RECOVERY FROM ACUTE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN THE LEWIS RAT

EARLY RESTORATION OF NERVE CONDUCTION AND REPAIR BY SCHWANN CELLS AND OLIGODENDROCYTES

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SUMMARY

Light and electron microscopic histological studies and electrophysiological studies were performed on Lewis rats with acute experimental allergic encephalomyelitis (EAE) induced by whole spinal cord or myelin basic protein to determine the mechanism of clinical recovery. In these animals, total clinical recovery from complete paraplegia may occur as early as 4 days after the onset of hindlimb weakness. These studies indicate that this recovery occurs at a time when there is restoration of nerve conduction in the peripheral nervous system (PNS) and central nervous system (CNS) and when most demyelinated fibres have been invested, and some partially remyelinated, by Schwann cells or oligodendrocytes in the PNS and CNS, respectively. These findings support the hypothesis that the neurological signs of acute EAE are due to demyelination in the PNS and CNS.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is a disease of the nervous system induced by inoculation with whole central nervous tissue, purified myelin basic protein (MBP) or myelin proteolipid protein, together with adjuvants (Hashim *et al.,* 1980; Raine, 1984). It is widely studied as an animal model of human central nervous system (CNS) demyelinating disease, particularly multiple sclerosis (MS). EAE may have either an acute or a chronic relapsing course, but each form produces the same neurological signs, namely tail paralysis and limb ataxia, weakness and paralysis. Acute EAE is a monophasic disease like the human disease, acute disseminated encephalomyelitis, while chronic relapsing EAE has a relapsing remitting course resembling MS. In chronic relapsing EAE there are large plaques of CNS demyelination as in MS. However, in acute EAE such plaques are rare or do not occur. In fact, because of reports of the absence of demyelination in some animals with neurological signs of EAE it has been suggested that the neurological deficits of acute EAE and of the first episode of chronic relapsing EAE are due not to demyelination (Hoffman *et al.,* 1973; Lassmann and Wisniewski, 1979; Panitch and Ciccone, 1981; Raine *et al.,* 1981; Simmons *et al.,* 1981, 1982; Kerlero de Rosbo *et al.,* 1985), but to other factors such as oedema (Paterson, 1976; Simmons *et al.,* 1982; Kerlero de Rosbo *et al.,* 1985; Sedgwick *et al.,* 1987) or an impairment of monoaminergic neurotransmission (Carnegie, 1971; White, 1984). It has also been suggested that neurological recovery from acute EAE is too rapid to be accounted for by remyelination, and therefore that demyelination cannot be the cause of the neurological signs (Panitch and Ciccone, 1981; Simmons *et al.,* 1981). However, these studies have either failed to use sensitive histological techniques to detect demyelination or have failed to examine the whole nervous system thoroughly, particularly the lumbar, sacral and coccygeal spinal cord and the peripheral nervous system (PNS), which is known to be involved in EAE. We have recently shown that

demyelination in the PNS and the CNS is an important cause of the neurological signs in acute EAE by demonstrating demyelination and nerve conduction abnormalities in the dorsal root ganglia of rabbits (Pender and Sears, 1982, 1984) and, to a lesser extent, of rats with EAE induced by whole spinal cord (Pender and Sears, 1986), at the ventral root exit zone of the spinal cord in rats with EAE induced by whole spinal cord (Pender, 1986a, 1988a) and in the dorsal roots, ventral roots and ventral root exit zone in rats with EAE induced by MBP (Pender, 1986b, 1987, 1988b). Rats with hindlimb weakness due to EAE induced by whole spinal cord or MBP were found to have a reduced hindfoot H reflex which can be accounted for by demyelination affecting the CNS and PNS components of the monosynaptic reflex pathway (Pender, 1988a, b). In this report the rapid neurological recovery in rats with acute EAE induced by whole spinal cord or MBP is shown to be associated with the restoration of nerve conduction in the PNS and CNS at the time of investment and remyelination of demyelinated fibres by Schwann cells and oligodendrocytes.

MATERIAL AND METHODS

Animals

Male Lewis rats (JC strain) were kept 5 to a cage and with an unrestricted cage supply of rat and mouse cubes and water.

Induction of EAE

MBP-induced EAE. MBP was prepared from guinea pig spinal cord (after removal of the spinal roots) by the method of Deibler *et al*. (1972). MBP in 0.9% saline was emulsified in an equal volume of incomplete Freund's adjuvant (Commonwealth Serum Laboratories, Melbourne, Australia) containing 4 mg/ml of added Mycobacterium butyricum (Difco). Under ether anaesthesia rats, 8-10 wks old, were inoculated with 0.1 ml of emulsion in one footpad of each hindfoot. The total dose of MBP was 50 μg/rat.

Whole spinal cord-induced EAE. The inoculum was a homogenate of equal volumes of a 30% suspension of guinea pig spinal cord (the spinal roots having been removed) in 0.9% saline and a suspension of 4 mg of killed and dried Mycobacterium butyricum per ml of incomplete Freund's adjuvant. Rats, 8-10 wks old, received 0.05 ml of inoculum in the footpad of each of the four feet or 0.1 ml in one footpad of each hindfoot.

Management of inoculated animals

The rats were examined daily from the seventh day postinoculation and the clinical signs were recorded using the previously published grading system (Pender, 1986b). Hindlimb weakness was graded thus: 0 = no weakness; 1 = slight dragging of the toes of both hindfeet; 2 = severe dragging of both hindfeet but not of the rest of the hindlimbs; 3 = severe dragging of both hindlimbs, often with both hindlimbs displaced to one side of the body; 4 = total paralysis of the hindlimbs. Electrophysiological studies were performed in terminal experiments on 10 rats with MBP-induced EAE (5 at the peak of hindlimb weakness and 5 that had just fully recovered from hindlimb weakness) and on 12 rats with whole spinal cord-induced EAE (5 at the peak of hindlimb weakness and 7 after recovery from hindlimb weakness). Histological studies were carried out on 5 rats with MBP-induced EAE (3 at the peak of hindlimb weakness and 2 during or immediately after recovery from hindlimb weakness) and on 5 rats with whole spinal cord-induced EAE

(4 at the peak of hindlimb weakness and 1 after recovery from hindlimb weakness). The last animal was studied electrophysiologically before it was perfused for histological study.

Controls

Normal male Lewis rats, 10-12 wks old, served as controls for the histological (2 rats) and electrophysiological studies (9 rats). As these studies were performed on the animals with EAE about 2 wks after inoculation, the control animals were the same age as the animals with EAE when studied.

Electrophysiological studies

Anaesthesia. Anaesthesia was induced with urethane (25% in 0.9% saline, intraperitoneal (i.p.), 5 ml/kg for controls, 4 ml/kg for rats with EAE undergoing H reflex studies but not laminectomy, and 4-5 ml/kg for rats with EAE undergoing laminectomy for ventral root and ventral root exit zone recordings). The dose of urethane/kg required to obtain a given depth of anaesthesia was lower in rats with EAE than in normal controls. Pentobarbitone sodium (i.p., 12 mg/kg) supplemented the urethane anaesthesia. The animals breathed spontaneously through a tracheostomy. At the beginning of each experiment 9 ml of Hartmann's solution (compound sodium lactate BP, Travenol) were given i.p.

M wave and H reflex recordings. The rat was mounted in an animal frame, and a metal box, through which water at 37°C was circulated, was placed under the animal. Because of the effects of anaesthetics on synaptic transmission it was important that, for the H reflex studies, an adequate depth of anaesthesia was maintained without depressing the corneal reflex. The left sciatic nerve was exposed in the posterior thigh and immersed in paraffin oil maintained at 37°C by radiant heat. Under these conditions the rectal temperature was 37°C-38°C. The freed sciatic nerve was lifted away from the volume conductor and stimulated in continuity with platinum electrodes 3 mm apart (cathode distal) delivering 0.1 ms square-wave voltage pulses at 1 Hz. Recordings were made with a 25 gauge needle electrode in the belly of the fourth dorsal interosseus muscle and with a reference 25 gauge needle electrode subcutaneously in the plantar aspect of the distal fourth digit of the left hindfoot.

Ventral root and ventral root exit zone recordings. Under urethane and pentobarbitone anaesthesia a T12-L4 laminectomy was performed and the left sciatic nerve was exposed in the posterior thigh as previously described (Pender and Sears, 1986). Radiant heat maintained the laminectomy and sciatic nerve pools at 37°C. The left L2-L6 dorsal roots were cut close to the spinal cord and displaced laterally. By freeing the two most caudal left denticulate ligaments from the dura and tying them to right paravertebral tendons, the spinal cord was rotated through 90° so that its ventral surface faced laterally to the left (*see* Pender, 1986*a*). The freed left sciatic nerve was lifted away from the volume conductor and stimulated in continuity with platinum electrodes 3 mm apart delivering 0.1 ms square-wave voltage pulses at 1.0 Hz. Volume conductor recordings were made with a 0.5 mm diameter silver ball electrode over one or more, in turn, of the left L4, L5 and L6 ventral roots (VRs), 1-3 mm distal to the respective ventral root exit zones (VREZs), and over the rostral parts of the VREZs. A reference electrode was placed on the right paravertebral region at the same level. At the end of each experiment the dissection was extended to confirm that the L4 and L5 spinal nerves always gave large contributions to the sciatic nerve and that the L3 and L6 spinal nerves gave small contributions.

Statistical analysis

Analysis of variance was used to compare the recordings from normal control rats with those from rats with hindlimb weakness due to MBP-induced EAE (Pender, 1988*b*), rats with hindlimb weakness due to whole spinal cord-induced EAE (Pender, 1988*a*) and rats recovering from each form of EAE to minimize the chance occurrence of statistically significant differences when multiple comparisons were made.

Histological studies

The methods have been described in detail previously (Pender, 1985, 1986*b*). Under ether anaesthesia the rats were perfused through the left ventricle with 0.9% saline followed by 2.5% (or 2%) glutaraldehyde/2% formaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3-7.4). The brain, optic nerves, spinal cord, dorsal and ventral roots, dorsal root ganglia, spinal nerves, sciatic and tail nerves were removed and immersed in fixative. The tissues were postfixed with 2% osmium tetroxide and were embedded either in HistoResin (LKB Bromma) or Epok 812 (Ernest F. Fullam, Schenectady, NY). HistoResin sections (2 μm) were stained with toluidine blue in phosphate buffer (pH 7.6) or with cresyl violet. Semithin (1 μm) Epok 812 sections were stained with toluidine blue for light microscopy, and ultrathin sections were double-stained with uranyl acetate and lead citrate and examined in a Philips 301 electron microscope.

RESULTS

Clinical findings

Distal tail weakness commenced 8-11 days after inoculation and was followed by flaccid tail paralysis, hindlimb weakness and sometimes hindlimb paralysis. Forelimb weakness occurred occasionally. The temporal profiles of hindlimb weakness in rats undergoing H reflex studies are shown in fig. 1. This shows that hindlimb weakness commenced earlier and lasted longer in rats with whole spinal cord-induced EAE than in rats with MBP-induced EAE. For both whole spinal cord-induced EAE and MBP-induced EAE the time course and severity of maximal hindlimb weakness were similar for animals studied at the time of peak hindlimb weakness and for those studied after recovery from hindlimb weakness. The ascending tail weakness was accompanied by an ascending impairment of tail nociception as previously described (Pender, 1986*b*). The recovery process began rapidly. Often there was complete recovery of tail nociception and of hindlimb motor function by 15 days after inoculation, but moderate tail weakness persisted for several days longer. By 20 days after inoculation there was usually only mild distal tail weakness. The shortest time for total clinical hindlimb recovery from complete paraplegia was 4 days after the onset of hindlimb weakness, and this was in a rat with MBP-induced EAE.
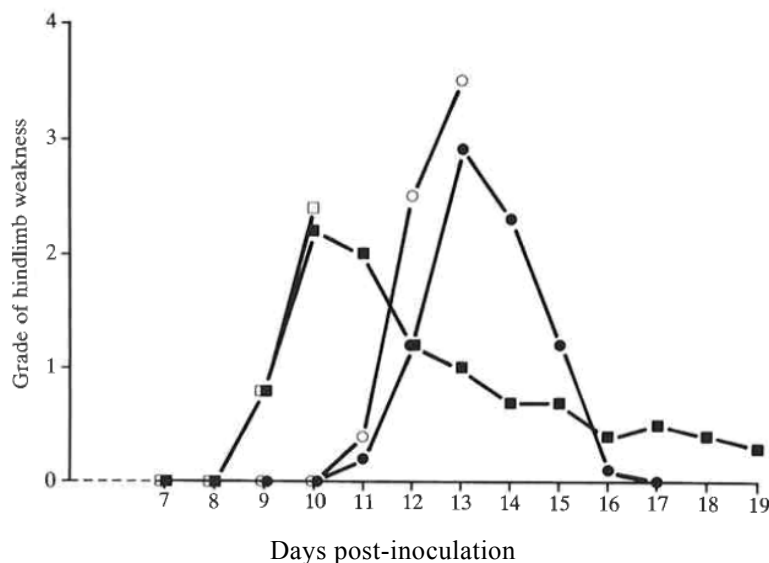
FIG. 1. Mean grades of hindlimb weakness plotted against days postinoculation in rats with whole spinal cord-induced EAE (squares) and rats with MBP-induced EAE (circles). The hindlimb weakness in the 5 animals undergoing H reflex studies at the peak of hindlimb weakness (open squares and circles) and in the 5 undergoing II reflex studies after recovery from hindlimb weakness (closed squares and circles) are shown for each type of EAE. If the grades differed between hindlimbs in the same animal, the mean of the two grades was used for that animal. The graphs show that hindlimb weakness commenced earlier and lasted longer in whole spinal cord-induced EAE than in MBP-induced EAE.

H reflex studies

Normal controls

The results of studies on the fourth dorsal interosseus H reflex and M wave elicited by direct sciatic nerve stimulation in normal controls are shown in figs 2, 3 and 4 and the Table. The H reflex was maximal at a lower stimulus intensity than that giving the maximal M wave. As the stimulus intensity was increased above that giving the maximal H reflex, the amplitude of the H reflex declined as a result of collision, in the ventral root, between the orthodromic impulses of the H reflex and antidromic impulses evoked by direct activation of sciatic nerve motor fibres. As the amplitude of the H reflex was greater after a period of no stimulation for several seconds, the maximal H reflex was usually recorded as the response to the first stimulus after a 5 s period of no stimulation. Selective sectioning and stimulation of individual lumbar spinal roots established that the H reflex of the fourth dorsal interosseus muscle is mediated through the L5 dorsal and ventral roots and to a lesser extent the L6 ventral root. The maximal reflex response obtained by sciatic nerve stimulation had the same amplitude as that elicited by selective L5 dorsal root stimulation, indicating that low intensity stimulation of the sciatic nerve did not result in significant collision in the ventral root. To assess the lumbar monosynaptic reflex pathway in EAE, direct sciatic nerve stimulation was used rather than L5 dorsal root stimulation because the former technique requires less anaesthesia (and hence less depression of synaptic transmission) and does not bypass the L5 dorsal root ganglion and distal dorsal root, which are sites of lesions in EAE. Under the controlled conditions used in these experiments the ratio of the peak-to-peak amplitude of the maximal H reflex to that of the maximal M wave (H/M ratio) has a narrow range in normal rats (fig. 2) and serves as a reliable indicator of the integrity of the monosynaptic

reflex arc. It also allows the assessment of function in relation to a specifically defined muscle group.
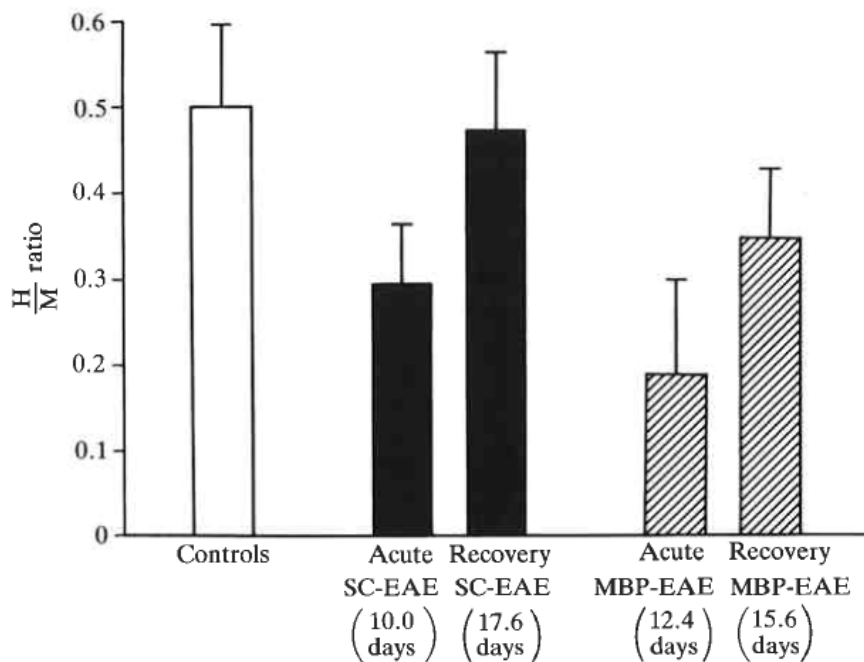

FIG. 2. Mean H/M ratios in normal control rats, rats at the peak of hindlimb weakness due to whole spinal cord-induced EAE (acute SC-EAE), rats after recovery from hindlimb weakness due to whole spinal cord-induced EAE (recovery SC-EAE), rats at the peak of hindlimb weakness due to MBP-induced EAE (acute MBPEAE) and rats after recovery from hindlimb weakness due to MBP-induced EAE (recovery MBP-EAE). There were 5 rats in each group. Mean values are shown with 1 SD. The mean day (postinoculation) on which the studies were performed in each group is bracketed. There were highly significant differences between each of the following groups: acute SC-EAE and controls *(P < 0.005)*; recovery SC-EAE and acute SC-EAE *(P < 0.005)*; acute MBP-EAE and controls *(P < 0.001)*; recovery MBP-EAE and acute MBP-EAE *(P = 0.01)*.

MBP-induced EAE

In MBP-induced EAE the H/M ratio was severely reduced at the time of maximal hindlimb weakness and returned towards normal during recovery (fig. 2). At the peak of hindlimb weakness (12-13 days after inoculation), the M wave was normal but the H reflex was markedly reduced in amplitude, without temporal dispersion, and was of slightly prolonged latency (Pender, 1988b; fig. 3, Table). As shown in the Table there was a highly significant reduction in the mean H/M ratio compared with that in normal controls. These findings indicate normal conduction in peripheral nerve but interruption of the monosynaptic reflex arc. On the first day of complete clinical recovery of hindlimb motor function (15-17 days after inoculation) the mean H/M ratio was significantly greater than in animals with maximal hindlimb weakness but was still significantly less than in normal controls (figs 2, 3; Table). The interval between the onset of the M wave and the onset of the H reflex was shorter in rats after hindlimb recovery than in rats with maximal hindlimb weakness, but this difference was not statistically significant (Table).

FIG. 3 *(left)*. Maximal H reflex (H) and maximal M wave (M) elicited in the left fourth dorsal interosseus muscle by sciatic nerve stimulation in a normal control rat (A), in a rat on the day of onset of hindlimb weakness due to MBP-induced EAE (B) and in a rat on the first day of full clinical recovery from hindlimb weakness due to MBP-induced EAE (C).

FIG. 4 *(right)*. Maximal H reflex (H) and maximal M wave (M) elicited in the left fourth dorsal interosseus muscle by sciatic nerve stimulation in a normal control rat (A), in a rat with hindlimb weakness due to whole spinal cord-induced EAE (B) and in a rat on the first day of full clinical recovery from hindlimb weakness due to whole spinal cord-induced EAE (C).

Whole spinal cord-induced EAE

The findings in rats with whole spinal cord-induced EAE were similar to those described above. At the peak of hindlimb weakness (10 days after inoculation) the M wave was normal and the H reflex was reduced (Pender, 1988*a*; fig. 4, Table). As shown in fig. 2 and the Table there was a highly significant reduction in the mean H/M ratio compared with that in normal controls. After hindlimb recovery (14-25 days after inoculation) the mean H/M ratio was significantly higher than in rats at the peak of hindlimb weakness and was not significantly different from that in normal controls (figs 2, 4; Table). The H reflex was temporally dispersed in 1 rat showing recovery, although the latency to onset of the H reflex was not prolonged.

TABLE. M WAVES AND H REFLEXES IN EAE

| | | Groups (mean ± SD) N=5 in each group | | | | Analysis of variance | Comparisons between means | | | | | |
| | | MBF-EAE | | SC-EAE | | | | | | | | |
Parameter	Controls	Acute	Recovery	Acute	Recovery	Value and significance	Acute MBP-EAE and controls	Recovery MBP-EAE and controls	Recovery MBP-EAE and acute MBP-EAE	Acute SC-EAE and controls	Recovery SC-EAE and controls	Recovery SC-EAE and acute SC-EAE
M amp [a] (mV)	4.6±1.1	5.7±1.3	5.9±1.4	4.6±1.6	3.2±1.2	$F=3.19$ $P < 0.05$	n.s. (P>0.05)	n.s	n.s.	n.s.	n.s.	n.s.
$\frac{\text{H amp}}{\text{M amp}}$	0.50±0.09	0.19±0.11	0.35±0.08	0.29±0.07	0.47±0.09	$F=10.44$ $P<0.001$	$P<0.001$	$P<0.025$	$P=0.01$	$P<0.005$	n.s.	$P<0.005$
M latency [b] (ms)	2.23±0.34	2.42±0.24	2.30±0.15	2.39±0.28	2.52±0.33	$F=0.84$ n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
M-H latency [c] (ms)	4.62±0.28	5.12±0.52	4.84±0.34	4.87±0.26	4.68±0.33	$F=1.49$ n.s.	$P<0.05$	n.s.	n.s.	n.s.	n.s.	n.s.

Maximal M wave (M) and maximal H reflex (H) elicited in fourth dorsal interosseus muscle by sciatic nerve stimulation in normal control rats, rats with hindlimb weakness due to MBP-induced EAE (acute MBP-EAE), rats that have recovered from hindlimb weakness due to MBP-induced EAE (recovery MBP-EAE), rats with hindlimb weakness due to whole spinal cord-induced EAE (acute SC-EAE) and rats that have recovered from hindlimb weakness due to whole spinal cord-induced EAE (recovery SC-EAE).

[a] Peak-to-peak amplitude. [b] Latency to onset of maximal M wave. [c] Difference between latency to onset of maximal M wave and latency to onset of maximal H reflex.

n.s. = not significant.

Ventral root and ventral root exit zone recordings

While the H reflex is a useful technique for studying nerve conduction in the entire lumbar monosynaptic reflex pathway, it has the disadvantage that it is also dependent on synaptic transmission. To assess nerve conduction directly, antidromically conducted compound action potentials evoked by sciatic nerve stimulation were recorded in the volume conductor over the lumbar VREZs and respective VRs in 2 rats showing hindlimb recovery (16 and 21 days after inoculation). In the normal control rat the VR response is a biphasic wave (positive, negative) with the negativity being greater than the initial positivity (figs 5A, 6A). The positivity is due to passive outward current driven by the approaching impulses, and the negativity is due to active inward current occurring during the rising phase of the action potential under the recording electrode. The VREZ response in the normal control rat is similar (figs 5A, 6A). In rats with hindlimb weakness due to whole spinal cord-induced EAE the VR recording is normal apart from a slight reduction in the conduction velocity of the peak negativity (Pender, 1988a). However, the VREZ recording shows a marked reduction in the
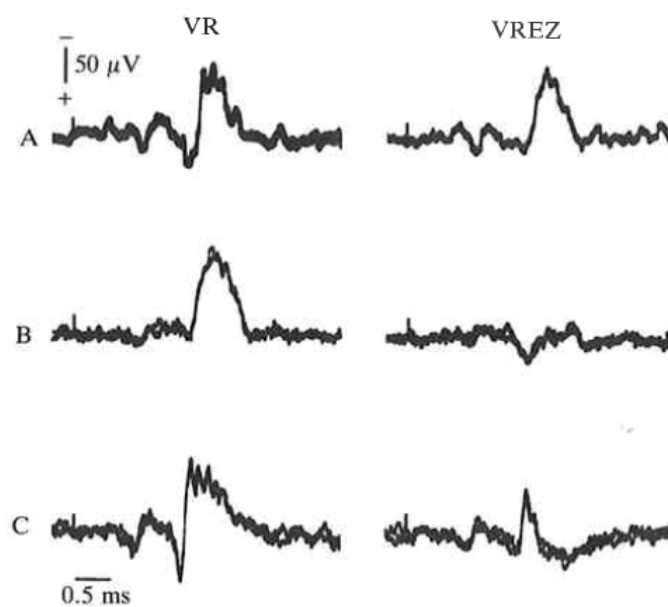

FIG. 5. Volume conductor recordings of the maximal L6 ventral root (VR) and L6 ventral root exit zone (VREZ) compound action potentials evoked by sciatic nerve stimulation in a normal control rat (A), in a rat with hindlimh weakness due to whole spinal cord-induced EAE (B) and in a rat 6 days alter full clinical recovery from hindlimb weakness due to whole spinal cord-induced EAF. (C).

amplitude of the negativity, indicating conduction block in many fibres at the VREZ (Pender, 1986a, 1988a; fig. *5B).* In rats showing hindlimb recovery, the recordings over some lumbar VRs showed temporal dispersion due to slowing of conduction (fig. *6B).* Such slowing probably indicates the restoration of conduction in previously blocked VR fibres, as was observed in the dorsal root ganglia of rabbits recovering from EAE (Pender and Sears, *1984).* VREZ recordings in rats with hindlimb recovery revealed either a temporally dispersed waveform with a reduced negativity (fig. *6B)* or a response with a negativity greater than the initial positivity (fig. *5c).* The former is a complex waveform probably due to phase cancellations resulting from slowed conduction in the peripheral VR as well as slowed conduction through the VREZ; the latter indicates restoration of conduction in many fibres at the VREZ.
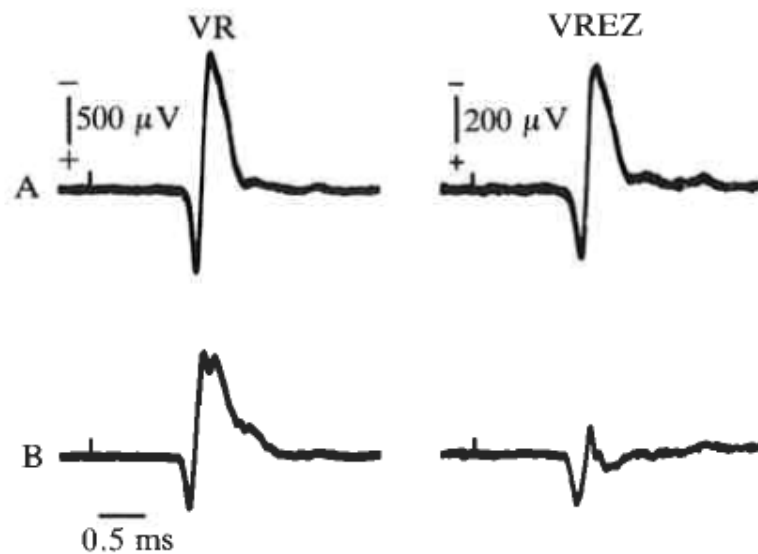
FIG. 6. Volume conductor recordings of the maximal L4 ventral root (VR) and L4 ventral root exit zone (VREZ) compound action potentials evoked by sciatic nerve stimulation in a normal control rat (A) and in a rat 6 days after full clinical recovery from hindlimb weakness due to whole spinal cord-induced EAE (B).

Histological findings

At the time of hindlimb weakness

In rats with hindlimb weakness due to whole spinal cord-induced EAE, there was perivascular and subpial inflammation and demyelination in the brainstem and spinal cord, and perivascular inflammation and demyelination in the dorsal root ganglia and dorsal and ventral spinal roots. There was minimal, if any, involvement of the spinal nerves and peripheral nerves. In the spinal cord the ventral root exit zones and the dorsal root entry zones were sites of predilection. The lesions in MBP-induced EAE were similar except that there was less demyelination in the spinal cord and minimal demyelination in the dorsal root ganglia (Pender, unpublished results). Electron microscopic examination showed invasion of the myelin sheath by macrophages containing myelin debris (fig. 7A) and association of demyelinated axons with such macrophages (fig. 7B). Somedemyelinated PNS axons were invested by debris-free Schwann cell processes as described below during the recovery phase; however, only a small proportion of the invested fibres, and these were all small diameter fibres, showed any significant promyelin stage, and there was no evidence of compact myelin formation. Several degenerating fibres were seen.

During/after recovery from hindlimb weakness due to MBP-induced EAE

To investigate the structural basis of functional recovery, it was essential to examine tissue during early recovery. Here the detailed histology in rats during, or immediately after, recovery from hindlimb weakness due to MBP-induced EAE (15 days after inoculation) is described. The reparative processes detailed below occurred in lumbar, sacral and coccygeal segments.
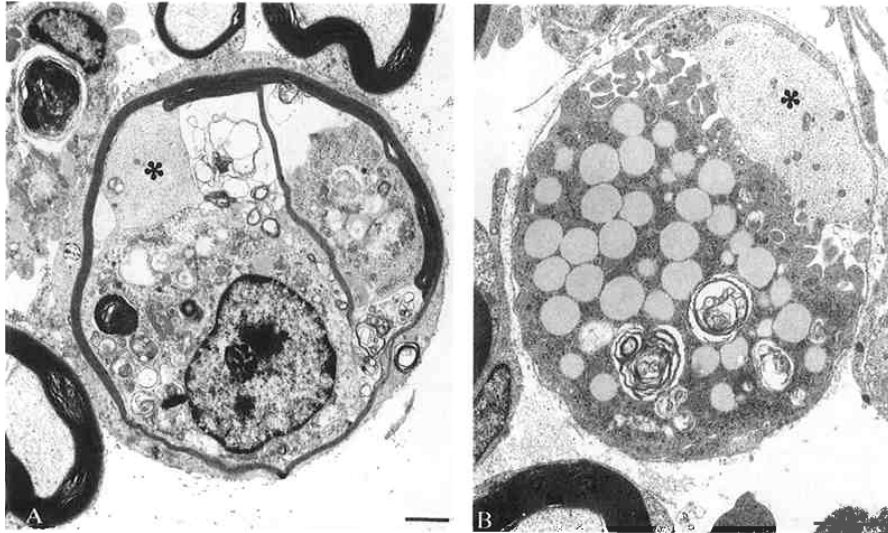
FIG. 7. Electron micrographs from a rat 2 days after the onset of tail weakness due to MBP-induced EAE (12 days after inoculation). A, first coccygeal dorsal root showing invasion of a myelin sheath by a macrophage containing myelin debris. The axon is shrunken (asterisk). B, S3 dorsal root showing a demyelinated axon (asterisk) closely associated with a macrophage containing myelin debris. Bars = 2 μm.

PNS. Light microscopic examination of the PNS showed demyelinated axons (fig. 8A), axons with inappropriately thin myelin sheaths, and some degenerating axons. Electron microscopic examination of the PNS showed that the majority of demyelinated axons were invested by at least one, and often several, layer(s) of debris-free Schwann cell processes (figs 8B, 9) or by thin compact myelin within a Schwann cell basal lamina (fig. 10). The maximum number of myelin lamellae was 8. That these lamellae were the product of new myelin formation and not the residue of partial demyelination was indicated by the criteria of Harrison *et al.* (1972) for remyelination. First, the configuration of the myelin resembled that seen during primary myelination in development. Secondly, unlike partially demyelinated fibres, the myelin lamellae were intact and were not invaded by debris-containing macrophages. Thirdly, thinly myelinated fibres with these characteristics of remyelination were seen only after a stage when completely demyelinated fibres were consistently found.
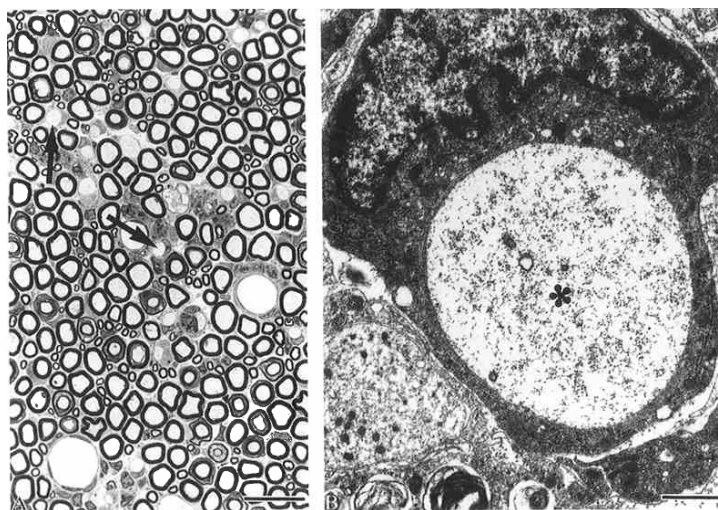

FIG. 8. A, transverse section through L5 ventral root of a rat recovering from complete paraplegia due to MBP-induced EAE, 15 days after inoculation. At this stage there was only mild hindlimb weakness. Demyelinated axons are present (arrows). Epok 812 section stained with toluidine blue. Bar = 25 μm. B, electron micrograph of L6 ventral root from the same animal showing investment of a demyelinated axon (asterisk) by a debris-free Schwann cell. Bar = 0.5 μm.

Occasionally there was dense undercoating of a region of demyelinated axolemma separated by a uniform intercellular cleft from a debris-free Schwann cell process loaded with mitochondria (fig. 11), suggesting the development of a new node-like axonal specialization, as described in amyelinated PNS fibres in the dystrophic mouse (Rosenbluth, 1979). In transverse sections such axonal specializations were restricted to short lengths of the circumference. In fig. 11 a node-like axolemmal specialization lies immediately adjacent to a region of exceedingly close apposition between the axolemma and the indenting Schwann cell process, a region reminiscent of the paranodal-type axolemmal specializations described in the same model (Rosenbluth, 1979). Macrophages containing myelin debris were also present in these lesions but, unlike the earlier lesions, their processes were separated from the demyelinated axons by debris-free Schwann cell processes, or new compact myelin, and a Schwann cell basal lamina.

FIG. 9. Electron micrograph of S4 dorsal root of a rat that had just fully recovered from tail nociceptive impairment and hindlimb weakness due to MBP-induced EAE, 15 days after inoculation. A demyelinated axon (asterisk) is invested by a Schwann cell. Bar = 5 μm.
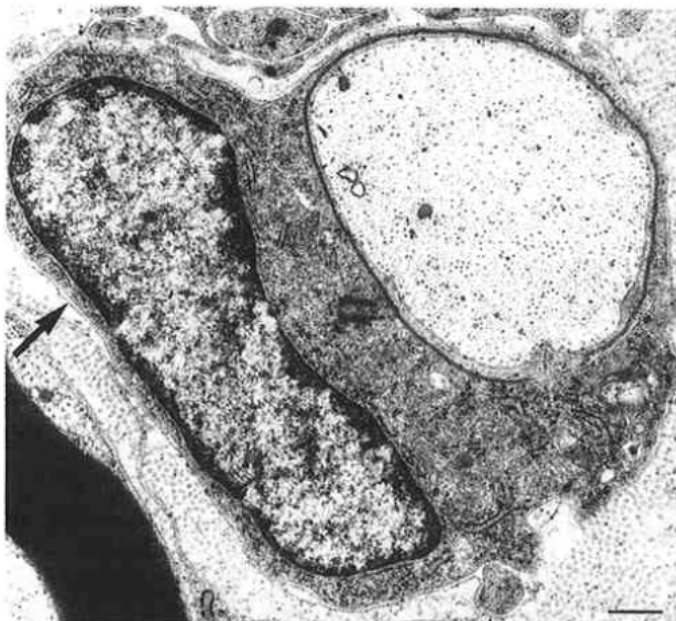

FIG. 10. Electron micrograph showing remyelination by a Schwann cell in the first coccygeal dorsal root of the same rat as in fig. 9. The basal lamina of the Schwann cell is visible (arrow). Bar = 0.5 μm.
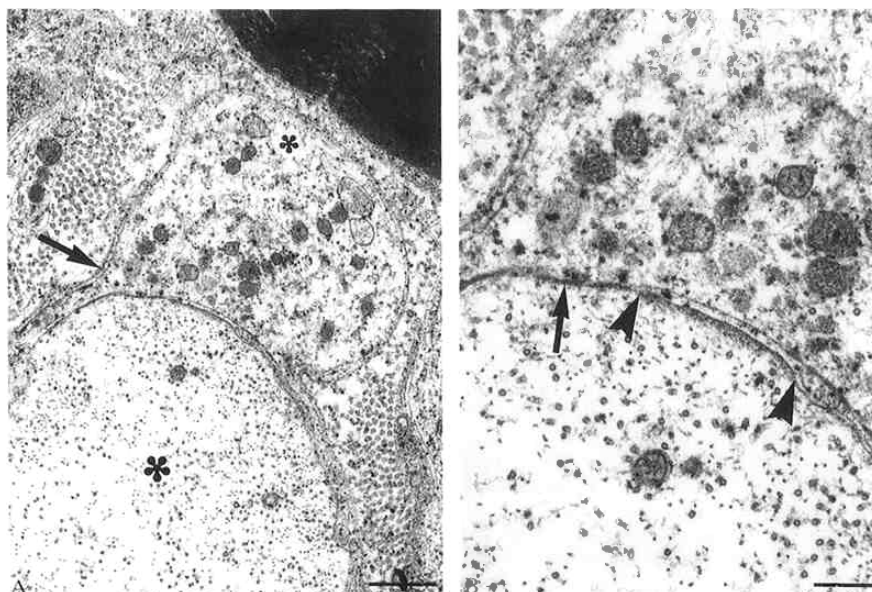
FIG. 11. Electron micrographs of L4 ventral root of a rat on the first day of full recovery from hindlimb weakness due to MBP-induced EAE, 15 days after inoculation. A, a demyelinated axon (large asterisk) is closely associated with a mitochondria-laden Schwann cell process (small asterisk) within a basal lamina (arrow). Bar = 0.5 μm. B, detail at higher power showing a region of subaxolemmal density (between arrowheads) where the axon is separated from the Schwann cell process by a relatively constant intercellular cleft. Adjacent to this region, the Schwann cell process is exceedingly closely apposed to the axolemma and indents it (arrow). Bar = 0.2 μm.

CNS. Light microscopic examination of the CNS showed demyelinated axons, some degenerating axons and resolution of inflammation in the spinal cord. Electron microscopic examination of the spinal cord showed demyelinated axons invested by debris-free glial processes without a basal lamina. It was sometimes difficult to distinguish between astrocyte and oligodendrocyte processes but at other times it was clear that demyelinated axons were invested by oligodendrocyte processes that could be distinguished from astrocyte processes by their denser cytoplasm and absence of intermediate filaments (fig. 12). Noninvested parts of the axolemma of oligodendrocyte-invested axons were often closely apposed to normally myelinated fibres (fig. 12). Occasionally there was compaction of an investing oligodendrocytic loop to form a major dense line. In the central tissue projection of the ventral root, an area normally myelinated by oligodendrocytes (Fraher and Kaar, 1986), there were demyelinated fibres invested by debris-free oligodendrocyte and astrocyte processes. Several of these fibres each had a region of subaxolemmal density in close association with an astrocytic process the membrane of which was separated from the axolemma by a uniform cleft. These regions were restricted to short lengths of the axonal circumference and closely resembled the node-like axolemmal specializations described in spinal cord demyelinated by ethidium bromide (Rosenbluth *et al.,* 1985). Some of these specializations lay immediately adjacent to paranodal-type axolemmal specializations closely associated with oligodendrocyte processes *(see* Rosenbluth *et al.,* 1985). The oligodendrocyte-invested fibres in the central tissue projection lay within a dense matrix of astrocyte processes enclosed within the glia limitans.
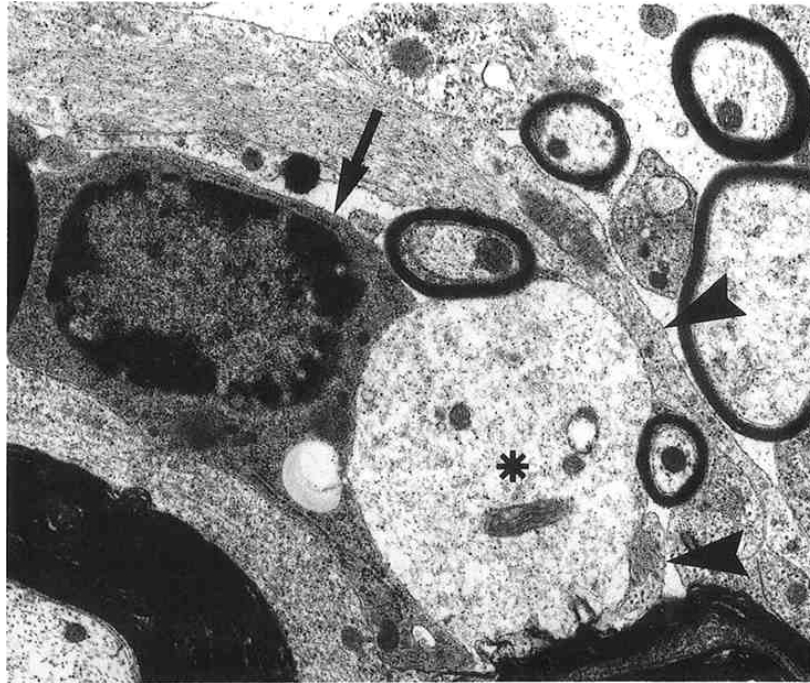
FIG. 12. Electron micrograph of a ventral column of the L5 spinal cord of a rat that had just fully recovered from hindlimb weakness due to MBP-induced EAE, 15 days after inoculation. A demyelinated axon (asterisk) is closely associated with an oligodendrocyte (arrow) and astrocytic processes (arrowheads). The demyelinated axolemma is also closely apposed to the outermost myelin lamellae of normally myelinated axons. Bar = 0.5 μm.

Around some axons the investing oligodendroglial loop was compacted to form a single myelin lamella. There was no evidence of investment or remyelination of CNS axons by Schwann cells, either in the spinal cord or in the central tissue projection.

After recovery from hindlimb weakness due to whole spinal cord-induced EAE

The following observations were made just after recovery from hindlimb weakness due to whole spinal cord-induced EAE (17 days after inoculation). The reparative processes described below were present in lumbar, sacral and coccygeal segments.

PNS. Light microscopic examination of the PNS showed demyelinated fibres, fibres with inappropriately thin myelin, and degenerating fibres in the dorsal root ganglia and spinal roots (fig. 13A). Electron microscopy of the PNS revealed Schwann cell investment and remyelination of demyelinated fibres as in the recovery phase of MBP-induced EAE, except that remyelination was generally more advanced, with the formation of up to 20 new myelin lamellae. The latter was probably because this study was made 2 days later than for MBP-induced EAE.
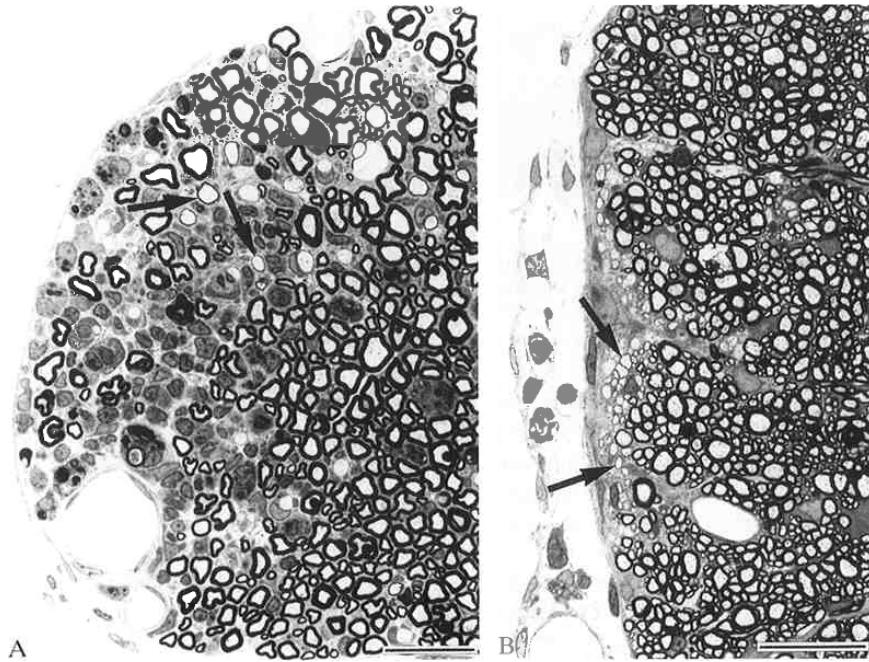
FIG. 13. A, transverse section through L6 ventral root of a rat that had just fully recovered from hindlimb weakness due to whole spinal cord-induced EAE, 17 days after inoculation. There is well established remyelination of many axons (arrows). B, transverse section through a ventral column of S4 spinal cord of the same animal. Many subpial axons (arrows) are being remyelinated. Epok 812 sections stained with toluidine blue. Bars = 25 μm.
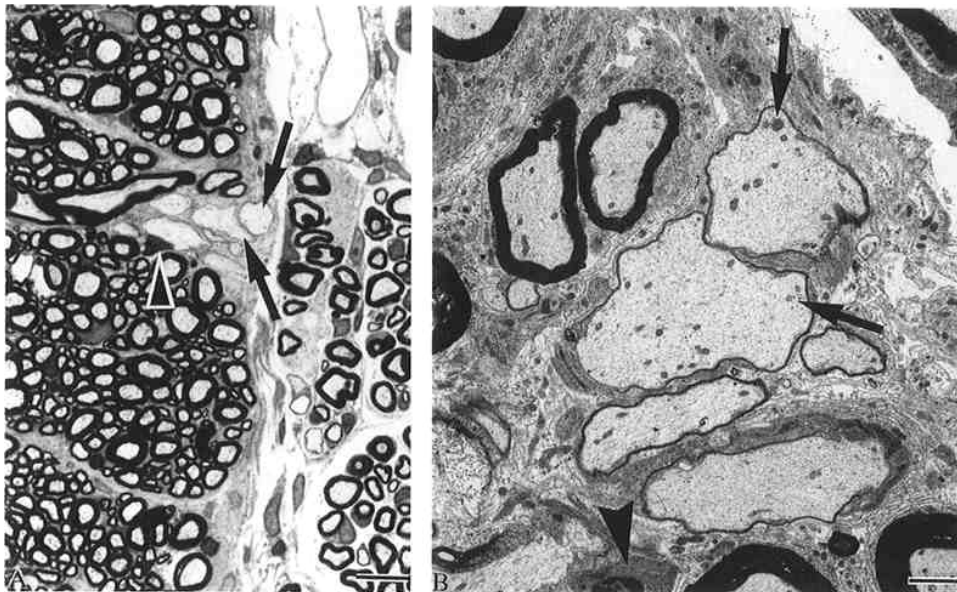

FIG. 14. A, transverse section through L5 ventral root exit zone of the spinal cord of the same rat as in fig. 13. Several fibres (arrows) in the CNS portion of the ventral root exit zone are being remyelinated. An oligodendrocyte (arrowhead) lies adjacent to one of these fibres. Epok 812 section stained with toluidine blue. Bar = 10 μm. B, electron micrograph showing detail in A. The same remyelinated fibres (arrows) and oligodendrocyte (arrowhead) can be seen. Bar = 2 μm.

CNS. Light microscopic examination of the CNS showed demyelinated fibres, fibres with inappropriately thin myelin, and degenerating fibres in the spinal cord, and resolution of meningeal and spinal cord inflammation (figs 13B, 14A). The changes were similar to those in the recovery phase of MBP-induced EAE with the exception that there was more widespread remyelination because whole spinal cord-induced acute EAE results in more spinal cord demyelination (Pender, unpublished results). Electron microscopy of the spinal cord showed that there were many fibres being remyelinated with up to 7 new myelin lamellae. Such remyelinated fibres were found in the VREZ of the lumbar spinal cord (fig. 14B), in other subpial regions of the spinal cord (fig. 15) and in the central tissue projections of the ventral roots. The newly formed myelin sheaths in the VREZ were much thinner than the thin sheaths that occur here in normal rats (Pender, 1988*a*) and thus could be distinguished from the latter. It was concluded that the remyelination of the CNS was performed by oligodendrocytes rather than by Schwann cells because: (1) the newly formed myelin was not encircled by the cytoplasm of the myelinating cell but originated by the compaction of microtubuleladen cytoplasmic loops which occupied only a small proportion of the fibre circumference; (2) there was no basal lamina around any of the remyelinated fibres; and (3) the remyelinated fibres were closely apposed to each other and to normal myelinated fibres as in the normal CNS (fig. 15B). There was no evidence of investment or remyelination of CNS axons by Schwann cells, even within the transitional VREZ. Most of the CNS fibres that were still demyelinated were invested by debris-free oligodendrocyte and astrocyte processes. As in the recovery phase of MBP-induced EAE there were some demyelinated axons invested by glial cells with node-like and paranodal-type axolemmal specializations.
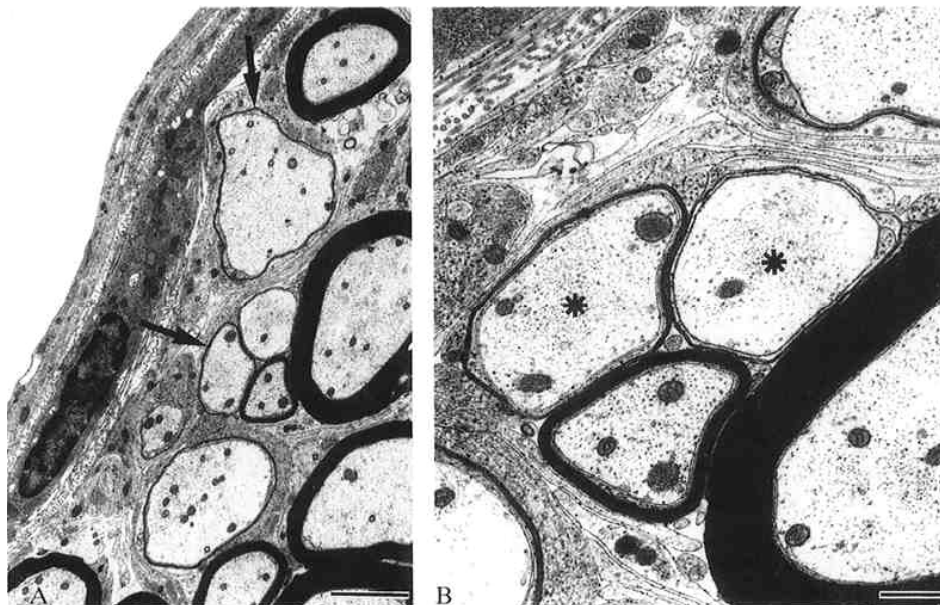

FIG. 15. A, electron micrograph showing remyelination of subpial axons (arrows) in a ventral column of L5 spinal cord of the same animal as in fig. 13. Bar = 2μm. B, high power detail of A showing apposition of newly formed myelin sheaths of 2 axons (asterisks) without an intervening basal lamina. Bar = 0.5 μm.

DISCUSSION

The present study demonstrates that, in acute EAE in the rat, rapid clinical recovery from hindlimb weakness is closely associated with the restoration of the hindfoot H reflex. The reduction in the amplitude, without temporal dispersion, of the H reflex in rats with hindlimb weakness due to EAE indicates either (1) conduction block in fibres mediating the reflex or (2) decreased synaptic transmission between the Ia afferent terminals and motor neurons. Demyelination and consequent conduction block in the L5 dorsal root ganglion, dorsal root, and dorsal root entry zone and L5 and L6 intramedullary ventral roots, ventral root exit zones (CNS and PNS) and extramedullary ventral roots can account for the depressed H reflex (Pender, 1988a, b). The lack of a major delay in the M-H latency could be accounted for by sparing of some of the fastest fibres. Demyelination of descending pathways in the brainstem and spinal cord could also alter the H reflex by increasing or decreasing excitability of the motor neuron pool. This may occur in whole spinal cord-induced EAE but seems unlikely in MBP-EAE, because of the paucity of CNS demyelination in the latter. The restoration of the H reflex during recovery is interpreted as indicating restoration of nerve conduction in CNS and PNS fibres mediating this reflex. This conclusion is supported by the temporal dispersion of the H reflex and the direct evidence of restoration of conduction in the ventral root and VREZ during recovery from whole spinal cord-induced EAE.

The recovery from hindlimb weakness and the restoration of the H reflex occur at a time when there is investment and remyelination of lumbar CNS and PNS axons by oligodendrocytes and Schwann cells, respectively. In the animals studied immediately after clinical hindlimb recovery there were considerably more demyelinated fibres invested by glial or Schwann cells than there were fibres with newly formed myelin. This suggests that conduction had been restored in fibres that were still demyelinated but which had been invested by debris-free glial or Schwann cells. Studies in noninflammatory models of PNS demyelination have shown that nerve conduction can be restored by the development of electrical excitability in demyelinated internodal axolemma as early as 4 days after the induction of demyelination (Bostock and Sears, 1978; Smith *et al.*, 1982). In the lysophosphatidyl choline model, restoration of conduction occurred when demyelinated fibres became closely associated with debris-free Schwann cells (Smith and Hall, 1980; Smith *et al.*, 1982), as was observed in the present study.

During recovery from EAE, areas of subaxolemmal density were occasionally observed in demyelinated axons in close association with astrocytic or mitochondria-laden Schwann cell processes. Such axolemmal specializations resemble the node-like specializations observed in regions of CNS demyelination induced by chronic EAE (Raine, 1978), cuprizone (Ludwin, 1980) and ethidium bromide (Blakemore and Smith, 1983; Rosenbluth *et at.*, 1985) and in amyelinated PNS fibres in the dystrophic mouse (Rosenbluth, 1979). It is possible that these subaxolemmal densities in rats recovering from acute EAE might be the morphological equivalents of the foci of inward current, termed `phi nodes', found in ventral root axons demyelinated by lysophosphatidyl choline (Smith *et at.*, 1982). A recent ultrastructural study of the spinal cords of rats and guinea pigs with acute EAE found no evidence for the development of phi nodes, but this study was performed in animals with hindlimb paralysis and not during recovery (Black *et al.*, 1987). Thus the development of electrical excitability in CNS and PNS demyelinated fibres invested by glial or Schwann cells may be the mechanism for the rapid clinical recovery in acute EAE. Remyelination by oligodendrocytes and Schwann

cells occurs rapidly and would make the restoration of conduction more secure (see Smith et al., 1981).

The present findings support the hypothesis that demyelination of the PNS and CNS is the cause of the neurological signs of acute EAE induced by inoculation with either whole spinal cord or MBP (see Pender, 1987). Restoration of conduction in the ventral roots and VREZs would contribute to the recovery from hindlimb and tail paralysis while restoration of conduction in the sacrococcygeal dorsal root ganglia, dorsal roots and dorsal root entry zones would explain the recovery of tail nociception. The possibility that lesions of descending brainstem and spinal cord pathways with subsequent recovery might contribute to the development and resolution of hindlimb weakness was not investigated. Pender and Sears (1984) presented evidence for the restoration of conduction by the development of electrical excitability in demyelinated axons of the dorsal root ganglia of rabbits recovering from acute EAE but electron microscope studies were not performed. In contrast to the good correlation between clinical recovery and glial/Schwann cell ensheathment-remyelination in the present study, there is a poor correlation between neurotransmitter system changes and the clinical course of chronic relapsing EAE in the Lewis rat (Krenger et al., 1986). The latter indicates that neurotransmitter system changes are not responsible for the temporary neurological signs, although axonal degeneration of monoaminergic neurons could of course contribute to persistent neurological deficits.

Previous studies have shown that remyelination occurs in the CNS and PNS of rats, rabbits and guinea pigs with EAE (Luse and McDougal, 1960; Bubis and Luse, 1964; Lampert, 1965; Prineas et al., 1969; Raine et al., 1969; Snyder et al., 1975; Madrid and Wiśniewski, 1978; Lassmann et al., 1980, 1986), in the CNS of mice with EAE (Brown et al., 1982; Raine et al., 1984) and in the PNS in experimental allergic neuritis (Ballin and Thomas, 1969). However, no previous study has defined the morphological changes associated with early recovery in EAE. In the present study, remyelination of the PNS was seen as early as 5 days after the onset of tail weakness, and remyelination of the CNS by oligodendrocytes was well advanced 9 days after the onset of tail weakness and possibly commenced as early as 6 days. Remyelination of CNS axons by Schwann cells has been observed in chronic EAE in the guinea pig (Snyder et al., 1975; Lassmann et al., 1980), mouse (Brown et al., 1982; Raine et al., 1984) and rat (Lassmann et al., 1980). In the present study, demyelinated CNS axons were rapidly invested and remyelinated by oligodendrocytes and there was no evidence of investment or remyelination of CNS axons by Schwann cells. This suggests that Schwann cells myelinate CNS axons only when there is active inhibition of oligodendrocytmediated remyelination.

This rapid remyelination by oligodendrocytes in acute EAE is of particular interest in view of the recently reported evidence of early massive remyelination in MS (Lassmann, 1983; Prineas et al., 1984). Glial ensheathment and remyelination may account for the rapid as well as the delayed recovery after relapses of MS. The rapidity of the recovery process described in the present study also has another important implication. The development of neurological signs in EAE and MS is likely to be determined not only by the degree but also by the time course of demyelination. Thus slowly evolving asynchronous demyelination may be less likely to cause dysfunction than rapid simultaneous demyelination in the same number of fibres. Therefore the clinical states of neurological deterioration, stability or recovery reflect a dynamic

process in which active demyelination predominates over, equals or is dominated by these recovery processes.

In conclusion, the present study indicates that clinical recovery from acute EAE is due to rapid investment and remyelination of demyelinated PNS and CNS fibres by Schwann cells and oligodendrocytes. Thus the mechanisms for production of, and recovery from, the neurological signs in acute EAE may be the same as in relapses of chronic EAE. This emphasizes the fact that knowledge of the pathogenesis and pathophysiology of acute EAE is essential for viewing the processes of chronic relapsing EAE in perspective.

ACKNOWLEDGEMENTS

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