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Review*

Glial–glial and glial–neuronal interfaces in radiation-induced, glia-depleted spinal cord

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ABSTRACT

This review summarises some of the major findings derived from studies using the model of a glia-depleted environment developed and characterised in this laboratory. Glial depletion is achieved by exposure of the immature rodent spinal cord to x-radiation which markedly reduces both astrocyte and oligodendrocyte populations and severely impairs myelination. This glia-depleted, hypomyelinated state presents a unique opportunity to examine aspects of spinal cord maturation in the absence of a normal glial population. An associated sequela within 2–3 wk following irradiation is the appearance of Schwann cells in the dorsal portion of the spinal cord. Characteristics of these intraspinal Schwann cells, their patterns of myelination or ensheathment, and their interrelations with the few remaining central glia have been examined. A later sequela is the development of Schwann cells in the ventral aspect of the spinal cord where they occur predominantly in the grey matter. Characteristics of these ventrally situated intraspinal Schwann cells are compared with those of Schwann cells located dorsally. Recently, injury responses have been defined in the glia-depleted spinal cord subsequent to the lesioning of dorsal spinal nerve roots. In otherwise normal animals, dorsal nerve root injury induces an astrocytic reaction within the spinal segments with which the root(s) is/are associated. Lesioning of the 4th lumbar dorsal root on the right side in irradiated or nonirradiated animals results in markedly different glial responses with little astrocytic scarring in the irradiated animals. Tracing studies reveal that these lesioned dorsal root axons regrow rather robustly into the spinal cord in irradiated but not in nonirradiated animals. To examine role(s) of glial cells in preventing this axonal regrowth, glial cells are now being added back to this glia-depleted environment through transplantation of cultured glia into the irradiated area. Transplanted astrocytes establish barrier-like arrangements within the irradiated cords and prevent axonal regrowth into the cord. Studies using other types of glial cultures (oligodendrocyte or mixed) are ongoing.

Key words: Astrocytes; oligodendrocytes; myelin; Schwann cells; x-irradiation.

INTRODUCTION

Interrelations among the various glial elements and between glial and neuronal elements within the central nervous system (CNS) are difficult to define because of their intimate structural associations. A model developed and characterised in this laboratory creates an environment in which genesis and differentiation of macroglia (astrocytes and oligodendrocytes) is altered, resulting in depletions of these populations and impairment of myelinogenesis. One result of this 'thinning out' or depletion of these glial populations is that relationships that are normally difficult to discern can be more readily visualised and examined. Further, the depletion of oligodendrocytes and astrocytes can, under certain circumstances, result in the

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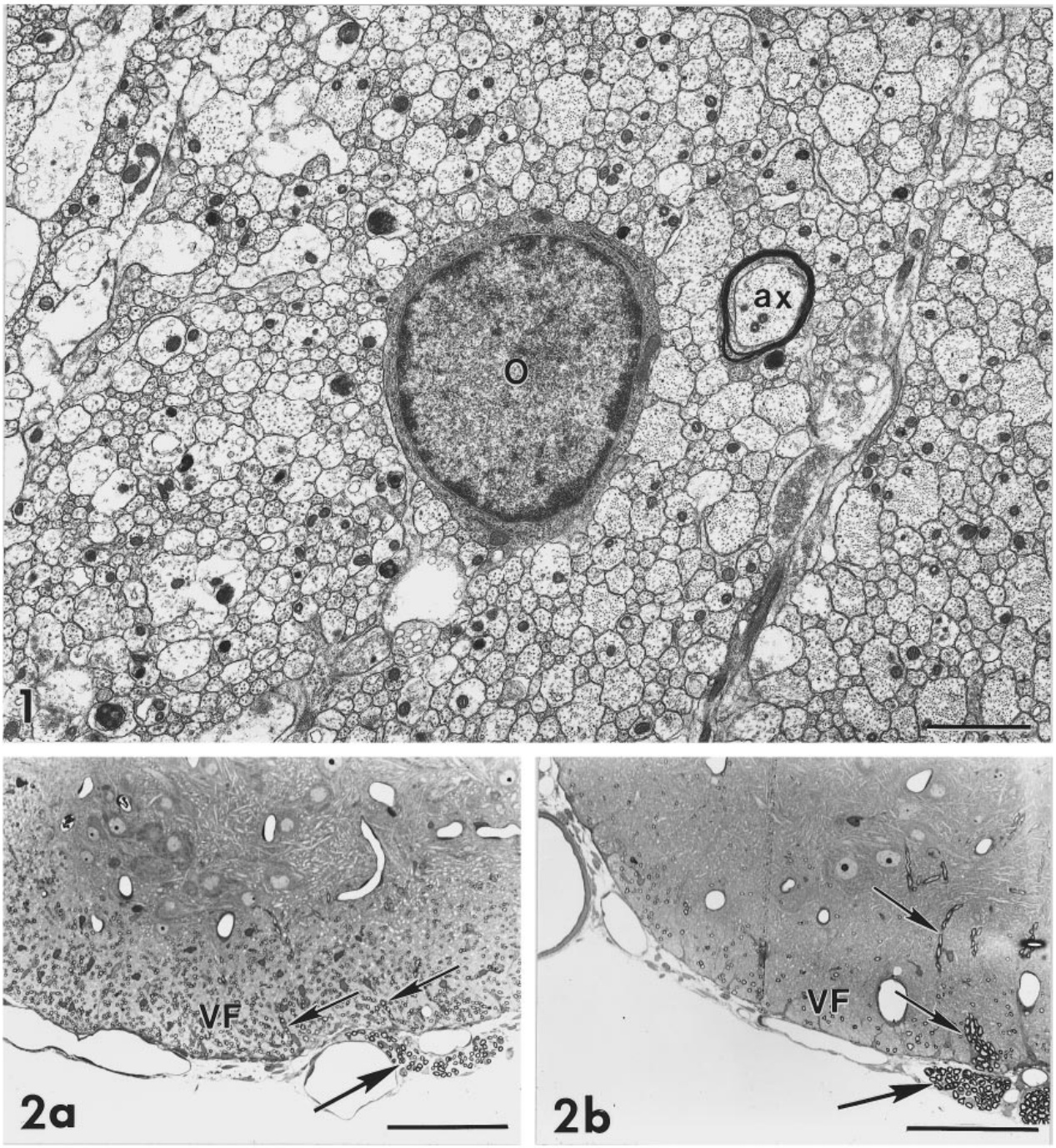


Fig. 1. Electron micrograph of the dorsal funiculus of a 5-d-old normal rat lumbar spinal cord. A presumptive oligodendrocyte (o) with a thin rim of dark cytoplasm is located in an expanse of small, nonmyelinated axons. The single myelinated axon (ax) is indicative of the sparcity of oligodendrocyte myelin in the normal dorsal funiculus at this time. Bar, 2 μm.

Fig. 2. Portions of ventral grey matter and ventral funiculus (VF) in sections of lumbar spinal cords from 13-d-old rats. By this age the white matter of the normal ventral funiculus (*a*) contains many axons (small arrows) myelinated by oligodendrocytes. In contrast, the white matter from the animal irradiated 10 d earlier (*b*) is hypocellular and contains many fewer myelinated axons. Note that the axons of motor neurons (small arrows) traversing this region are myelinated. The ventral roots (large arrows) in both animals contain myelinated axons. Thick (1 μm) toluidine blue-stained plastic sections. Bars, 100 μm.

induction of the presence of Schwann cells and myelin of the peripheral type within the CNS. This review summarises glial–glial and glial–neuronal interfaces detailed in earlier publications and includes aspects of these interrelations not previously reported.

INDUCTION OF THE GLIAL-DEPLETED STATE

The area of nervous system in which the glia-depleted state has been studied most intensely is the lumbo-sacral spinal cord of the rat. This reduction in glia is

brought about by exposure of the spinal cord to ionising radiation during the early postnatal period, i.e. 5 d of age or younger. For most studies discussed in this review the lumbosacral spinal cord is exposed to 40 Gy of soft x-rays on the 3rd postnatal day. The physical characteristics of the radiation beam and the manner of administration have been detailed in earlier publications (Gilmore, 1963*a*; Heard & Gilmore, 1980; Gilmore et al. 1982; Gilmore & Sims, 1986). Lesser amounts of radiation can be used to induce this glial depletion, but the duration of this state is abbreviated due to recovery of the glial population. Exposure to 20 Gy, for example, results in a marked loss of glia with a return to an essentially normal appearing state by 1 mo following irradiation (Gilmore, 1966). The nature of x-ray beams capable of inducing the glial depletion is not limited to that used in this laboratory; other investigators have achieved glial depletions using x-ray beams that are quite different both in source and in quality (Beal & Hall, 1974; Blakemore & Patterson, 1975). Finally, x-radiation is not the only type of ionising radiation capable of inducing the glial depletion; earlier investigations utilized high energy protons (Gilmore, 1964, 1965).

GENERAL CHARACTERISTICS OF THE GLIAL-DEPLETED STATE

Glial cells are relatively few in number in the lumbosacral spinal cord through the 1st 5 d postnatally, and the white matter contains few myelinated axons (Fig. 1). By the end of the 2nd postnatal week the numbers of glial cells in the white matter undergo a dramatic increase (Gilmore, 1971*b*) and myelination is well under way (Fig. 2*a*) (Gilmore, 1963*b*; Heard & Gilmore, 1985). Irradiation (40 Gy) at 3 d of age, however, results in a marked loss of glial cells from both white and gray matter with a consequent state of hypomyelination that is clearly evident by the end of the 2nd postnatal week (Fig. 2*b*). These changes are consistent and predictable and thus constitute a glial-depleted condition that can serve as a background upon which further studies can be designed.

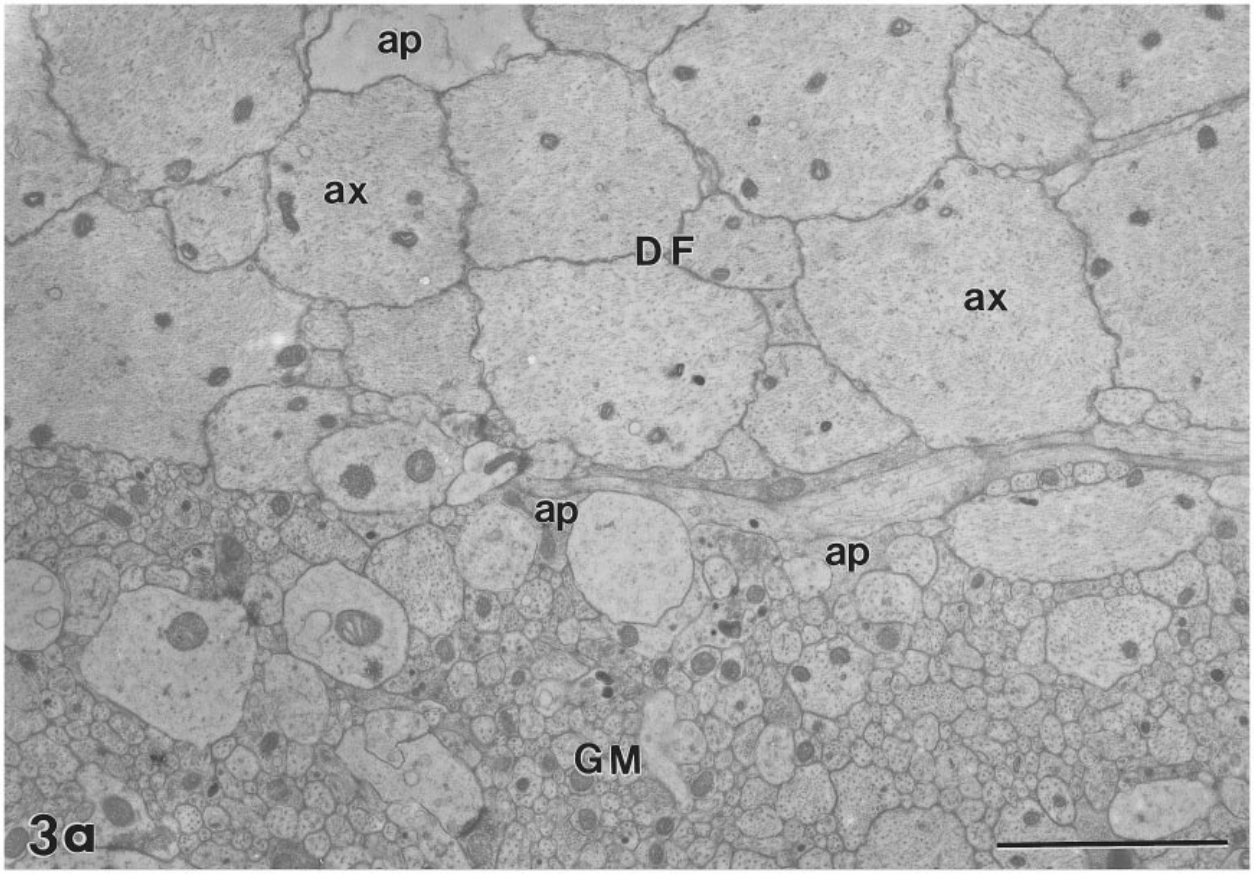
FEATURES OF WHITE MATTER DURING EARLY PERIOD OF GLIAL DEFICIENCY

The 3 wk period following irradiation is an interval during which axonal growth continues in spite of the absence of a normal population of glia (Black et al. 1985). Figure 3*a* illustrates a portion of the dorsal funiculus and adjacent grey matter containing large naked axons of a diameter that would normally be heavily myelinated at this age (23 d). There are few oligodendrocytes available to carry out this function, and this condition exists well into adulthood in these animals. Consequently, when the few remaining oligodendrocytes myelinate nearby axons, the thickness of the myelin sheath is much less than that anticipated, based on the axonal diameter (Fig. 3*b*). This observation suggests that the oligodendrocytes that are present myelinate more axons than normal and possibly compensate for this increased metabolic demand by maintaining fewer myelin lamellae than normal for axons of a given diameter.

The decreased number of glia throughout the white matter creates a situation that makes it possible to visualise and follow a given glial cell process much more readily than when the normal complement of cells and processes is present. Of the observations made, one of the more interesting ones is the relationship of astrocyte processes to the nodes of Ranvier even when myelination is so limited and the population of astrocytes is so sparse (Sims et al. 1985*b*). This relationship of astrocytes to nodes in the CNS was described by Hildebrand (1971*a*, *b*), who suggested that these astrocyte processes play a role in nodal physiology. His observations have since been confirmed by others and the evidence for participation of astrocytes in the functioning of central nodes has been strengthened (Black & Waxman, 1988). Certainly, the presence of astrocyte processes at the nodes in this markedly glial-deficient environment (Sims et al. 1985*b*) supports the idea that this relationship is not one of chance.

INTRASPINAL SCHWANN CELL DEVELOPMENT—DORSAL PORTION OF IRRADIATED REGION

A consistent finding in lumbosacral spinal cords of animals exposed to 40 Gy is the development of Schwann cells in the dorsal funiculi and dorsal gray matter (Gilmore & Duncan, 1968; Gilmore, 1971*a*; Gilmore et al. 1982; Sims & Gilmore, 1983, 1989; Gilmore & Sims, 1986). These intraspinal Schwann cells become evident in almost all animals during the 3rd week after irradiation and have been observed as early as 9 d following exposure (Gilmore, 1971*a*). Autoradiographic analysis of spinal cords from animals injected with tritiated thymidine revealed that these Schwann cells are not derived exclusively from extraspinal sources but are capable of proliferating within the intraspinal environment (Gilmore, 1971*a*; Gilmore et al. 1982) . Further ultrastructural analysis revealed that these intraspinal Schwann cells, as their



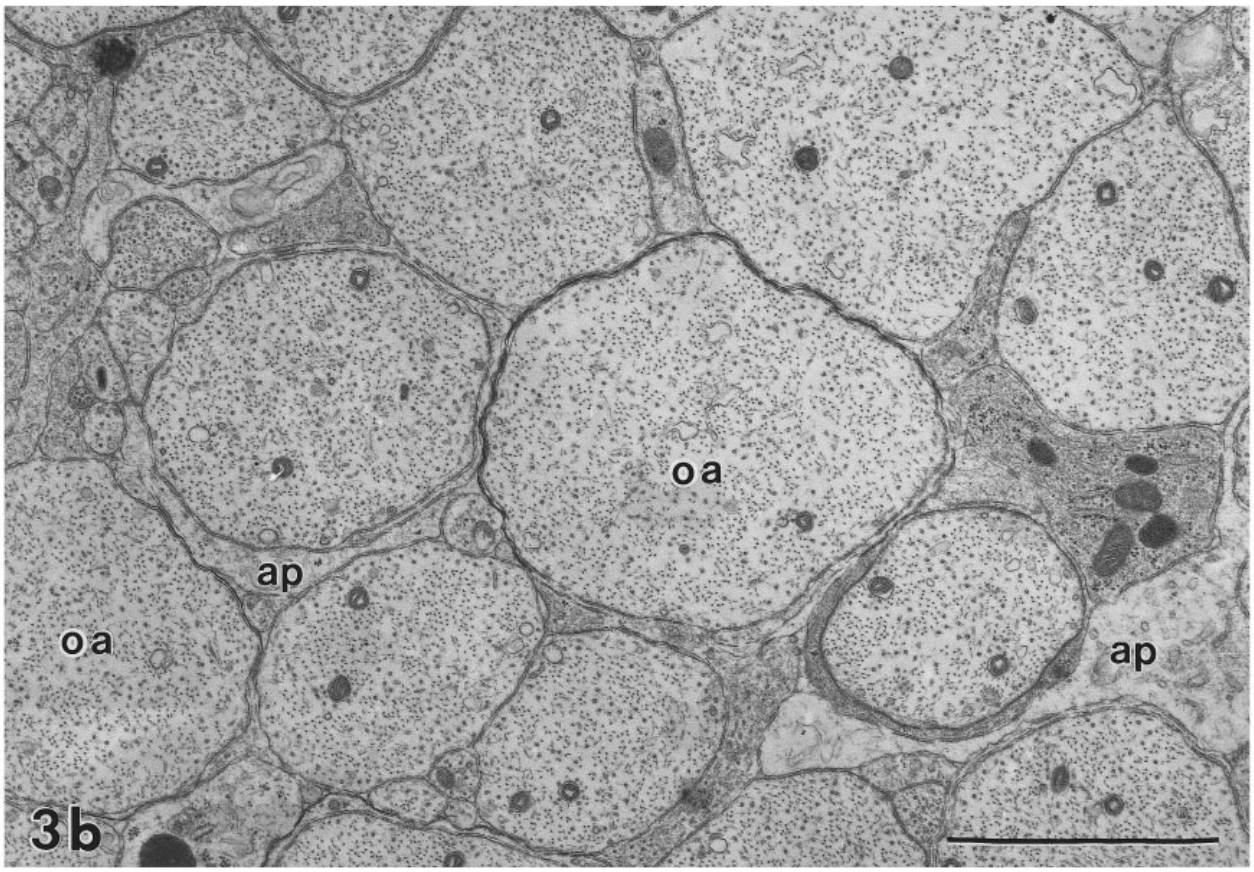


Fig. 3. For legend see opposite.

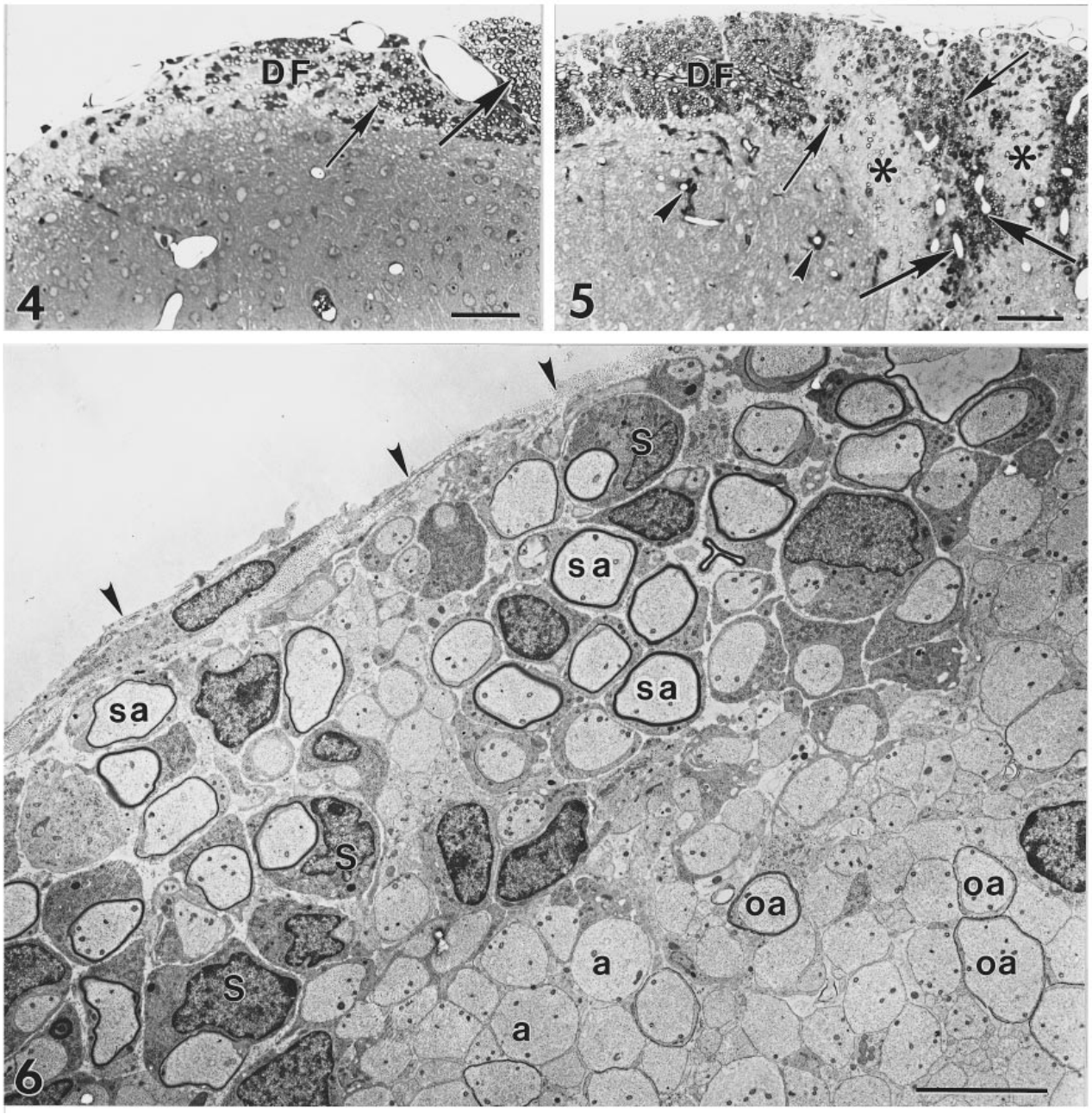
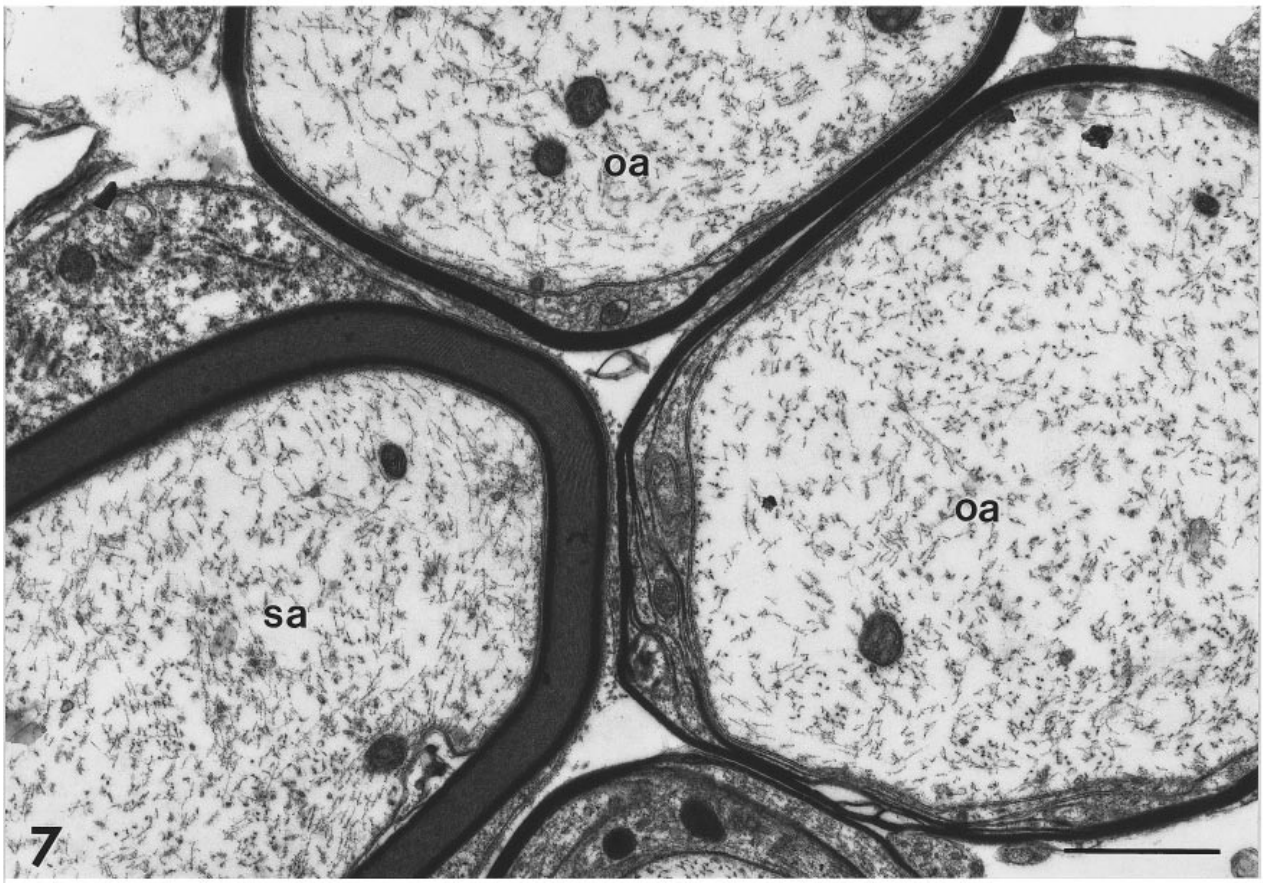


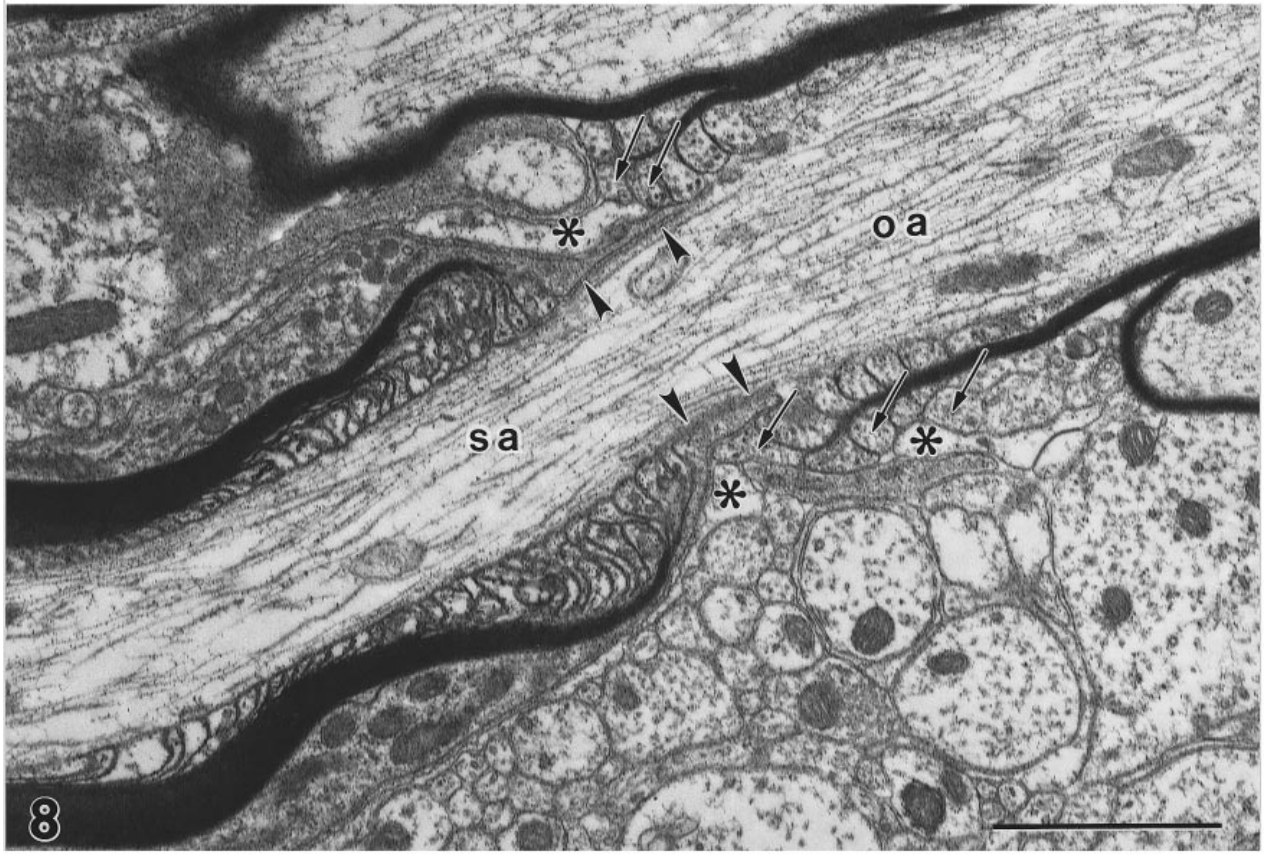
Fig. 4. Dorsal funiculus (DF) and adjacent gray matter from the lumbar spinal cord of 19-d-old irradiated rat. Note the area of high cellular density (small arrow) at the lateral extent of the dorsal funiculus adjacent to the dorsal spinal nerve root (large arrow). This area of high cell density includes many Schwann cells and associated myelinated axons. Bar, 50 μm.

Fig. 5. Medial portion of dorsal funiculus (DF) and adjacent grey matter from the lumbar spinal cord of a 25-d-old irradiated rat. Note the areas of high cell density (small arrows) due to the presence of Schwann cells. Many myelinated axons are seen within these areas in contrast to the glia-depleted areas (*) of the dorsal funiculus. Schwann cells appear to be clustered around blood vessels (large arrows) and in the grey matter (arrowheads), suggesting that the vessels may provide a route for migration. Bar, 50 μm.

Fig. 6. A relatively low power electron micrograph of the medial portion of the dorsal funiculus depicting regions of diverse myelin formation in a 23-d-old irradiated rat. Axons myelinated (sa) by Schwann cells (S) are present near the cord surface (arrowheads). Deeper within the cord is an area of large calibre axons that remain nonmyelinated (a) or are thinly myelinated by oligodendrocytes (oa). Bar, 4 μm.

Fig. 3. Electron micrographs of lumbar spinal cords from 23-d-old irradiated rats. (a) includes portions of the dorsal funiculus (DF) and adjoining grey matter (GM). Note that the large calibre axons (ax) remain nonmyelinated due to the radiation-induced loss of oligodendrocytes. Few astrocyte processes (ap) are present in either the funiculus or the grey matter. (*b*) shows a region of the dorsal funiculus in which the oligodendrocytes that survive form extremely thin myelin sheaths on large calibre axons (oa). Astrocyte processes are present but reduced in number. Bar, 2 μm.





Figs 7, 8. For legend see opposite.

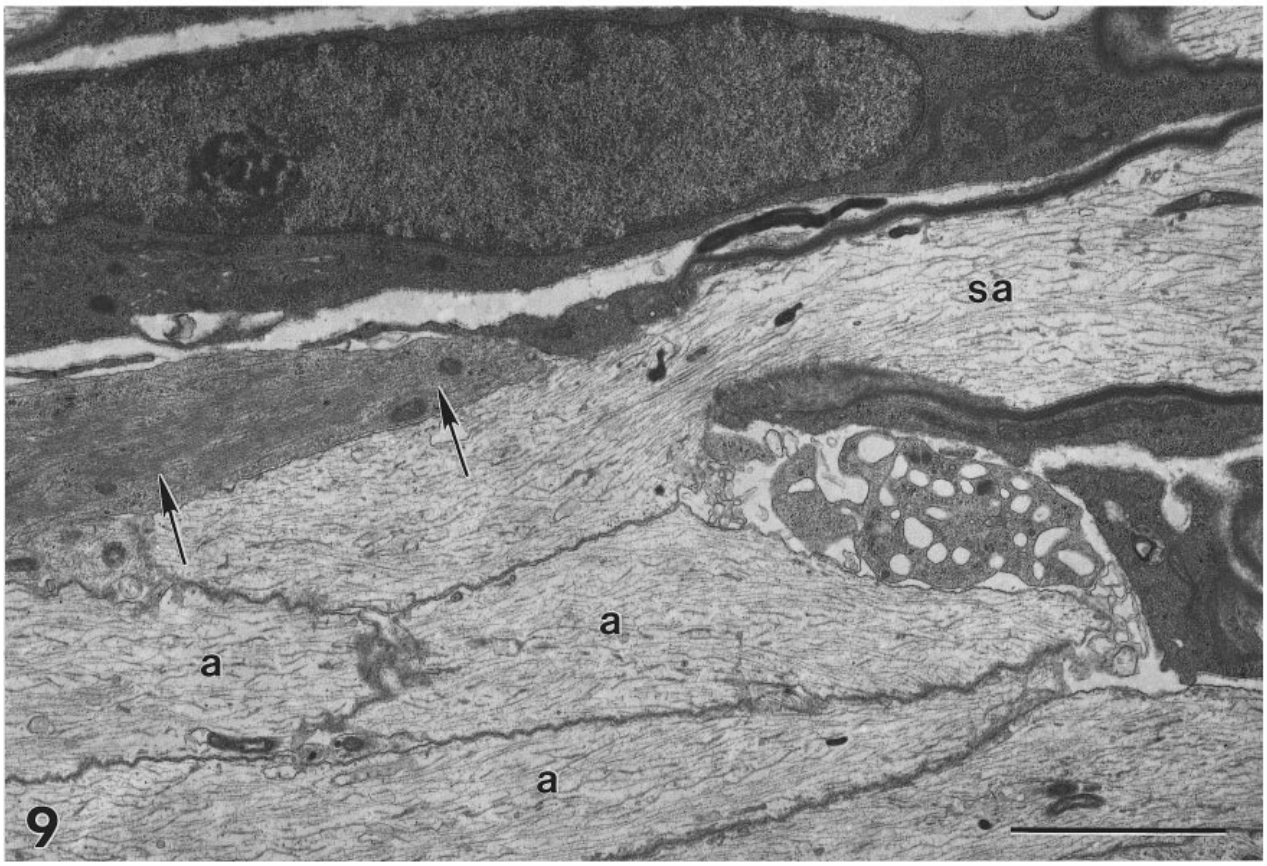
peripheral nervous system counterparts, can either myelinate or ensheath axons (Gilmore & Duncan, 1968; Gilmore et al, 1982; Sims & Gilmore, 1983; 1989; Gilmore & Sims, 1986) . The areas occupied by these Schwann cells are highly cellular (Figs 4, 5) when compared with the cellularity of the normal CNS. When examined electron microscopically these areas are found to be a hybrid milieu containing a few central glia as well as Schwann cells (Fig. 6). In spite of the cellular density of the Schwann cell-occupied regions, many naked axons remain, as seen in Figure 6. Normal-appearing astrocyte processes are observed in apposition to Schwann cells or Schwann cell myelin (Sims & Gilmore, 1983). In some instances a group of oligodendrocyte-associated axons is observed to be separated from a group of Schwann cell-associated axons, as seen in Figure 6. In other instances Schwann cell-myelinated and oligodendrocyte-myelinated axons are in direct contact and appear to be entirely compatible, even into adulthood (Fig. 7). A pronounced characteristic of the oligodendrocyte-myelinated axons is that the myelin sheath continues to be thinner than normal and markedly thinner than Schwann cell-myelinated axons based on axonal diameter. This represents a persistence of the state observed in younger, irradiated animals (Fig. 3*b*).

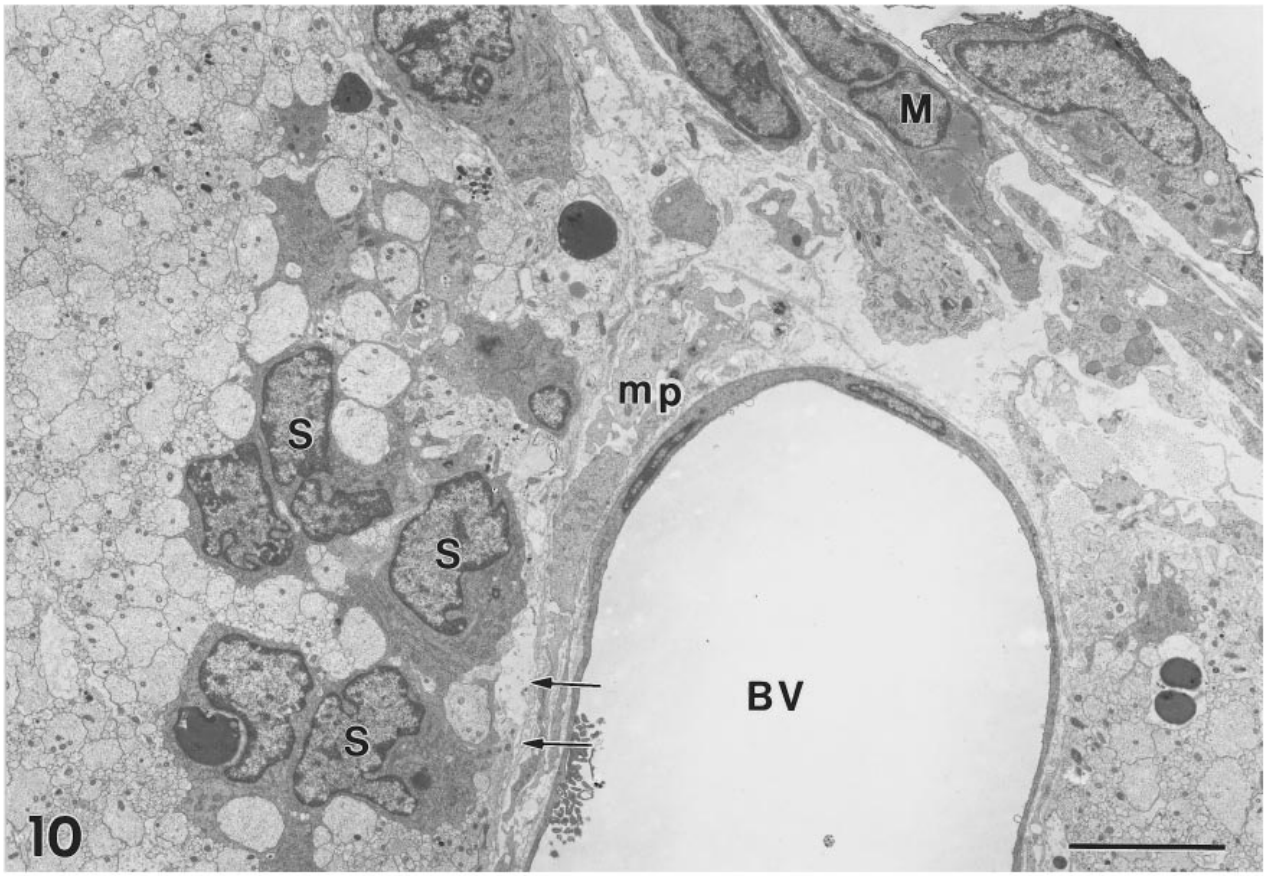
The regions occupied by the intraspinal Schwann cells, as already discussed, actually represent a mixture of both central and peripheral glia. Thus, nodes of Ranvier might be expected where one paranodal region is formed by an oligodendrocyte and the other by a Schwann cell, as occurs at the transition zone between the central and the peripheral nervous systems. Such nodal areas have been observed well within the substance of the spinal cord (Fig. 8) (Gilmore & Duncan, 1968; Sims et al. 1985*b*). These mixed nodal areas have astrocyte processes associated with them, as do nodes bordered by segments of oligodendrocyte myelin in the CNS of normal animals (Hildebrand, 1971*a*, *b*; Black & Waxman, 1988), and in this irradiated model (Sims et al. 1985*b*). Fingers of Schwann cell cytoplasm share contact with the axolemma in the nodal regions within this CNS environment in the same pattern as they do in the peripheral nervous system. In some instances (Fig. 8) Schwann cell processes cover the axolemma of a mixed node, while the ever-present astrocyte process is in direct contact with the terminal loops of the oligodendrocyte paranodal process, which is a normal occurrence at CNS nodes. The interesting question that arises from the observations of the mixed nodes in the irradiated situation is the function of the astrocyte process in these instances where the nodal axolemma is entirely covered by Schwann cell processes. Perhaps the maintenance of central myelin in this situation is dependent upon this astrocyte–oligodendrocyte interrelationship.

Several routes by which the Schwann cells access the CNS environment within this model have been considered. The earliest appearance of these cells is generally in the region of the dorsal root transition zone, and from there the cells appear to spread medially through the dorsal funiculus and into the dorsal grey matter. This pattern is evident in Figures 4 and 5 which show the highly cellular, Schwann cell-occupied area located near the root transition zone in the younger animal (Fig. 4), whereas in the older animal (Fig. 5) the Schwann cells occupy the entire dorsolateral portion and are also present medially. Such a pattern strongly supports a path of migration along the incoming dorsal root axons (Gilmore & Duncan, 1968; Gilmore et al. 1982; Gilmore & Sims, 1986). This is a reasonable route of access given that Schwann cells normally myelinate the extraspinal portion of dorsal root axons. If alterations in the astrocyte population compromise the barrier properties of the glia limitans on the dorsal surface of the spinal cord and if, concurrently, the central, oligodendrocyte-derived myelin fails to develop (Fig. 9), this route of migration becomes readily available. Interruptions in the continuity of the glia limitans on the dorsal surface of the irradiated region of the spinal cord are noted as early as 10 d following irradiation (Sims et al. 1985*a*). Such interruptions, combined with the state of hypomyelination already discussed, establish the 2 major conditions considered to be necessary for this route of entry. These 2 conditions are illustrated in Figure 9 where only a single astrocyte process is evident and where, in addition to a lack of oligodendrocyte myelin, there is also an absence of the complex of astrocyte processes normally associated with the dorsal root transition zone. In addition to

Fig. 7. Electron micrograph of a region of mixed myelin types in a 63-d-old irradiated animal. Compare the thicknesses of the myelin sheaths between the Schwann cell-myelinated axon (sa) and the oligodendrocyte-myelinated axons (oa), noting the very thin sheath produced by the oligodendrocytes given the large diameter of the axon. Bar, 1 μm.

Fig. 8. Electron micrograph of a node with mixed myelin types in a 48-d-old irradiated animal. This node is located in the dorsal horn, presumably on a primary afferent axon. Schwann cell processes myelinate a segment of the axon (sa) and cover the nodal axolemma (arrowheads). The paranodal region (arrows) of the oligodendrocyte-myelinated segment of axon (oa) is contacted by astrocyte processes (*). Bar, 2 μm.





Figs 9, 10. For legend see opposite.

possibly following incoming dorsal root axons, Schwann cells appear to migrate into the spinal cord through gaps in the glia limitans at sites distant from the dorsal root transition zone (Sims & Gilmore, 1983). This route of entry is similar to that used by Schwann cells which migrate into the CNS from segments of peripheral nerve placed into the subarachnoid space (Blakemore, 1977, 1984). A 3rd possible route associated with loss of the barrier properties of astrocytes is a perivascular migration route. Aggregates of Schwann cells have been observed in perivascular positions within the irradiated immature spinal cord (Gilmore & Sims, 1986). The location of aggregates in both white and grey matter in Figure 5 is strongly suggestive of a possible perivascular route. Routes of migration of Schwann cells in other models (Raine et al. 1978; Blakemore, 1984; Duncan et al. 1988; Franklin & Blakemore, 1993), some of which involve exposure to radiation, have been described to involve perivascular spaces. The perivascular route is clearly demonstrated in Figure 10 where Schwann cells occur in proximity to a vessel at the spinal cord surface which lacks a covering of astrocyte processes. The vessels in this region also lack a complete investment by astrocyte processes so the Schwann cells are directly apposed to the vessel wall (Fig. 11). Thus it appears that Schwann cells enter the CNS environment in instances in which the integrity of the astrocytic interfaces is compromised, a point stressed in the review by Franklin & Blakemore (1993).

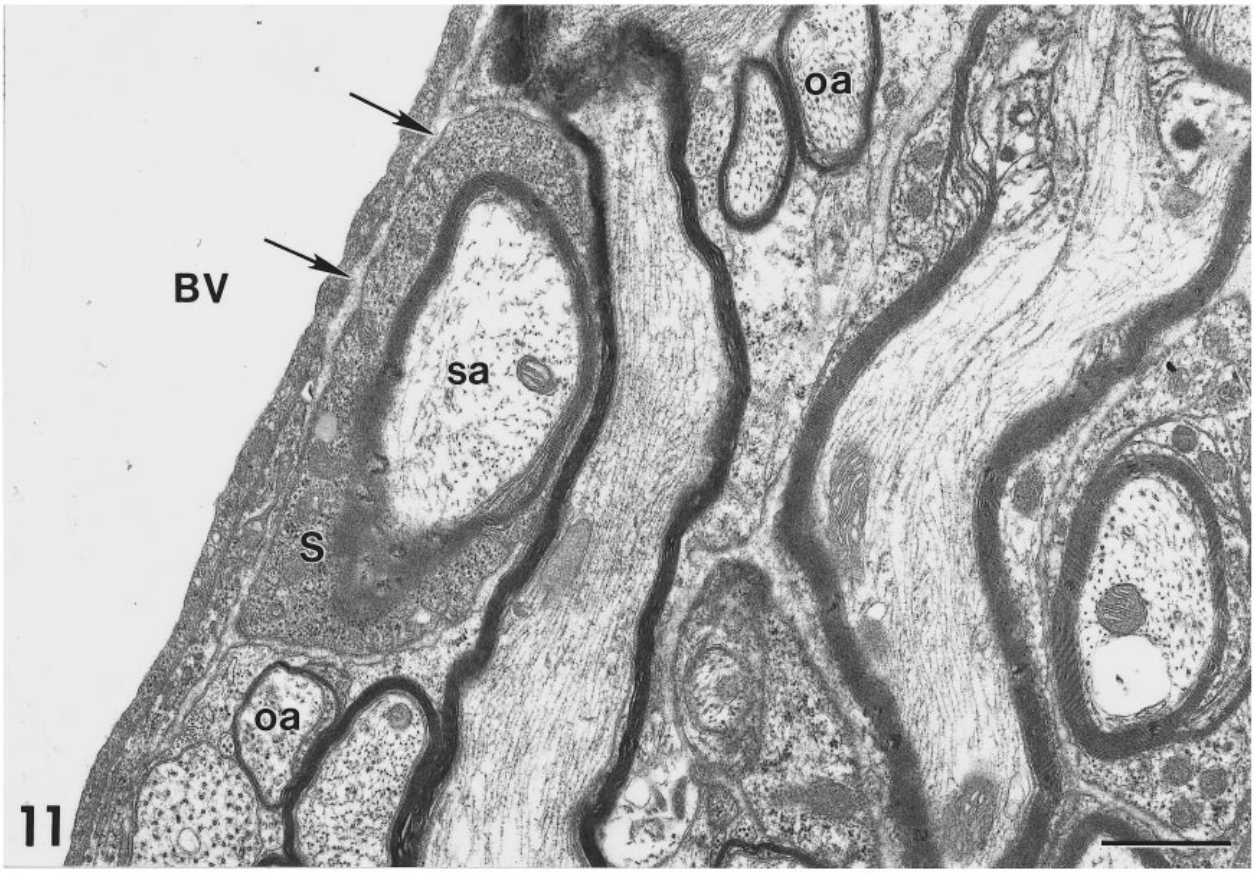
INTRASPINAL SCHWANN CELL DEVELOPMENT—VENTRAL PORTION OF IRRADIATED REGION

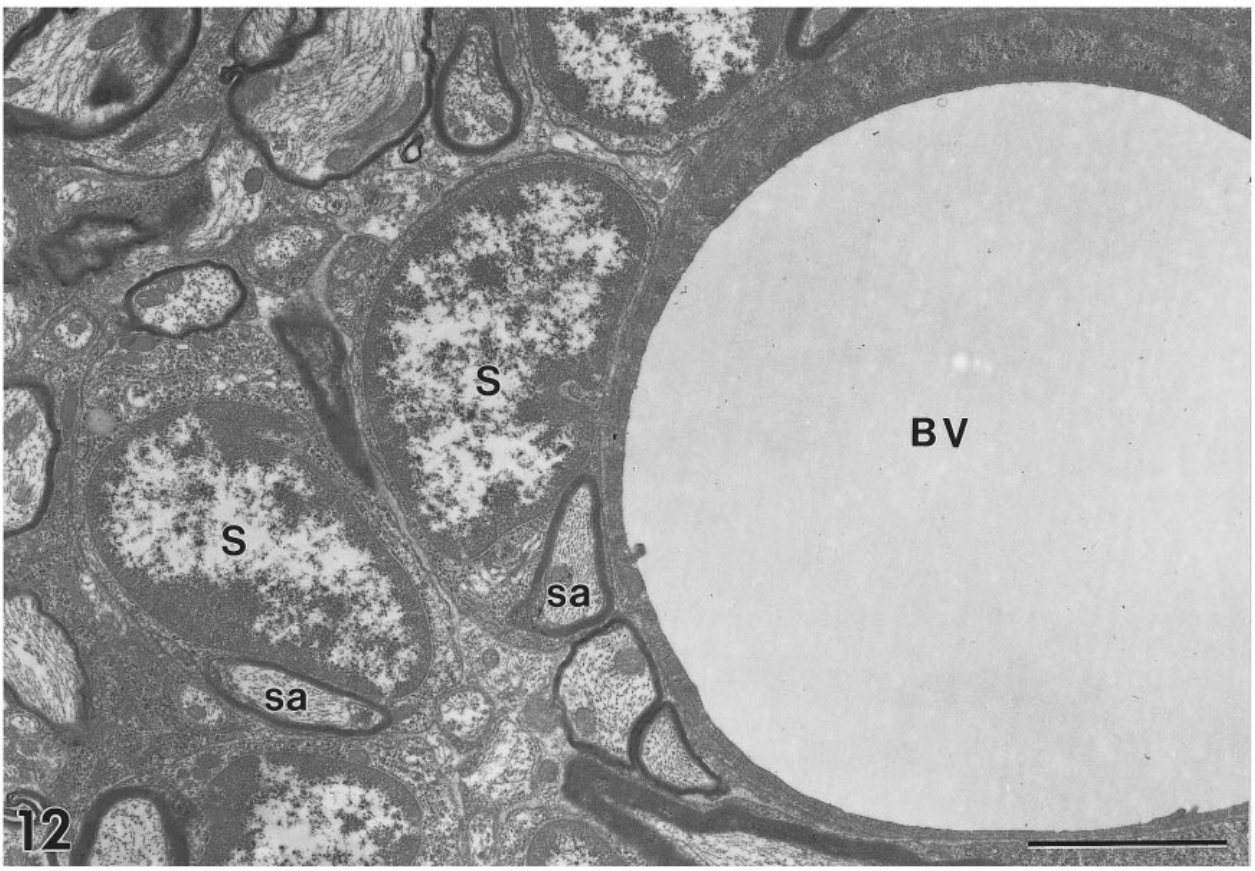
The development of intraspinal Schwann cells as a result of early postnatal exposure to radiation is not restricted to the dorsal portion of the irradiated length of spinal cord. Development in the dorsal portion, as just described, occurs within the first 3 wk following exposure. At later intervals, usually 2 or more months following irradiation, aggregates of Schwann cells can be identified in the ventral portion of the irradiated region (Gilmore et al. 1993). In general, the ventrally located Schwann cell aggregates differ from those observed dorsally. One of these differences is that the ventrally located Schwann cells occur in only 40% of the animals, in contrast to their presence in the dorsal portion of the spinal cord in nearly all of the irradiated animals. An even more striking contrast is the location, which for the ventrally located Schwann cells is predominantly in the grey matter with often little involvement of the white matter. Only rarely is it possible, even with use of serial sections, to document continuity between Schwann cells in the ventral grey matter and the ventral spinal nerve root. In general, the ventrally located aggregates tend to be very small and are situated adjacent to or around small blood vessels (see figs 5 and 6 in Gilmore et al. 1993), a feature shared with the dorsally situated Schwann cells (Fig. 5). Another feature shared with perivascular aggregates dorsally is that the vessels with which these Schwann cells are associated lack a complete investment of astrocytic end-feet, resulting in direct apposition between Schwann cells and vessel walls (Fig. 12).

The ventrally located Schwann cell aggregates become quite sizeable in some instances and occupy a significant portion of the ventral spinal grey matter (see figs 3 and 4 in Gilmore et al. 1993). In these instances, the Schwann cells appear to surround or encapsulate individual neuronal perikarya (Fig. 13). The intimate association between Schwann cells and neuronal perikarya raises important issues regarding the fate of synaptic contacts on the neuronal surface. Light microscopic examination of spinal cord sections immunostained with the antibody, synaptophysin, reveals immunoreaction product around neuronal perikarya and along primary dendrites. Ultrastructural examination reveals a variety of relationships between Schwann cells and neuronal perikarya or their primary dendrites, including contact between the 2 cell types with no intervening basal lamina but with a cleft of 40–80 nm, or contact with the presence of an intervening basal lamina or a layer of axon terminals (Fig. 13). These findings confirm that approximation of these 2 cell types does not preclude the presence of synaptic contacts on the neuronal perikarya and their primary dendrites.

Fig. 9. Electron micrograph of a sagittal section through the dorsal root entry zone of a 23-d-old irradiated rat. Axons (a) that have entered the spinal cord remain nonmyelinated due to the loss of oligodendrocytes which normally would have formed thick myelin sheaths on them by this time. Note that the axon on which a Schwann cell forms a myelin segment (sa) passes deep to an astrocyte process (arrows) forming part of the glia limitans in this region. Bar, 2 μm.

Fig. 10. Electron micrograph from a 28-d-old irradiated rat. A large blood vessel (BV) at the dorsal surface of the cord provides a route for Schwann cells (S) to enter the cord and begin to myelinate axons within the region. Meningeal cells (M) and their processes (mp) cover the cord and partially invade the space between vessel and the glial processes associated with the vessel. The Schwann cells may enter the cord when this glial lining is disrupted, as in the region indicated by the arrows. Bar, 4 μm.





Figs 11, 12. For legend see opposite.

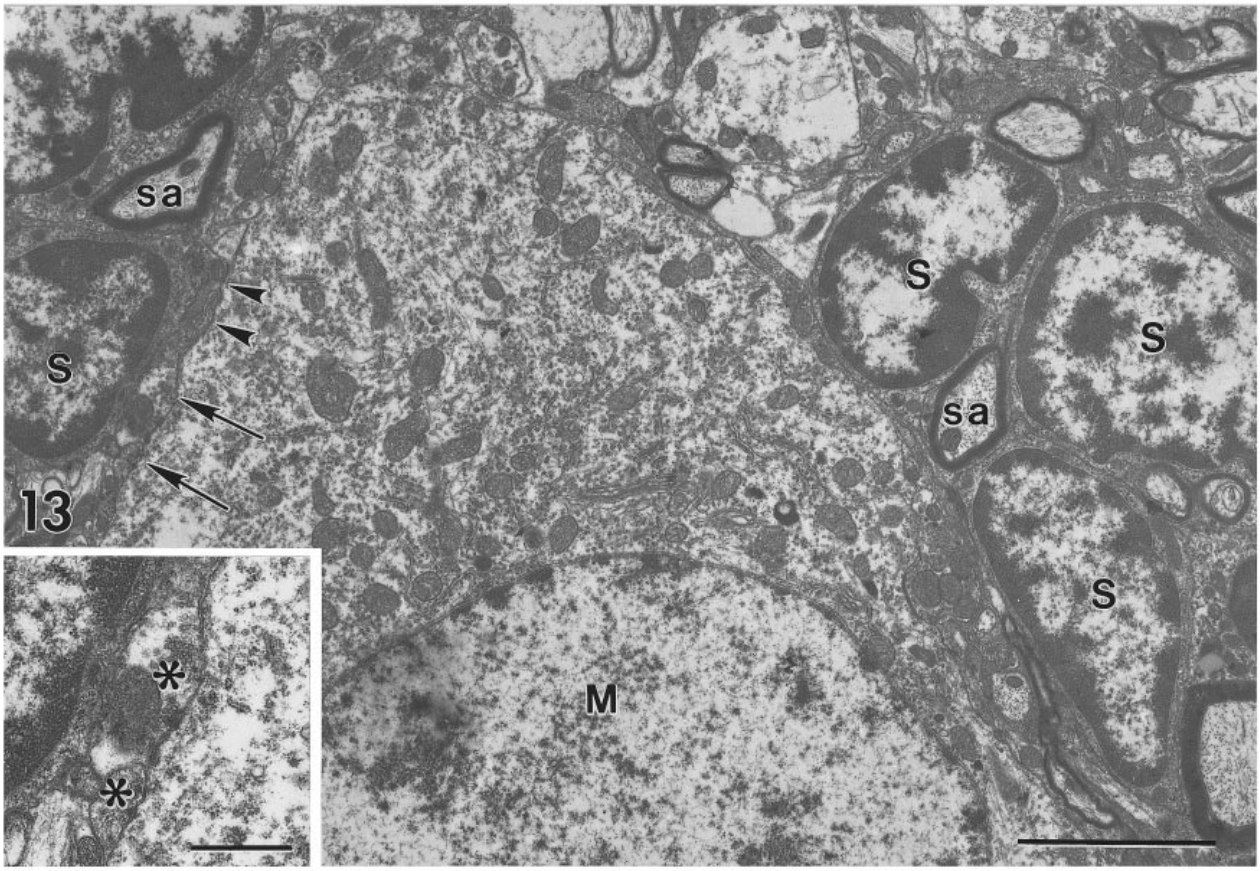
The relationships between Schwann cells and neuronal perikarya and dendrites in the irradiated spinal ventral grey matter bear many similarities to those normally occurring between the receptive surfaces of neurons and astrocytes (Peters et al. 1991). Under normal circumstances, astrocyte processes segregate or isolate receptive fields on neurons and in doing so segregate axon terminals synapsing on neuronal perikarya. The various relationships observed between membranes of the intraspinal Schwann cells and neurons suggest that the Schwann cells may be performing some of the tasks normally carried out by astrocytes. This idea is further strengthened by instances in which the intraspinal Schwann cells are noted to be interposed between a neuronal perikaryon and a blood vessel without any intervening astrocyte processes. Thus the positioning of Schwann cells directly between vessel and neuronal perikaryon may enable them to transport materials between these structures. Investigations of possible transport functions are in progress.

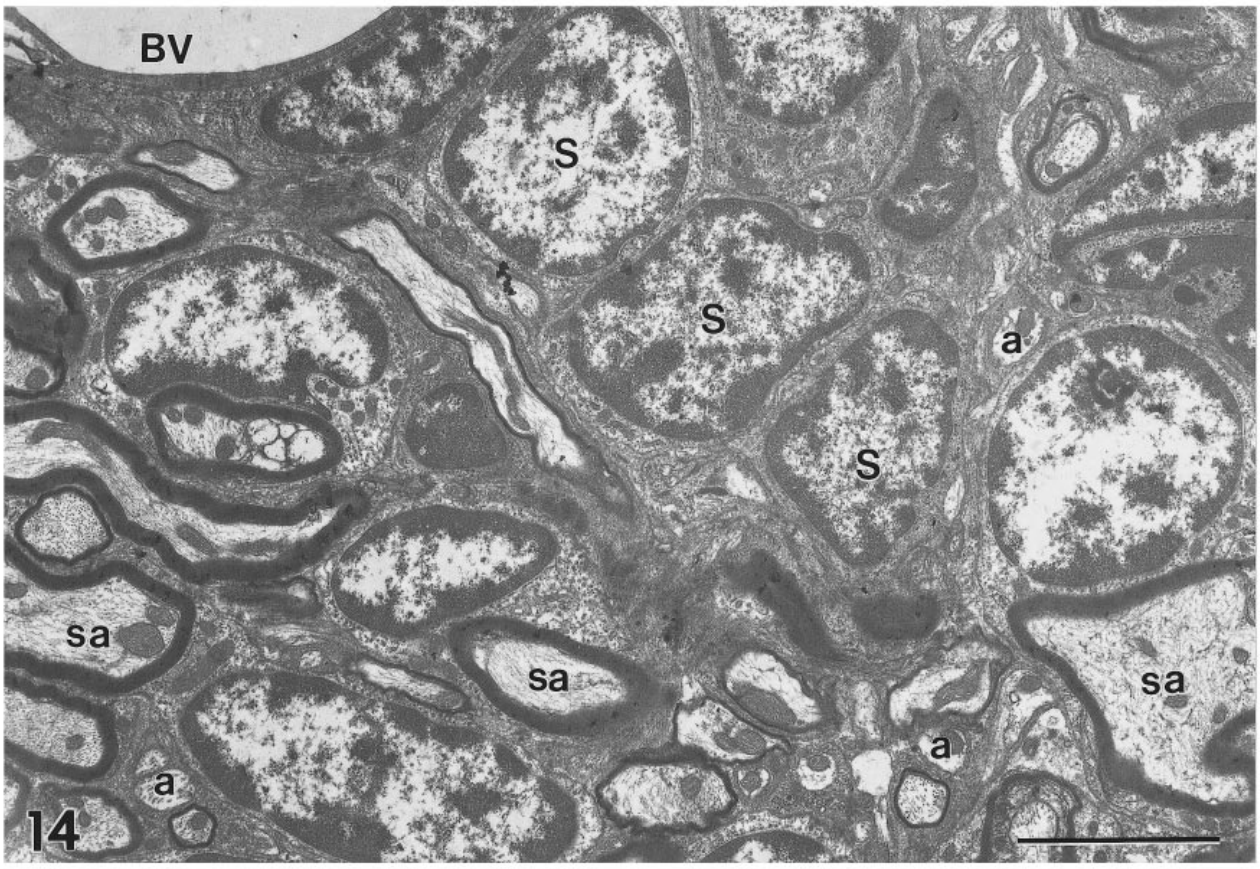
Finally, the Schwann cells located in the ventral grey matter share in common with the dorsally situated Schwann cells the capability of myelinating or ensheathing axons (Figs 12–14). Although axons within the ventral grey matter are capable of interacting with Schwann cells to effect myelination or ensheathment, it is rare to observe Schwann cell myelination of the intraspinal portion of axons of motor neurons which emerge from the spinal cord to enter the ventral root. The myelination by Schwann cells which occurs in the ventral grey matter months following the radiation exposure is in all likelihood a primary myelination rather than a remyelination. In support of this is the absence of any evidence of demyelination and/or myelin degradation in the spinal grey matter at these longer postirradiation intervals. The marked alteration in oligodendrocyte development observed in the irradiated white matter is probably also paralleled in the grey matter. If so, it is possible that the grey matter includes naked axons which have the potential to become myelinated. Perhaps these axons, in a manner not yet understood, exert a tropic influence on Schwann cells. Based on their studies of remyelination models, Franklin & Blakemore (1993) have proposed that demyelinated axons may be the source of a diffusable chemotactic substance capable of signalling migration of Schwann cells from remote sites. Perhaps axons that are mature but remain naked due to loss of myelinating cells, as in the model used in this laboratory, may also serve as the source of a chemotropic stimulus.

The origin(s) of the Schwann cells which populate the ventral grey matter and the pathway(s) by which they do so are not yet defined. Whereas migration along the spinal nerve roots is a pathway dorsally, this is not the case ventrally. As noted above, it is rare to find Schwann cells associated with the intraspinal portions of axons which give rise to the ventral roots. Further, the incidence of Schwann cell aggregates in the ventral spinal cord is much higher in the grey than in the white matter (Gilmore et al. 1993). Interruptions in the glia limitans on the dorsal surface of the spinal cord are considered to provide a pathway for migration of Schwann cells into the spinal cord (Blakemore & Patterson, 1975; Sims & Gilmore, 1983; Sims et al. 1985*a*; Duncan et al. 1988). Examination of the glia limitans on both dorsal and ventral surfaces of the spinal cord during the 1st 2 postnatal weeks reveals the presence of interruptions in the glia limitans dorsally but not ventrally (Sims et al. 1985*a*). Further, the ventral glia limitans in both nonirradiated and irradiated animals is thicker and is composed of more astrocyte processes than that on the dorsal surface. A study nearing completion in this laboratory focuses on the question of whether changes in the glia limitans on the ventral surface of the spinal cord at intervals later than 2 mo following irradiation can provide a pathway for ingress of Schwann cells. Findings to date reveal no significant differences in thickness of the glia limitans with intervals up to 100 d following irradiation. The number of astrocyte processes forming the glia limitans is, however, slightly decreased in the irradiated animals, a finding which correlates with the ultrastructural observation of thicker, swollen astrocyte processes in this group. In spite of these changes, the continuity of the glia limitans has never been observed to be interrupted. A 3rd pathway for migration is the vasculature, and the frequent association of the ventral Schwann cell aggregates with blood vessels (Figs 12, 14) (Gilmore & Sims, 1986; Gilmore et al. 1993) strengthens the view

Fig. 11. Electron micrograph of blood vessel (BV) in the dorsal aspect of the spinal cord from an irradiated rat. The Schwann cell (S) adjacent to the vessel myelinates an axon (sa). Note that the vessel lacks the normal complement of perivascular astrocyte processes in the space (arrows) between the vessel and the Schwann cell. Oligodendrocytes myelinate smaller axons (oa) in the region. Bar, 1 μm.

Fig. 12. Electron micrograph of blood vessel (BV) in the ventral region of spinal cord from a 63-d-old irradiated rat. Schwann cells (S) adjacent to the vessel myelinate small axons (sa). As in Figure 11, there are no astrocyte processes interposed between the vessel and the Schwann cell. Bar, 3 μm.





Figs 13, 14. For legend see opposite.

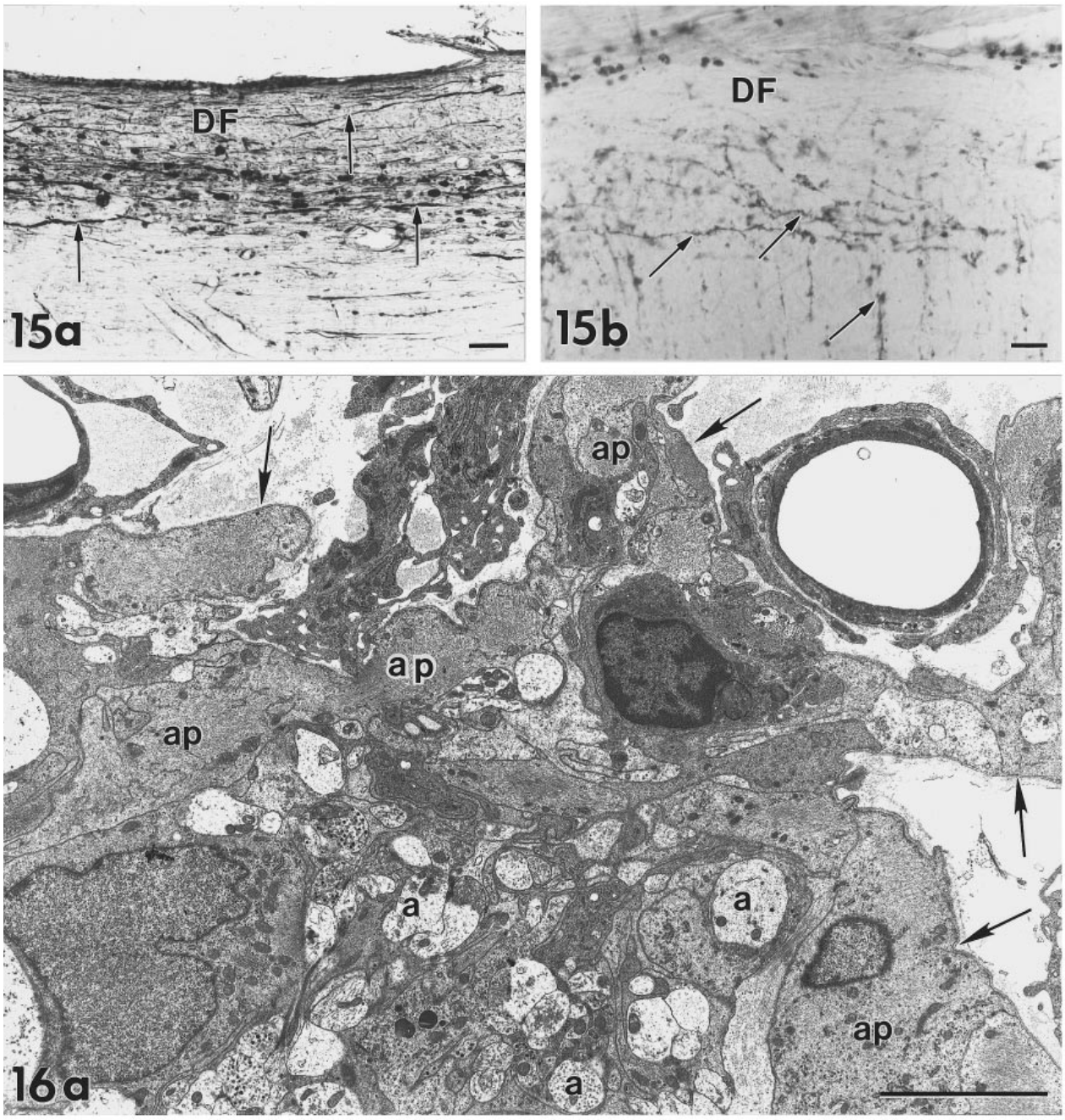
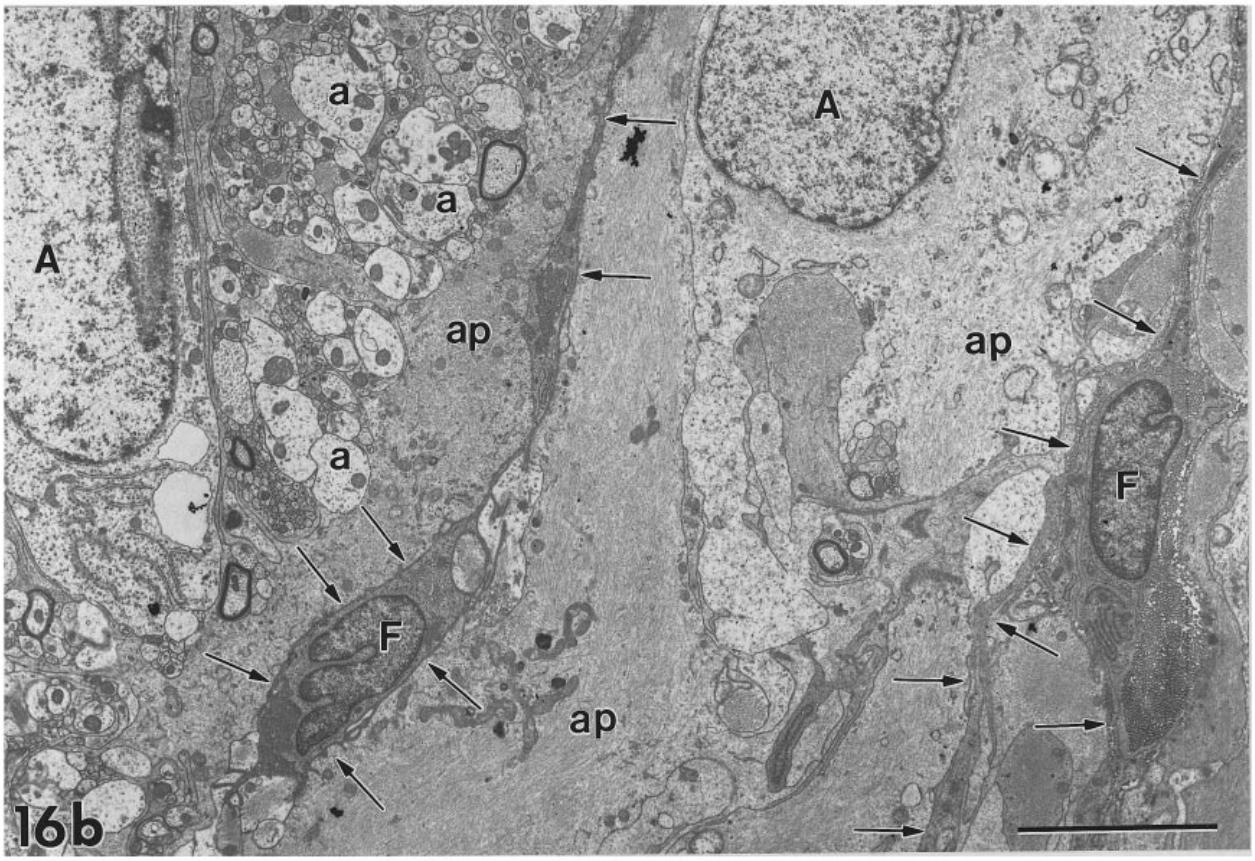


Fig. 15. Sagittal sections through the dorsal funiculi (DF) and adjacent grey matter showing HRP-labelled, dorsal nerve root axons that have regrown into spinal cords of irradiated animals following a crush-freeze lesion of the L4 dorsal root. The tracer, HRP, was placed on the root at a site distal to the lesion. (*a*) is an animal lesioned at 43 d of age and labelled with HRP 80 d later. Note the abundance of anterogradely labelled axons (arrows) in the dorsal funiculus. (*b*) is an animal lesioned at 23 d of age and labeled with HRP 30 d later. HRP-labelled axons can be seen in laminae 3 and 4 of the gray matter (arrows). Vibratome sections, 40 μm in thickness. Bar, 50 μm.

Fig. 13. Electron micrograph of a motor neuron (M) in an 83-d-old irradiated rat. Schwann cells (S) surround most of the neuronal cell body and in doing so cover synapses (arrows) or directly contact portions of the plasma membrane (arrowheads). Some perineuronal Schwann cells myelinate axons (sa) in the region. Bar, 2 μm. Inset: higher magnification of the Schwann cell-covered synapses (*) in the region indicated by the arrows. Bar, 0.5 μm.

Fig. 14. Electron micrograph of a large cluster of Schwann cells adjacent to a blood vessel (BV) in the ventral spinal cord 103 d following irradiation. Schwann cells (S) appear to spread into the ventral grey matter from sites adjacent to vessels. In these situations they often myelinate the larger calibre axons (sa) or ensheath axons (a) of smaller calibre. These regions are generally devoid of astrocyte processes. Bar, 2 μm.



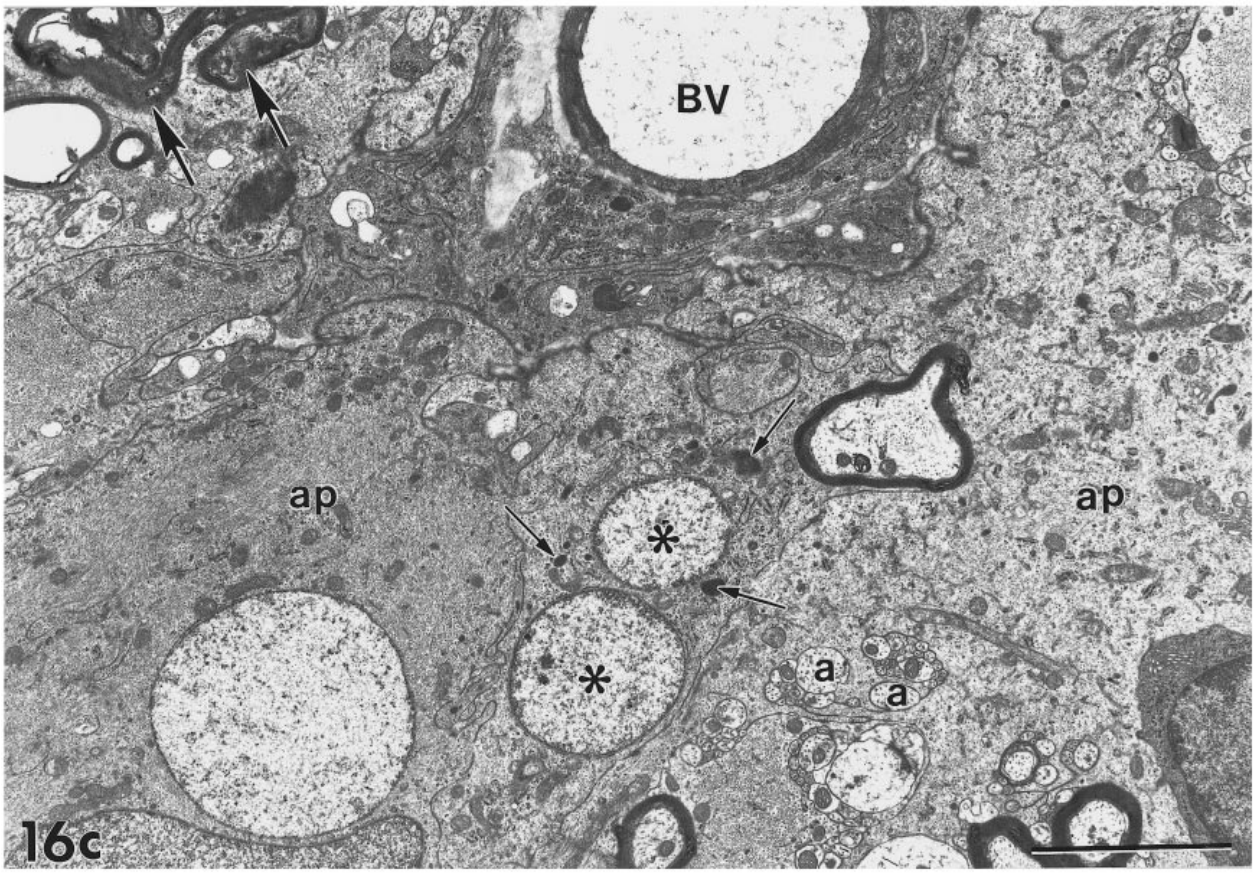


Fig. 16. For legend see opposite.

that blood vessels play key roles. Blood vessels as pathways for migration of Schwann cells in other experimental situations and models are well documented (Raine et al. 1978; dal Canto & Barbano, 1984; Harrison, 1985; Sasaki and Ide, 1989; Brook et al. 1993; Raisman et al. 1993; and others).

REGROWTH OF DORSAL SPINAL NERVE ROOT AXONS INTO THE ALTERED GLIAL ENVIRONMENT OF THE IRRADIATED SPINAL CORD

Injured dorsal root axons are known to grow robustly through the peripheral nervous system environment within the root but generally fail to enter or regrow within the CNS environment of the spinal cord (Reier et al. 1983; Liuzzi & Lasek, 1987). This failure of substantial regrowth of regenerating dorsal root axons within the CNS environment, along with the limited regrowth of CNS axons within that environment, is generally attributed to the glial constituents—astrocytes and/or oligodendrocytes (Reier, 1987; Reier & Houle, 1988; Schwab & Caroni, 1988; Liuzzi, 1990; Rudge & Silver, 1990; Schnell & Schwab, 1990; Cadelli et al. 1992). The glia-depleted environment of the irradiated spinal cord presents an interesting opportunity to explore astrocytic reactions to nerve root injury (Sims & Gilmore, 1992) and to test whether regrowth of dorsal root axons into the spinal cord is enhanced in the irradiated animals (Sims & Gilmore, 1994*a*, *b*). The L4 dorsal spinal nerve root was selected since its site of continuity with the spinal cord is well within the irradiated length. Astrocytic responses were examined in animals lesioned 20 d following irradiation (23 d of age) when the dorsal root entry zone contains few astrocytes or their processes (Fig. 9). The animals were allowed to survive for 60 d after lesioning, an interval which permits close comparison with changes described by others in root-lesioned but otherwise normal rats (Nathaniel & Nathaniel, 1973, 1977; Murray et al. 1990). These responses differ significantly between nonirradiated and irradiated animals. The response in the nonirradiated group includes formation of a thick scar composed of many layers of astrocyte processes, along with extension of the astrocytic response into the root. In contrast, a thin glia limitans consisting of only 1 or 2 layers of astrocyte processes is present in the root-lesioned, irradiated rats. This confirmation of the ineffectiveness of the remaining astrocyte population in mounting a significant response to the dorsal root injury then led to investigations of the ability of the lesioned L4 dorsal spinal nerve root axons to regrow into the CNS environment (Sims & Gilmore, 1992, 1994*a*, *b*). The timing of the nerve root injury in these studies varied from 9 to 47 d following irradiation. Animals lesioned up to 14 d represent a group in which dorsal root regrowth, if it occurs, would be into a glia-depleted environment. Animals lesioned later than 14 d following irradiation represent a condition in which Schwann cells are present within the dorsal white matter, as described above, where they can possibly exert a positive influence on dorsal root regrowth. Ultrastructural findings and results obtained using either anterograde (Fig. 15) or retrograde tracing methods demonstrate that injured dorsal root axons are not only capable of entering the spinal cord environment but also of growing into regions where they are known to occur in the normal spinal cord (Fig. 15) (Molander & Grant, 1986; Rivero-Melián & Grant, 1990). This is in contrast to the situation in nonirradiated animals in which regrowth into the spinal cord is essentially absent. Further, the tracing methods revealed a trend which strongly suggests that regrowth is enhanced with a lengthening of the interval between irradiation and root injury (Sims & Gilmore, 1994*a*). From these data it is inferred that the presence of Schwann cells at the later postirradiation intervals exerts a positive influence on regrowth of dorsal root axons into the CNS environment.

Recent and ongoing investigations continue to explore aspects of regrowth of dorsal nerve root axons into the irradiated spinal cord. The rationale for these investigations takes advantage of the state of glial depletion which exists at 20 d following irradiation

Fig. 16. Electron micrographs of lumbar spinal cords from 35-d-old irradiated rats that have received microinjections of cultured astrocytes. These cultures were established from 3-d-old rat spinal cords and were maintained for 8 d. Injections were made on d 20 following irradiation and the animals were perfused 12 d later. (*a*) shows the injection site on the dorsal surface of the cord. Many astrocyte processes (ap) are present and some appear to surround axons (a) or participate in formation of the glia limitans (arrows). (*b*) depicts the injection site in the dorsal grey matter. Astrocytes (A) and their processes (ap) are abundant and surround many of the axons (*a*). Fibroblasts (F) are surrounded by astrocyte processes which appear to create a barrier (arrows) between the fibroblasts and neuronal elements. These fibroblasts were either contaminants of the culture or migrated inward from the pia mater along the needle track. (*c*) is a region of the dorsal grey matter in an animal in which the L4 dorsal nerve root was lesioned at the time of injection. Astrocyte processes (ap) are abundant and surround axons (a) and a blood vessel (BV). An occasional astrocyte appears to be binucleate (*). The injected astrocytes usually contain lysosomes (small arrows) but do not appear to participate in removal of the degenerating myelin (large arrows) resulting from the root lesion. Bar, 4 μm.

and attempts to bias the environment by transplanting cultured central glial cells into it at the time the root is lesioned. Data analysed to date reveal that the injected astrocytes located near the surface of the spinal cord form a thick scar (Fig. 16*a*), creating a distinct astrocytic barrier which contrasts sharply with the situation occurring in irradiated, root-lesioned animals not injected with cultured astrocytes (Sims & Gilmore, 1992). These injected cells, when located at levels of greater depth within the spinal cord, establish relationships characteristic of indigenous astrocytes. For example, they form a barrier around fibroblasts (Fig. 16*b*) which probably enter the substance of the spinal cord along the track created by the needle used for injection. Note in Figure 16*b* that astrocyte processes are integrated with axons and separate them from the connective tissue elements. In addition, these injected astrocytes become associated with blood vessels (Fig. 16*c*). In general, the injected astrocytes carry out the roles anticipated from indigenous astrocytes. Regarding regrowth of injured dorsal root axons into the CNS environment in this experimental situation, preliminary data indicate that this reconstitution of astrocytic barriers by the cultured cells blocks regrowth of axons following dorsal nerve root lesion. Ongoing and future studies involve the transplantation of other types of cultured glia (oligodendrocytes or mixed astrocytes and oligodendrocytes) into the irradiated spinal cord in order to assess their roles in supporting or blocking regrowth of injured dorsal root axons in the CNS environment.

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