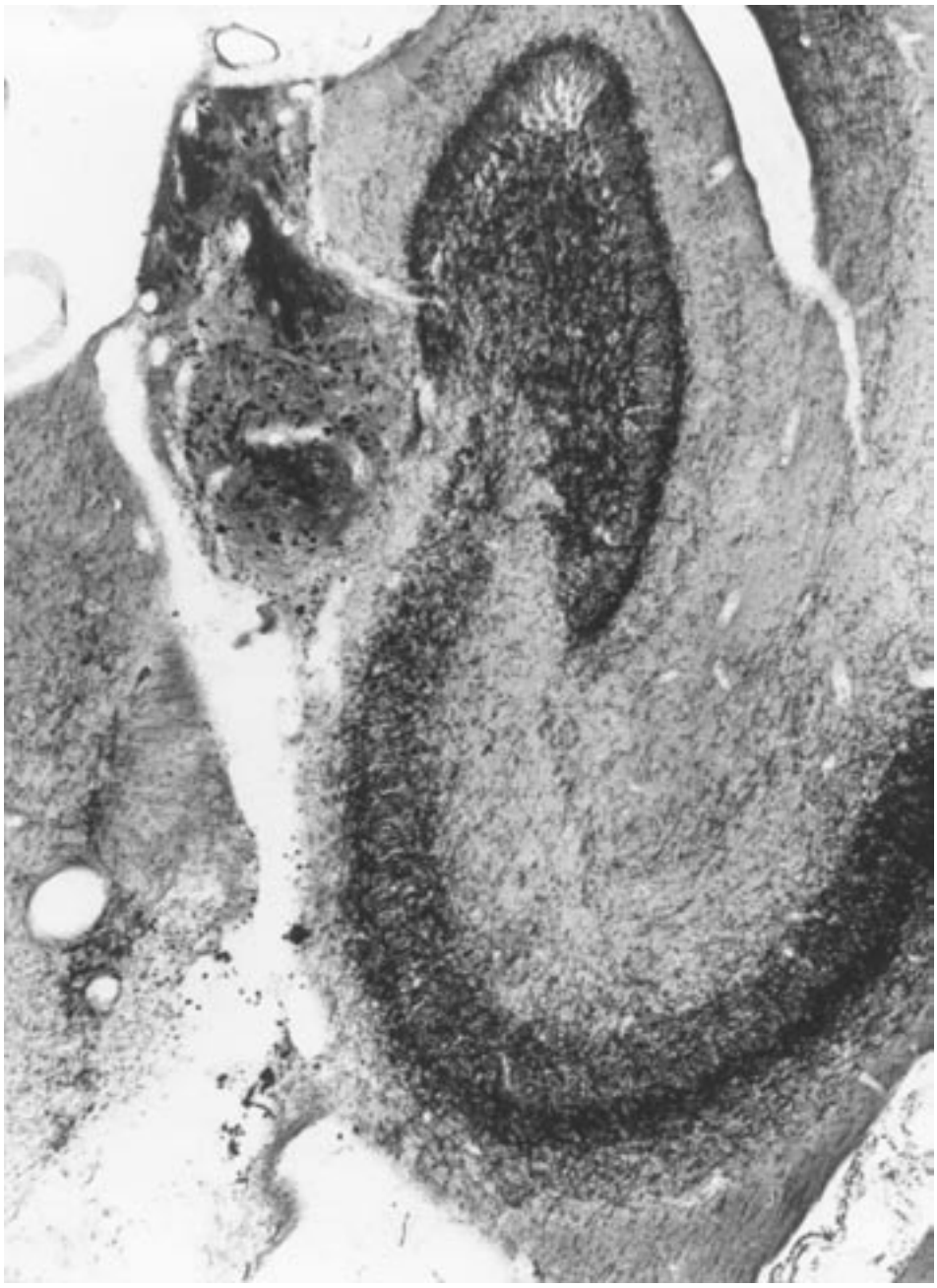


Fig. 7.16

A close-up of fig. 7.15 demonstrating the neurons with their dendrites and axons. (x 275)

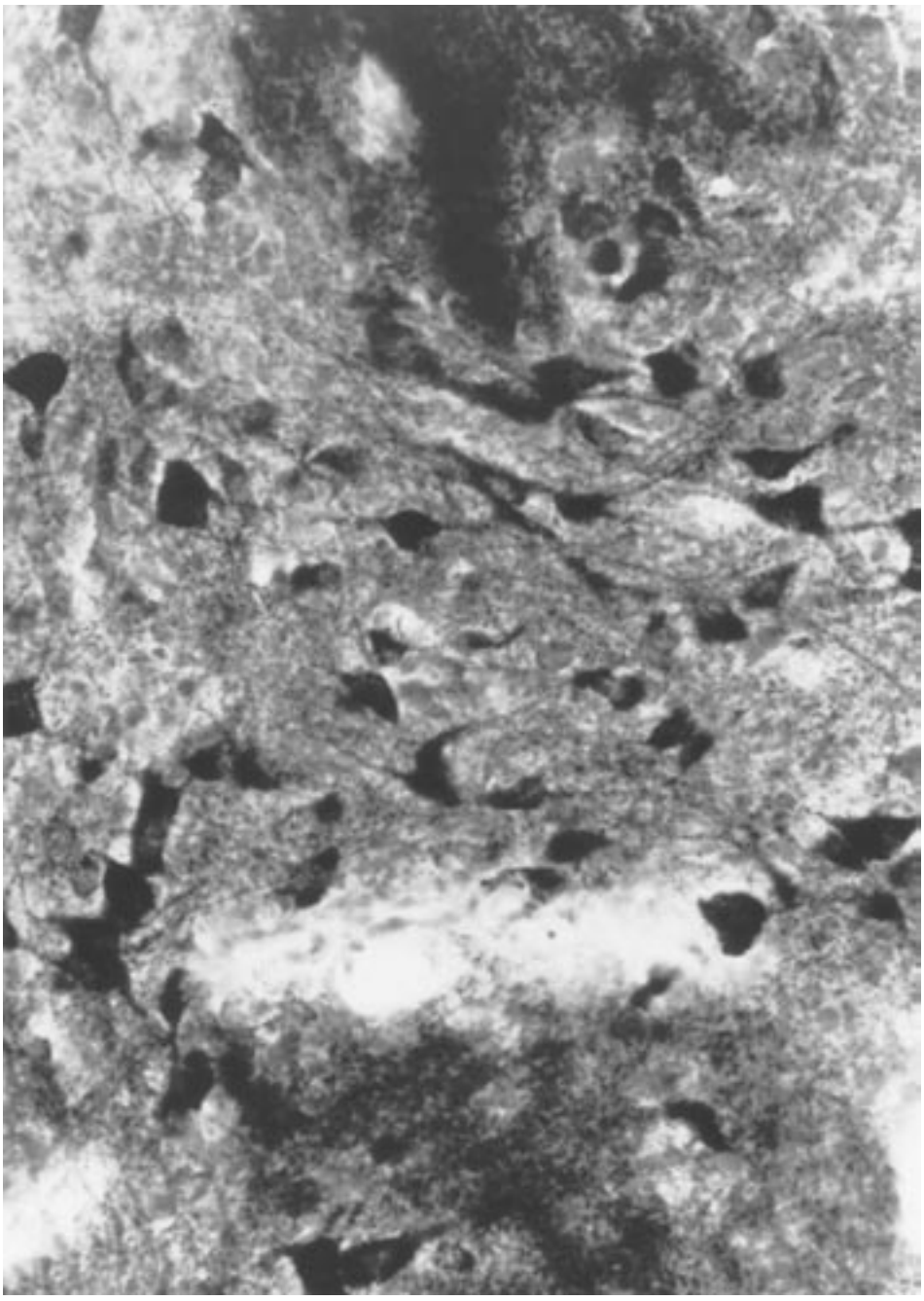


Fig. 7.17

A coronal section of an E -14 spinal graft to the hippocampal area. Lots of fibers are seen in this Nauta-stain. The anatomical integration is good but it is not possible to conclude anything about ingrowth into the host by axons or dendrites, or whether there is any synaptical activity between the transplant and the host, (x 44)

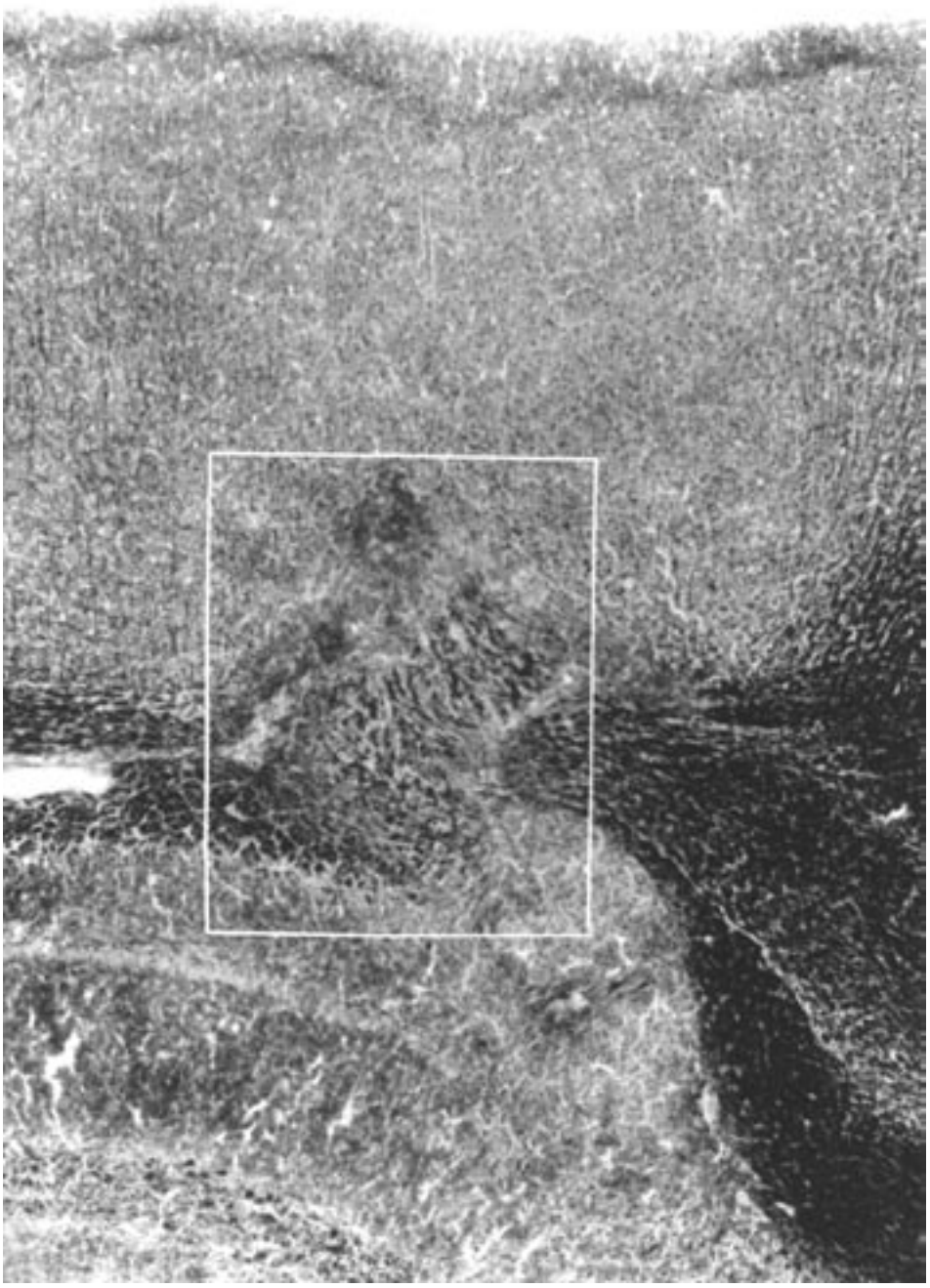


Fig. 7.18
The same as fig. 7.17 in a larger magnification. (Nauta x 108)

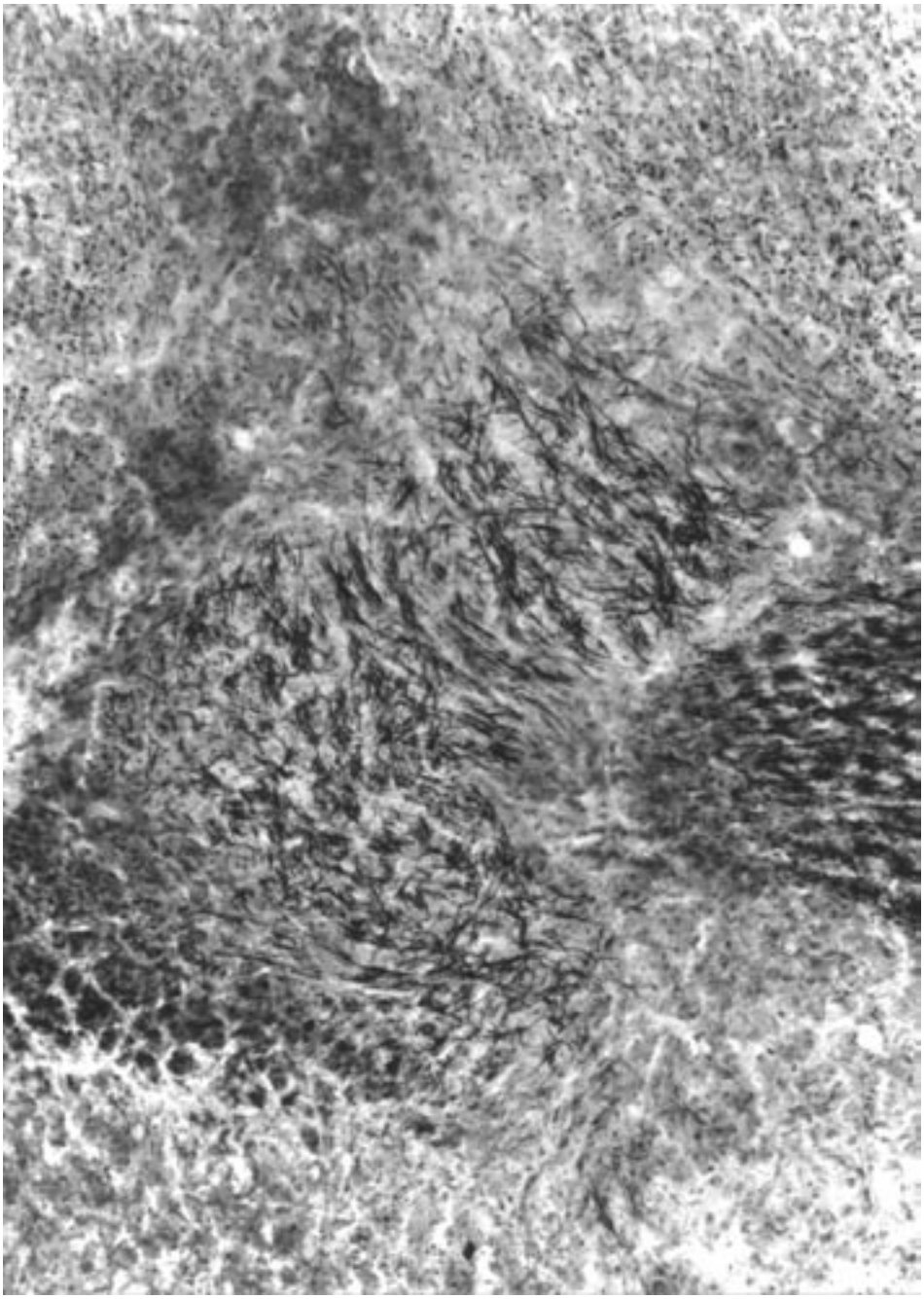


Fig. 7.19

This shows a spinal transplant situated in the subarachnoidal space of the spinal cord. The transplant is surrounded by spinal fluid. A lesion was made in thepia to facilitate ingrowth and outgrowth of axons and dendrites between transplant and host. In the upper right corner just above the black frame a nerve root is seen, demostratingt he growth of the transplant after six weeks. Originally the transplant 1x2 mm large and now seen to be one fifth of the thickness of the host spinal cord. The stain is a cell body staining with thionin. Fig. 7.20 shows the black frame enlarged. (x20)

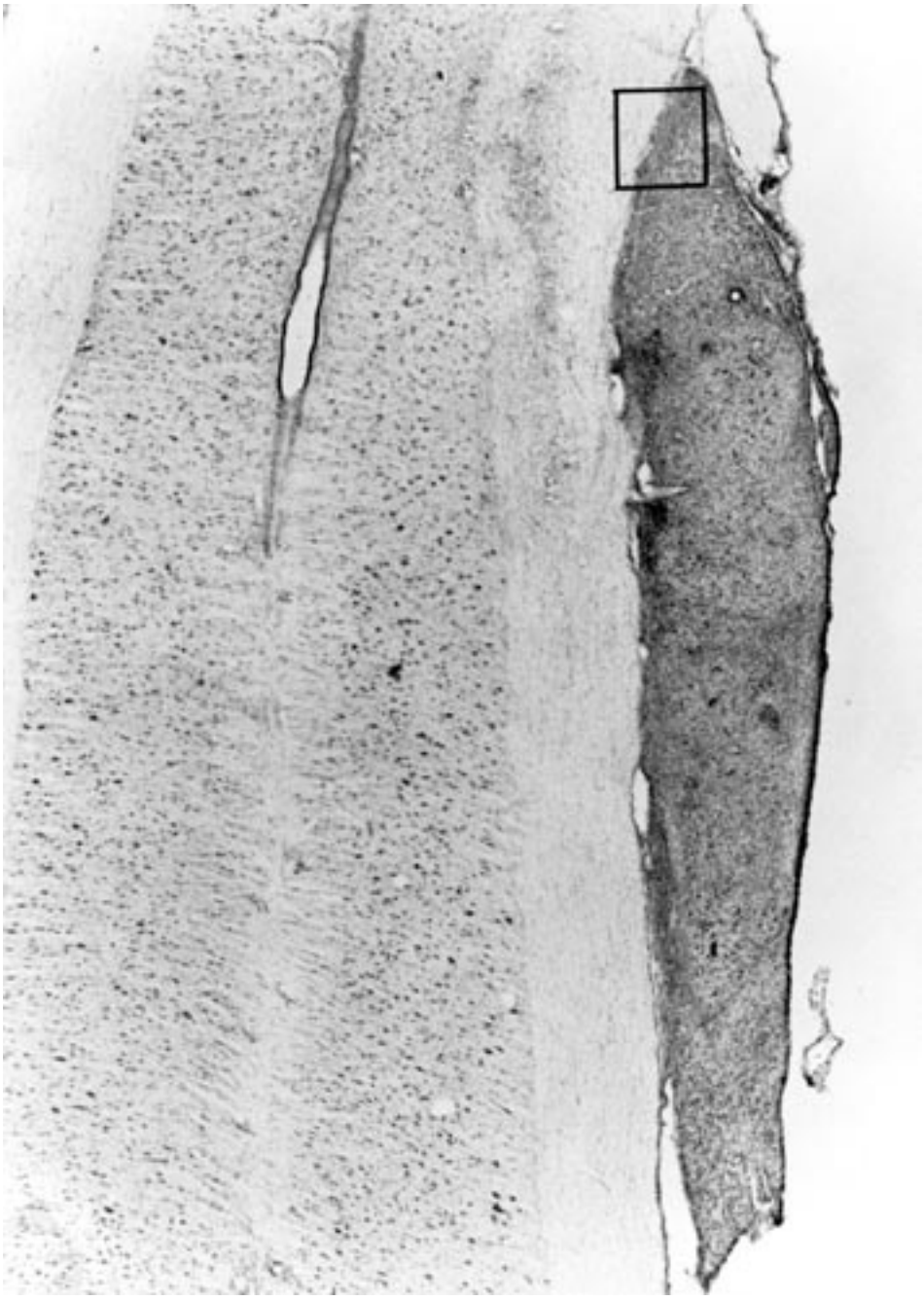
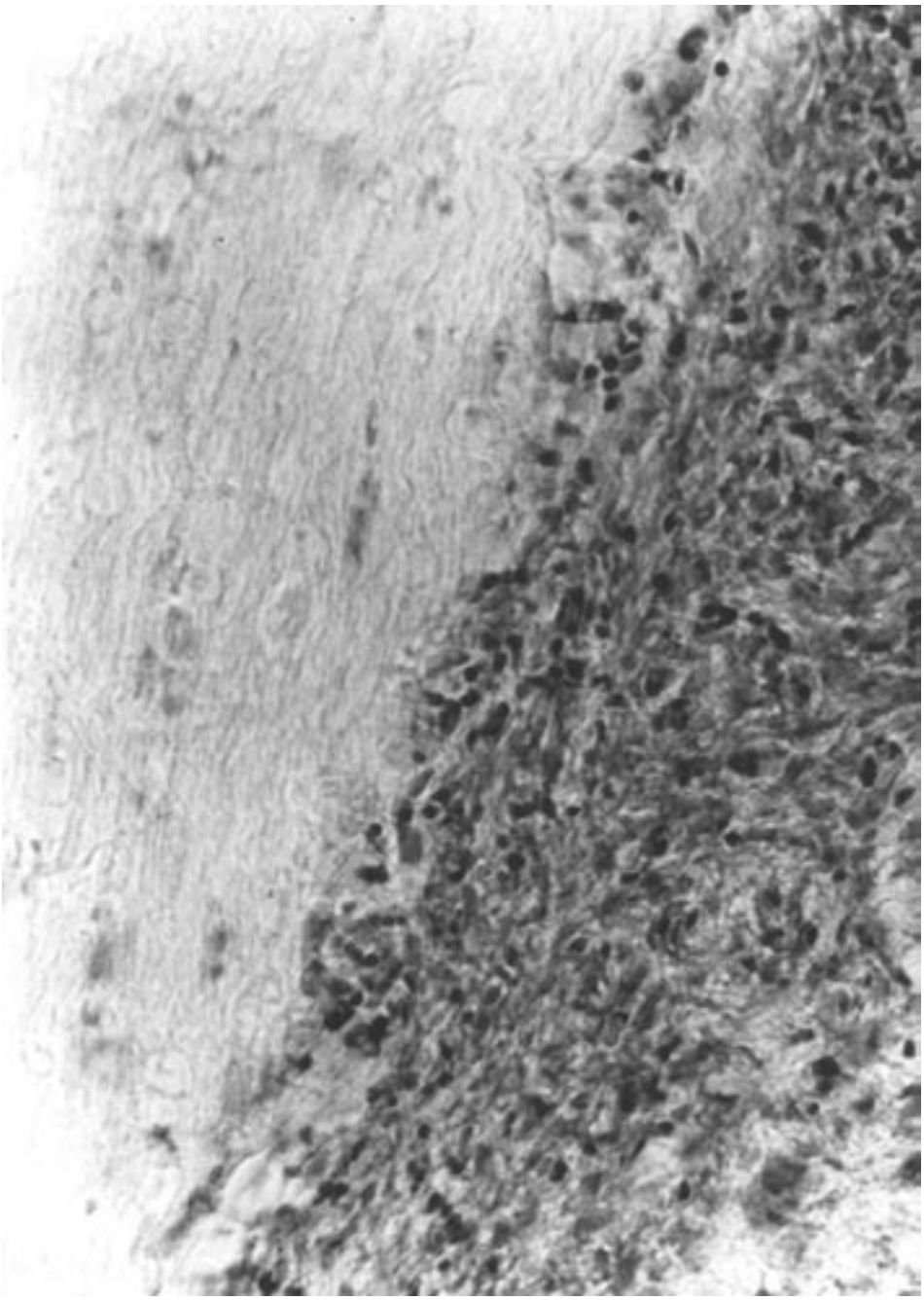


Fig. 7.20

The interface between the transplant and the white matter of the host spinal cord. Thionin staining. Survival time six weeks, (x 275)



7.21

A spinal transplant placed in the subarachnoidal space next to the white matter of the host spinal cord. It is an ACHE-stain showing large neurons which are distinct ACHE-positive.
Fig. 7.22 is the white frame enlarged, (x 44)

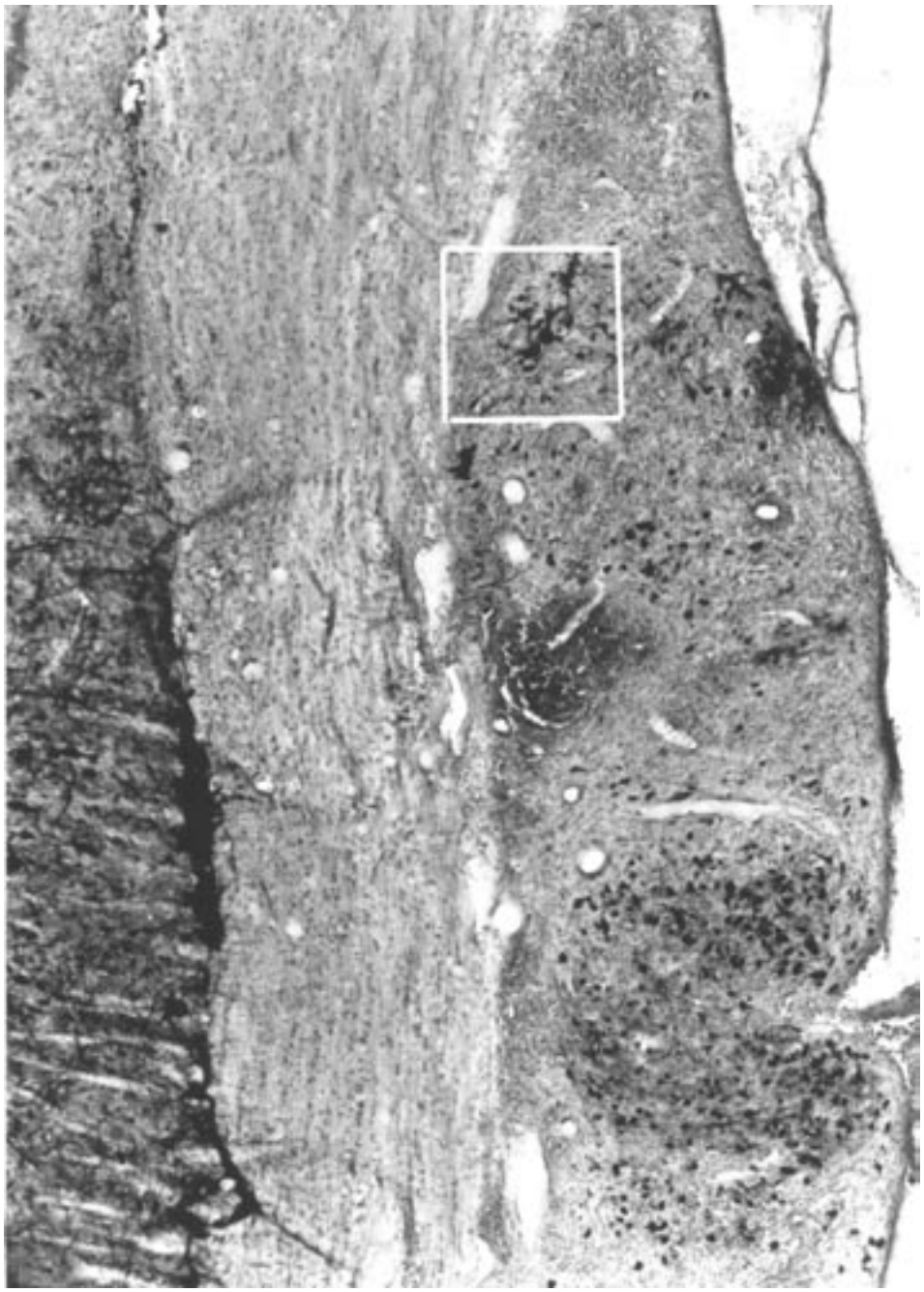


Fig. 7.22

Large ACHE-positive neurons with axons and dendrites in the interface zone of the spinal cord transplant and the subarachnoidal space of the host spinal cord, (x 275)

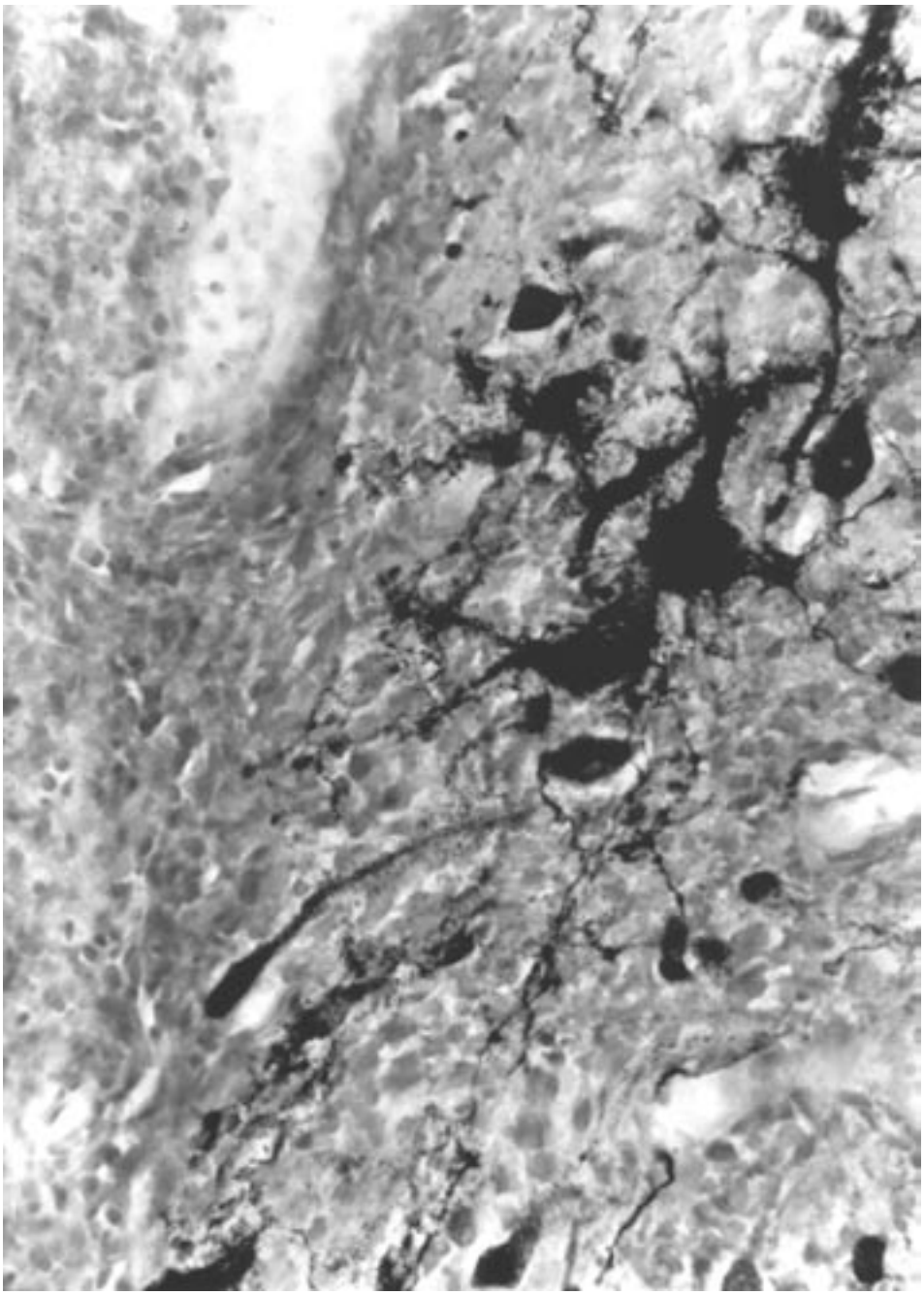


Fig. 7.23

A Nauta-stain of the same transplant as in fig. 7.21. The fibers in the interzone benveen the transplant and the host are dearly seen. The interaction or the ingrowth of the axons from the spinal cord or from the transplant is not seen in this figure, (x 198)

Fig. 7.24

From the same transplant as fig. 7.23. Another place in which the interface between the transplant and the host is seen. At the area where there is contact between the transplant and the host there seems to be an accumulation of cells and fibers. Furthermore it is seen how the fibers from the transplant are growing into the white matter of the host. The area is enlarged in fig. 7.25. (Nauta x 108)

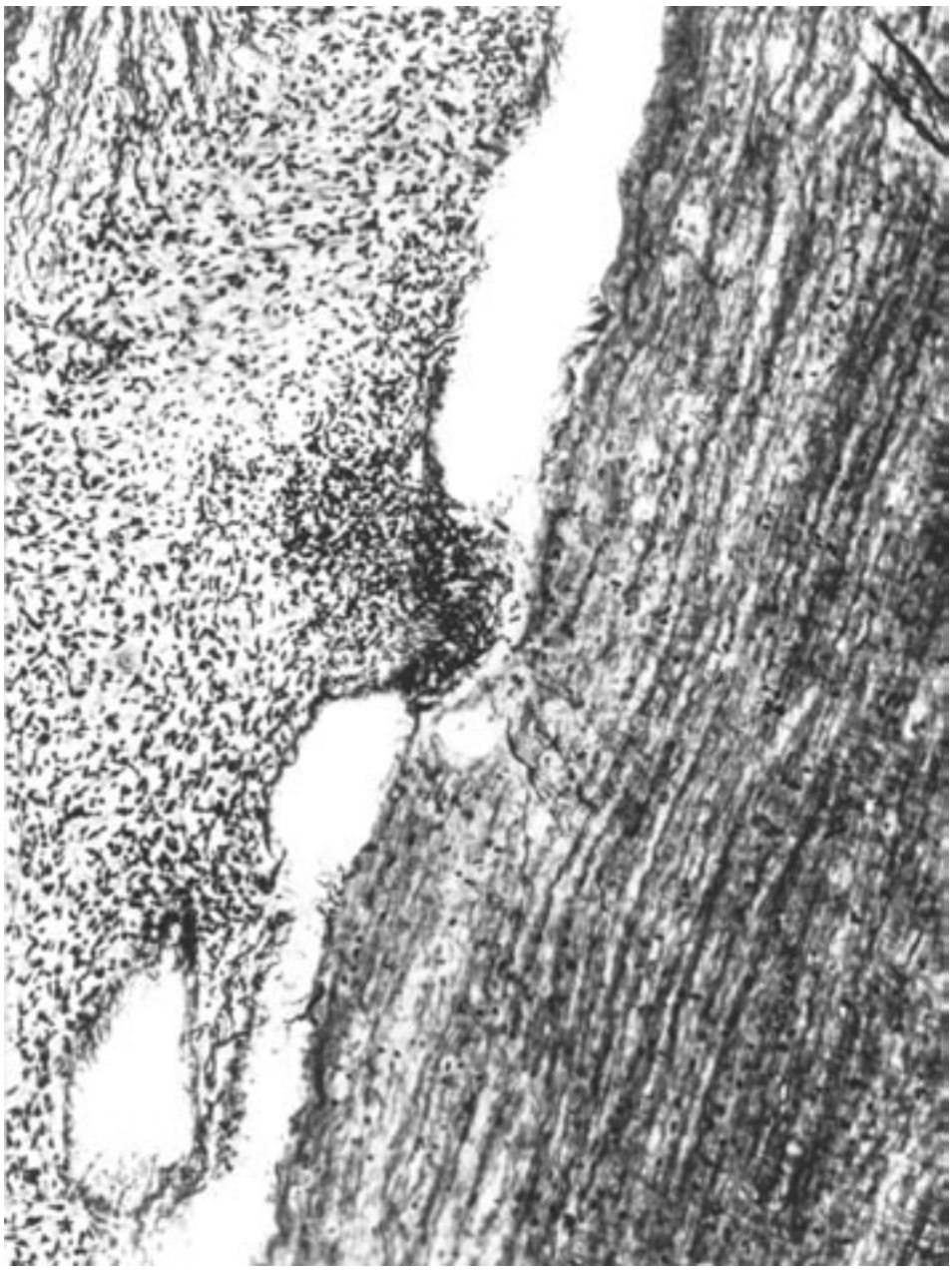


Fig. 7.25

Fibers from the transplant growing into the host white matter. It seems without any doubt that this should not be the case. However, the question is whether a synaptical connection exists between the fibers from the transplant and the fibers from the host. Could such fibers be used as a relay in a spinal cord lesion of the adult rat? (x 275)

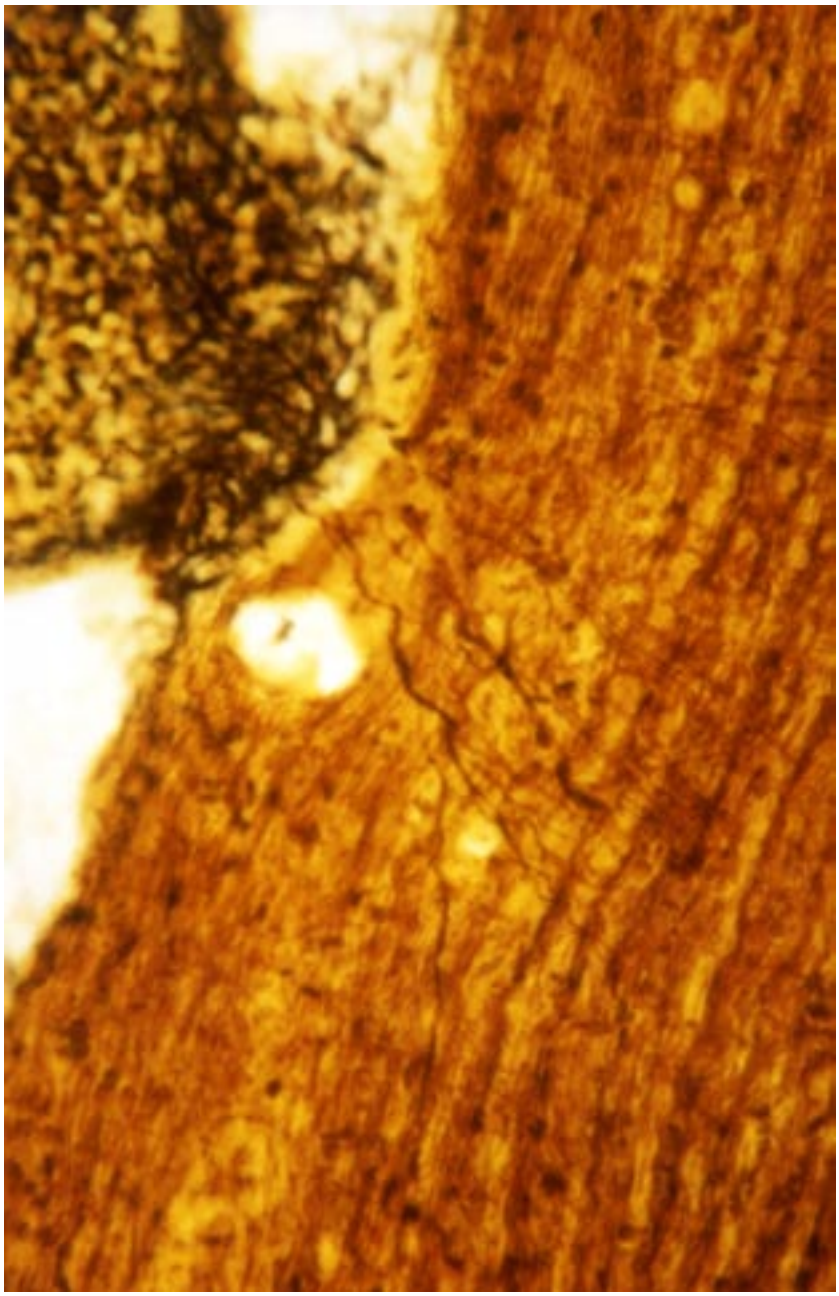


Fig. 7.26

This shows an E 15 intraspinal transplant in the spinal cord. ACHE stain. Survival time six weeks. It is clearly seen how the transplant has been situated between the two dorsal cornu in the white matter, preferably on the right side of the rat. The size of the transplant is much bigger than when it was inserted.

Fig. 7.27 shows the black frame in a larger magnification, (x 54)

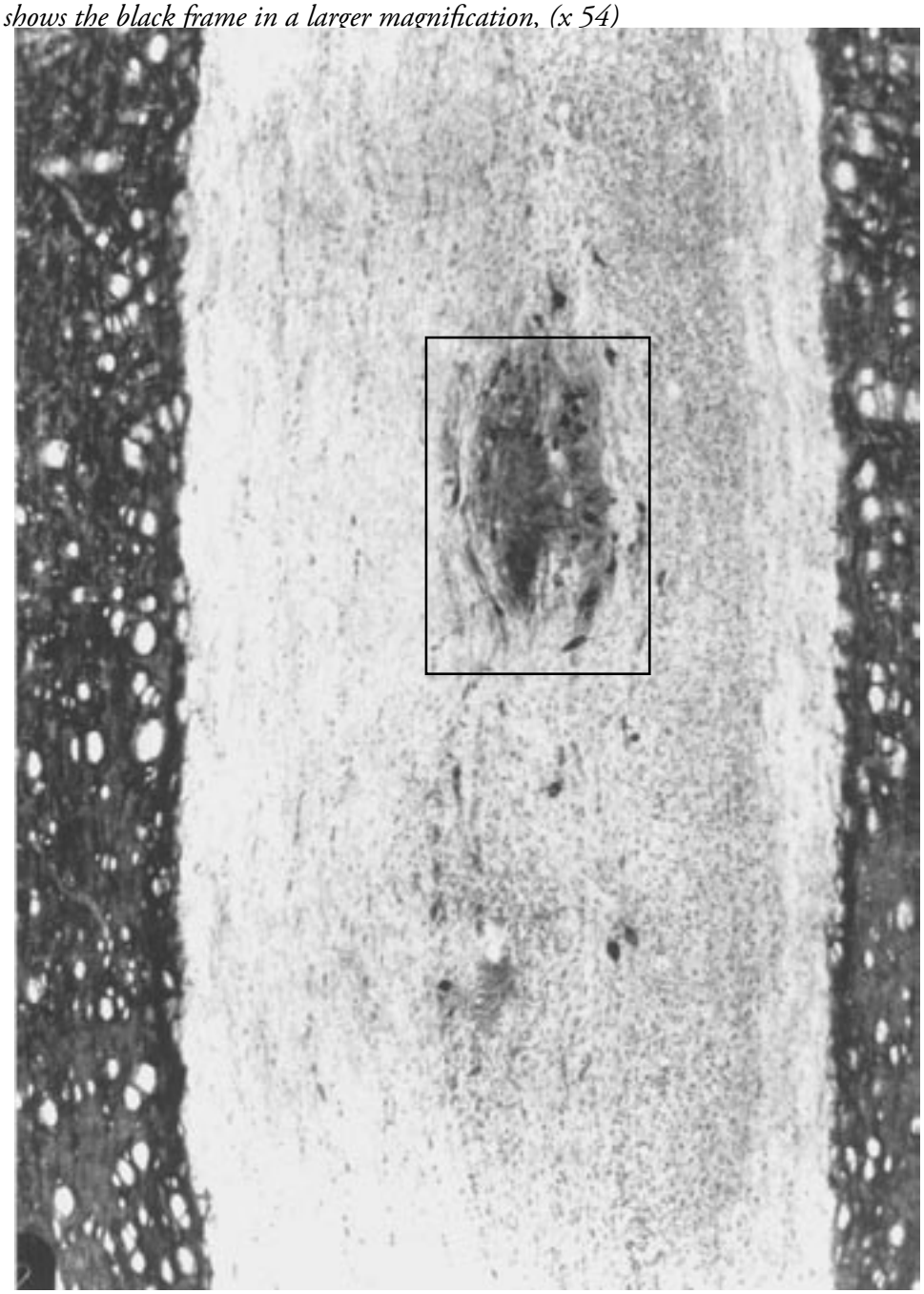


Fig. 7.27

The cell stain thionin shows clearly many large mature neurons of the transplant situated in the white matter of the host. Again it cannot be told whether a synaptical connection between the transplant and the host exists. It is the nature of neurons, axons and dendrites to make synaptical connections. If they are made, it seems logical that they would function as well. The growing and maturation is probably not just a question of bloodvessels, nutrition and oxygen, but it may also comprise a certain extension of a functional level. At least, the transplant was excellently anatomically integrated, although it did not show any sign of an organotypic organization, (x 275)

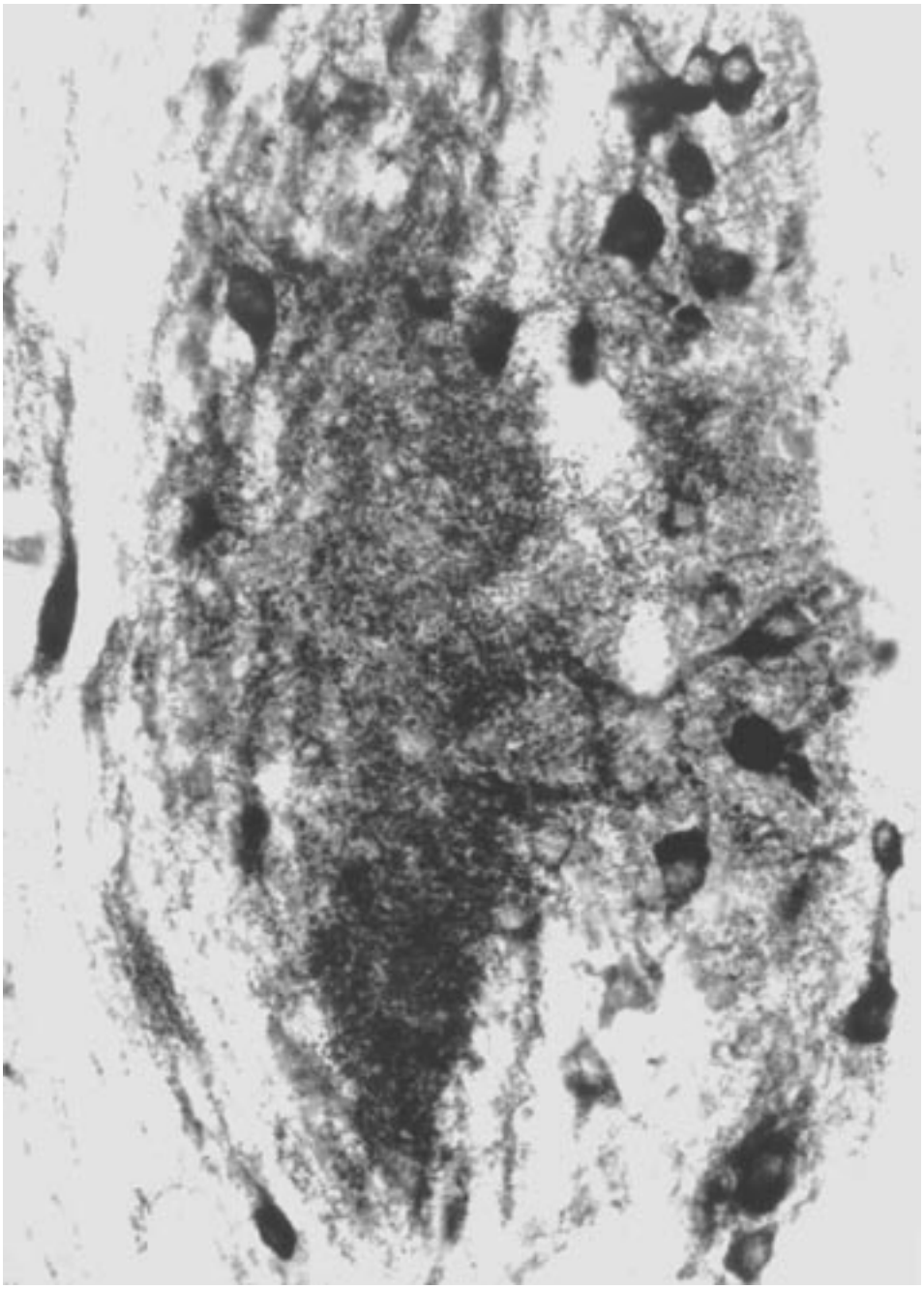
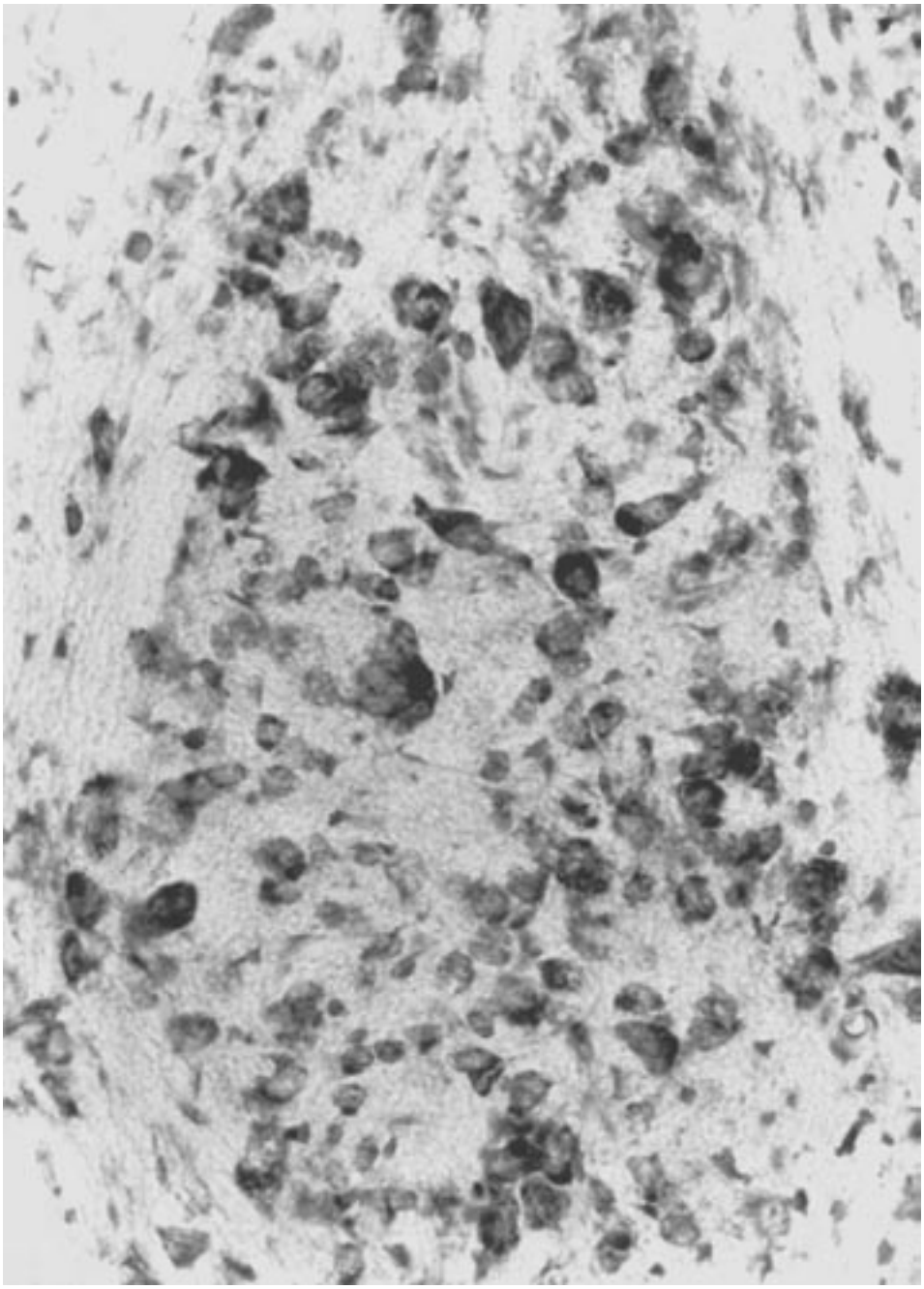


Fig. 7.28

The same transplant as shown in fig. 7.27 but now in a Thionon-stain. (x 108).



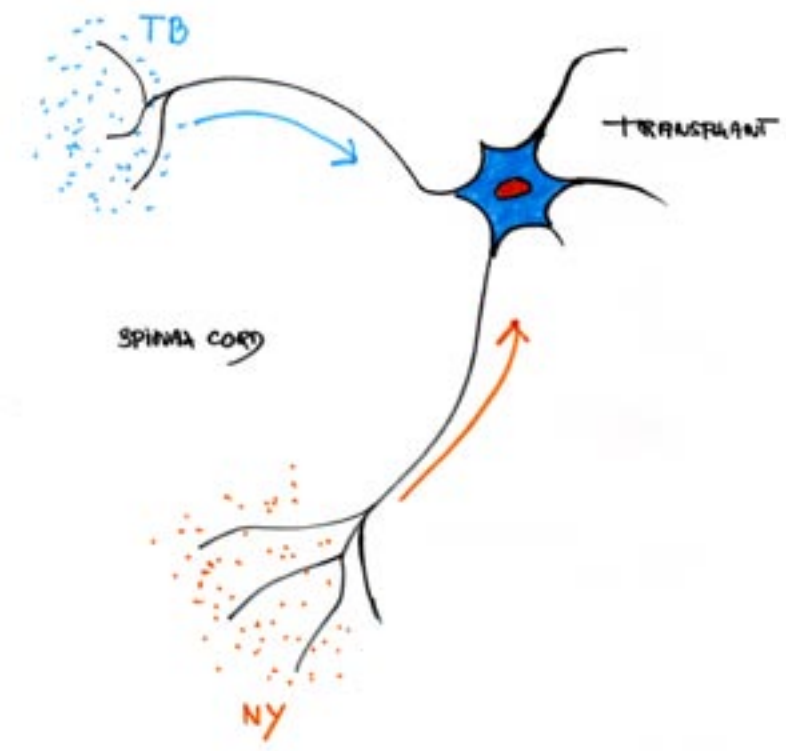
Double Labeling with True Blue and Nuclear Yellow

In order to clarify the growth and extension of the axons deriving from the transplants invading the host, a series of experiments was made with True Blue and Nuclear Yellow. Six weeks after the transplantation had taken place, True Blue was injected 5 mm proximally to the transplant at the right side of the spinal cord where all the transplants were situated. 5 mm distally from the transplant, Nuclear Yellow was injected. The injection was performed with a Hamilton syringe attached to a holder and 0.2p 1. was injected at each level. As the rats were not curarized, the injections were difficult to handle because of the jumping reflex of the rat when the needle was passed through the arachnoidal pia membrane into the spinal cord. After three days the rats were transcardially perfused and the sections were examined under a fluorescense microscope.

Some of the transplants were stained by either True Blue or Nuclear Yellow or both. Due to the extension of the fluorescence in the cells of the transplant and the host, it was not possible to determine whether what could be seen was in fact an active tracer transport in the transplants in fibers extending from the transplant into the host. The phenomenon could also be due to a leaking or diffusion of the tracers.

The tracing was carried out in both spinal cord transplants to the hippocampal area of the brain and to the spinal cord (N = 30).

In none of the transplants to the spinal cord was there any evidence of a retrograde transport in the neurons extending into the host. This again shows that the difficulties in carrying out spinal cord transplants are much greater than with fetal brain transplants which today present excellent results.



Benedicte Dahlerup

8
Cultures

Cultures

In 1970, Hiromichi Aihara [2] wrote in Brain and Nerve, a Japanese journal, that to avoid the up-filling of collagenous scar tissue in the gap between severed spinal cord, he transplanted cultured cerebellar cortex with neurons into 49 adult female dogs. In the dogs he made a suboccipital craniectomy and removed a portion of the cerebellar cortes. This was cultured in a CO_2 incubator at 37° centigrade for a week prior to autografts. As the original paper was in Japanase no further information about the culture technique can be given. The spinal cord in the dogs was then resected from 5-10 mm in length at the Th. 12 level, and the cultured cerebellar cortex was grafted into this gap. In his article Aihara says that 16 among 49 dogs could be followed for more than two months after the operation. In four dogs the function of the spinal cord recovered about three months after the operation. To prove the regeneration of axons in the spinal cord a cystometrogram was recorded, and in those four dogs who were able to walk after the operation the cystometrogram was near to normal.

Three of the four dogs who were able to walk after the operation revealed regenerating nerve fibers in the gap of the spinal cord. One dog who was paraplegic for nine months after the spinal cord transection, had another operation with cultured cerebellar cortex after the scar tissue excision of the first operation. Three months after the second operation the dog regained its ability to walk, and seven months after the operation the spinal cord was dissected for microscopic examination.

This showed the spinal cord to be narrowed by invading scar tissue. However, regenerating nerve fibers could also be demonstrated and the connections between both ends of the severed spinal cord were detected. Another dog who could walk two months after the second operation became akward after five months and six months after the operation lost the ability to walk. At autopsy it was revealed that the gap in the spinal cord was filled up with collagenous scar tissue and regenerating nerve fibers could not be found. In some of the dogs who did not recover function of the spinal cord, only a few nerve fibers were found in the invading scar tissue. These nerve fibers were considered to be the so-called abortive regenerating nerve fibers (after Cajal) [183].

Aihara writes:

> *"From now this study will be continued to establish as the operative method for human spinal cord injury".*

Kao, Shimizy, Perkins and Freeman [133] in 1970 in the Journal of Neurosurgery publishedan article of "experimental use of cultured cerebellar cortical tissue to inhibit the collagenous scar following spinal cord transection". In this article only the avoidance of a collagenous scar tissue is discussed. In Experimental Neurology in 1974, C.C. Kao has another article on comparison of the healing

processes in the transected spinal cord grafted with autogenous brain tissue, sciatic nerves and nodose ganglions. In this study no evidence of a regeneration in the host nervous tissue could be found.
In Spinal Cord Reconstruction (eds.: C.C. Kao, R.P. Bunge and Poul J. Reier) from 1983 [128], the experiments of Aihara are presented by Yoshefusa Shimizu. This time the series comprises 54 dogs. A movie was made of one of the dogs who had started to walk. Spinal walking could be ruled out because the dogs were completely paraplegic for a period from five to nine months before the grafting of the cultured cerebellar cortex was performed. Thus spinal walking as well as incomplete spinal cord transection were out of question.

To my knowledge no results of similar experiments have been published since 1983. To continue such investigations I made two series of experiments of autografting to prevent most of the problems of graft rejection.

Personal experiments

A series of cultures from five weeks old female wistar rats was made. The cerebellar cortex was cultured in slices in a CO_2 incubator at 37° for a week with the purpose of autografting to the spinal cord, however after a whole week not a single neuron had survived.Consequently cultures were stopped, and it was decided to performed the cerebellar grafts directly from a grown-up female Wistar rat to newborns (allografts). A craniectomy was made on an adult rat and while in deep anaesthesia a small tissue block was taken out of the cerebellar cortex and immediately grafted into the hippocampal area of newborn rats (n = 8) anaesthetized by hypothermia. The adult rat was kept alive. It had no apparent neurological deficits. After five weeks the newborns were perfused transcardially with a sulphide solution and their brains examined in series of 30 mm thick cryostate sections stained with thionin and ACHE. There was no evidence whatsoever of any survival of the grafts. All eight rats had developed varying degrees of hydrocephalus. In some areas of the brain, especially at the site of the previous graft in the right hippocampal region, the brain showed signs of necrosis with vacuoles. There were ACHE-containing cells that looked like large mononuclear macrophages containing lipofuschin. The macrophages were seen in the toluidin-blue stains in the same area as the leucocytes. Several surviving glia cells were seen at the site of grafting and in the surrounding tissue, suggesting that they survive and migrate from the site of grafting.

As there was no evidence of surviving neurons in these allotransplants, two important possibilities are to be considered:

1. That postnatal cerebellar neurons at any age simply die after grafting.
   This is a well-known neurobiological phenomenon.

2. That they are rejected because of the immunological tissue reaction.

To investigate whether the "death" of the cerebellar grafts or at least the death of neuronal cells (the glial cells might survive and migrate into the host brain) could be due to the poor survival of adult cerebellar tissue or perhaps to an immunological rejection of the grafts. It was tried to reduce the histo-incompatibility between donor and recipient. A donor rat having just given birth to nine rat pubs was anaesthetized and a craniectomy was performed. A small block of cerebellar tissue was removed and cut into nine pieces. These pieces were immediately grafted into the right hippocampal area of the newborns. The donor rat was sutured, and she did not show any signs of neurological deficits and continued to feed the newborns. All nine newborns did well after the operation.

After 17 days, the animals were perfused transcardially with 4% paraformaldehyde solution, soaked overnight in 30% sucrose and cut into 30 mm thick cryostate pieces. The brains were examined in a toluidin stain and an ACHE stain.

Again the same picture appeared. The rats showed signs of various degrees of hydrocephalus, and at the site of grafting there was still necrosis with vacuoles and large ACHE-containing cells. These cells resembled mononuclear macrophages containing a material resembling lipofuschin. There were no neurons in the toluidinblue stain. Within and around the site of grafting, leucocytes could be seen. Glia-reaction, especially in the site of the graft and around it, indicated that the leucocytes had migrated away from the site of grafting. To me this is an unknown reaction for which I have no explanation.

Thus, it was demonstrated that such kinds of grafts are not very successful and apparently the Japanese came to the same conclusion since there is no evidence that the promising series of experiments has been confirmed in Japan or in other countries since 1970. However the idea of an autograft is sound because the ethical aspects of using embryoes are avoided and the immunological problems eliminated. On the other hand it seems likely that this is not the way to go. It is a neurobiological fact that neurons which are grafted after birth will die in the host.

Reference: 2, 43, 66, 84,.85,127, 128,170,183.

9 Scar Tissue

Trauma to the spinal cord results in the formation of a connective scar tissue containing; dense collagenous matrix, fibroblasts and macrophages. The connective tissue response is almost always accompanied by gliosis and cystic formations.

In 1922, Cajal [183] reported that following transection of the mammalian spinal cord, a short segment of cord tissue immediately adjacent to both cord stumps became necrotic and separated spontaneously from the stumps. He called this phenomenon a spinal cord, autotomy. This phenomenon was studied elaborately by Kao and Chang in 1977 [129] who found the autotomy as described by Cajal to be closely linked to the lysosomal cellular lytic process; they also found that the lytic process did not start at the cut-ends of the cord, but at some distance from the cut-ends within the spinal cord stumps. The result is that there is a correlation of lysosomal activity and cord cavitation.

Barret, Donati and Guth [10] in their study of astroglial response to spinal cord injury found the gliosis from the spinal cord lesion to be the result of a degeneration of the long ascending and descending fiber tracts. They also found that if the injured neurites could be induced, to cross the site of injury, there was a possibility of regenerative axonal growth beyond the lesion.

In 1981, Krikorian, Guth and Donati [140] suggested the use of a synthetic dural sheath of dacryl and silicon to cover the site of transection of the spinal cord. This should prevent the invasion of fibroblasts from giving rise to the connective scar tissue which they find to derive from connective tissue components of the injured bone and muscle tissue adjacent to the spinal cord. They support their statement by the findings of very little formation of dense connective tissue in brain lesions. The Dacryl and CNS silicon dural sheath in their experiments were placed extramedullary and prevented the formation of dense connective tissue in the spinal cord parenchyma.

My own observations revealed no differences in the formation of the scar tissue whether the spinal cord including the pia mater and the arachnoid membrane was covered by a plastic film sheath, aluminium foil or human dural sheath from Germany. (This was used in the hospitals until a patient in the United States got Jacob Kreutzfeldt's disease after which the use of human dural sheath has been prohibited). Whenever a foreign body was placed upon the spinal cord on which a laminectomy had been performed, and the arachnoid had been injured together with the spinal cord, this foreign body was completely imbedded in dense connective scar tissue and sometimes it migrated away from the lesion in the spinal cord. Dense connective tissue from bone and periost also sometimes merged into the spinal cord. Fig. 3.2 clearly demonstrates glial reactivity at the site of a spinal cord injury without a graft. In this figure the rat had not been immobilized by orthopaedic methods as the one in figure 3.3. The reason why there sometimes is formation of glial scar tissue and sometimes formation of connective scar tissue has not yet been satisfactorily explained. In 1986 Kesslak, Nieto-Sampedro, Globus and Cotman [137] showed that transplants of purified astrocytes promoted behavioural recovery after frontal cortex ablation. They showed that transplants

of purified rat brain astrocytes could facilitate behavioural recovery to the same degree as transplants of embryonic brain. This is very interesting, because other authors such as Liuzzi and Lasek in 1987 in their observations suggested that mature astrocytes may stop the intrinsic tendency of axons to elongate through their effect on the axon tip. Kesslak et al. state that a delay in transplantation did help the transplants to survive probably by stimulating axons to sproute through a local host-transplant synaps formation. They write:

> *"Astrocytes are known to produce neurotrophic and neurite promoting factors in both vitro and probably in vivo and could facilitate functional recovery or acting as a continuous source of these proteins. Impacted glial cells would increase a survival of injuried host neurons and accelerate sprouting and reactive synapto genesis.*
>
> *They could also bind and remove the excess excitotoxic aminoacids liberated after injury. An additional beneficial effect of the glial cells may be to help the restoration of ionic balance in the damaged region. Thus implanted astrocytes may provide a comparative healthy and stable environment that would help damaged neurons to recover and undamaged ones to assume new functional roles."*

They conclude that:

> *"Astrocyte transplants probably act as a source of trophic signals but in addition may act by normalizing the neuronal environment. Astrocyte proliferation is one of the initial events after injury of the central nervous system and the formation of the so-called glial scar has traditionally been considered deleterious. The effect of astrocyte transplants on behavioural recovery after cortical lesions emphasizes the beneficial aspect of astrocvte proliferation and its potential usefulness for central nervous system repair"* [146].

As already mentioned, Liuzzi and Lasek in 1987 published an article in Science called "Astrocytes block axonal regeneration in mammals by activating the physiological stop pathway" [146]. They found that the regenerating sensory axons in the dorsal root of adult mammals are stopped at the junction between the root and spinal cord by reactive astrocytes. In the dorsal root regeneration model there is no connective scar tissue and axonal growth is in a purely astroglial environment and can thus be studied. At the interface between root and spinal cord the regenerating axon tip encounters a substrate consisting almost entirely of reactive astrocytic processes. In this interface the growth cone stops and forms stationary axon terminals thereby indicating that mature astrocytes stop the intrinsic physiological pathway which normally operates when axons form synaptic contacts with target neurons or peripheral receptors. The axon terminals were completely incapsulated by astrocytic processes which were identified by numerous intermediate filaments.

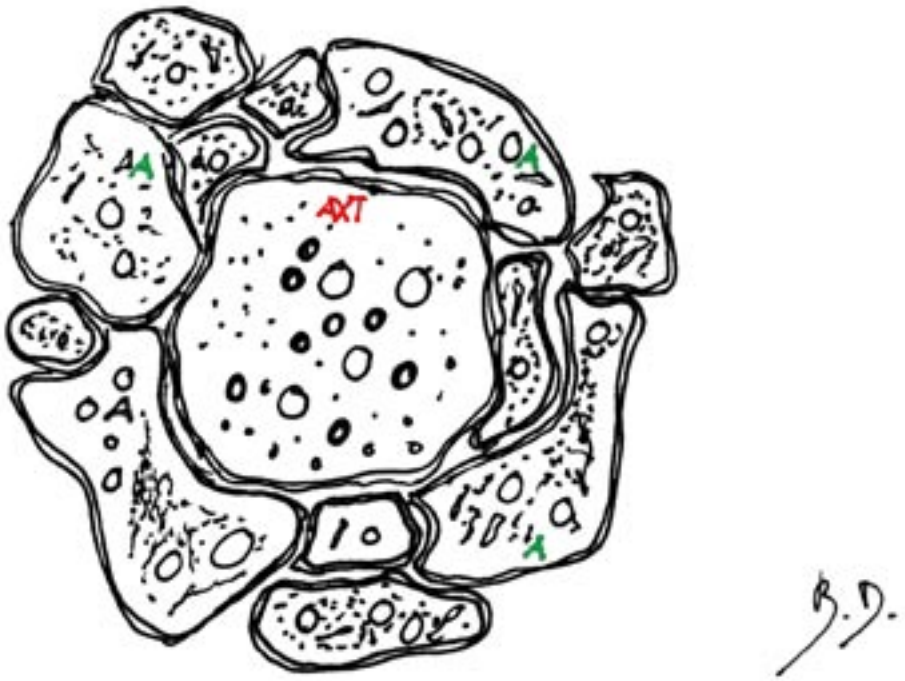
The authors conclude that regenerating axons can be stopped by two mechanisms: 1. By activating the physiological stop pathway that is built into the axon, and 2. By physically obstructing the advance of the axon tip.

In conclusion, glial processes seal off the synaptic membranes within 24 hours after the trauma.

Fig. 9.1

According to Liuzzi and Lasek (1987) astrocytes block axonal regeneration in mammals [146]

This drawing *numerous small mitoch*



AXT: Axon terminal **A: Astrocytic Process**

Fig. 9.2

Shows a single neuron in a transplant labelled with Tru Blue injected in the host
This result shall, though, be taken with much precaution, because the staining of the neuron might not be from retrograde transport of True Blue but might be due to diffusion of the tracer. To be certain the investigation has to be supplied with electrophysiological examinations.

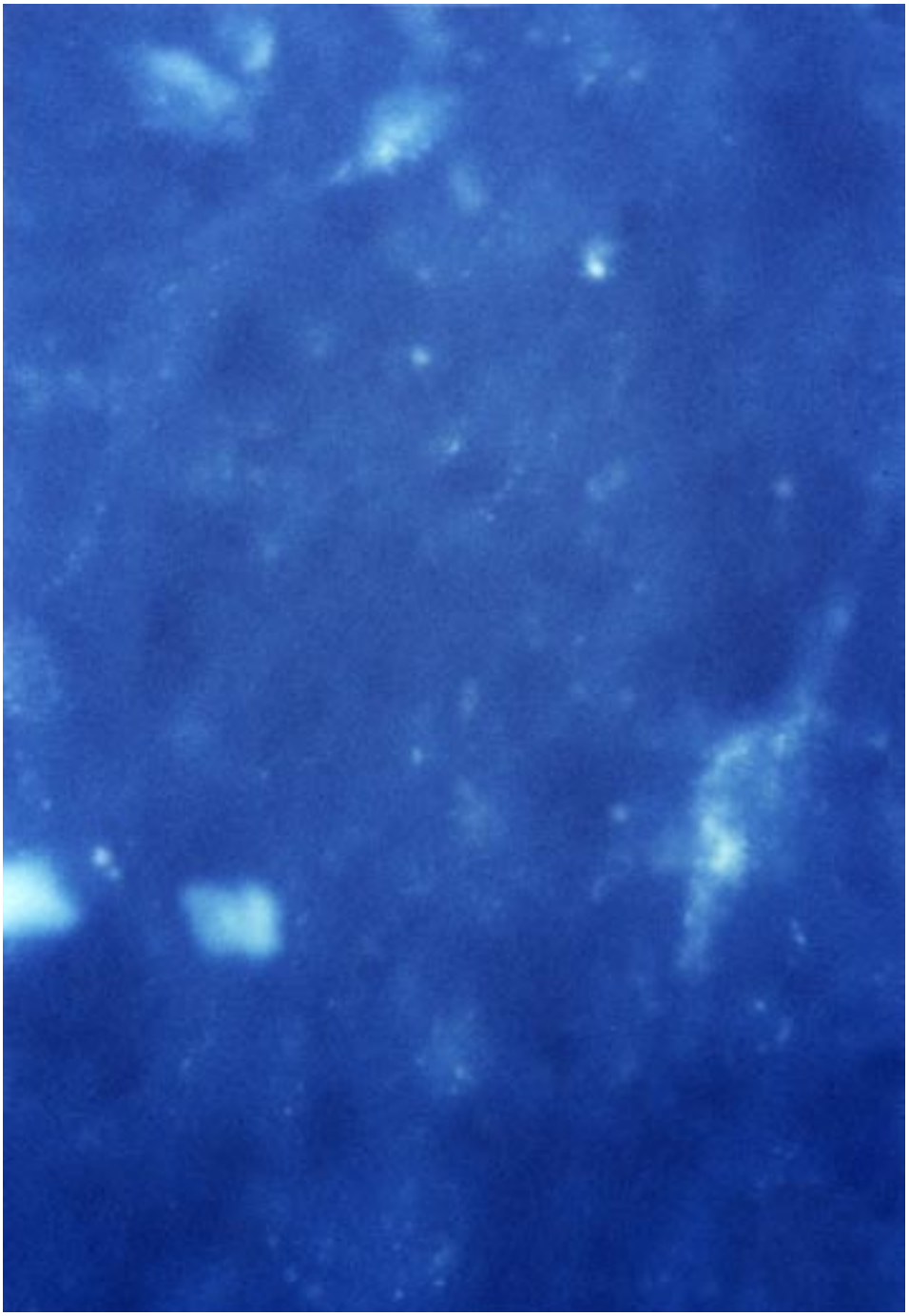


Fig. 9.3

This shows the gray substance of the host injected with both Nuclear Yellow and True Blue. The figures 9.2 and 9.3 are both from the same spinal cord.

Fig. 9.4

A large transplant that has grown about 1000% or more. Again this spinal cord has been injected with True Blue cranial to the transplant and Nuclear Yellow caudal to the transplant. The next two figures show the result. (ACHE-stain).

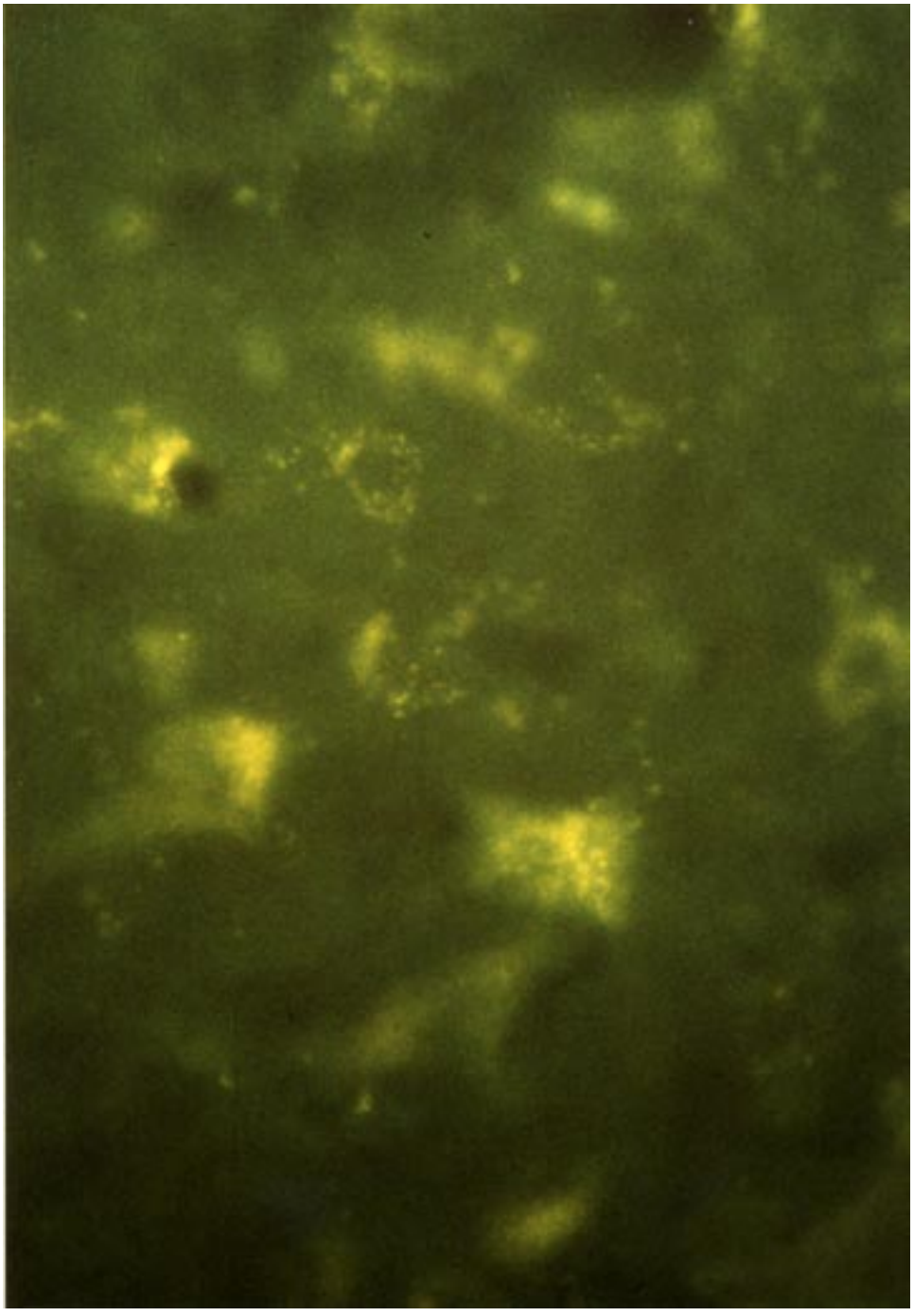


Fig. 9.5

This shows a group of cells in the transplant stained with Nuclear Yellow. This might not be due to active retrograde transport in the transplant, it could be due to autoflourescens.

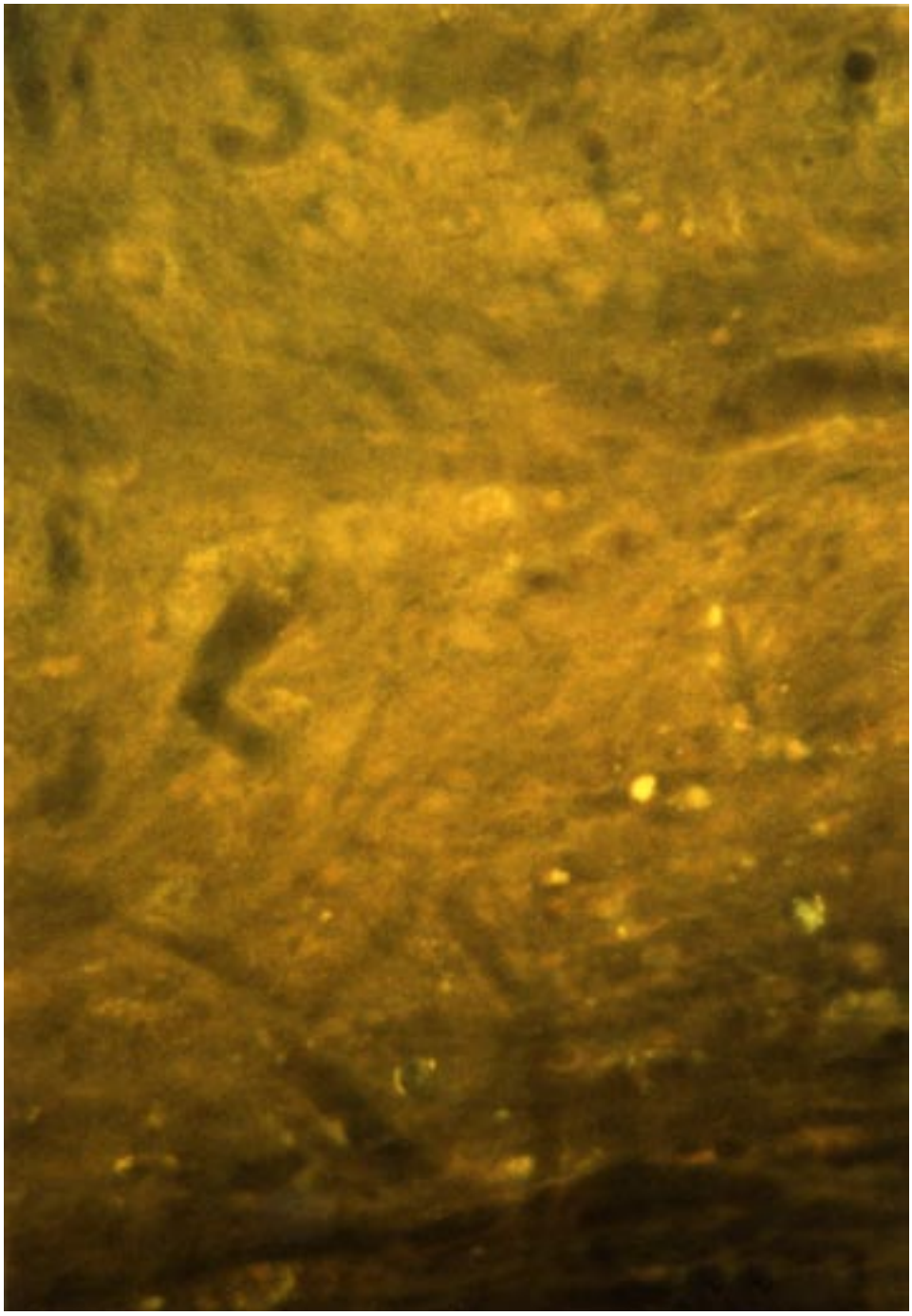


Fig. 9.6

The borderzone between the transplant and the host where the cells in the host show double labelling with Nuclear Yellow and True Blue.
It is never certain to determine whether the tracing of neurons in the transplant is due to active retrograde transport in the dendrites, or whether it is actually leaking or diffusion of the flouchrome from the host into the transplant when the injection is made. This differentiation needs electrophysiological examinations to be carried out.

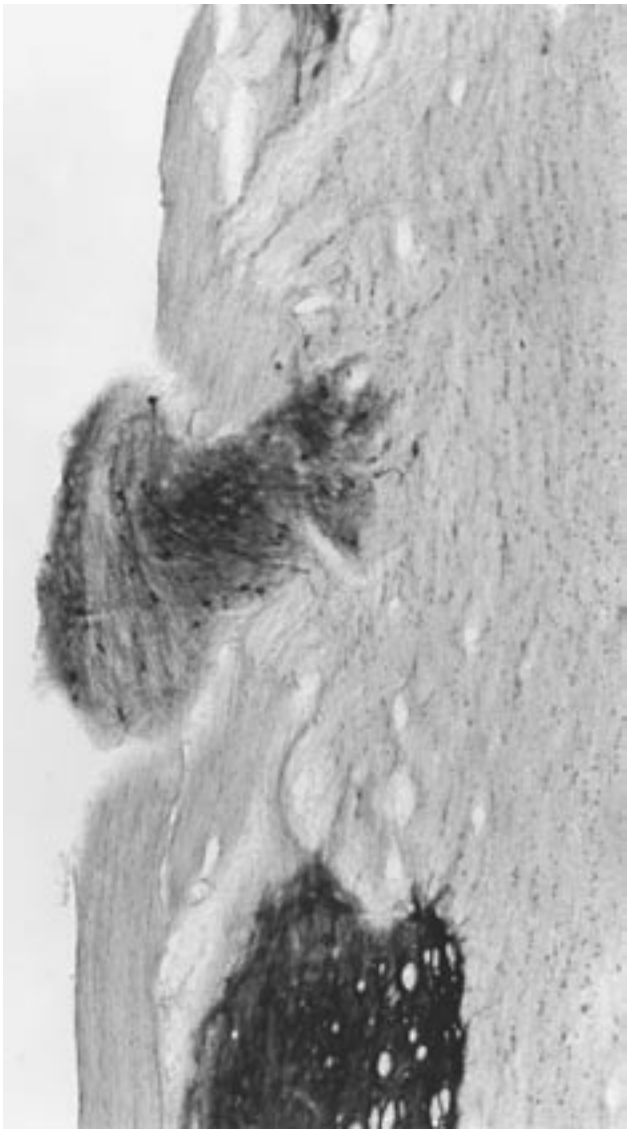


Fig. 9.7

Another transplant, not so extensive as the previous one. (ACHE-stain)

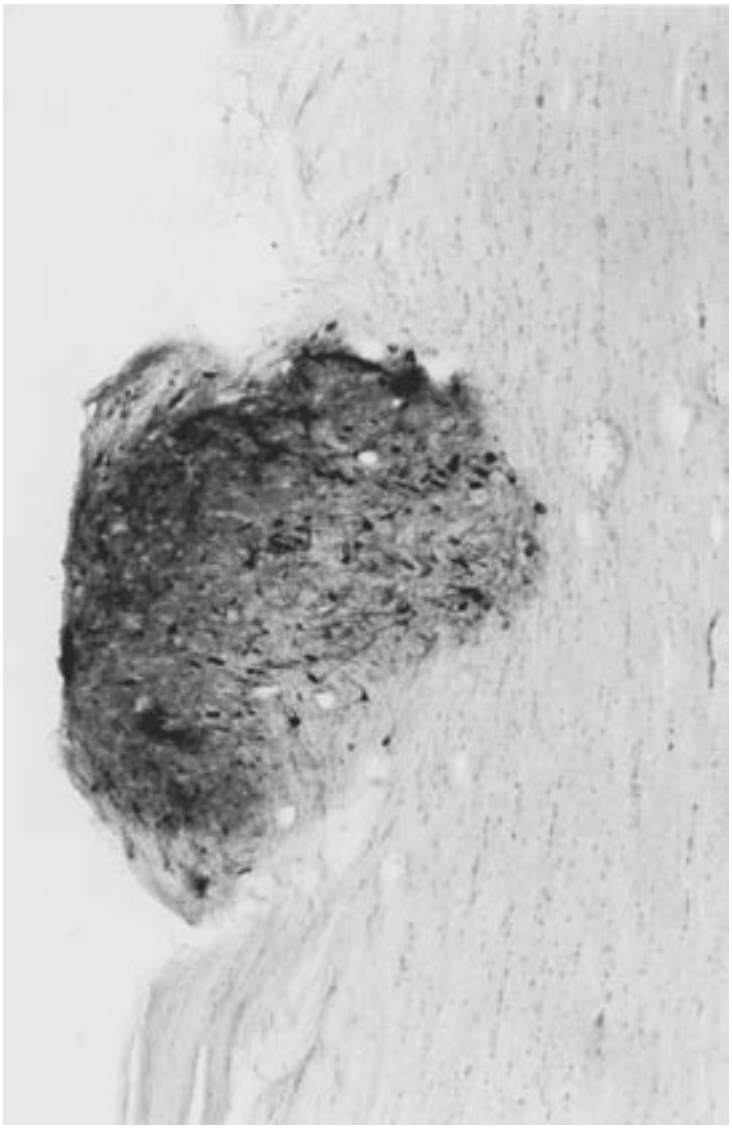


Fig. 9.8

A higher magnification of the previous transplant.

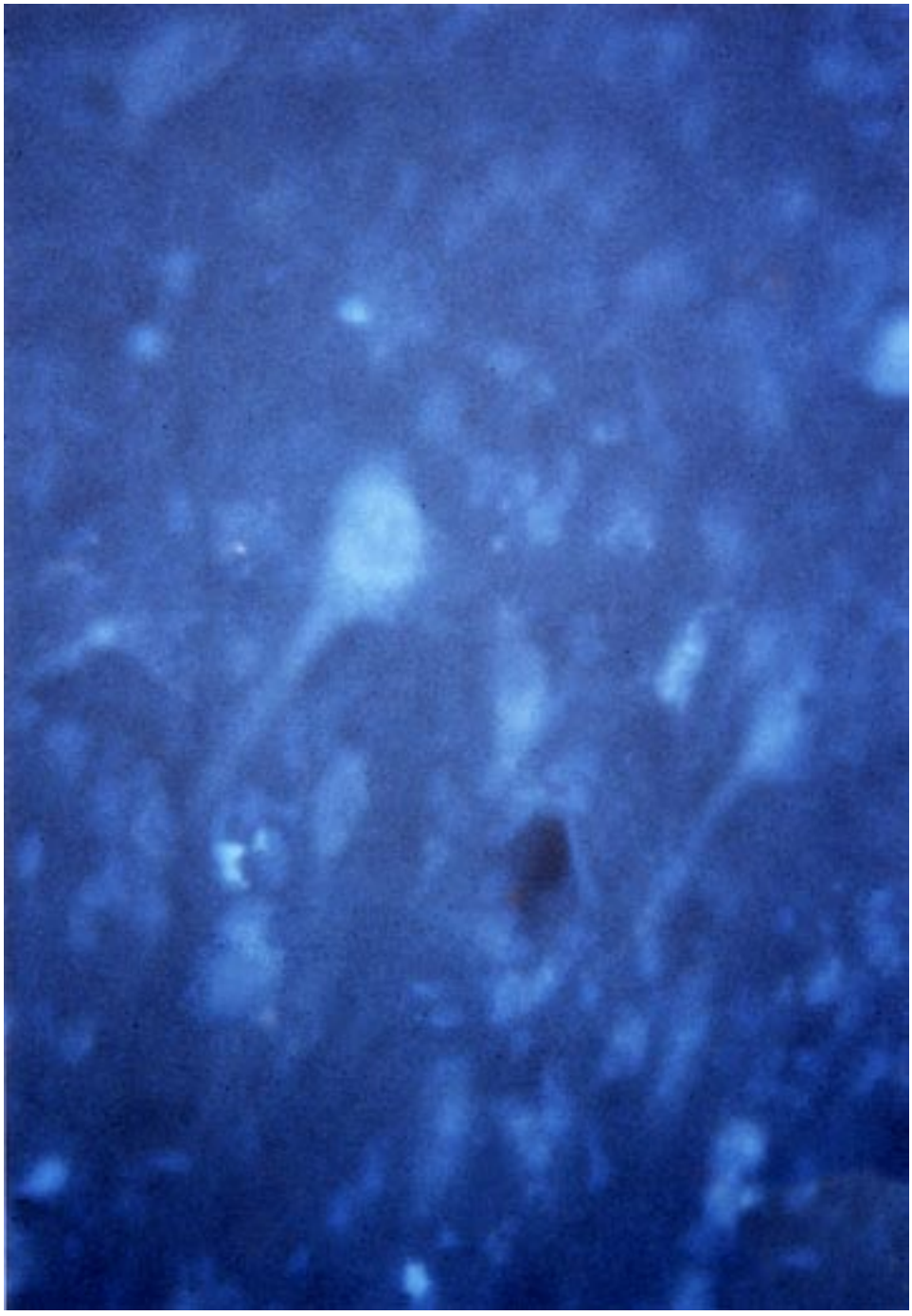


Fig. 9.9

Fluoro-Gold has been injected caudal to the transplant shown in fig. 9.8. The figure shows Fluoro-Gold labelled cells within the transplant, but again this might come from diffusion and not from active retrograde transport in the dendrite.

Astroglia	1. Fibrous - grey substance. 2. Protoplasmic - white substance. The astocytes have the shape of a star and form the scar tissue.
Microglia	Derives from the pia mater and has phagocytic properties.
Oligodendroglia	A transitional cell similar to the Schwann cells. They are closely related to both neurons and vessels and form the myelin (as the Schwann cells in PNS).
Cerebellar glia	Feather cells. Are in close relation to the Purkinje-cells of the molecular layer.
Peripheral glia	Schwann cells, from the myelin sheaths of peripheral nerves.

Neuron	-	Cell body, axon and dendrites.
Axon	-	Concerned with conduction and transmission (myelinated or unmyelinated).
Dendrite	-	Concerned with receiving synapses (myelinated).

In 1967, L. Wolman [226] made a study on post traumatic regeneration of nerve fibers in the human spinal cord, especially in relation to intramedullary neuromas. This is the most comprehensive study of the traumatized human spinal cord. The material consisted of 76 spinal cords from cases of traumatic paraplegia surviving from 6 hours to 43 years after the injury. He found that well-developed axon regeneration appeared in or near the damaged segment in at least 12 cases. He found small bundles of fine axons usually well-myelinated and ensheathed with Schwann cells. The Schwann cells were probably of peripheral origin. In three cases some of the bundles consisted of a mass of whirled and intertwined fine myelinated fibers resembling an amputation neuroma.

Wolman found the regenerating fibers in four main situations:

> *"1. In the thickened pia arachnoid on the posterior or posterolateral aspect of the cord adjacent to the posterior root entry zone. When they were numerous hey extended to the posterior surface to the midline or laterally to the attachment of the ligamentum denticulatium.*
>
> *2. In the thickened pia arachnoid in the anteromedian flssura where they appeared either as widely separated small bundles or closely packed larger fascicles. Occasionally they were present on the anterolateral aspect of thecord near the site of attachment of the anterior nerve roots.*
>
> *3. In the spinal cord which was replaced widely separated by a loosely arranged fibroglial meshwork and surrounded by a dense glial layer around the periphery of the cord remnant. Histiocytes were seenfrequently in the loose intervening tissue and bundles were accompanied and adherent to the wall ofblood vessels and a few were in the perivascular spaces.*
>
> *4. When there was a large cystic cavity in the spinal cord the regenerating axons were found around the wall usually in the damaged segments."*

Such fibers are seen both above and below the site of maximum damage.

These axons are usually called curling axons because the axons curl up in the wall of the cavities and show a tendency to regenerate but not an ability to penetrate the wall of the cavity or the cavity itself.

Wolman concluded that such neuromas do not originate from dysplastic foci but from regeneration not only of perivascular nerve plexes but also from many other damaged extra-and intramedullary neurons.

Holmes in 1915 [118] and Bailey in 1960 [8] found that in human pathological material aninjured cord forms either a single cavity or dense connective scar tissue surrounded by cavities. They have also described the presence of preserved fibers within the necrotic tissue in human spinal cord

specimens obtained shortly after trauma because the lytic processes do not start at the cut-ends of the cord but at some distance form the cut-ends within the spinal cord stumps. Therefore, intact axons and preserved fibers at the very end of the original cord transection can be present when the cellular lytic processes have already developed within the spinal cord stumps.
These investigations clearly show that the axons within the human spinal cord have a tendency to regenerate. Scar tissue and cavitation prevent this regeneration. In order to obtain the optimum result of outgrowing axons, the scar tissue should be removed and the stumps of the cord approximated. This shows scientists and doctors which pathway to follow. In previous chapters fetal spinal cord transplants have been dealt with but one possibility has not yet been mentioned, i.e. the growth factors. Maybe this is altogether the way to head either with or without transplantation of fetal tissue combined with external fixation of the spinal cord to keep the cut and severed ends in close approximation to promote axonal outgrowth.

Reference: 1, 8,11,14,77,118, 129, 136, 137,138, 140, 146, 226.

10
Trophic Agents, Nerve Growth Factors and Steroids

Trophic Agents, Nerve Growth Factors and Steroids.

Numerous therapeutic agents have been used to prevent scar tissue and promote axonal regeneration. Fetal grafts are well known to diminish the development of scar tissue. To avoid oedema and to stabilize the white matter of the spinal cord in the presence of central hemorrhagic lesions, steroids are reported to be useful by preserving the cellular and vascular membrane integrity. Hyperosmotic agents increase the rate of urinary excretion and is therefore useful in mobilizing extracellular fluid when this is present as tissue oedema following injury. Antiadrenergic compounds have also been proposed because noradrenalin is released following spinal cord trauma and can accumulate to high and even toxic quantities. Antifibrionytic therapy has been suggested to reduce the amount of bleeding and carbon anhydrase inhibitors to reduce cerebrospinal fluid pressure while increasing $_{p}CO_{2}$ [62].

Trophic agents

The narcotic antagonists 27 have been thought to ameliorate post traumatic ischemia in experimental spinal contusion and still maintain a sufficient bloodsupply to the spinal cord, although there is a moderate drop in systemic arterial pressure. Dimethyl sulfoxid exhibit cellular membrane protection especially to the myelin sheaths and axons as it reduces tissue swelling and extracellular fluid retention. Hyperbaric oxygen and hypothermia will relieve the cellular hypoxia and lower the metabolic and oxygen requirements of the neurons. Pyrogens and hormons have been administered to adult animals with spinal cord transection. The main effect of bacterial pyrogens is an alteration of the character of the tissue at the severed ends of the cord in the gap where the glial membrane formation was inhibited. Thyrotropin-releasing hormone (TRH) is a partial physiological opiate antagonist which reverses the anatonomic effects of opiods without altering the analgesia [172]. The endogenous opiods released following spinal trauma probably reduce the spinal cord blood flow. (Faden et al.) [72].

Treatment with TRH will prevent paralysis in fetuses with spinal cord injuries.

Other experiments showed the effect of adrenocorticotrophic hormon (ACTH) to be similar to that of piromen. The regenerating nerve fibers traversing the transection site in the spinal cord of cats were

found to conduct impulses for a short distance beyond the lesion. Some spinal cats who had received piromen therapy began to exhibit improved involuntary movements of the hind limbs after the operation (C. F. Windle) . When using milipore filter membrane, Campbell, Basset, Husby and Nobak found that the axons grew along; the membrane and across the transection site. After some time the milipore membrane became calcified which terminated the usefulness of this type of operation.
Campbell also used cryo-surgery by freezing a narrow segment of the spinal cord at the transection site. No scar formation was seen and the frozen segment became richly vascularized. Immunosuppressive drugs have also been used to suppress autoimmune response to proteins released in the traumatized spinal cord. The pyrogens and the ACTH were supposed to have suppressive effects. The results concerning the use of immunosuppressive drugs are inconclusive. Enzymes have been used [92]. Hyaluronidase, trypsin and elastase to improve conditions forneural regeneration in the severed spinal cord of adult female rats. The enzymes were administered intrathecally, at the transection site and intramuscularily. The results of the treatment showed that treated rats lived longer than untreated ones, the incidence of infection was lower and also bladder-control appeared earlier in the treated rats. Furhermore, a reduction in the scar formation improved the vascularization and reduced the number of cavities in the region of the lesion. Even conduction of electrical impulses was observed (in the clinical results from Moscow). Because of encouraging results clinical treatment was tried in patients with chronical stabilized injury. However invetigation from United States could not confirm the results from Russia. To improve spinal cord injuries, other agents have been also used: X-rays to diminish the scar formation in adult female dogs, treatment with triiodothyronin which appeared to have a beneficial effect on the central nervous system lesions, and conchanavalin A (a lechtin which binds glycoproteins) to reduce microcavity formation. Furthermore, the use of chemotherapy has been examined although without convincing effects.

Nerve growth factors

Nerve growth factor [139, 159] has been applied to see if growth of transected axons in the central nervous system of the spinal cord in rats would have the same effect as the regeneration of axons as it has on the peripheral and sympathetic sensory neuron growth. The findings turned out be most successful, the nerve growth factor able to stimulate central axonal fibers previously destroyed by transection.

At the site of the lesion neurotrophic factors with increased vascularization at the injury site and astrocytes become reactive. This means that they stabilize the ionic environment, remove excess excitatory amino acids and produce trophic factors (at least in vivo). Thus such trophic mechanism may accelerate recovery from injury by acting on the surrounding host cells. It will restore the capacity of regeneration in the environments in which the axons are loacted rather than the intrinsic capacities as thought by Cajal. The reason is that the axonal outgrowth in peripheral nerves are over a much longer distance than those in the spinal cord.

Up til now no scientific work has been published on the survival of neurotrophic neurons and neurit promoting sprouting factors in the spinal cord following injury. Numerous papers have been published on the neurotrophic and neurit promoting factors in the brain following injury [52, 53, 159, 160, 161, 162, 165,] injuries of the central nervous system induces the production of neurotrophic factors which support the survival of both peripheral and central neurons in cell cultures. Trophic

activity in gelfoam extracts placed in wound cavities of neonatal and adult rats showed the activity two hours post lesion to be negligible and it remained low during the first four days after the lesion. After that time the concentration began to rise by day 6 and reached a maximum at day 16 after the injury (Nieto-Sampedro, Kesslak, Gibbs and Cotman 1988)[161]. This suggests that the neurotrophic factors or the cells producing them are transferred from the tissue to the gelfoam. Furthermore, the induction of neuronotrophic activity by a neural damage correlates excellently with the glial activation which produces a strong glial reaction and thus a gliosis. The grafting of a wound gelfoam which had stayed for five days in a brain wound of another animal, actually did accelerate the behavioural recovery.

Many neuronotrophic substances can be demonstrated in the brain and it has been shown that some of them cause the death of spinal cord neurons in a 24 hour cultured assay. The chemical identity of such toxic activity, however, is still unknown. (Nieto-Sampedro et al.) [160].

It might be that a spinal cord graft is not the answer to spinal cord regeneration following trauma. Possibly the graft absorb and use all the neurotrophic factors for its own growth thereby not promoting the regeneration of the host axons. It also seems as if the brain derived nerve growth factors possess toxic activities which might even stop the neuronal outgrowth. Perhaps the ideal situation is immediate application of spinal cord derived neurotrophic factors at the site of injury. This might prevent the heavy gliosis from taking place.

For some reason the formation of gliosis does not take place to the same extent in the brain as in the spinal cord. Therefore it is much easier to perform successful grafts to the brain than to the spinal cord.

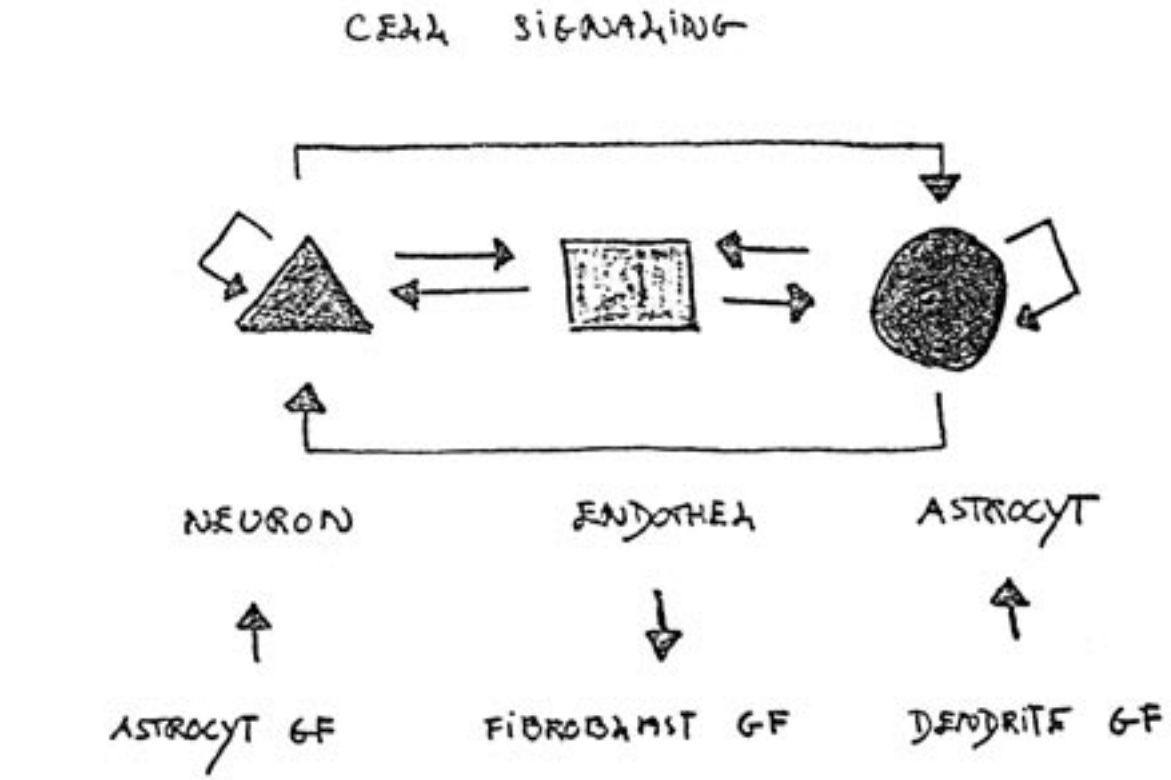
Steroids

Recently, special interest has developped in intensive glucocorticoid medication to animals with injured spinal cord. It has been shown that a high dosage of glucocorticoid given intravenously may facilitate the neuronal excitability and the impulse conduction; it may even improve the blood flow and preserve the ultrastructure of the cord through a reduction of the injury induced free radical-catalyzed lipid peroxidation. Such reduction might even prevent the ischemia related tissue degeneration.

Glucocorticoid enhances excitability of the central nervous system and augments both single and repetitive spinal monosynaptic reflex transmission as well as polysynaptic reflex transmission. The resting membrane potential becomes hyperpolarized by glucocorticoids at an average of 4mV. On the injured neurons which are depolarized glucocorticoids support their resting potential, with the result that the ability to conduct impulses ismaintained. This facilitates the myelinated axonal conduction and reduces the anatomical destruction and the reflex function of the injured spinal cord [103].
It is of the outmost importance that the blood supply to the injured spinal cord is sufficient in order to maintain the necessary energy metabolism especially supported by oxygen. In the injured spinal cord hypoxia is induced because of the vasogenic edema. It has been shown that high doses of glucocorticoid enhance the blood flow of the injured central nervous system. The mechanically induced vasospasm is a result of a massive release of vasoconstrictor amines, microvascular platelet

aggregation, and endothelial degeneration. Glucocorticoid also reduces the vasoconstrictor effects of norepinephrine and epinephrine. It depresses the alpha adrenergic receptor sensitivity and enhances the beta-receptor responsiveness of the spinal arterioles. Glucocorticoid also interferes with

Polypeptide Growth Factors



NERVE GF : EPIDERMAL GF
FIBROBLAST GF
GLIA GF
PLATELET GF

SURVIVOR FACTORS
DIFFERENTIATION FACTORS
NEUROTROFIC FACTORS

After S. Gammeltoft 1987.

the activation of the prostaglandin system. The prostaglandins possess vasoconstrictor properties and also tend to increase the formation of thromboxanes which are extremely potent vasoconstrictors and promoters of platelet aggregation. Normally the action of thromboxanes is counteracted by products of the vascular endothelium but following spinal cord injury the endothelial lipid peroxidation may lead to a decrease of the products of the vascular endothelium especially the prostacyclin, leaving the action of thromboxanes to be unopposed. In case of decrease in the spinal vascular lipid peroxidation the synthesis of prostacyclin would counteract the ischemia induced by the thromboxanes. Glucocorticoids will cause a dilatation of the small arteries and counteract the endotoxin-induced increase of the cerebrovascular resistance resulting in an enhanced blood flow to the injured spinal cord.

The most striking effect a high dose of glucocorticoid has shown is a decrease of the spinal cord lipid peroxidation and the tissue degeneration following trauma to the spinal cord. The central nervous tissue contains 40% of lipids in contrast to the extraneuronal tissue which contains only 5 to 10% of lipids. For this reason the central nervous system is very sensitive to lipid peroxidation reactions which involve free radical attacks on unsaturated fatty acids within myelin, neuronal, and micro-vasculature membranes. Therefore alterations in membrane lipids have serious consequences for the function of the membrane of the cells and the organelles within the cells. Injury-induced tissue hypoxia generates free radicals which initiate peroxidative reactions within the lipid bilayer of the membranes of the cells and organelles. Iron and copper complexes catalyze lipid peroxidation reactions that induce necrosis of the contusioned spinal cord. This leads to disruption of the membrane function to a point where impulsive generation and axonal conduction can no longer occur. Among the enzymes within the membrane which are inhibited by a lipid peroxidation the most important is the activity of the electrogenic sodium pump $(Na^+ + K^+)$ATPase. A large dose of glucocorticoid will reduce the lipid peroxidation and thus enhance $(Na^+ + K^+)$ATPase activity in the injured spinal cord. It has also been shown that when a large dose of glucocorticoid is given 1 hour prior to trauma, there is less lipid peroxidation in the spinal cord than when glucocorticoids have not been given. Thus the self-perpetuating process resulting in tissue death can be either diminished or even stopped[102].

In laboratory experiments it has been shown that glucocorticoids should be given intravenously as soon as possible after the trauma and in sufficiently high dosage to be effective. Glucocorticoids do not have any antioxidant properties unless given in sufficient dosage i.e. 30 mg/kg. An effect has been demonstrated after 5 minutes, 30 minutes and 60 minutes after trauma - even up to 8 hours. An early administration will give the best results consisting in

1. an antioxidant effect,

2. a prevention of thromboxane-formation,

3. decreased vascular responsiveness to vaso-active neurotransmitters and

4. a direct vasodilator action.

It may thus be concluded that large doses of glucocorticoids can significantly prevent the development of a post traumatic ischemia of the spinal cord white matter. However, it is not able to reverse the decrease in blood flow if this has occurred.
A substance even more potent than the glucocorticoids is the non-glucocorticoid 21-aminosteroid tirilazad mesylate (U74006F) [105], which will potently and effectively inhibit post traumatic, postischemic and hemorrhage-induced brain lipid peroxidation. Not only does it possess antioxidant properties, it also has been demonstrated to reduce the vasogenic edema of CNS-injury.

The tirilazad mesylate was also observed to preserve tissue vitamin E levels significantly after spinal cord injury. Vitamin E is utilized to quench lipid peroxyl radicals. Tirilazad mesylate destroyes the lipid peroxyl radicals but preserves the vitamin E, resulting in an inhibition of lipid and a maintenance of the vitamin E. Pretreatment of cats with high doses of vitamin E will antagonize post traumatic spinal cord ischemia and enhance neurological recovery.

The neurological recovery of cats given sufficiently high doses of glucocorticoid or 21-aminosteroid has been shown to be enhanced, especially if the treatment with the steroids had been given early after the trauma.

A random study of high doses of methylprednisolone (Upjohn) in the treatment of acute spinal cord injury in human beings has been carried out, but the clinical effectiveness is uncertain. Patients with spinal cord lesions were all given methylprednisolone 30 mg/kg as a bolus dose followed by an infusion of 5,4 mg/kg pr hour for 23 hours, resulting in a total of 10,8 grams of steroids given to a 70 kg patient. Today such treatment is obligatory in The United States, and is begun at the site of the accident. Treatment started later than 8 hours after injury has no effect. As demonstrated in laboratory animals. No proven side effects of such high dose, short-term use of glucocorticoids has been noticed, except occasionally gastrointestinal bleeding in patients with a history of peptic ulcer [27].

NBQX: AMPA Receptor Antagonist

To prevent neuronal death extensive studies on neuroprotective agents have been carried out. The CNS group at Novo Nordisk has especially been looking at the effect of the AMPA receptor antagonist NBQX. Glutamate release plays a major role in neuronal death.

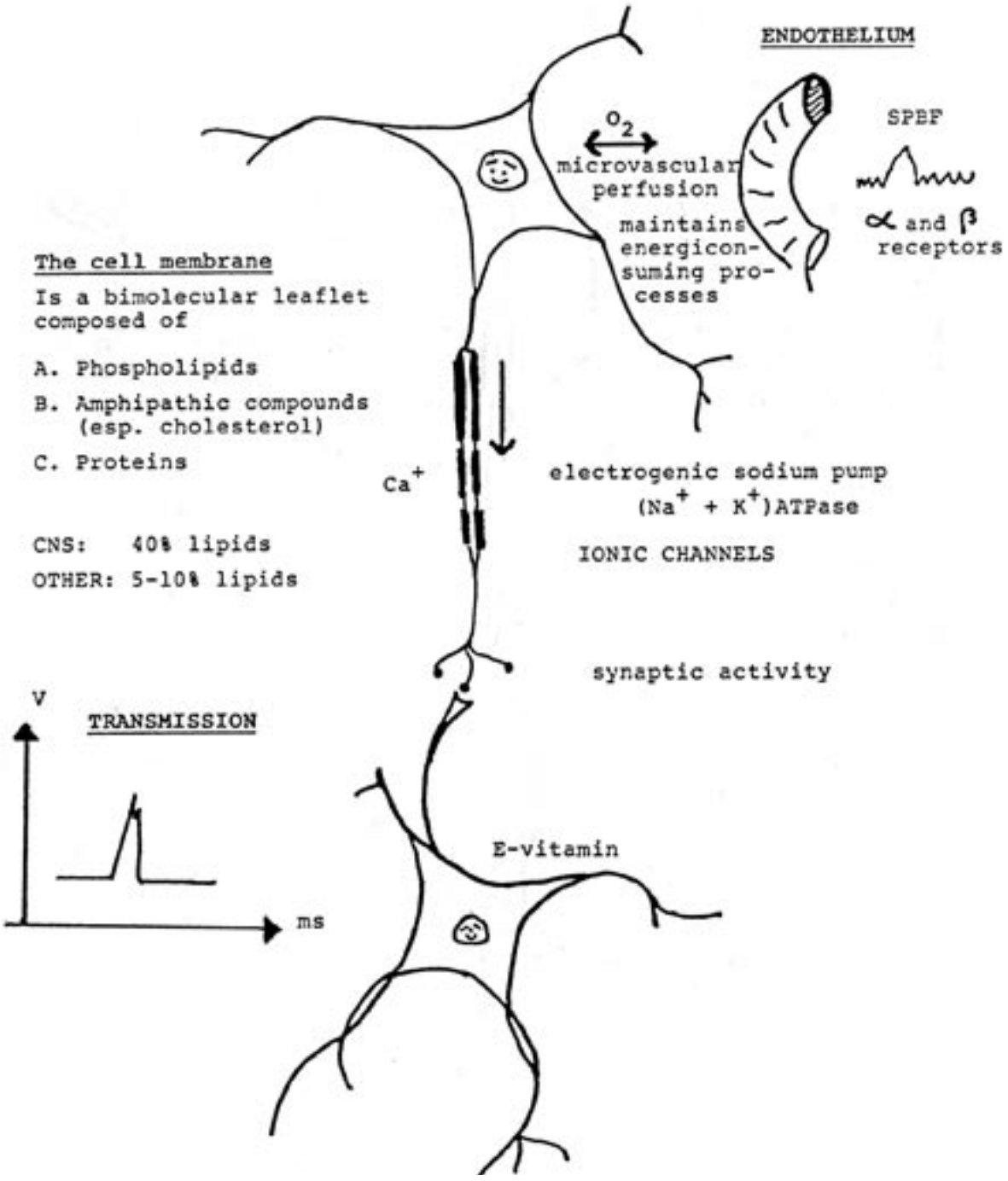
Glutamate produces excitation of practically all cells in the mammal CNS. In the spinal cord it is especially concentrated in the grey matter, where lipid peroxidation processes start when trauma has occured. Glutamate deporalizes neuronal membranes in association with an increase in Na+ and K+ conductance which may result in a CA^{+} displacement and that alters the cell membrane-permeability.

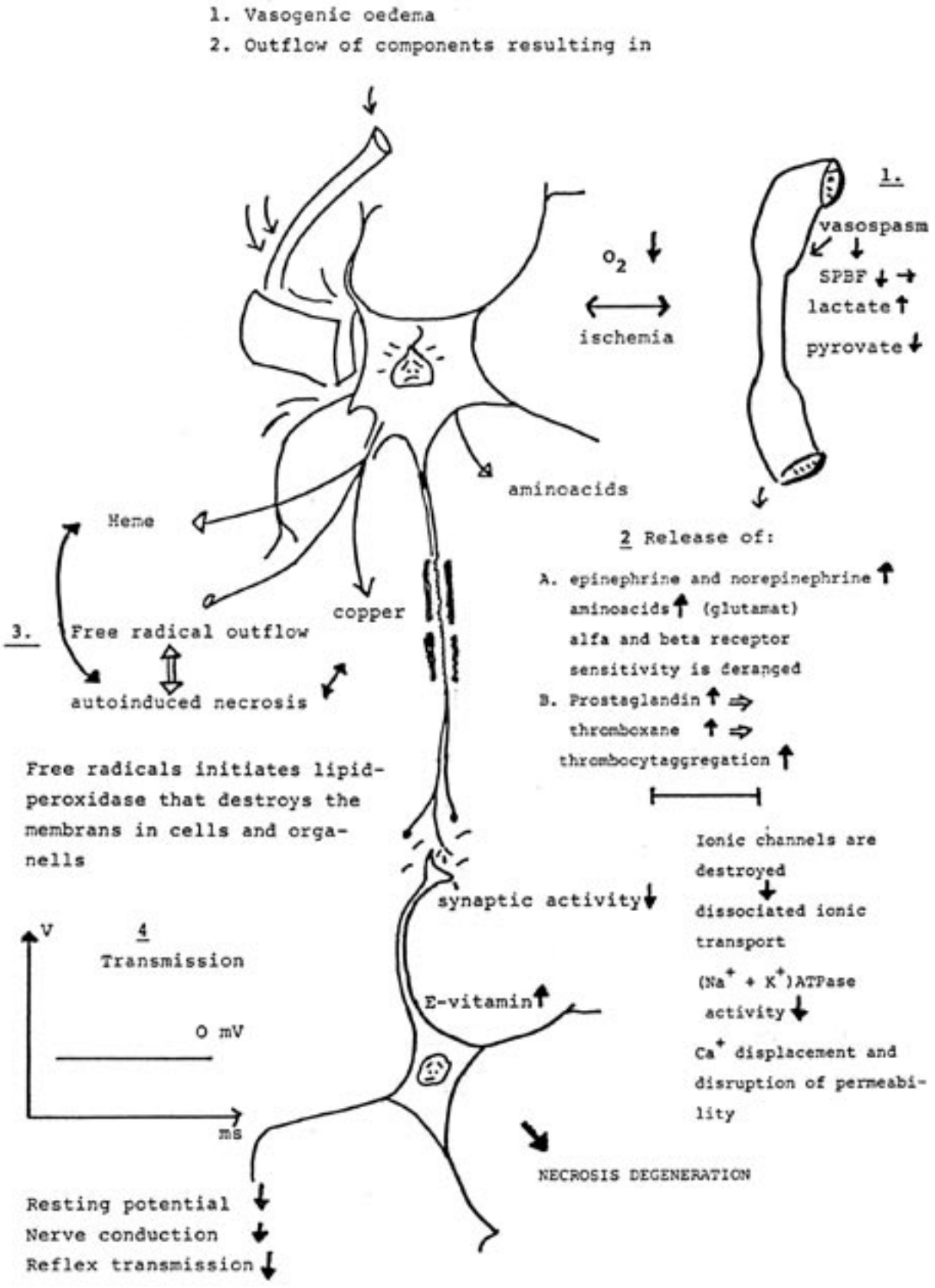
Glutamate interacts on the AMPA receptor among others in the cell membrane. For this reason the AMPA receptor antagonist has been proven to be neuroprotectant in global ischemia in the rat and the gerbil. The exact mode of the anti-ischemic action is at present unknown, but further studies are being conducted [177].

FUNCTION: Transmission of electrical impulses through neuron and axon to tissue.

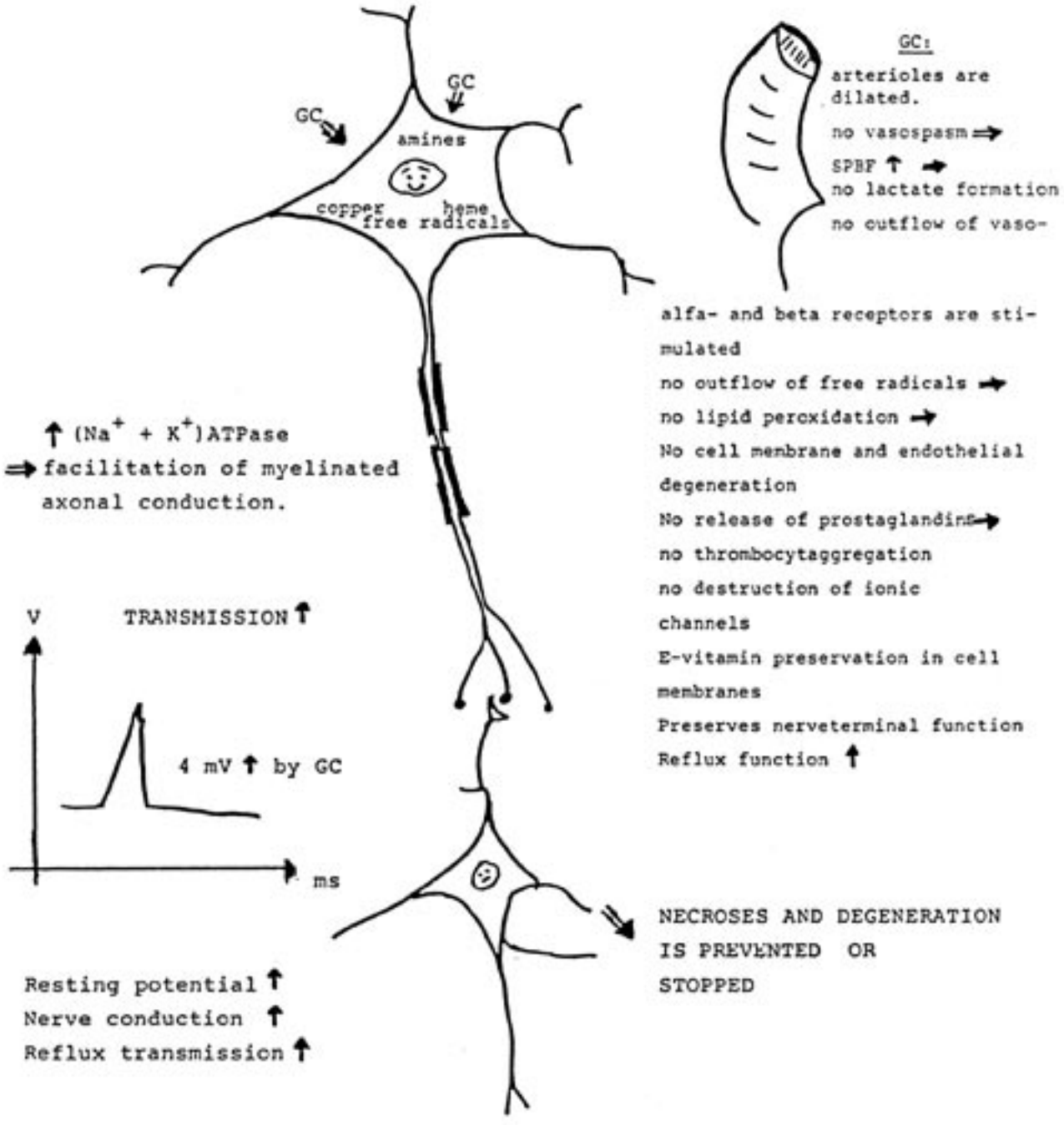
DEMANDS OF SURVIVAL:

1) To maintain sufficiant Spinal Blood Flow (SPBF).
2) To prevent ischemia - related tissue degeneration.
3) To support excitability of neurons




1. Vasogenic oedema
2. Outflow of components resulting in
1.
vasospasm
SPBF
lactate
pyrovate
O_2
ischemia
aminoacids
Heme
copper
3.
Free radical outflow
autoinduced necrosis
2 Release of:
A. epinephrine and norepinephrine
aminoacids (glutamat)
alfa and beta receptor
sensitivity is deranged
B. Prostaglandin
thromboxane
thrombocytaggregation
Free radicals initiates lipid-
peroxidase that destroys the
membrans in cells and orga-
nells
Ionic channels are
destroyed
dissociated ionic
transport
$(Na^+ + K^+)$ATPase
activity
Ca^+ displacement and
disruption of permeabi-
lity
synaptic activity
V
4
Transmission
0 mV
ms
E-vitamin
NECROSIS DEGENERATION
Resting potential
Nerve conduction
Reflex transmission

GC prevents lipidperoxidation in cell membranes by acting on the archways of polyunsaturated fatty acids created by unsaturated double bonds in the cis configuration. In the peroxidation process the double bonds are changed from cis to trans and the effect of GC is thereby arrested.



Summary

In summary, there are three objectives for the use of neuronal spinal cord transplants for repair:

1) circuit reconstruction with bridging the lesions by replacing neurons,

2) transmitter replacements within the cholinergic and monoaminergic system and

3) provision of trophic factors.

The survival factors prevent host nerve cell death, the growth factors promote host axonal development and regeneration and the steroids prevent lipid peroxidation processes. This takes place by either establishment of nerve cell connections or secretion or diffusion to the surrounding host tissue.

Reference: 4, 25, 27, 28, 29, 46, 47, 52, 53, 62, 68, 72, 79, 88, 89, 91, 92, 95, 101, 102, 103,104, 105,110, 139, 153, 159,160,161, 162, 163,172, 213, 224.

11

Spinal Injury Research Yields a Glimmer of Hope

By WALTER SULLIVAN

SCIENTISTS building on work by generations of researchers are seeing signs of progress on one of medicine's most poignant challenges: the restoration of nerve function after spinal injury.

In experiments, chiefly with rats, researchers are seeking ways to combat the paralysis that follows damage to the spine. In an effort to carry nerve signals over damaged areas, they have transplanted nerves and human placental membranes into the animals. They have also inserted cells from the spine of a rat fetus into the severed spine of an adult rat, hoping to restore the broken link.

In many cases the transplanted tissue survived. In some it seemed to be establishing links with the severed nerves or providing a channel for nerve regeneration.

Aware that thousands of paraplegics and other victims of spinal injury grasp at any hints of a cure, virtually all researchers in this field warn that, despite encouraging advances, no solution is in sight. None of the experiments has succeeded in restoring spinal function.

Nevertheless, the results have reawakened hope that it may some day become possible to restore some degree of muscle function and sensation lost through damage to the spine.

In amphibians, lampreys and other lower animals, as well as in the mammalian fetus, a damaged spine can regenerate. But in adult mammals this is only true of peripheral nerves, such as those carrying sensory information or muscle commands. They are distinct from the central nervous system, which includes the spine and brain.

Each peripheral nerve fiber is protected by a sheath that can survive crushing and provides a channel through which a replacement nerve can grow. Such sheaths are absent in the central nervous system.

If the multitude of peripheral nerve fibers forming the sciatic nerve between spine and leg is locally severed and then rejoined, according to William J. Freed of the National Institute of Mental Health in Washington,

Continued on Page C6

— and the spinal

— appeared that sensory — erves from that part of the spine beyond the cut were growing into the transplant. None of these nerves showed signs of growing across the gap, but he said he hoped that ultimately transplants could be used to relay at least some nerve impulses.

A different approach, using human placental tissue as a "passive bridge," was described by Dr. Fred H. Gage and his colleagues at the University of California in San Diego in a recent issue of Science. The amnion membrane — a portion of the placenta, or afterbirth, formed of collagen, the protein laminin and other substances, but containing no cells — has been tested and it has been found that the side of the membrane rich in laminin encourages regeneration of nerves from the central nervous system.

In one experiment the multiple nerve links between two parts of the rat brain — the hippocampus and septum — were severed and an amnion membrane from a human placenta was placed in the gap. After seven or eight weeks, Dr. Gage explained in a telephone interview, nerve fibers from the septum had grown across the membrane "bridge," had re-entered the hippocampus and were headed for "appropriate locations" there.

Tests of Restored Function

He said memory and learning tests are now under way to see if such nerve growth leads to any restoration of the function that was lost when the nerves were severed. Probably because the transplanted material contains no cells, it has not led to rejection by the host's immune system.

— Etiske prob

THE NEW YORK TIMES, TUESDAY, JULY 14, 1987

Torsdag 9. juli 1987

POLI

1. sektion 5

Spinal Injury

Transplantation af hjernevæv

LONDON: Svenske

Human afterbirth becomes avai able at every delivery and is no mally discarded. Its use would not b accompanied by the legal and ethical problems associated with using fetal tissue.

Several laboratories are seeking ways to restore function where pa ralysis results from a break in the link between spine and muscles. One group has reported that a fiber from the spine has grown along an im planted peripheral nerve and rees tablished a sufficient link with mus cle to produce contractions under electrical stimulation.

Furthermore, restoration of sexual responses in male rats whose spines had been severed was described at the Rochester meeting. Dr. Alain Privat, an Institute of Biology in Montpellier, France, told how cells from the brain of the rat fetus that evolve into the brain and spine were inserted into an adult spine beyond the point where it had been severed.

Two to three months later it was found that fetal cells of the type that produce serotonin, a substance controlling nerve function, had survived and begun to establish contact with appropriate nerve cells within the spine.

Isolating the lower spine, Dr. Privat said, had killed the nerve cells producing serotonin. Without it, male rats were unable to achieve an ejaculation. This was partly corrected in those with transplants that produced serotonin.

While the severed spine cut off signals from the brain, its lower, disconnected part was able to perform local reflexes controlled by serotonin. Eventually, Dr. Privat hopes, such transplants may help paraplegics achieve some measure of sexual activity.

Fetal Tissue From Animals

An alternative to the use of human fetal tissue for spinal repair would be fetal tissue from animals. To test such a procedure in reverse Swedish researchers have transplanted human fetal tissue into animal brains. Likewise pig embryo tissue has been inserted into rats brains. The transplanted cells survived, but a drug had to be given to neutralize the recipient's immune system and avoid rejection of the transplant.

Some specialists fear such drugs could make the recipient vulnerable to infection by bacterial contaminants, since it would be difficult to insure that the fetal tissue was free from bacteria.

In describing the advantages of using human amnion membranes, the San Diego group pointed out that such transplants persisted in rat brains even though no anti-rejection drugs were used.

Ethics

Almost any organ can be transplanted. Kidneys, livers, pancreas, lungs, hearts, bone marrow, corneas, bones and even penis.

Some years ago, quite a sensational case took place in Italy. A little girl suffered from leukaemia and the doctors told the parents that only a bone marrow transplant from a sister or brother would save her life. Since it was their only child, the parents decided to have another child with the purpose of giving the daughter a bone marrow transplant. This child became a donor of bone marrow to his sister. This was in the spring of 1985.

Transplants of medullary tissue from the adrenal gland in patients with Parkinson's disease have taken place in many parts of the world, especially in Sweden, Mexico and Japan. Only transitory rewarding effects were seen after the transplantation of adrenal medullary tissue to the putamen. After a few months, all signs of clinical improvements had disappeared. (Backlund 1987) [7]. For that reason scientists in Sweden decided to make a transplant from aborted human fetuses, aborted in the eighth to eleventh week of pregnancy, into immunosuppressed rodents intraocularly and intracranially. Central monoamine neurons from locus coeruleus and from substantia nigra survived grafting to the eyechamber and produced nerve fibers that innervated the host iris. The human fetal parietal cortex developed intraocularly and became innervated by sympathetic nerve fibers from the host rat iris. Raphe pallidus, hippocampus, cerebellum and the spinal cord survived and developed in the rat eye. It was observed how dorsal root ganglia and adrenal medulla chromaffin cells formed nerve fibers. The human heart muscle maintained its rhythmic contractions after transplantation to the eye, and it was even innervated by the rat sympathetic nerves. Intracranial grafts of human dopamin neurons to rats with 6-hydroxydopamin induced degeneration of the nigro striatal dopamin pathway survived and developed in cavities overlying striatum. In 1986 such observations were presented at a poster session in Marseille in France by Stromberg, Bygdeman, Olson and Seiger [203].

The same Swedish scientists have now made human fetal transplants in patients with Parkinson's disease. The results were presented at the 700th meeting of The Danish Neurosurgical Society in Copenhagen in april 1991. In Birmingham in Great Britain 25 patients with Parkinson's disease have been operated on until now, some with bilateral fetal transplants with very promising results. The operations are still ongoing.

Hoping to help patients with severe diabetes mellitus, tissue from fetal pancreas has been taken from prostaglandin-induced abortions (twelfth to eighteenth week) and transplanted into patients. The fetal tissue was injected into the liver of the patient via the vena porta or directly into the spleen. It was hoped that injected beta-cells from the fetal pancreatic tissue would then become attached to the net of capillaries in the liver and the spleen and start to produce insulin. In one of seven patients there were objective signs that they had started to produce insulin, although only 5% of the normal production was achieved. If the grafted tissue could produce 10-15% of the normal production this would be of significant help to diabetic patients. (Läkartidning) 145.

Because science has now reached the point of using fetal tissue in order to help severely handicapped patients live a life without symptoms of Parkinson's or diabetes, the Swedish Medical Association asked an ethical delegation to work out some rules for the use of aborted fetal tissue for grafting. The resolution consists of six points:

1."The tissue must only be taken from embryos."

One is concerned here with the age of the fetus. If it is too old and thus too developed it must not be used. In experiments that have already taken place it was shown how the grafted fetal cells will have the best growing conditions when grafted at the ninth postcoital week.

2."The operations must only take place in accordance with the law of transplantation. If the tissue is taken from human fetuses the woman who is having the abortion should be informed and accept the planned transplantation."

Thus it is the duty of the doctor to inform the woman and ask her if she will allow her fetus to be used for the purpose of transplantation.

3. "The transplantation should be passive in accordance with abortions obtained by routines and it should not influence how, when and why the abortion take place. There must be no connection between the donor (aborting woman) and the recipient".

This is a very important point. There are certain aspects to fear if one thinks of the consequences invoted in successfull fetal transplants for instance into the central nervous system. Paraplegics and tetraplegics would be willing to pay any sum of money to any woman who would produce and sell fetuses so the handicapped could regain motor and sensory function. Paraplegic women could possibly make their own fetuses and paraplegic men might pay a woman to have a fetus. Women with no scruples at all might even take advantas of such situations if doctors also without scruples would accept such activities. For this reason, great precautions to separate the donor and the recipients should be taken. Women with sick children are willing to go very far to help their sick child and perhaps even make a fetus with the aim of a fetal transplant to the sick child. As mentioned earlier, this situation has already taken place in Italy where a child was produced to gain bone marrow for another child. Where should the lines be drawn?

4."Only isolated nerve cells or fragments from the central nervous system may be used for transplants. Parts or entire organs from fetuses should not be used for transplantation".

The reason for this rule is to prevent the transplantation of spirits and souls since we do not know where the soul is located.

5. "All staff shall be informed".

It is absolutely necessary that all persons involved in such a process as abortion for transplanting and the grafting procedure are informed of what is going on. No secrets or misinterpretations

should be possible.

> *6."Every project which has to do with transplantation of fetal tissue must be approved to committee which deals with the ethical aspects of the research".*

An open discussion of every research project should take place to have an ethical discission of the pertinence of the projects when dealing with human fetuses.

These are the six directives from the Swedish Ethical Delegation. (Läkartidningen) [145].

In my opinion the time has come to perform fetal grafting in patients who have suffered a spinal cord trauma with paraplegia. Although functional synapses between host and graft in the spinal cord have not been clearly demonstrated in animals, there is no reason to believe that transplanted neurons in the spinal cord behave differently from those transplanted to the brain, where there has been proof of functional host-graft synapses.

Furthermore it is not necessarily the function of the synapses which is desired, perhaps only the prevention of scar tissue formation and the action of neurotrophic factors to promote axonal regeneration. There is still a long way to go, but if surgeons feel that, at this early stage, it is justifiable to make spinal cord transplants, their patients should be fully aware that they do participate in an experiment which to the presen has not produced any results in anamal experiments. To use this kind of treatment in thoracic spinal cord lesions can do no harm, quite to the contrary, a lot of good might happen and for the time being this is the best we can offer. With all the different kinds of operations that have been performed on human beings with spinal cord lesions including implantation of peripheral nerves into the injured spinal cord, there is no doubt that fetal grafting would have been attempted if the product had been called foeto-mix and it had been synthetic.

The consideration would have been minor if a total tear of the spinal cord had been shown by the MR-scan and at the operation.

It can give rise to some speculation why scientists are prepared to do fetal grafting in patients with Parkinson's disease. The operation is a craniectomy, where healthy brain tissue is penetrated to reach the basal ganglia in the lateral walls of the ventricles. No experimental surgery has to my knowledge been done on monkeys.

It is of outmost importance, if permission to use fetal tissue for grafting into human beings is granted, that Danish scientists unanimously agree that the time has come to carry out these operations.

Spinal cord fetal graft operation in human beings is a simple operation that any neurosurgeon who is familiar with operations upon the spine and the spinal cord can performe. That will not be a heroic performance.

I do not wish, though, that any premature attempts are made if that will stop or delay future research of based on greater knowledge. However, we do have to think about starting at some point and at least to make a change in the law of transplantation of organs, so that the law will include fetal tissue.

When a new technique is adopted, problems will always arise no matter how many operations have been performed upon animals. Techniques and knowledge will always improve over the years, but someday we do have to take the responsibility and operate on a patient that we can communicate with and therefore examine properly. Maybe painful dysaesthesias will occure in transplanted patients. We know that from neurotization in peripheral nerves. One will not be able to discover this in animals. If the patients are in great discomfort a spinal cord transection can be performed again, if this is desired.

The complications following kidney transplants and heart transplants were numerous at the beginning, and this will also be the case with fetal transplants, but organ transplants are now well established routine operations in human beings and fetal transplants willalso be one day.

If patients are willing to take the risk and benefit to science with their body as previous patients have done, future patients will be grateful for their help.

Considerations should also be taken of the animals who suffer paraplegia and pay with their lives for this kind of research. To diminish their sufferings the animals from my experiments were at the beginning only severed enough to become paraplegic. I soon discovered that it was not necessary to transect the spinal cord completely to gain meaningful results. There are many controversial views of therights of animals in medical research. Medicine would not be nearly as advanced in proper treatment of numerous diseases if it had not been for animal experiments. Today patients receive help because of all those animals who contributed their lives. Had animals not been used, it would be a terrible stroke to reseach and future patients.

Of course this is a matter of personal belief. In January 1987, representative Robert J. Mrazed introduced a new article to the pet protection Act of 1987. It contains the following restrictions:

> *"In general any person obtaining or using for any research purpose any animal acquired directly or indirectly from any animal shelter shall not be eligible to receive federal furds under any grant or research protocol of the National Institute of Health". (New York, USA).*

In my research I have been using rats. I find it necessary, but I certainly do not like it.

If experiments should be continued to the benefit of man it will be absolutely necessary to use mammals. The best animal would be a monkey, but cats, dogs and pigs could also be used. To use mammalians will not only be extremely expensive, but in my opinion also unethical.

Do we, human beings, have the right to put a monkey into a cage and make it paraplegic in order to benefit ourselves? I personally do not think so.

As long as we can use our own bodies, and have our free will to decide whether we want to participate in experiments to our own benefit I would prefer this.

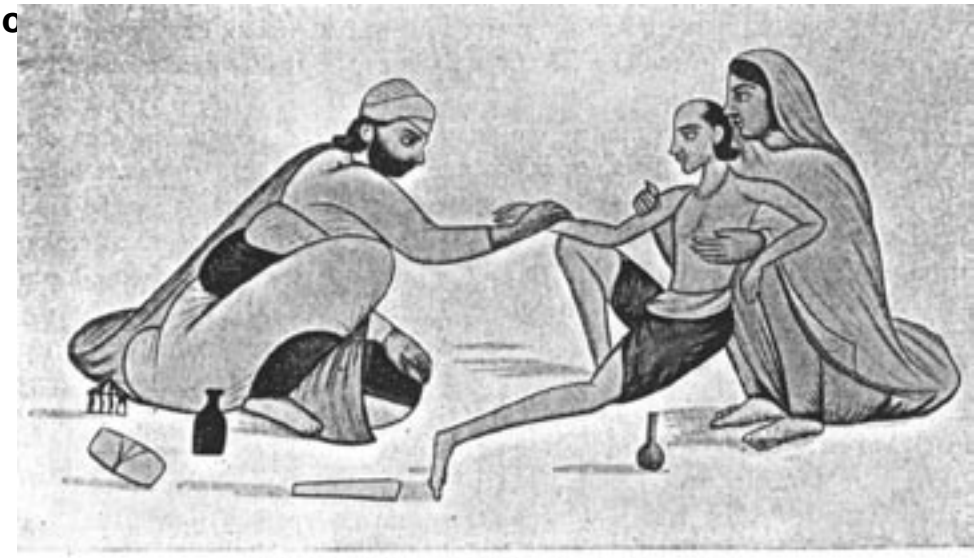
We are the sick beings, **we** are the ones who want the treatment.

I can see no harm, in the use of fetuses once aborted only a justification of an act that should not have

been effectuated in the first place. In my belief the body is a shelter for the soul. If the body is removed the soul is free to find another one to live in. In Denmark, 20,000 abortions are performed each year.

12
Conclusion

The do



Lægen føler Pulsen. Indisk Maleri fra ca. 1820.

The goals of CNS transplants have not yet been achieved but since 1890, quite a lot has happened.

As Guth, Brewer, Collins, Goldberger and Perl wrote in 1980 [55] in the Editorial Commentary in Experimental Neurology it is true that it is not necessary to carry out experiments infinitely once the facts are certain and the technique has been learned. The present study summarizes the knowledge until 1990, and at the same time comprises the results and ideas of my own research. Furthermore, some corrections concerning the meninges are made to the anatomy of the rat and the human being.

It is my hope that this work will be continued and that still more will be learned to facilitate the life of para- and tetraplegics.

Future prospects will be to continue the grafting of fetal spinal cord in rats and probably also in primates. As I have stressed earlier, it is not necessary to severe the spinal cord of the rats completely. The animals do not need to have neurological deficits, because there is no correlation between the extension of the spinal cord lesion and the neurological rehabilitation. Yet, the rat cannot be compared to man. Once a human being has a complete spinal cord lesion with para- or tetraplegia which persists for more than 24 hours, the condition is often, but not always, perma-

nent. This is not true as regards the rat. To ensure the neurons in the grafted tissue do originate from fetal grafted tissue, the graft should be labelled in the pregnant donor mother prior to the transplantation. The very operation on the host animal should take place with the animal anaesthetized, intubated and curarized, and the lesion should be made with a toxic agent by injections four weeks prior to the grafting. Thus, the complications of edema and the expelling of the transplant are prevented and scar tissue can be removed. If a spinal cord derived neurotrophic factor is found this should be applied to promote axonal outgrowth from the host or to prevent gliosis from the graft. After six weeks the graft has grown to its optimal size and the tracing can thus be made with a lectin or a substance which is known to have transsynaptic transport abilities (like the tetanus toxin). If a tracer is injected into the graft, it should be possible to find the tracer in the sensory cortex of the host animal. Furthermore, the tracer should also be found at the motor endplates if the grafted neuron is a part of the motor unit. So far, however axons from grafted tissue have been seen only to grow a few mm into the host spinal cord.

To apply the present knowledge to a patient with a spinal cord trauma, my suggestion would be to operate on paraplegic patients either within 24 hours after the trauma or after six weeks when there is absolutely no evidence of improvement and the lesion has proven to be complete with a total loss of all motor and sensory functions distal to the site of the lesion in the spinal cord. The basis of this work leads to the following conclusion in the treatment of spinal cord lesions:

1. On suspicion of a spinal cord lesion high dose glucocorticoid treatment should be administrated as soon as possible after the trauma, preferably within the first hour of the trauma. In due time glutamate receptor antagonists to prevent ischemic neuronal cell death probably also will be availabel.

2. On admission plane X-ray, CT and MR (if possible), should be carried out to clarify theextent of the trauma and to decide and establish the correct immediate treatment.

3. Possibly surgery

A. If the bony lesions require.

B. If there is a suspicion of a severed spinal cord that might need a fetal transplant; treatment with cyclosporin should be started if a fetal transplant is carried out.

4. Physical exercises and mobilisation from day one.

5. All the above should take place in a spinal cord center where there is immediate cooperation between the patient, the surgeon, the urologist, the physiotherapist, the psychologists and the social workers, so that nothing is delayed and lost unnecessarily, andthe utmost expertise is offered from day one by a unanimous team throughout the country.

If surgery is needed for stabilization of the spine it should take place within the first 24 hours after the accident. The vasogenic edema and the auto-destruction of the neuronal tissue by lipidperoxidation processes should be prevented by the steroids. To prevent formation scar and the glial processes from sealing off the synaptic sites on the axons and dendrites a fetal graft should be applied. No grafting is needed when the cord is not severed, but has only suffered from contusion. The

graft should be prepared in the operating theatre by neurobiologists, either as blocks or as a suspension which can easily be applied with a syringe. Cultures may also be used. The problem is to get enough fetal material and which gestational age should be used.
When the graft has been applied and the dura is closed, orthopedic procedures should be carried out to stabilize the spine.

A fetal grafting operation will not be possible after 6 weeks if osteosynthesis has been performed by a posterior procedure. Furthermore all scar tissue then has to be removed to get clean synaptical sites.

As long as man has been walking on earth a paraplegic has been paraplegic and a tetraplegic has been tetraplegic. Despite much research and a lot of trials nothing has changed these facts. In doing fetal grafting we have nothing to lose and everything to gain.

I am convinced that we will succeed in treating severe spinal cord lesions - someday!

13
Summary in Danish

Denne afhandling er ment som et oplæg til at foretage føtale, neurale transplantationer fra aborteret fostervæv til patienter med totale, spinale, medullære tværsnitslæsioner og deraf følgende para- eller tetraplegi.

Der er, såvidt muligt, gjort rede for behandlingen af spinale læsioner fra lidelsen første gang er beskrevet ca. 3000 år BC til nu. Udviklingen i behandlingen omfatter både dyreforsøg og behandling af patienter. Blandt de ældste værker er Edwin Smith's Papyrus, som blev købt i Luxor i Ægypten i 1862 og senere trykt i Chicago i 1930. Oversigten omfatter de gamle ægyptere, grækerne, med Hipokrates i spidsen, byzantinernes, tyrkernes og arabernes fremgangsmåder, overvejende med traktion og manipulation af columnafrakturer. I Europa begyndte man i 1500-tallet at foretage laminektomier inspireret af byzantinerne. Immobilisering af patienter med columnafrakturer er også kendt gennem tiderne lige fra Hieronymus' top-til-tå-skjold fra 1500-tallet til Perry og Nickels Halo-vest, som første gang blev præsenteret i 1959.

Regeneration af spinalt medullært væv blev første gang beskrevet af Charles Edouard Brown-Séquard (1817 - 1897), som overskar medulla spinalis på duer, marsvin og kaniner. Han fandt, at axonerne til en vis grad regenerede efter overskæring, og at der dannedes arvæv efter medullære læsioner. Efter denne opdagelse begyndte lægerne i USA at foretage myelorrhaphier på mennesker med spinale læsioner, første gang i 1902. Herefter forsøgte en række læger sig med myelorrhaphier med mere eller mindre gode resultater. Man forsøgte allerede i 1902 at transplantatere medullært væv, hvilke første gang er beskrevet af dr. Shirres i 1902, hertil brugte man medulla spinalis fra en hund. Vævet blev indsat langs en medullær tværsnitslæsion i det håb, at det medullære væv kunne fungere enten som bro eller relæ for de ødelagte menneskelige axoner. Den første transplantation af medulla spinalis fra et menneske blev foretaget i 1944 i St. Louis i USA og beskrevet al Woolsey, Mincklar, Rezende og Klemme. Det medullære væv blev udtaget fra en afdød og lagt i 10% formalin i 12 dage, herefter renset i 24 timer med sterilt vand, og derefter anbragt i 70% alkohol for at transplantatet skulle være sterilt. Det blev indsat i en 16-årig dreng med en total tværsnitslæsion efter en skudlæsion. Drengen døde 5 måneder senere uden, at der var opnået noget funktionelt resultat af transplantationen. Ved sektionen opdagede man, at neuroner i centralnervesystemet kan regenerere, da man fandt såvel ascenderende som descenderende fibre groende fra patientens hvide substans ind i transplantatet. Man fandt også, at udvæksten af fibre fulgte vaskulariseret bindevæv i tragter, men at udvæksten stoppede, når fibrene skulle igennem overgangen fra transplantat til vært. Selve transplantatet var veipræserveret. Efter dette ene forsøg er der ikke beskrevet flere i litteraturen. Herefter gik man over til at foretage operationer med andet materiale, interkostalnerver, muskler og plastikfibre.

Enkelte ortopædkirurgiske procedurer er gennemgået, specielt som det gøres i dag efter de nyeste principper under hensyntagen til Alf Briegs forsøg, hvor man skal undgå traktion al medullært væv for at hindre, at der kommer cystiske kaviteter indeholdende lysosomale enzymer.

Anatomien i medulla spinalis er herefter gennemgået, dels makroskopisk, dels mikroskopisk, og der er foretaget sammenlignende undersøgelser mellem rottepræparater og føtale, humane spinale præparater. Det påvises tillige, at foretages der laminektomi på en rotte fjernes dura, og man kommer direkte ind på arachnoidea, som i dag normalt blandt forskere kaldes dura. Det er ikke tilfældet. Arachnoidea er en avaskulær gennemsigtig hinde hos mennesker såvel som hos rotter. Når man derfor taler om duras helende virkning på spinale læsioner hos rotter, taler man i virkeligheden om arachnoideas helende virkning. Disse undersøgelser er dokumenteret ved egne forsøg.

Herefter gennemgås de dyreeksperimenter, som skal ligge til grund for vor videre behandling af patienter med spinale læsioner. Den første, som foretog en transplantation af væv fra centralnervesystemet, var W. Gilmann Thompson, som i 1890 foretog kortikale transplantationer på hunde. Hans beretning er gengivet i sin helhed, da den er historisk interessant. Denne artikel ligger til grund for de følgende mange arbejder, hvor der er foretaget føtale transplantationer af væv fra centralnervesystemet til voksne dyr, på hvilke der er lavet læsioner, såvel cerebralt som spinalt. Der er desuden foretaget uendeligt mange studier af centralnervesystemets regeneration, hvor specielt Ramon Y Cajal har beskrevet degeneration og regeneration i centralnervesystemet i sin store bog, der udkom i 1928. Ikke alle forskere kunne dog påvise, at der var regeneration ved spinale læsioner hos rotter, og specielt i 1940'erne var det en fremherskende teori, at regeneration ikke fandt sted. Thulin og Bunge var i 1972 de første som foretog transplantationer af føtale neuroner fra medulla spinalis til en voksen medulla med godt resultat. Herefter er det gået slag i slag, og specielt svenskerne Nygren, Olson og Seiger, som 11977 foretog føtale transplantationer fra hjernestammen til medulla, og inderen Das, boende i USA, foretog transplantationer af neocortex til medulla spinalis. Bernstein fra USA foretog ligeledes spinale fØtale transplantationer. Disse forskere viste alle, at der var gode resultater med overlevende transplantater og udvækst af axoner og dendritter. Hvad man derimod endnu ikke har vist med fuldstændig sikkerhed i medulla spinalis er, om der er funktionelle synapser mellem transplantat og vært og omvendt. Det er påvist i cerebrum, og efter min opfattelse skulle det være utænkeligt, at neuroner, axoner og dendritter ikke skulle udvise samme egenskaber spinalt som cerebralt. Det er dog ikke lykkedes helt at vise dette endnu, da det er ualmindeligt svært at foretage sådanne transplantationer, bl.a. fordi man pga. størrelsesforholdene ikke kan foretage sikre fluorescensundersøgelser med anterograd og retrograd transport, ej heller elektrofysiologiske undersøgelser. De forskere, som i dag forsøger at undersøge forholdene i medulla, specielt med henblik på at få motorisk funktion efter føtal transplantation, er bl.a. Barbara Bregman og Poul Reier i USA samt gruppen i Lund bestående af Anders Björklund, Nornes og Pontén. Franskmanden Privat mener at have påvist, at der er en mindre fremgang i motorisk aktivitet efter føtale transplantationer til rottens medulla, påvist ved både evoked potentials aktivitet og ved lokomotoriske undersøgelser.

Dette arbejde blev påbegyndt i håb om at kunne bidrage til undersøgelserne omkring væksten af føtale transplantater på medulla spinalis på rotter, specielt med undersøgelser af udvækst af axoner og dendritter samt synaptisk aktivitet. Det var kun muligt at vise, at transplantaterne blev uddifferentieret, voksede og udsendte axoner og dendritter ind i værtsvævet. Forsøget blev foretaget med føtalt væv udtaget fra forskellige dele af centralnervesystemet, hvorefter det blev flyttet hen til andre dele af centralnervesystemet for at undersøge om det føtale væv var cytospecifikt.

Forsøgene blev foretaget med såvel vævsblokke som kulturer og suspensioner. Det viste sig, at det neuronale væv var cytospecifikt, og at det godt kunne gro i andre dele af centralnervesystemet, samt at det udviklede sine oprindelige karakteristika. Axoner og dendritter voksede ligeledes pænt ud i værten. Det var ikke muligt at påvise funktionelle synapser.

Et af de store problemer ved spinale læsioner er dannelsen af arvæv. Ved overskæring af medulla spinalis kommer der dels "sprouting", og herefter nekrose samt hvad Cajal kaldte medulla spinalis autotomi. Dette hænger sammen med den lysosomale, cellulære lytiske aktivitet, som resulterer i kavitetsdannelser ved læsionen. Desuden dannes der sekundært gliose, hvorved problemet er, at gliacellerne, indenfor 24 timer efter traumet, dækker de læderede axoner og neuritter, specielt udsendes der pseudopodier, som dækker de synaptiske områder. Det er af denne grund, at en føtal transplantation skal foretages indenfor 24 timer efter traumet, såfremt det drejer sig om en overskæring, ellers hvis man foretrækker at transplantere sekundært, da skal man fjerne alt arvæv, således at der kommer friske læsionsflader uden glia dække, på grund af gliaproliferation. Det kan gennemføres, såfremt det drejer sig om en thoracl medullær læsion. Kesslak, Nieto-Sampedro, Globus og Cotman har påpeget at astrocytter producerer neurotrofiske og neuritdannende faktorer, foruden at de er med til at restaurere ion-balancen i de ødelagte områder. Forfatterene konkluderer, at unge astrocytter således hjælper til ved axonregenerationen, hvorimod de ældre astrocytter blokerer den axonale regeneration. Det er vist, at føtale transplantater er med til at hindre dannelsen af arvæv.

Et utal af forskellige behandlinger og stoffer er blevet brugt i forsøg på at hindre arvævsdannelsen ved medullære læsioner samt til at fremme axonal regeneration. Behandlingen skal i første omgang hindre dannelsen af det vasogene Ødem, og herefter hindre udslip af toksiske stoffer fra cellerne, som udløser en kaskade af degenerative processer, som fører til autodestruktion af centralnervesystemet i de læderede områder. Af hensyn til behandlingen er det nødvendigt at skelne mellem en overrivning af axoner og ødelæggelse af neuroner, der kræver føtalt transplantat, og en kontusion, der ligesom overrivningen provokerer et vasogent Ødem, og destruktion af cellemembranen pga. udslip af frie oxygen radikaler, som initierer lipid peroxidase-processen. En kontusion kræver ikke et føtalt transplantat, men produkter som kan modvirke enten de toksiske stoffer, der slipper ud ved cellemembranødelæggelsen eller som kan modvirke de destruktive processer, som initieres herved. Blandt de stoffer man i tidens løb har foresøgt at behandle med kan nævnes hyperosmolære agens, anti-adrenerge stoffer, antifibrinolytiske stoffer, morfin- antagonister, hypertermi, hyperbar oxygen, pyrogener, hormoner, endogene opiater, immunosuppressiv terapi og enzymer. Desuden har man forsøgt sig med røntgenbehandling for at mindske arvævsdannelsen. Efter at man fandt vækstfaktorerne og de neurotrofiske faktorer (NGF og BDGF), har man også forsøgt at behandle med disse for at fremme den neuronale regeneration.

Steroider har være brugt gennem mange år, uden at man har kunnet påvise nogen effekt at disse, før man begyndte at behandle med højdosis glukokortikoid-injektioner intravenøst på katte, som man udsatte for et spinalt traume. Man har fundet, at glukokortikoideme og 21 -non-aminosteroiderne (lazaroiderne) modvirker effekten af de frie oxygen radikaler, således at lipidperoxidaseprocessen standses, idet glukokortikoidet indkoopereres i dannelsen af de umættede fedtsyrer. Effekten af behandlingen er vist på dyr, og i USA har man også på mennesker med spinale læsioner påbegyndt behandlingen med methyiprednisolon 30 mg pr kg den første time, dette fortsættes i 23 timer, således så der gives i alt 11 g i løbet af i døgn. Den funktionelle effekt af dette er endnu ikke med sikkerhed påvist.

Yderligere arbejder går ud på at finde et neuroprotektivt stof, og man arbejder her med AMPA og receptorantagonisten MBQX, hvilket i dyreforsøg er vist at virke neurobeskyttende, også selv om det gives flere timer efter traumet. Glukokortikoiderne skal gives så hurtigt som muligt, helst indenfor i time efter traumet.

Konklusionen af dette arbejde er, at det er vist, at føtale transplantater vokser, uddifferentieres og højst sandsynligt også danner synapser i medulla spinalis. I cerebrum er disse synapser funktionelle, og det skulle være underligt om ikke det føtale neuronale væv skulle have samme kvalifikationer i medulla spinalis. Jeg mener ikke man kan komme videre med undersøgelser på rotter, men er nødt til at gå over til aber, evt. hunde eller grise, men dette mener jeg er uetisk.

Jeg mener derfor, at man med den nuværende viden må begynde at foretage neuronale transplantationer på mennesker, som har været udsat for spinale traumer, og som selv kan tage stilling til, om de vil modtage disse transplantater på forsøgsbasis. Jeg mener ikke, at det er nødvendigt at udsætte højerestående dyr for denne voldsomme læsion, for at vi mennesker kan få glæde af en behandling man direkte og uden at skade patienten kan udføre på mennesker, som i forvejen har pådraget sig lidelsen, og som man i øvrigt i udlandet foretager på patienter med Parkinsons syge i basalganglinierne.

Man kan vælge enten at transplantere indenfor de første 24 timer efter traumet, hvilket specielt vil være nødvendigt i de tilfælde, hvor en osteosyntese også vil blive udfort eller man kan vente 6 uger. Er en osteosyntese udført, vil det være yderst vanskeligt senere at foretage en føtal transplantation. Ønsker man at vente med en føtal transplantation til man er overbevist om, at patienten er paraplegisk, skal alt arvæv fjernes, hvor-efter det føtale transplantat indføres i det læderede område i medulla. Problemet med dette er at få en tilstrækkelig mængde føtalt væv.

For at behandle disse patienter optimalt, og med de behandlingstilbud vi har i dag, mener jeg, patienterne tidligst muligt skal have højdosis methylprednisolon, hvilket vil sige helst indenfor i time efter traumet. Der skal foretages konventionelt røntgen, CT-skanning og MR-skanning. På mistanke om overrivning af medulla, skal der indenfor 24 timer foretages føtal transplantation samt stabiliserende osteosyntese. Man opnår således at hindre autodestruktion, fremme axonal regeneration, hindre arvævsdannelse, og fysioterapi med mobilisering kan startes praktisk taget dagen efter operationen.

Dette mener jeg, at den fremtidige behandling må være, indtil vi bliver klogere, da vi ikke har andet at tilbyde disse patienter. Siden Imhoteb har vi ikke været i stand til at tilbyde nogen form for behandling, som har kunnet bedre tilstanden. Vi må give disse patienter den optimale behandling, som vi kender den i dag, selvom forskerne måske ryster på hovedet af os om 20 år. Patienterne kan af egen fri vilje beslutte, om de vil være med til en forsøgsbehandling, og de kan ikke blive skadet mere end de er i forvejen.

Jeg mener, at man skal starte med at operere 25 patienter indenfor 24 timer efter traumet med føtale transplantationer, og ligeledes tilbyde 25 totalt paraplegiske og anæstetiske patienter operation med fjernelse af arvæv og føtal transplantation, for at se om der kommer nogen bedring i deres tilstand.

De etiske aspekter omkring abortvæv og recipient er diskuteret i det sidste kapitel. Det må altid være helt klart, at der ikke må være nogen som helst forbindelse mellem donor og recipient, og der må naturligvis aldrig nogensinde være penge mellem giver og modtager.

Hermed overgives afhandlingen til Københavns Universitet og til den centrale videnskabsetiske komité, som dokumentation for den viden vi i dag har, og som basis for det grundlag man i dag kan starte en ny behandling af spinale medullære læsioner på.

14
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