

# Demyelination and remyelination in the dorsal funiculus of the rat spinal cord after heat injury

MAKOTO SASAKI\* and CHIZUKA IDE

Department of Anatomy, Iwate Medical University School of Medicine, Uchimaru 19-1, Morioka, 020, Japan

Received 16 May 1988; revised 22 September and 14 November 1988; accepted 22 November 1988

## Summary

Part of the dorsal funiculus of the adult male rat (Wistar) spinal cord was treated for 1 h at the thoracolumbar level by running hot water, at approximately 48–50°C, through a polyethylene tube 2 mm in diameter in contact with the dura. Animals were fixed 1 day to 4 weeks later and the spinal cords were examined by light and electron microscopy. The affected area in the dorsal funiculus was approximately 1 mm long and less than 1 mm wide at the dorsal surface, and varied from 0.4 to 0.7 mm in depth.

Within 3 days after treatment, almost all the myelin sheaths in the affected area were degraded, leaving the axons denuded, and at the same time astrocyte endfeet at the glial limiting membrane were swollen and partly destroyed. Almost all the denuded axons remained intact, exhibiting no noticeable morphological changes. There was evidence of a moderate vasogenic oedema, but minimal signs of haemorrhage in the lesion. Seven days after treatment, many immature Schwann cells but no oligodendrocytes were found between the denuded axons. By 2 weeks many of the denuded axons were remyelinated, and by 4 weeks almost all of those axons located near the pial and perivascular surfaces had been remyelinated by Schwann cells, while most of those located in the deep and marginal zones bordering the adjoining intact areas were remyelinated by oligodendrocytes. Longitudinal sections revealed that at nodes of Ranvier PNS-type myelin sheaths were apposed by either intact or newly formed CNS-type myelin sheaths. A typical glial limiting membrane was not reformed beneath the pial surface, but an inconspicuous one was found between the PNS- and CNS-type fibre areas.

## Introduction

Recently, therapeutic hyperthermia in localized or whole-body applications has been used as a new method of cancer therapy. In focal hyperthermia, the tissue is heated with microwave or ultrasound transducers to about 40°C, a temperature which damages tumour cells but does not affect the surrounding normal tissue (Hahn, 1982). Changes in the normal brain and spinal cord tissues resulting as an adverse effect of clinical or experimental hyperthermia have been studied only sporadically in the past three decades (Barnard *et al.*, 1956; Fajardo, 1984; Lyons *et al.*, 1986). In a clinical study (Douglas *et al.*, 1981) on the myelopathy induced by whole-body hyperthermia at 41.8°C for 6 h, severe demyelination, axonal and glial cell degeneration, and vascular congestion were noted to occur in the white matter, and swelling and chromatolysis of the neurons were seen in the gray matter.

Several experimental studies of hyperthermia (Barnard *et al.*, 1956; Britt *et al.*, 1983; Lyons *et al.*, 1984) have also demonstrated that acute changes, including myelin sheath breakdown, vasogenic oedema and

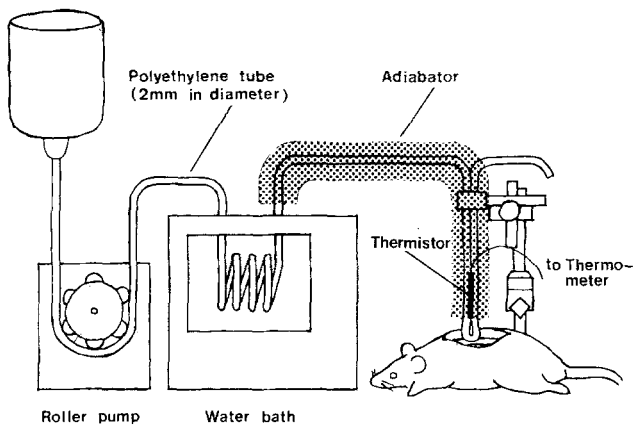
haemorrhage, were found mainly in the white matter after heat treatment at a temperature of over 42°C for about 60 min. These studies were done by light microscopy only, and no ultrastructural study has so far been reported with regard to the effects of hyperthermia on normal CNS tissues.

To investigate more precisely the histological changes induced in CNS tissues after high temperature treatment, heat injury was induced in the rat spinal cord by transdural heat application, and early degradation and subsequent repair of the affected tissues were examined by light and electron microscopy.

## Materials and methods

Thirty male rats (Wistar strain), weighing 200–300 g, were used. The animals were anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital, 100 mg/kg body weight), and the thoracolumbar region of the spinal cord was exposed through a rectangular window made by partial laminectomy. A polyethylene tube, 2 mm in diameter, was bent and the tip of the bent end was placed on the

\* To whom correspondence should be addressed.



**Fig. 1.** Schematic drawing of an experimental design for heat injury. Water was circulated by a flow roller pump through a 2 mm-diameter polyethylene tube. The water was warmed to approximately 60°C in the water bath so that the temperature of the thermocouple at the bent tube could be kept at 48–50°C. The tube was wrapped with adiabatic material, and the tip of the bent tube was in contact with the dural surface of the spinal cord.

dura mater using a stereotaxic device. Hot water, heated in a water bath (Yamato BK33), was circulated through this tube. The temperature was maintained at 48–50°C for 1 h, as monitored with a thermocouple (1.0 mm in diameter) inserted between the proximal and distal limbs of the bent tube, 10 mm away from its tip (Fig. 1). Room temperature was kept at 28–30°C, and the tube, except the bent tip, was shielded by adiabatic material to prevent cooling by air. The pressure on the dura exerted by the tube was not so great as to interrupt the blood flow in the dorsal vein of the spinal cord, as checked under a dissecting microscope. Control animals were operated on and treated in the same manner, except that hot water was not circulated through the tube in two animals, and hot water at 40–45°C was circulated in five animals.

The animals were killed 1 (n=4), 3 (n=4), 5 (n=3) and 7 (n=4) days, and 2 (n=4) and 4 weeks (n=4) after the operation by perfusion through the heart with a fixative

containing 2.0% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer. The treated parts of the cord were excised, together with the adjacent cranial and caudal segments and stored overnight in the same fixative. Specimens were cut into small blocks, and were postfixed in 1.0% osmium tetroxide solution in 0.1 M cacodylate buffer for 2 h, dehydrated through a graded series of ethanol, and embedded in Epon 812.

Ultrathin sections were cut on a LKB Ultratome and observed in a JEOL-100B electron microscope after double staining with uranyl acetate and lead citrate. Semithin sections 1 µm thick were stained with 1% toluidine blue and observed by light microscopy.

## Results

The rats did not show any gait or other behaviour disturbance after the operation. The lesion resulting from the heat treatment was confined within the dorsal funiculus of the spinal cord. The affected area was approximately 1 mm long, and less than 1 mm wide at the dorsal surface of the spinal cord. The depth of the lesion varied from 0.4 to 0.7 mm.

At every post-treatment stage only a few degenerated axons were observed in the dorsal funiculus at levels cranial to the lesion, indicating that the axons themselves were not damaged by the heat treatment.

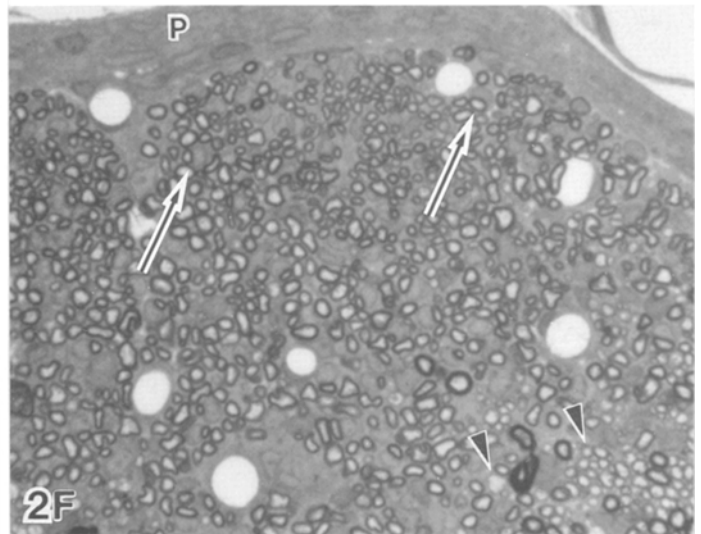
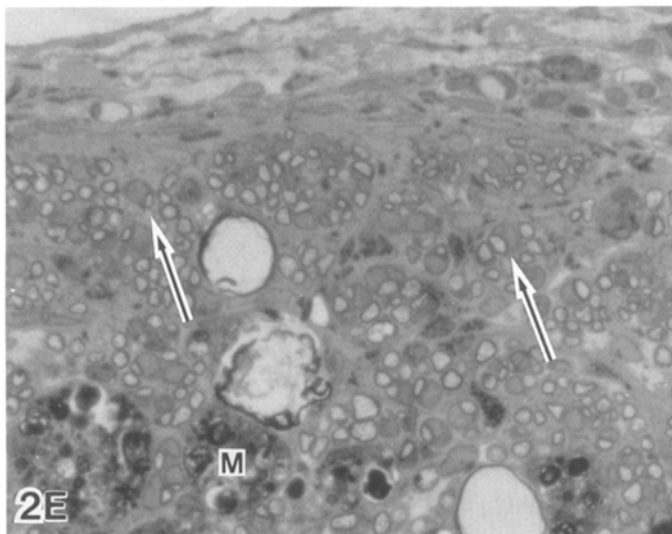
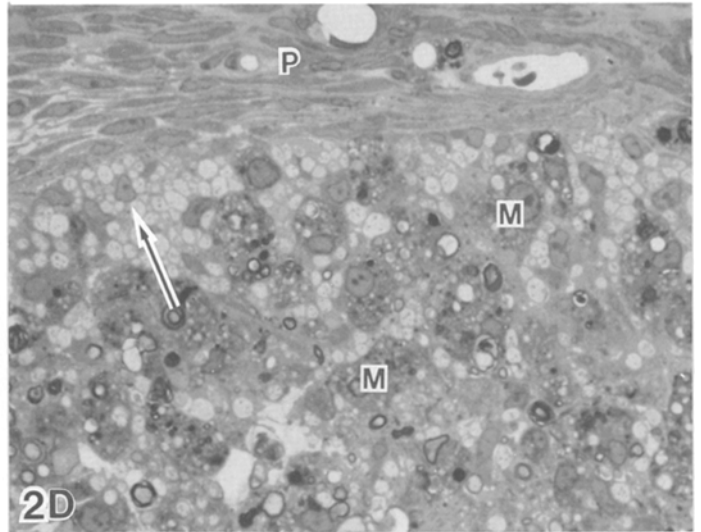
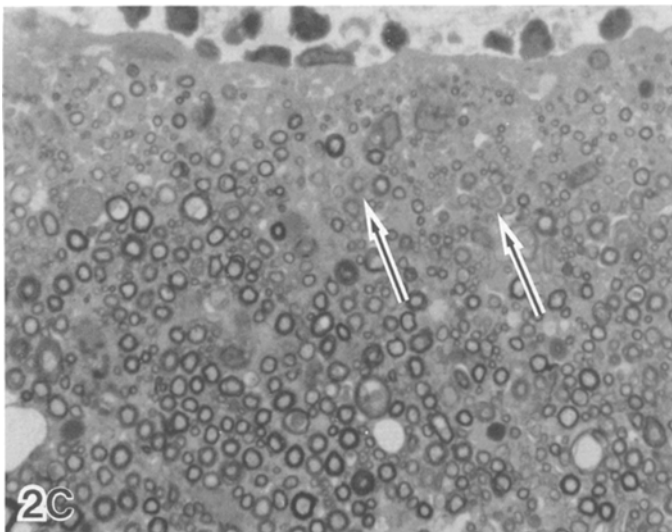
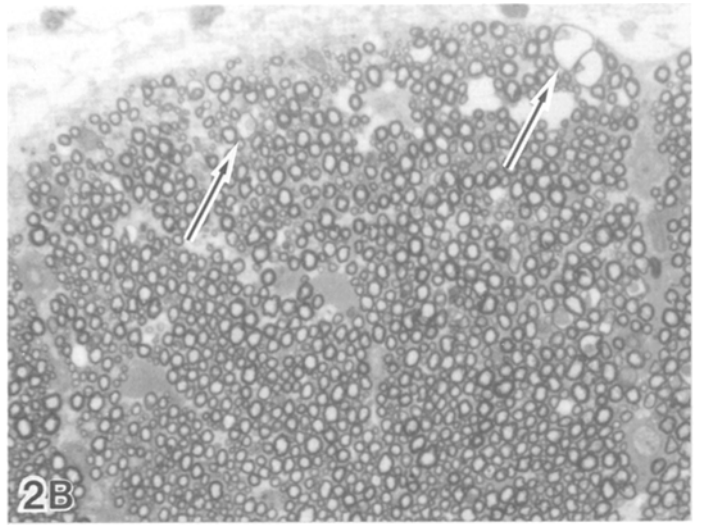
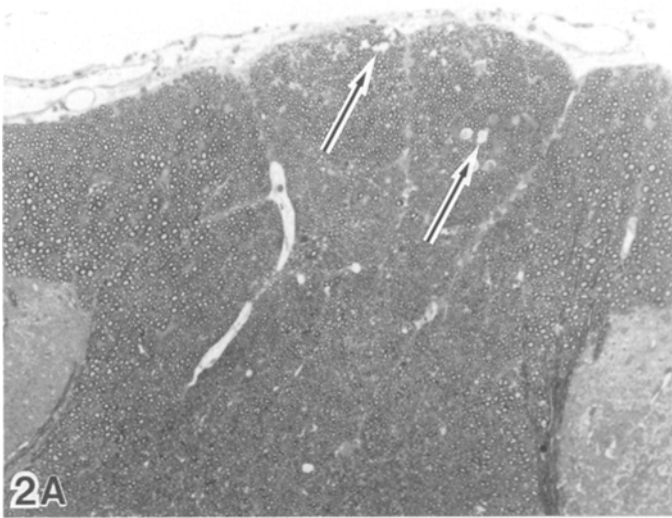
In the control cases the dorsal surface of the spinal cord was deformed to some extent, presumably due to compression by the tube. However, no focus of demyelination was found in the dorsal funiculus.

### 1 day after treatment

A few fibres whose myelin sheaths were degraded in the treated part of the dorsal funiculus were noted by light microscopy. Myelinated fibres located near the pial surface appeared loosely packed owing to the increase of extracellular spaces (Fig. 2A, B).

Electron microscopy showed that myelin sheath lamellae of some myelinated fibres were loosened or even broken up into irregular structures (Fig. 3). In some fibres the internal loops of myelin sheaths were

**Fig. 2.** Light micrographs showing the thoracolumbar dorsal funiculus after heat injury. (A) One day after treatment. No significant change can be seen in the dorsal funiculus except for some damaged foci (arrows) which are devoid of myelinated fibres. × 140. (B) Higher magnification of part of Fig. 2(A) showing focal breakdowns of the myelin sheaths (arrows) near the pial surface of the dorsal funiculus. In addition, myelinated fibres in this area seem to be loosened due to an increase of extracellular spaces. × 560. (C) Three days after treatment. The disintegration of the myelin sheaths has advanced: foci of myelin sheath breakdown have increased in number and, in addition, some myelin sheaths have lost their opacity (arrows). The area of myelin sheath degradation has become larger. × 560. (D) Seven days after treatment. Almost all the axons near the pial surface are completely demyelinated. Many macrophages (M) are scattered between them. A few dark round cells (arrow) other than macrophages are found near the surface. Proliferation of the pial cells has occurred, making a thick pial layer (P) on the affected spinal cord. × 560. (E) Two weeks after treatment. Almost all the fibres have been thinly myelinated by Schwann cells (arrows). Although not shown in this micrograph, axons are myelinated by oligodendrocytes in the deep and border zones of the intact areas. Some macrophages (M) are present in the affected area. × 560. (F) One month after treatment. Axons near the pial or perivascular surface have become thickly myelinated by Schwann cells (arrows), whereas axons in the deep zones bordering on the intact areas are thinly myelinated by oligodendrocytes (arrowheads). A thick pial cell layer (P) is found on the surface of the affected area. × 560.





**Fig. 3.** One day after treatment. Electron micrograph showing the initial stages of myelin sheath degradation. Several myelinated fibres in this micrograph are in the process of demyelination while most axons are intact. The myelin sheath degradation at the upper left corresponds to the focal myelin sheath breakdown as seen in Fig. 2(A, B), while the myelin sheath decomposition at the lower right corresponds to the low opacity myelin sheath seen in Fig. 2C. Astrocyte processes (As) at the GLM are disrupted, while the basal laminae (arrowheads) are preserved. The interaxonal spaces are considerably expanded and contain fibrin deposits (arrow). A presumptive macrophage (M) appears in the affected area.  $\times 9400$ .

markedly swollen. These were features of the early stages of myelin sheath degradation which finally led to the complete demyelination of the affected fibres. The extracellular spaces between the axons were enlarged and contained proteinaceous oedema fluid and electron-dense deposits of fibrin. Astrocyte processes, including endfeet of the subpial glial limiting membrane (GLM), were partly swollen and disrupted, but the basal lamina was preserved as a continuous sheet covering the entire surface of the spinal cord. Oligodendrocyte processes forming the myelin sheath outer loop disappeared from the degenerating myelin sheaths. In the affected area astrocytes and oligodendrocytes contained many vacuoles in their cytoplasm suggesting early degenerative changes. The

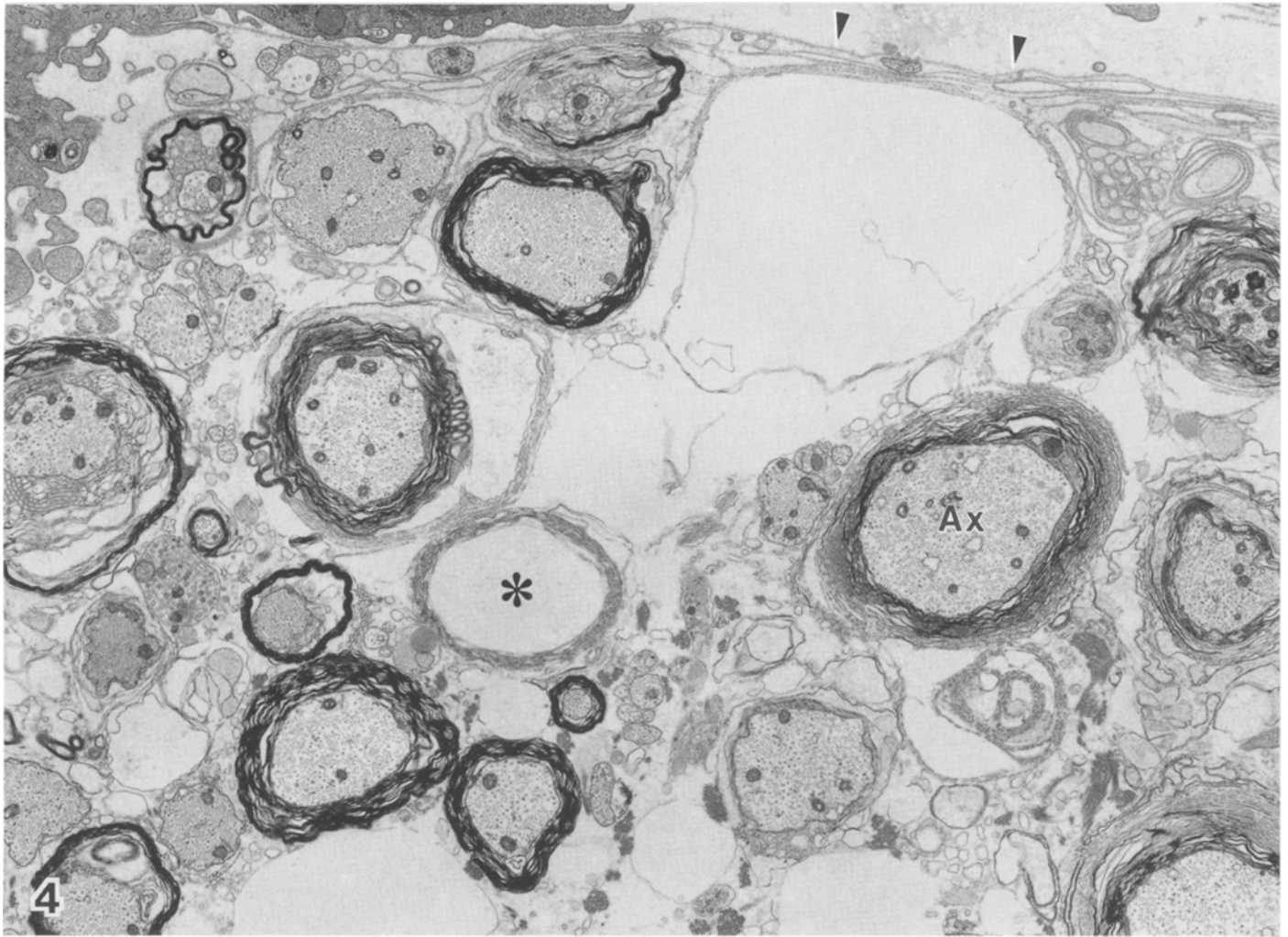
vessel walls appeared undamaged and no haemorrhage occurred in the lesion.

#### *3 days after treatment*

Light microscopy showed that most fibres located near to the pial surface had myelin sheaths with much less opacity, reflecting advanced myelin lamella disintegration and breakdown. Extracellular spaces between the axons were further enlarged, and some macrophages were found in such interaxonal spaces. No haemorrhage was noted (Fig. 2C).

Electron microscopy showed that many myelin sheath lamellae were split extensively into irregularly arranged sheets and had partially disintegrated into vesicular structures, losing their original electron





**Fig. 4.** Three days after treatment. This electron micrograph shows a later stage in myelin sheath degradation with interlamellar splitting of all the fibres. Some axons (asterisk) appear to have been damaged by heat injury, but most seem to be intact (Ax). Astrocytes at the GLM beneath the pia are totally destroyed, but the basal laminae are largely preserved (arrowheads). Extracellular spaces of the affected area have become much more conspicuous than in Fig. 3.  $\times 8600$ .

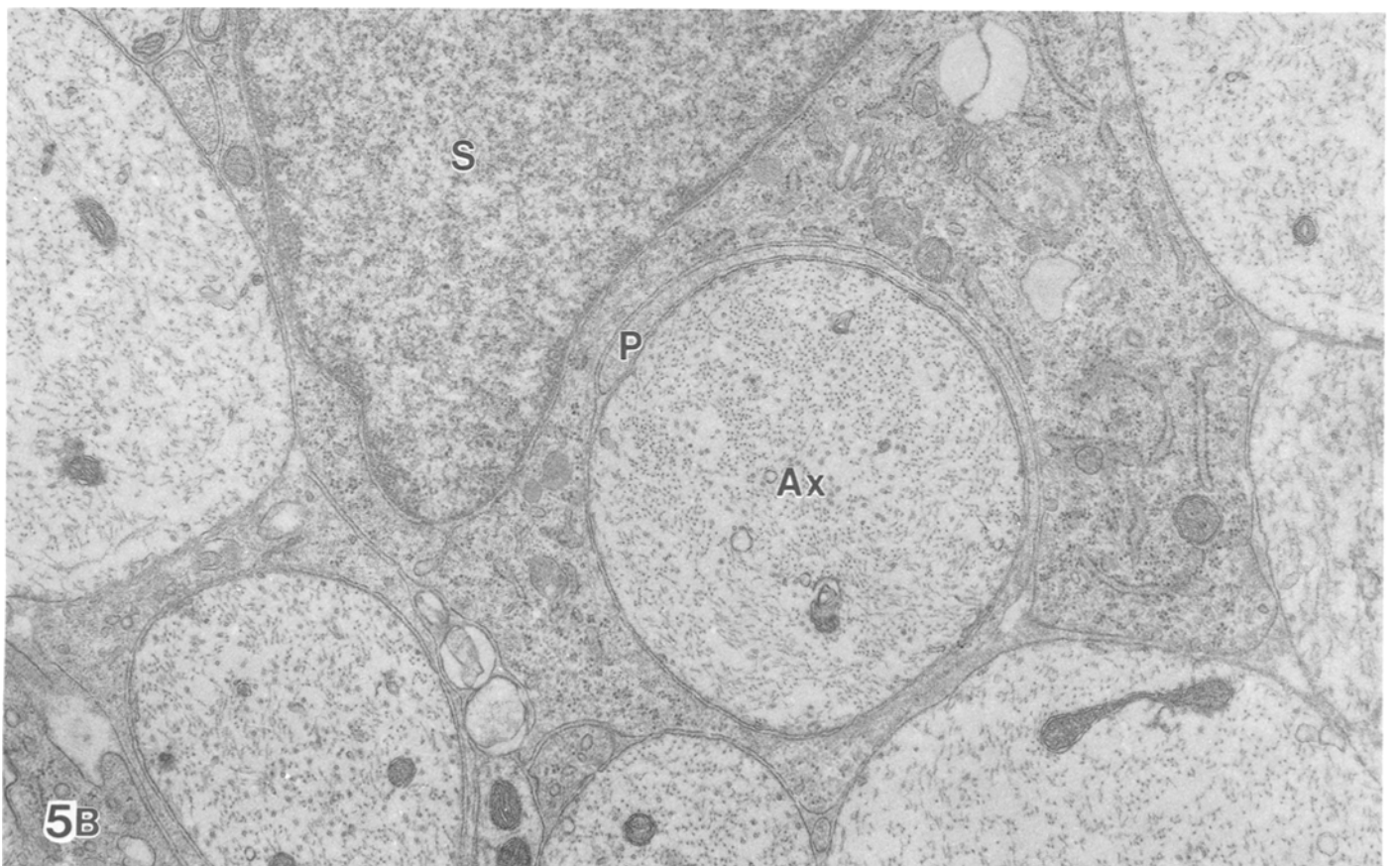
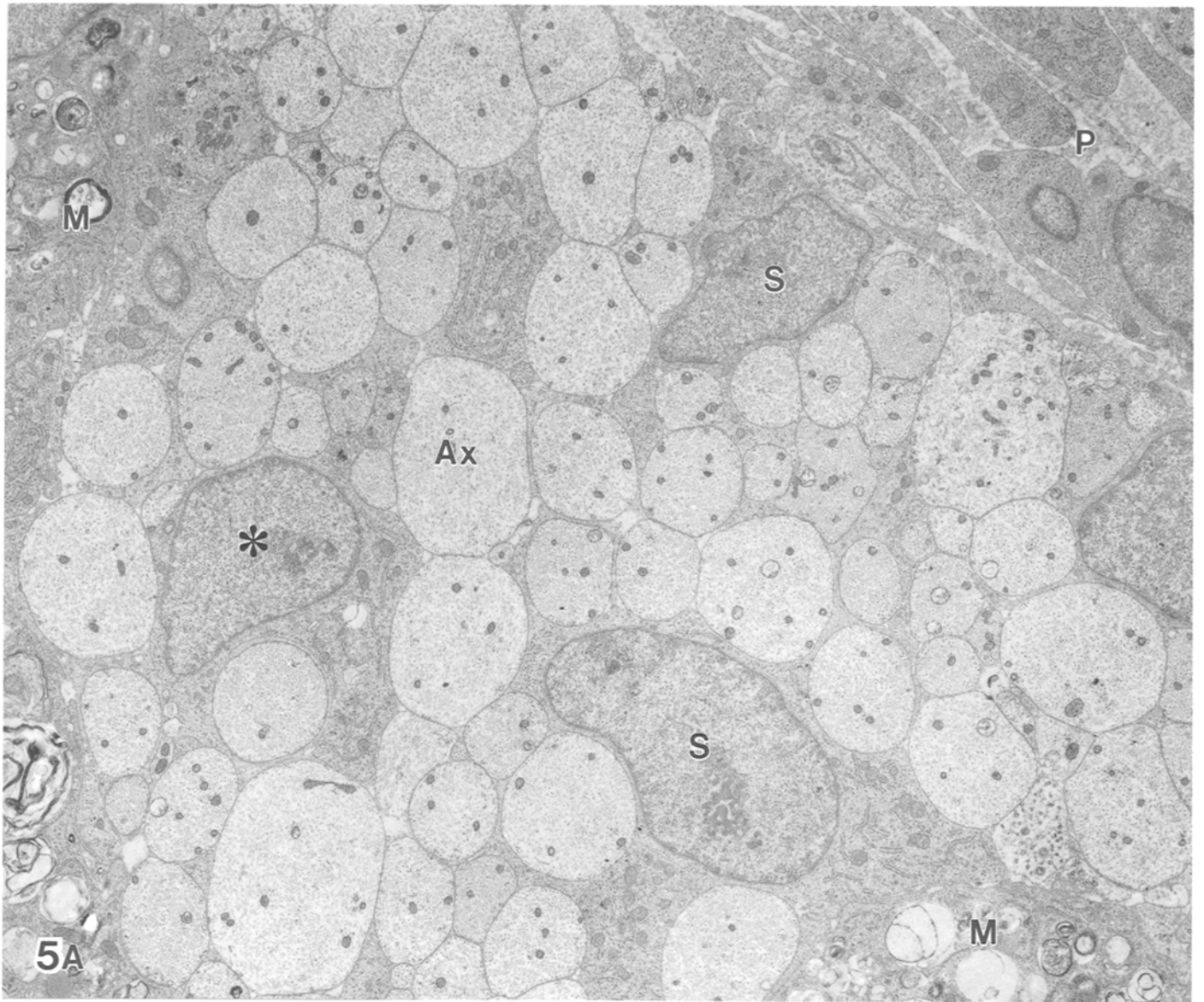
opacity (Fig. 4). Other myelin sheaths, while retaining densely packed lamellae, exhibited a greatly decreased electron opacity. Axons whose myelin sheaths had become degraded appeared normal. Astrocyte processes including endfeet of the GLM were entirely disrupted and were not visible. However, the basal laminae of the GLM remained intact separating the CNS from the surrounding connective tissue. No oligodendrocyte cell bodies or processes were found in the areas near the surface or in the centre of the lesion. Fragments of degraded myelin sheaths, as well as fibrin, were scattered in wide interaxonal spaces, and macrophages were phagocytosing cellular debris.

#### *7 days after treatment*

Numerous denuded axons were identified by light microscopy as various-sized bundles within the heat-

injured areas. Macrophages containing myelin debris were scattered between such denuded axons. Spindle-shaped cells proliferated and made a dense cell layer above the affected area (Fig. 2D).

By electron microscopy almost all axons were completely demyelinated and closely packed (Fig. 5A). A notable finding was the presence, between the demyelinated axons, of cells with round nuclei which contained no myelin debris. These cells often extended cytoplasmic processes around axons in the manner of developing Schwann and glial cells, suggesting that they were immature Schwann or glial cells migrating through the interaxonal spaces (Fig. 5B). Cells identifiable as astrocytes and oligodendrocytes were not observed within the affected area at this time. The GLM basal lamina at the pial surface was largely destroyed. Thus naked axons and associated imma-



ture Schwann cells were in direct contact with the pial connective tissues at the pial surface. Spindle-shaped non-neural cells were numerous throughout the pial layer.

#### *2 weeks after treatment*

Most axons near the pial surface appeared thinly myelinated by light microscopy, while most axons located toward the deep or marginal zones of the lesion remained denuded. Also noted were many round cells with relatively dark cytoplasm, intimately associated with the thinly myelinated axons. Spindle-shaped non-neuronal cells and bundles of collagen fibres formed a thick pial layer on the surface of the injured cord. Macrophages containing myelin debris were still present in the lesion (Fig. 2E).

By electron microscopy, many axons were noted near the pial surface covered mostly by very thin, but occasionally by relatively thick myelin sheaths (Fig. 6). It was evident that the round cells were associated 1:1 with the axons, and that they were covered by basal lamina, features typical of myelinating Schwann cells. In the marginal zone bordering the intact area, some axons were thinly myelinated by oligodendrocyte processes (Fig. 7): the myelin sheath had an interlamellar periodicity of about 130 Å. At this stage oligodendrocytes tended to be in contact with or near these CNS-type fibres. Astrocytes also appeared in the marginal zone bordering the intact area.

#### *1 month after treatment*

By light microscopy it was determined that almost all the axons located near the pial and perivascular surfaces were covered by thick myelin sheaths. Schwann cells were associated with axons in a 1:1 relationship. In the deep or marginal zones bordering the intact tissue, most axons had thin myelin, which, unlike Schwann cell myelin, had no cytoplasmic layer or basal lamina on the myelin sheath surface, indicating that oligodendrocytes had made the myelin sheaths around them. Blood vessels were abundant throughout the lesion but macrophages were no longer found in the injured area (Fig. 2F).

By electron microscopy it was determined that almost all the axons near the pial and perivascular surfaces were myelinated by Schwann cells (Fig. 8). The myelin sheaths were 0.5–1 µm thick and had an interlamellar periodicity of about 150 Å. Unmye-

linated axons were wrapped in a bundle of 2–3 fibres by a common Schwann cell, as in unmyelinated fibres of the PNS. Schwann cells were invested with basal laminae associated with a few collagen fibrils on their interstitial surfaces. On the other hand, in the deep and marginal zones bordering the intact area almost all the axons were myelinated by oligodendrocytes (Fig. 9). Such CNS-type myelin sheaths were extremely thin (about 0.1 µm thick), and had an interlamellar periodicity of about 130 Å.

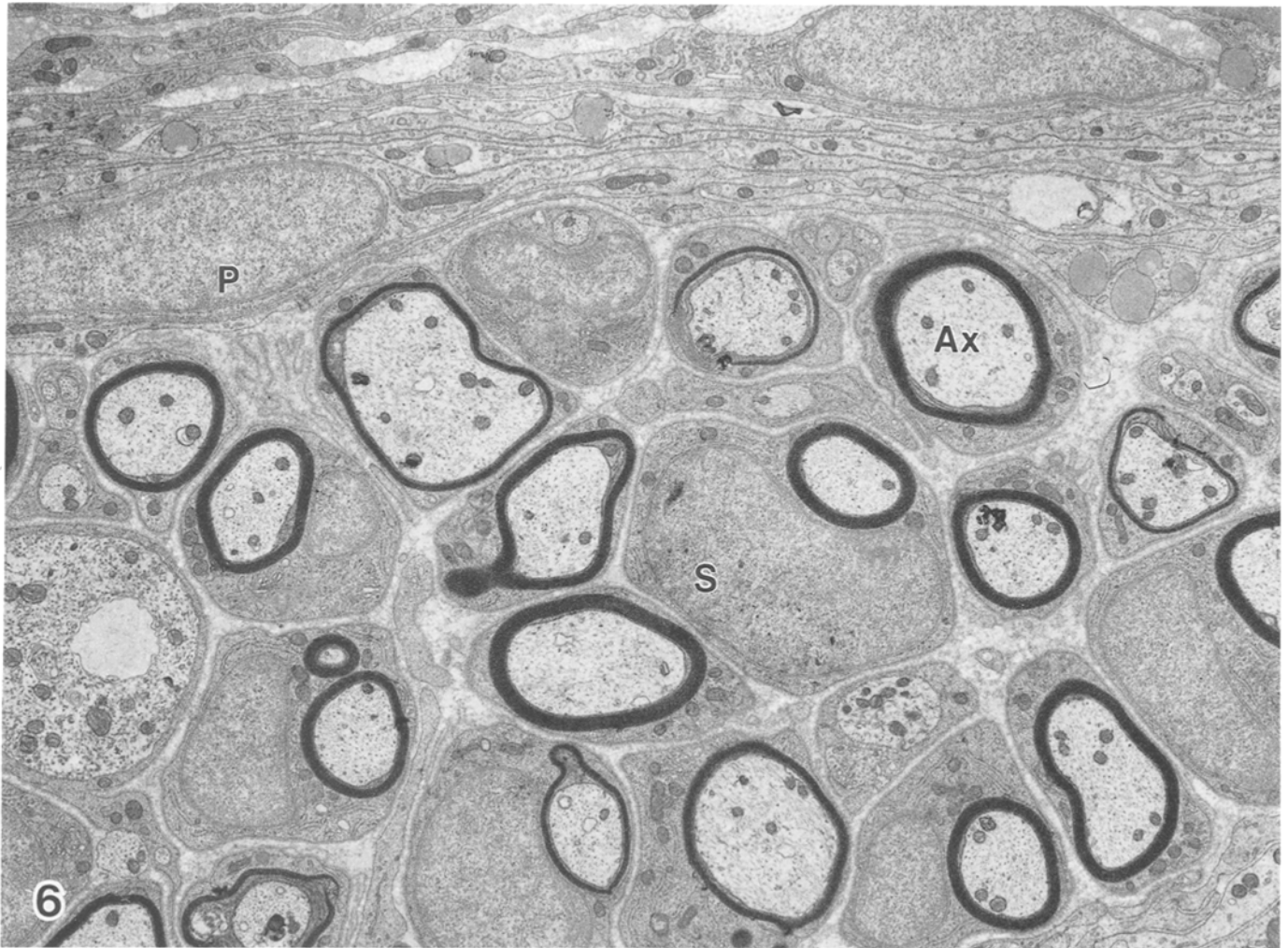
At the oligodendrocyte- and Schwann cell-myelination transition areas, PNS- and CNS-type fibres intermingled with one another with no clear line of demarcation (Fig. 10). In such transitional zones, the oligodendrocyte-myelinated fibres were incompletely covered by the astrocyte processes; therefore CNS-type myelin sheaths and oligodendrocyte processes were directly contiguous to Schwann cell basal laminae or collagen fibrils.

Peculiar dark cells, which had previously appeared as spindle-shaped cells, proliferated to form a thick layer on the pial surface of the lesion. No astrocytic layer had developed between this 'pial' cell layer and the underlying neural tissue. Blood vessels within the Schwann cell myelinated areas lacked an astrocytic covering; these vessels were thus of similar appearance to those found in the PNS. A few macrophages containing myelin debris were still observed in the affected area.

In longitudinal sections, internodes of CNS-type remyelinated axons were so short that the entire internode could be followed in one section, whereas those of PNS-type internodes were longer and only part of an internode was traceable in one section. Nodes of Ranvier of the PNS-type had a normal structure, while those of a CNS-type occasionally exhibited abnormally wide gaps. Oligodendrocytes sometimes formed a thin myelin sheath on their own cell body. Newly formed PNS- or CNS-type myelin sheaths were apposed to the adjacent intact CNS myelin sheaths at nodes of Ranvier. Sometimes a single axon was remyelinated by oligodendrocytes and by Schwann cells forming alternate PNS- and CNS-type myelin sheaths (Fig. 11). Usually only a few astrocyte processes covered these newly formed nodes of Ranvier. Unlike the normal PNS-CNS transition in the nerve root, lateral loops of oligodendrocytes were directly contiguous with Schwann cell processes at the node (Fig. 11, inset.).

**Fig. 5.** One week after treatment. (A) All the axons (Ax) near the pial surface are demyelinated. Immature cells (S) and macrophages (M) are scattered between them. The basal laminae of the GLM have disappeared and spindle-shaped pial cells (P) are present at the surface, with which axons and associated immature cells are in direct contact. × 5600. (B) Enlargement of part of Fig. 5A (asterisk). An immature cell (S) with a narrow process (P) invests a denuded axon (Ax) in the manner of a Schwann cell, but has no basal lamina. × 21 000.





**Fig. 6.** Two weeks after treatment. This electron micrograph shows part of the dorsal funiculus at the pial surface. All the axons (Ax) in this area are myelinated by Schwann cells (S) covered by basal laminae. Proliferation of pial cells has occurred on the surface of the affected area.  $\times 7500$ .

## Discussion

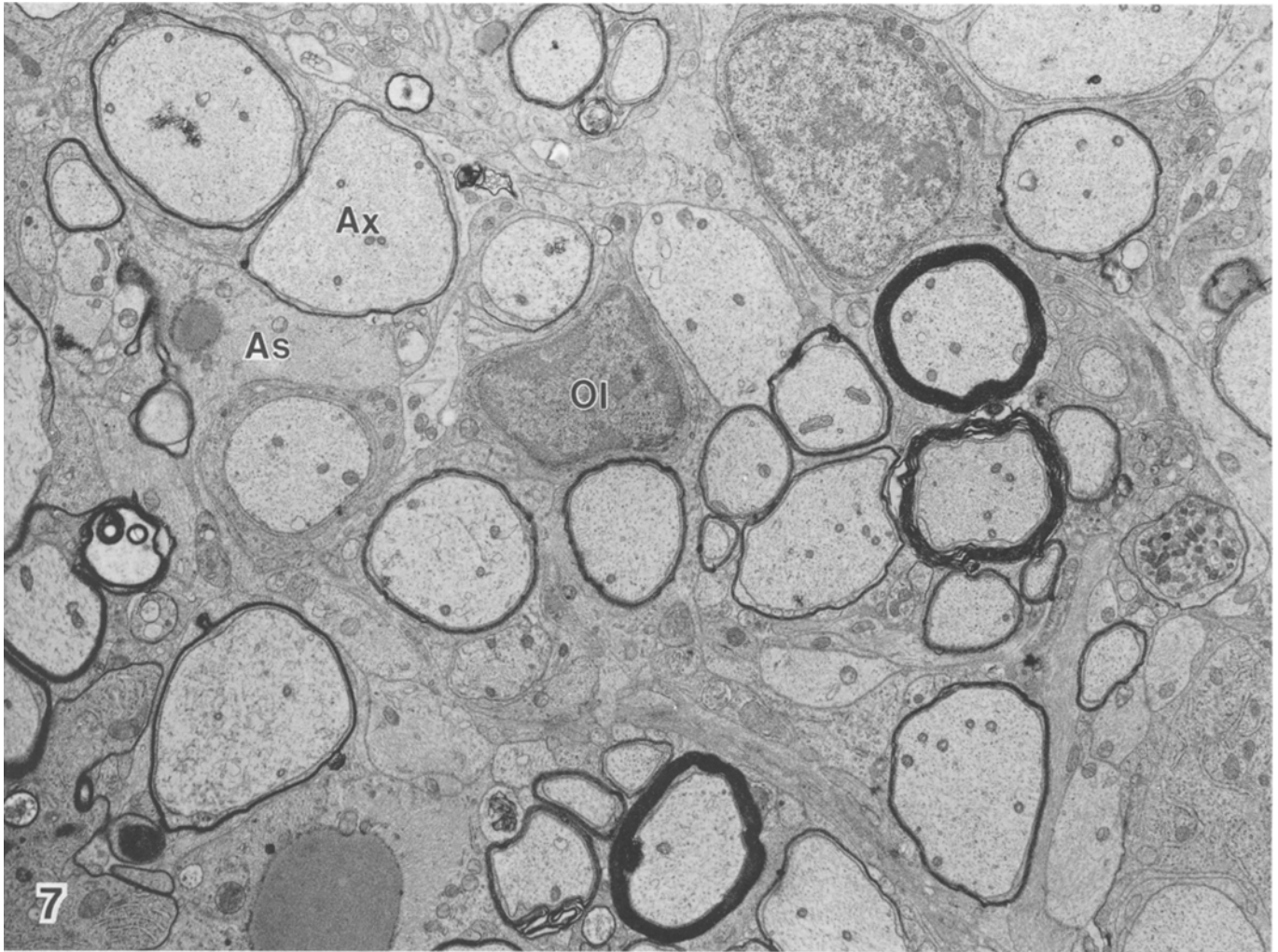
The present study has documented the morphological changes in the spinal cord injured by heat treatment, as observed by light and electron microscopy. Using hot water circulating in a tube, we made a small (less than  $1 \text{ mm}^3$ ), well-confined lesion in the dorsal funiculus of the rat spinal cord. According to previous reports (Britt *et al.*, 1983; Lyons *et al.*, 1984), the temperature threshold for cell damage in the CNS is  $42\text{--}43^\circ \text{C}$  for a duration of 50–60 min. In the present study the tube was kept at  $48\text{--}50^\circ \text{C}$  on the surface of the dura: this elevated the temperature in the tissue high enough to cause damage to glial cells but not axons.

The spinal cord is susceptible to compression: demyelination as well as axonal degeneration can

occur following mild compression (Gledhill *et al.*, 1973; Harrison *et al.*, 1975). In the present study, demyelination was not found in the dorsal funiculus of any control animals, including those exposed to a temperature of  $40\text{--}45^\circ \text{C}$ . Even in cases in which spinal cords were accidentally deformed by compression, no demyelination nor axonal degeneration was found in the spinal cord indicating that demyelination was not the result of compression but the direct result of heating.

### *Demyelination and glial cell damage by the heat injury*

In the present study the widespread demyelination occurred without any noticeable damage to the axons. Most myelin sheaths were degraded by splitting of the myelin lamellae as was reported by Hirano (1972),



**Fig. 7.** Two weeks after treatment. This electron micrograph was taken from the deep zone bordering the intact area of the affected dorsal funiculus. Almost all the axons (Ax) in the area are thinly myelinated by oligodendrocytes (Ol), while a few axons remain unmyelinated. Proliferation of astrocyte processes (As) has occurred in the spaces between these axons.  $\times 7200$ .

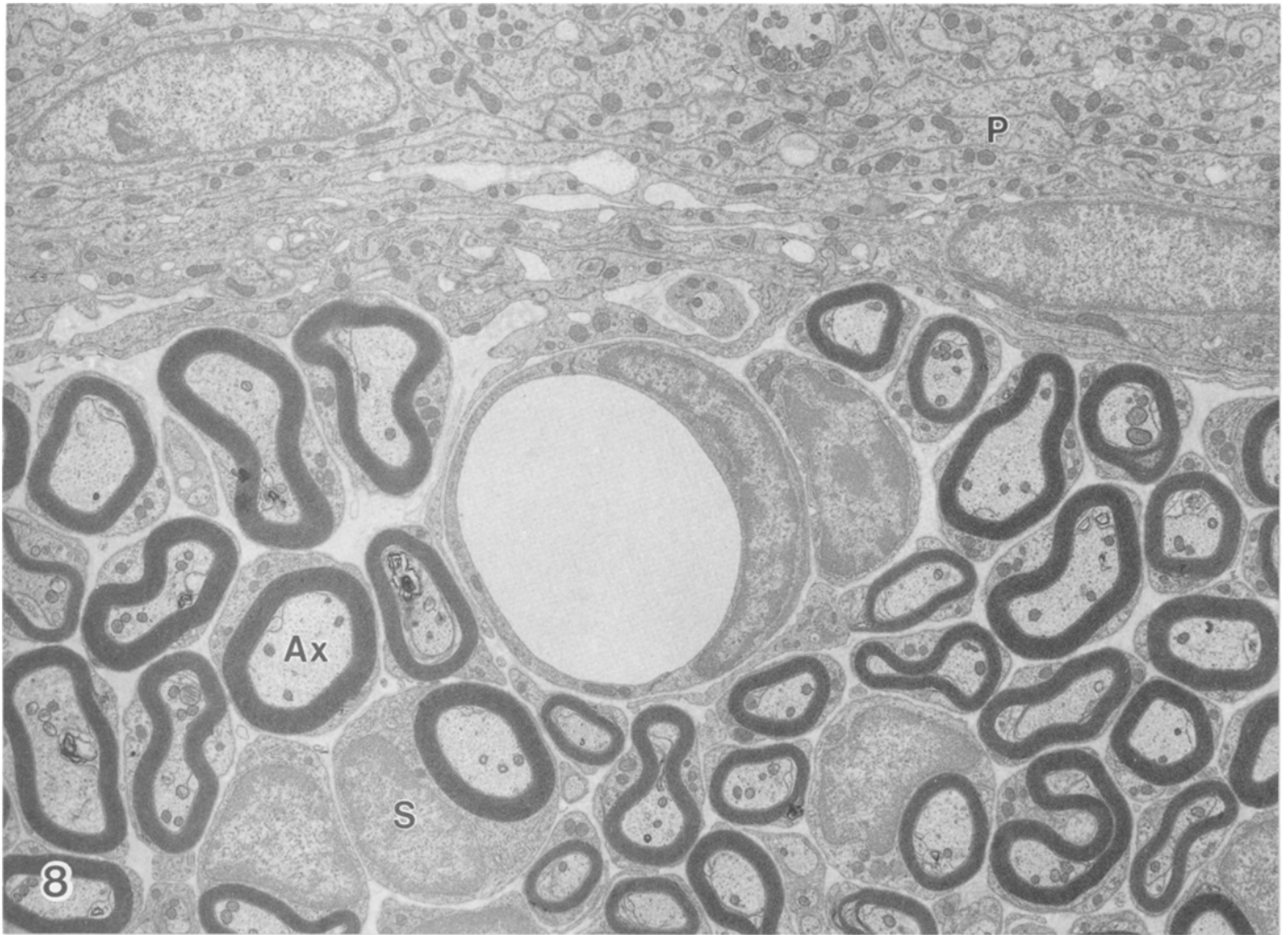
while others initially only lost their electron opacity while retaining their original compact lamellae. Myelin sheath changes of the latter type have also been found in the cryo-treated spinal cord (unpublished observations). Vulnerability of the myelin sheath to heating can probably be related to the high lipid content and structural characteristics of the membrane. Raison *et al.* (1971) utilizing electron spin resonance demonstrated that heating gives rise to changes in the lipid components of the plasma membrane. Similarly, the organizations of membrane-incorporated proteins are considered to be easily altered by heating (Hahn, 1982).

The finding that the glial cells and associated myelin sheaths were more vulnerable to heat treatment than axons is in contrast to previous studies using ultrasound for heating (Fry *et al.*, 1955; Barnard *et al.*, 1956)

which claimed that axons were more vulnerable to heat injury than glial cells. The discrepancy in the vulnerability between axons and glial cells between their studies and our own may be due to the different methods employed. The conductivity and absorption rate of the heat in the CNS tissues would be different in the two methods which could produce different effects on axons and on glial cells.

Primary demyelination in the CNS occurs in multiple sclerosis and experimental infection of Theiler's murine encephalomyelitis (TME) virus (Dal Canto & Lipton, 1980; Dal Canto & Barbano, 1984) as well as in other experimental injuries using lysophosphatidylcholine (LCP) (Blakemore, 1976), 6-aminonicotinamide (6-AN) (Blakemore, 1975), Cuprizone (Ludwin, 1978) and CSF barbotage (Bunge *et al.*, 1960), whereas in other experimental injuries such as allergic enceph-





**Fig. 8.** One month after treatment. This electron micrograph shows the subpial area of the lesion. All the axons (Ax) are thickly myelinated by Schwann cells (S). There is no typical GLM at the surface which is covered by a thick cell layer (P).  $\times 7400$ .

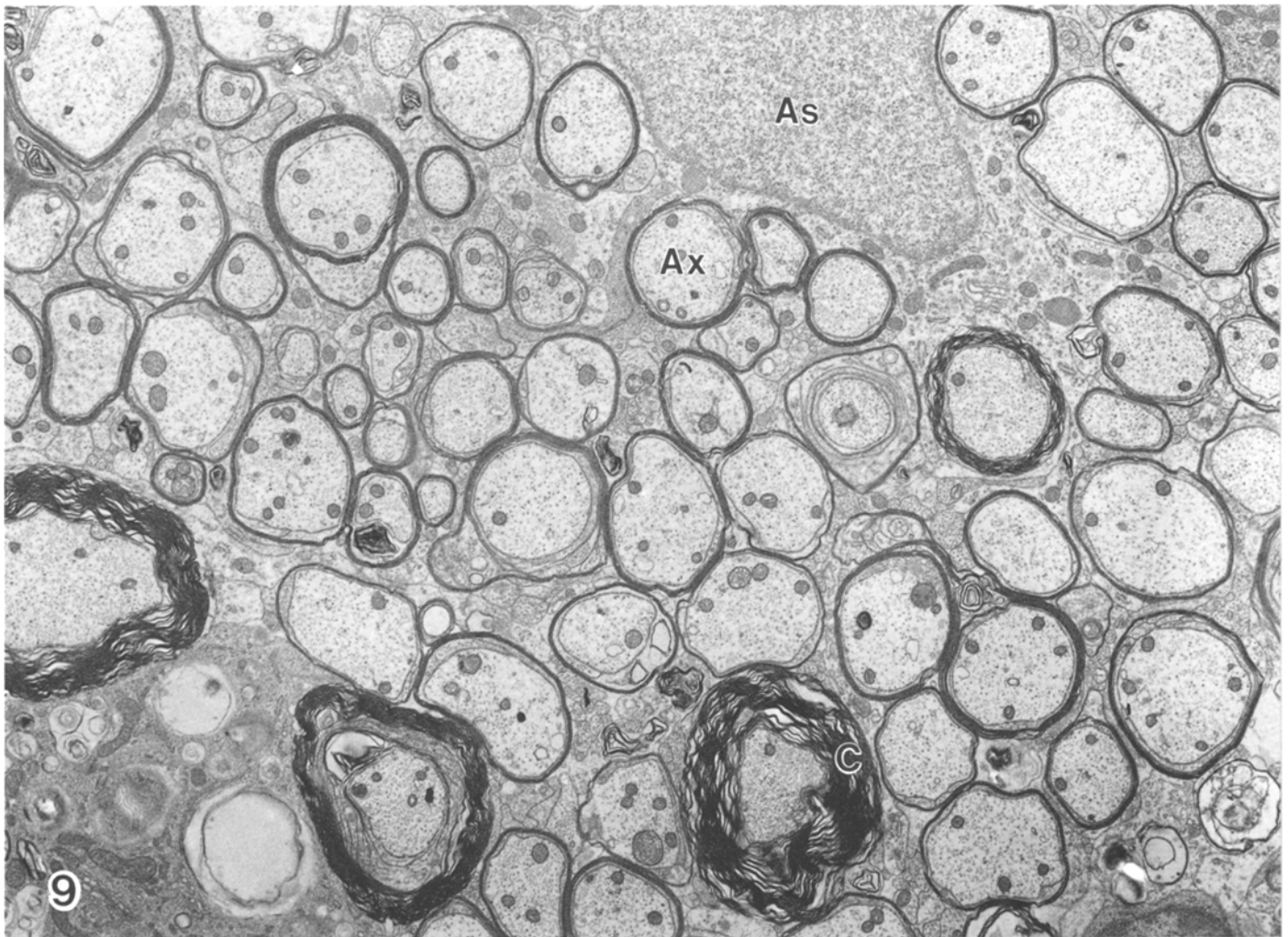
phalomyelitis (EAE) (Lampert, 1967), diphtheria toxin injection (Harrison *et al.*, 1972), and impact injury (Griffith & McCulloch, 1983), axons also undergo degenerative changes. In these latter disorders severe vasogenic oedema and local haemorrhage occur, which are considered to be harmful to axons, and thus may be partly responsible for axonal degeneration. It seems that heat treatment does not produce damage of sufficient severity to the blood-brain barrier (BBB) so as to fatally affect the axons, although permeability of damaged blood vessels seemed to increase to a certain degree (Mueller, 1979).

#### *Remyelination by Schwann cells and oligodendrocytes*

It was shown in the present study that demyelinated axons are effectively remyelinated by either oligo-

dendrocytes or Schwann cells. The same pattern of remyelination by oligodendrocytes and Schwann cells has also been noted in CNS lesions induced by the murine corona (JHM) virus (Nagashima *et al.*, 1979), the TME virus (Dal Canto & Barbano, 1984), and cryo-treatment (Collins *et al.*, 1986; our unpublished observations) as well as by X-ray irradiation of the infant spinal cord (Gilmore & Duncan, 1968; Blakemore & Patterson, 1975; Gilmore & Sims, 1986). Remyelination by Schwann cells is predominant in 6-AN (Blakemore, 1975) and ethidium bromide injected CNS (Blakemore, 1982; Graça & Blakemore, 1986), whereas remyelination by oligodendrocytes is prominent in ordinary EAE (Lampert, 1965, 1967) and Cuprizone treatment (Ludwin, 1978).

It should be noted that Schwann cell-remyelination was dominant in the subpial and perivascular areas,



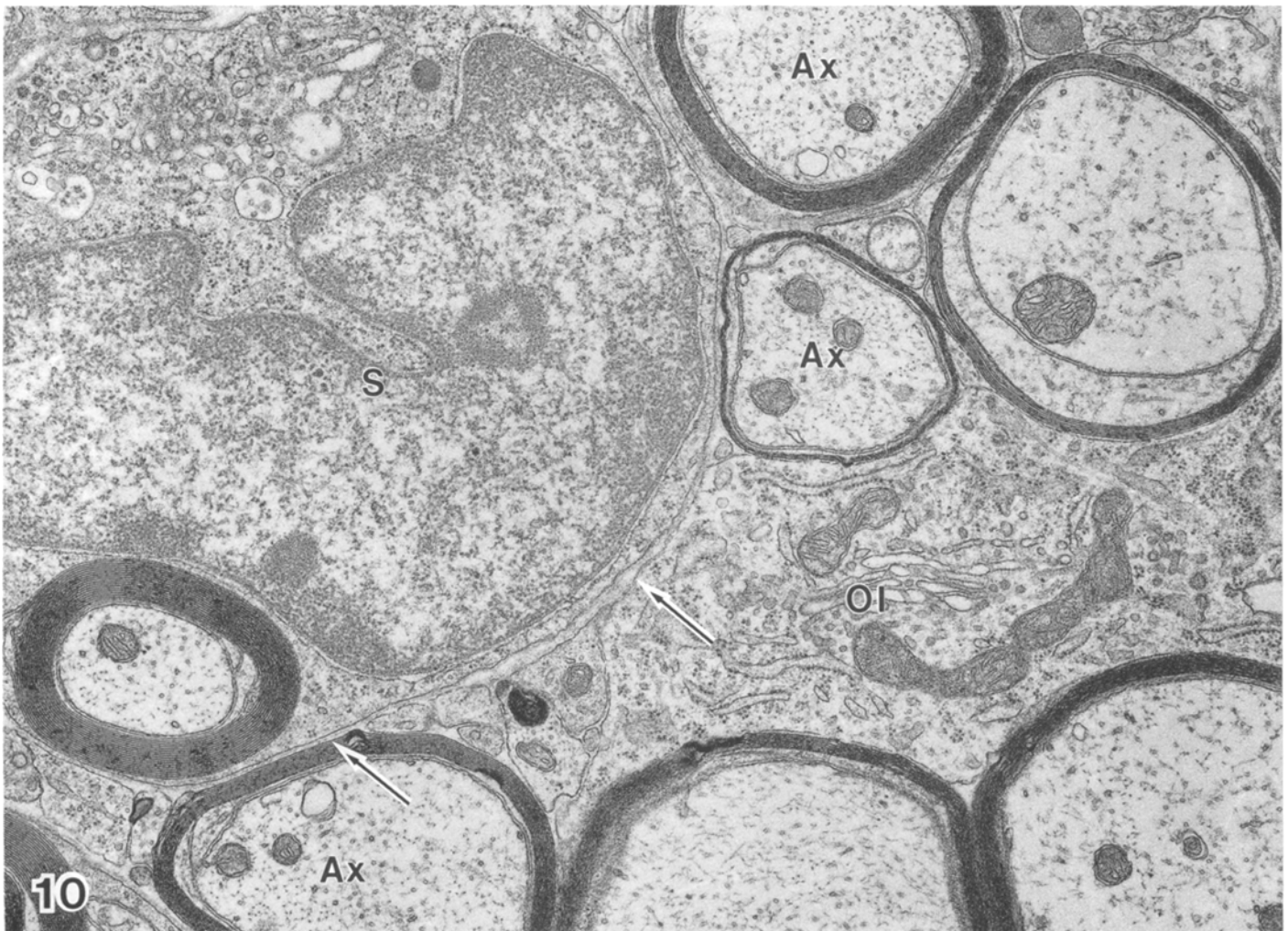
**Fig. 9.** One month after treatment. This electron micrograph shows the deep zone bordering the intact areas. Most axons (Ax) have CNS-type myelin sheaths, that is, they have been myelinated by oligodendrocytes, but their myelin sheaths are still very thin. The axons with thick myelin sheaths (C) are those that were unaffected by heat injury. As: astrocyte.  $\times 7800$ .

whereas oligodendrocyte-remyelination was conspicuous in the deep and border areas of a lesion. The appearance of Schwann cells in the CNS has been controversial and various prerequisites for ectopic Schwann cells in the CNS have been proposed which include (1) breakdown of the GLM, (2) absence of astrocytes, and (3) presence of denuded axons (Blakemore, 1983; Sims & Gilmore, 1983; Harrison, 1985; Gilmore & Sims, 1986).

As described by previous authors (Blakemore, 1983; Sims & Gilmore 1983; Harrison, 1985), the breakdown of the subpial and perivascular GLM might be the most critical factor for the appearance of Schwann cells. Thus in Cuprizone (Ludwin, 1978) and ordinary EAE (Lampert, 1965) injuries in which the GLM is retained intact, few Schwann cells appear in the lesion. Harrison (1985) thinks that Schwann cells

invade the CNS tissue through damaged GLM at the perivascular and subpial surfaces. Astrocytes therefore seem to block the invasion of Schwann cells. No astrocytes were found in CNS lesions where axons were exclusively myelinated by Schwann cells (Hammang *et al.*, 1986). In contrast, Schwann cells are rarely seen at the sites where astrocyte processes have proliferated throughout the CNS lesions as a result of LPC and 6-AN treatment, and JHM virus and TME virus infection (Blakemore, 1975, 1976; Nagashima *et al.*, 1979; Dal Canto & Lipton, 1980), as well as Schwann cell implantation into normal nervous tissue (Blakemore *et al.*, 1986).

Denuded axons seem necessary for Schwann cells to invade the CNS and proliferate. It was reported that naked axons have a mitogenic influence on Schwann cells *in vitro* (Wood & Bunge, 1975). Harrison (1987)



**Fig. 10.** One month after treatment. This micrograph was taken at the transitional zone between the Schwann cell-myelinated area and the oligodendrocyte-myelinated area. Oligodendrocyte-myelinated axons (Ax) and oligodendrocyte cytoplasm (Ol) both directly abut (arrows) on the basal laminae of the Schwann cells (S) without any intervening astrocyte processes.  $\times 23\,000$ .

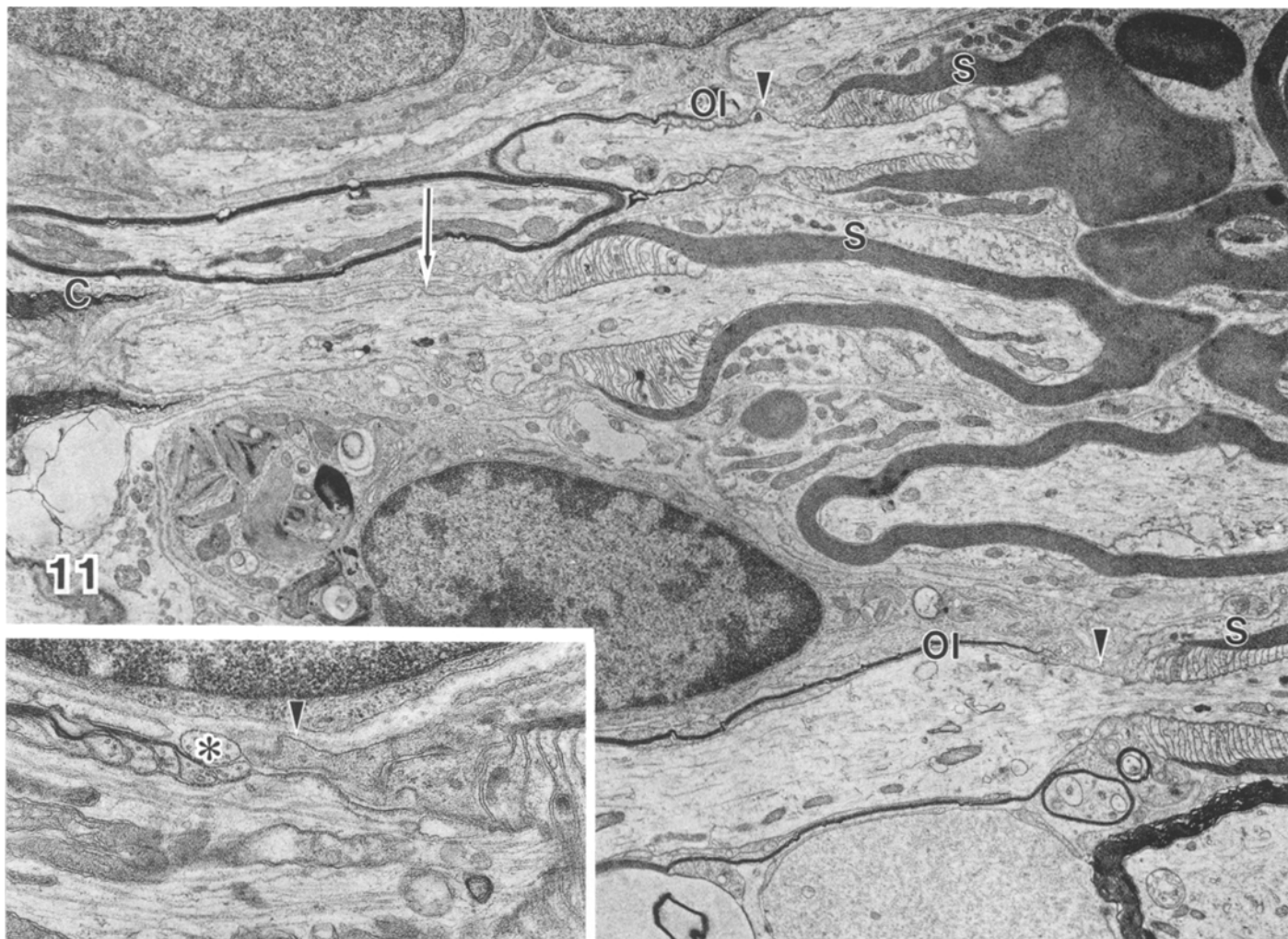
demonstrated by autoradiography that Schwann cell division is only initiated and promoted by direct contact with denuded axons. It has also been suggested that ensheathment of denuded axons by oligodendrocytes or astrocytes eliminated mitogenic influences of naked axons on Schwann cells (Sims & Gilmore, 1983).

Where are the sources of ectopic Schwann cells? Since many ectopic Schwann cells are found near the nerve root entry, it is supposed that Schwann cells in the root could move along the denuded axons into the injured CNS (Gilmore & Duncan, 1968). However, even when the roots and their entry zones are preserved undamaged, many Schwann cells have been seen in the midline of the dorsal funiculus, far from the root entry (Gilmore & Sims, 1986). On the other hand, Schwann cells of the perivascular autonomic nerves (Ghatak *et al.*, 1973) and of small nerves

located in a pial layer (Blakemore, 1983) could proliferate and migrate into the lesion. Though the sources of ectopic Schwann cells could not be clearly identified, the present study indicates that damage to glial cells as well as the GLM at the pial and perivascular surfaces might be the most important prerequisite (Blakemore *et al.*, 1986).

Oligodendrocytes remyelinate denuded axons after Cuprizone intoxication (Ludwin, 1987, 1988) and other CNS lesions including chronic relapsing EAE (Raine & Traugott, 1985) and TME virus infection (Dal Canto & Barbano, 1984). In these studies, as in the present study, remyelination by oligodendrocytes occurred in deep areas of the lesion, where there might be no influence of the damaged GLM. On the other hand, there is evidence that astrocytes can co-operate to some extent with oligodendrocytes in the facilitation of myelination (Blakemore, 1975, 1984;





**Fig. 11.** One month after treatment. This electron micrograph shows a longitudinal section from the caudal part of the lesion. A myelin sheath newly formed by Schwann cells (S) makes a node of Ranvier (arrow) at the junction with a thick CNS-type myelin sheath (C) which presumably survived the heat treatment. There are two fibres that are partly myelinated by Schwann cells (S) and partly by oligodendrocytes (Ol), forming new nodes of Ranvier between them (arrowheads).  $\times 7600$ . Inset: a high-powered micrograph showing a similar node of Ranvier taken from other section. A lateral loop (asterisk) of the oligodendrocyte is contiguous with a Schwann cell process (arrowhead). No astrocyte process is interposed between them.  $\times 28\,000$ .

Harrison, 1985). In the present study, however, there were relatively greater numbers of oligodendrocytes in the CNS-PNS border zone in which astrocytes and their processes were sparse. This finding indicates that oligodendrocytes can myelinate denuded axons in the presence of even only a few astrocytes.

#### Acknowledgements

The authors wish to thank Dr T. Ushiki and Dr K. Tohyama for their kind suggestions in the preparation of manuscript. The authors also thank Professor Paul Langman for his suggestions concerning English usage.

#### References

- BARNARD, J. W., FRY, W. J., FRY, F. J. & BRENNAN, J. F. (1956) Small localized ultrasonic lesions in the white and gray matter of the cat brain. *Archives of Neurology and Psychiatry* **75**, 15–35.
- BLAKEMORE, W. F. (1975) Remyelination by Schwann cells of axons demyelinated by intraspinal injection of 6-aminonicotinamide in the rat. *Journal of Neurocytology* **4**, 745–57.

- BLAKEMORE, W. F. (1976) Invasion of Schwann cells into the spinal cord of the rat following local injections of lysolecithin. *Neuropathology and Applied Neurobiology* **2**, 21–39.
- BLAKEMORE, W. F. (1982) Ethidium bromide induced demyelination in the spinal cord of the cat. *Neuropathology and Applied Neurobiology* **8**, 365–75.
- BLAKEMORE, W. F. (1983) Remyelination of demyelinated spinal cord axons by Schwann cells. In *Spinal Cord Reconstruction* (edited by KAO, C. C., BUNGE, R. P. & REIER, P. J.), pp. 281–91. New York: Raven Press.
- BLAKEMORE, W. F. (1984) The response of oligodendrocytes to chemical injury. *Acta Neurologica Scandinavica* **70** (suppl. 100), 33–8.
- BLAKEMORE, W. F., CRANG, A. J. & CURTIS, R. (1986) The interaction of Schwann cells with CNS axons in regions containing normal astrocytes. *Acta Neuropathologica (Berlin)* **71**, 295–300.
- BLAKEMORE, W. F. & PATTERSON, R. C. (1975) Observations on the interactions of Schwann cells and astrocytes following X-irradiation of neonatal rat spinal cord. *Journal of Neurocytology* **4**, 573–85.
- BRITT, R. H., LYONS, B. E., POUNDS, D. W. & PRIONAS, S. D. (1983) Feasibility of ultrasound hyperthermia in the treatment of malignant brain tumors. *Medical Instrumentation* **17**, 172–7.
- BUNGE, R. P., BUNGE, M. B. & RIS, H. (1960) Electron microscopic study of demyelination in an experimentally induced lesion in adult cat spinal cord. *Journal of Biophysical and Biochemical Cytology* **7**, 685–96.
- COLLINS, G. H., WEST, N. R. & PARMELY, J. D. (1986) The histopathology of freezing injury to the rat spinal cord. A light and electron microscope study. II. Repair and regeneration. *Journal of Neuropathology and Experimental Neurology* **45**, 742–57.
- DAL CANTO, M. C. & LIPTON, H. L. (1980) Schwann cell remyelination and recurrent demyelination in the central nervous system of mice infected with attenuated Theiler's virus. *American Journal of Pathology* **98**, 101–22.
- DAL CANTO, M. C. & BARBANO, R. L. (1984) Remyelination during remission in Theiler's virus infection. *American Journal of Pathology* **116**, 30–45.
- DOUGLAS, M. A., PARKS, L. C. & BEBIN, J. (1981) Sudden myelopathy secondary to therapeutic total-body hyperthermia after spinal-cord irradiation. *New England Journal of Medicine* **304**, 583–5.
- FAJARDO, L. F. (1984) Pathological effects of hyperthermia in normal tissues. *Cancer Research (Suppl.)* **44**, 4826–35.
- FRY, W. J., BARNARD, J. W., FRY, F. J. & BRENNAN, J. F. (1955) Ultrasonically produced localized selective lesions central nervous system. *American Journal of Physical Medicine* **34**, 413–23.
- GHATAK, N. R., HIRANO, A., DORON, Y. & ZIMMERMAN, H. M. (1973) Remyelination in multiple sclerosis with peripheral type myelin. *Archives of Neurology* **29**, 262–7.
- GILMORE, S. A. & DUNCAN, D. (1968) On the presence of peripheral-like nervous and connective tissue within irradiated spinal cord. *Anatomical Record* **160**, 675–90.
- GILMORE, S. A. & SIMS, T. J. (1986) The role of Schwann cells in the repair of glial cell deficits in the spinal cord. In *Neural Transplantation and Regeneration* (edited by DAS, G. D. & WALLACE, R. B.), pp. 245–69. New York: Springer-Verlag.
- GLEDHILL, R. F., HARRISON, B. M. & McDONARD, W. I. (1973) Demyelination and remyelination after acute spinal cord compression. *Experimental Neurology* **38**, 472–87.
- GRAÇA, D. L. & BLAKEMORE, W. F. (1986) Delayed remyelination in rat spinal cord following ethidium bromide injection. *Neuropathology and Applied Neurobiology* **12**, 593–605.
- GRIFFITHS, I. R. & McCULLOCH, M. C. (1983) Nerve fibres in spinal cord impact injuries part 1. Changes in the myelin sheath during the initial 5 weeks. *Journal of the Neurological Sciences* **58**, 335–4.
- HAHN, G. M. (1982) *Hyperthermia and Cancer*. New York: Plenum Press.
- HAMMANG, J. P., DUNCAN, I. D. & GILMORE, S. A. (1986) Degenerative changes in rat intraspinal Schwann cells following tellurium intoxication. *Neuropathology and Applied Neurobiology* **12**, 359–70.
- HARRISON, B. M. (1985) Schwann cell and oligodendrocyte remyelination in lysolecithin-induced lesions in irradiated rat spinal cord. *Journal of the Neurological Sciences* **67**, 143–59.
- HARRISON, B. M. (1987) Schwann cells divide in a demyelinating lesion of the central nervous system. *Brain Research* **409**, 163–8.
- HARRISON, B. M., GLEDHILL, R. F. & McDONALD, W. I. (1975) Remyelination after transient compression of the spinal cord. *Proceedings of the Australian Association of Neurologists* **12**, 117–22.
- HARRISON, B. M., McDONALD, W. I. & OCHOA, J. (1972) Central demyelination produced by diphtheria toxin: an electron microscopic study. *Journal of the Neurological Sciences* **17**, 281–91.
- HIRANO, A. (1972) The pathology of the central myelinated axon. In *The Structure and Function of Nervous Tissue* Vol. 5 (edited by BOURNE, G. H.), pp. 73–162. New York: Academic Press.
- LAMPERT, P. W. (1965) Demyelination and remyelination in experimental allergic encephalomyelitis. *Journal of Neuropathology and Experimental Neurology* **24**, 371–85.
- LAMPERT, P. W. (1967) Electron microscopic studies on ordinary and hyperacute experimental allergic encephalomyelitis. *Acta Neuropathologica (Berlin)* **9**, 99–126.
- LUDWIN, S. K. (1978) Central nervous system demyelination and remyelination in the mouse. An ultrastructural study of Cuprizone toxicity. *Laboratory Investigation* **39**, 597–612.
- LUDWIN, S. K. (1987) Regeneration of myelin and oligodendrocytes in the central nervous system. *Progress in Brain Research* **71**, 469–84.
- LUDWIN, S. K. (1988) Remyelination in the central nervous system and the peripheral nervous system. In *Advances in Neurology* vol. 47: *Functional Recovery in Neurological Disease* (edited by WAXMAN, S. G.), pp. 215–54. New York: Raven Press.
- LYONS, B. E., BRITT, R. H. & STROHBEHN, J. W. (1984) Localized hyperthermia in the treatment of malignant



- brain tumors using an interstitial microwave antenna array. *IEEE Transactions on Biomedical Engineering BME-31*, 53-62.
- LYONS, B. E., OBANA, W. G., BORCICH, J. K., KLEINMAN, R., SINGH, D. & BRITT, R. H. (1986) Chronic histological effects of ultrasonic hyperthermia on normal feline brain tissue. *Radiation Research* **106**, 234-51.
- MUELLER, S. M. (1979) Increased blood-brain barrier permeability during hyperthermia in the awake rat. *Transactions of the American Neurological Association* **104**, 81-3.
- NAGASHIMA, K., WEGE, H., MEYERMANN, R., TER MEULEN, V. (1979) Demyelinating encephalomyelitis induced by a long-term corona virus infection in rats. *Acta Neuropathologica (Berlin)* **45**, 205-13.
- RAINE, C. S. & TRAUOGOTT, U. (1985) Remyelination in chronic experimental allergic encephalomyelitis and multiple sclerosis. In *The Pathology of the Myelinated Axon* (edited by ADACHI, M., HIRANO, A. & ARONSON, S. M.), pp. 229-275. New York: Igaku-Schoin.
- RAISON, J. K., LYONS, J. M., MEHLHORN, R. J. & KEITH, A. D. (1971) Temperature-induced phase changes in mitochondrial membranes detected by spin labeling. *Journal of Biological Chemistry* **246**, 4036-40.
- SIMS, T. J. & GILMORE, S. A. (1983) Interactions between intraspinal Schwann cells and the cellular constituents normally occurring in the spinal cord: an ultrastructural study in the irradiated rat. *Brain Research* **276**, 17-30.
- WOOD, P. M. & BUNGE, R. P. (1975) Evidence that sensory axons are mitogenic for Schwann cells. *Nature* **256**, 662-4.