Journal of Neuroimmunology (1987) 15 (1): 11-24. http://dx.doi.org/10.1016/0165-5728(87)90003-8

Demyelination and Neurological Signs in Experimental Allergic Encephalomyelitis

M.P. Pender

Abstract

Because of the reported absence of demyelination in some animals with neurological signs of experimental allergic encephalomyelitis (EAE), it has been suggested that these signs are not due to demyelination. The present study demonstrates that there is ample demyelination in the central nervous system (CNS) and peripheral nervous system (PNS) to account for the neurological signs in rats with myelin basic protein (MBP)-induced acute EAE as well as in rats and rabbits with whole-spinal-cord-induced acute EAE. The main reasons for failure to detect demyelination in animals with neurological signs of EAE appear to be inadequate histological techniques and incomplete examination of the nervous system, particularly the PNS and the lumbar, sacral and coccygeal segments of the spinal cord.

Keywords*:* central nervous system; demyelination; experimental allergic encephalomyelitis; myelin basic protein; neurological signs; peripheral nervous system

Introduction

Experimental allergic encephalomyelitis (EAE), an autoimmune disease of the nervous system, is widely studied as an animal model of multiple sclerosis (MS), a human central nervous system (CNS) demyelinating disease of unknown aetiology (Raine 1984). The clinical status is the main guide to the progress of both EAE and MS, and the suppression of or improvement in the neurological signs of EAE by therapeutic agents (Alvord et al. 1965; Levine and Sowinski 1980) provides the basis for the use of these agents in MS. In MS, CNS demyelination contributes significantly to the production of neurological signs (McDonald 1974). However, because of reports of absent or minimal demyelination in some animals with neurological signs of acute EAE or the first attack of chronic relapsing EAE, it has been suggested that the signs of EAE are not due to demyelination (Hoffman et al. 1973; Lassmann and Wisniewski 1979; Panitch and Ciccone 1981; Raine et al. 1981; Simmons et al. 1981, 1983; Kerlero de Rosbo et al. 1985). Furthermore, it has been stated that the recovery of function in rats with EAE is too rapid to be accounted for by remyelination, and that demyelination is therefore not responsible for the neurological signs (Panitch and Ciccone 1981; Simmons et al. 1981). There have even been reports of neurological signs of EAE in the absence of any demonstrable histological lesions of the nervous system (Paterson 1972, 1982). Thus the neurological signs of EAE have been attributed to oedema (Paterson 1976; Simmons et al. 1982, 1983) or an impairment of monoaminergic neurotransmission (Carnegie 1971; White 1984).

It is clearly important to determine whether demyelination contributes to the production of neurological signs in EAE as it does in MS. This paper describes the histological findings in the CNS and the peripheral nervous system (PNS) of rabbits and rats with whole-spinal-cord-induced acute EAE and in rats with myelin basic protein (MBP)-induced acute EAE. The involvement of the dorsal root ganglia (DRGs) in rabbits and rats with whole-spinal-cord-induced acute EAE has been previously reported (Pender and Sears 1984, 1986).

Materials and methods

Animals

Lewis rats, bred by the Animal Breeding Establishment of the John Curtin School of

Medical Research (JC strain) were kept in cages of five and fed rat and mouse cubes arid water ad libitum. New Zealand white male rabbits were obtained from one source, kept individually and fed rabbit-guinea pig pellets and water ad libitum.

Induction of EAE

(1) *In rats (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* (Difco) per ml of incomplete Freund's adjuvant (Commonwealth Serum Laboratories, Melbourne, Australia). Under ether anaesthesia rats, 8-10 weeks old, were inoculated with 0.05 ml of inoculum in the footpad of each of the four feet.

(2) *In rats (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 containing 4 mg/ml of added *Mycobacterium butyricum.* Rats, 8-10 weeks old, received 0.1 ml of emulsion in a footpad of each hindfoot. The total dose of MBP was 50 µg/rat.

(3) *In rabbits.* Each batch of inoculum was prepared by mixing 5 g of rabbit spinal cord (the spinal roots having been stripped away) with 7 ml of incomplete Freund's adjuvant (Difco), 125 mg of H37 Ra heat-killed *Mycobacterium tuberculosis* (Difco) and 3 ml of 0.9% saline. Under sodium pentobarbitone anaesthesia, rabbits, 4-6 months old and weighing 2.5-3.5 kg, were given intradermal injections of 0.1 ml of inoculum into each of four sites in the nuchal region.

Histological studies

Histological studies were performed on four rats (two male, two female) with whole-spinal-cord-induced EAE, three male rats with MBP-induced EAE and seven rabbits with whole-spinal-cord-induced EAE. These studies were done 2-3 days and 1-5 days after the onset of neurological signs in the rats and rabbits respectively. Two normal rats and two normal rabbits served as controls. The methods have been described in detail previously (Pender and Sears 1984, 1986; Pender 1985) and will be presented here briefly. Under ether anaesthesia the animals 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). In several rabbits 10% formol saline or formol calcium was used instead of glutaraldehyde/ formaldehyde. The brain, optic nerves, spinal cord, dorsal and ventral roots, dorsal root ganglia (DRGs), spinal nerves, sciatic and (rat) tail nerves were removed and immersed in fixative. Paraffin sections were stained with haematoxylin and eosin, luxol fast blue and cresyl fast violet, or chromoxane cyanin-R and neutral red. Rabbit tissues fixed with glutaraldehyde/ formaldehyde were post-fixed with 1% osmium tetroxide, embedded in Araldite, sectioned (0.5 µM) and stained with toluidine blue. Most of the rat tissues were post-fixed with 2% osmium tetroxide, embedded in HistoResin (LKB Bromma), sectioned (2 µm) and stained with toluidine blue in phosphate buffer (pH 7.6) or with cresyl violet. Some rat osmium-fixed tissues were embedded in Epok 812 (Ernest F. Fullam, Schenectady, NY). Semi-thin (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

In rats with whole-spinal-cord- or MBP-induced EAE, distal tail weakness commenced 8-14 days after inoculation and was followed by flaccid tail paralysis, symmetrical hindlimb weakness and sometimes hindlimb paralysis. Forelimb weakness occurred occasionally. These signs were accompanied by weight loss. Most rats survived and by 20 days after inoculation were clinically normal apart from mild tail weakness.

Rabbits developed neurological signs 12-30 days after inoculation. The neurological signs were accompanied by weight loss and consisted of lateral splaying, ataxia and hypotonia of the hindlimbs and forelimbs, and loss of the knee jerks, followed by limb weakness, impairment of limb nociception, perineal soiling, paradoxical breathing and slowing of respiration (see Pender and Sears 1984). Some rabbits showed neurological improvement commencing 2-5 days after the onset of neurological signs. Such recovery was usually incomplete.

Histological findings

(1) In rats. Both demyelination and inflammation were present in the brain-stem, spinal cord, DRGs, dorsal roots and ventral roots in all rats with MBP-induced EAE as well as in all rats with whole-spinal-cord-induced EAE (Figs. 1-5). The inflammatory infiltrate was predominantly mononuclear and was located in the meninges, in the subpial region and around blood vessels. Polymorphonuclear leukocytes and extravasated erythrocytes were often present in these lesions, particularly in MBP-induced EAE. Demyelination was much more readily detected in HistoResin and Epok 812 sections than in paraffin sections. Myelin debris was present in macrophages in some of the demyelinating lesions. Axons and neuronal cell bodies were generally preserved, although occasional degenerating axons were seen in the spinal roots and spinal cord, and in one animal there was damage to sacral motor neurone cell bodies in the vicinity of severe inflammation. Oedema was also present around some vessels in the CNS and PNS. Glial mitotic figures were often found in both the grey and white matter of the spinal cord.

The spinal cord involvement increased caudally, the sacrococcygeal segments being the most severely affected. Lesions were present in the subpial and deep white matter (Figs. 1 and 2) and also in the grey matter. Of the subpial regions, the ventral root exit zones and the dorsal root entry zones (Fig. 1) were most consistently affected. The ventral horns, dorsal horns and intermediate regions of the grey matter were frequently infiltrated with inflammatory cells. Demyelination was also present in the grey matter but was more difficult to recognize than in the white matter because of the presence of the normal unmyelinated dendrites. Intracellular myelin debris indicated demyelination in grey matter lesions. In contrast to the spinal cord and brainstem, the cerebral hemispheres were not involved. The cerebellum was mildly affected.

In rats with whole-spinal-cord-induced EAE the DRG involvement increased caudally, it being very severe in the sacrococcygeal segments (see Pender and Sears 1986). However, in rats with MBP-induced EAE the DRG involvement did not increase caudally and was mild in all segments. In both whole-spinal-cord-induced EAE and MBP-induced EAE, demyelination was frequently present in the lumbar, sacral and coccygeal ventral and dorsal roots (Figs. 3 and 4). Small diameter fibres were demyelinated as well as large ones. Diligent searching was not required to demonstrate demyelination. For example, in MBP-induced EAE, almost every examined 1-2 μm transverse section of individual dorsal or ventral roots showed at least some demyelinated fibres. The spinal nerves and the sciatic and tail nerves were either normal or minimally affected.

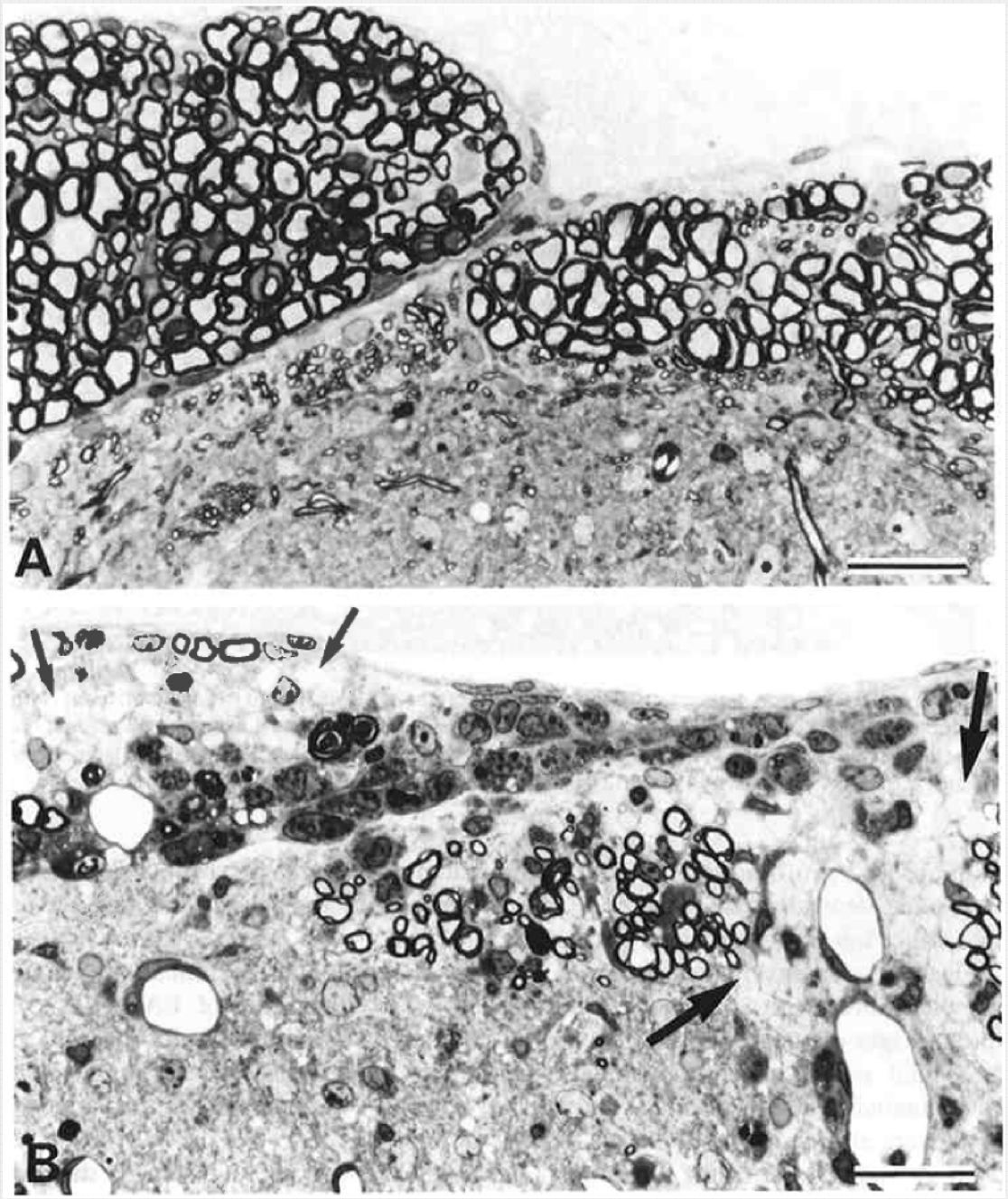

Fig. 1. Transverse sections through dorsal root entry zones of the S3 spinal cord segments of a normal control rat (A) and of a rat with whole-spinal-cord-induced EAE, 2 days after the onset of neurological signs (B). In the latter there are demyelinated axons in the PNS part of the dorsal root entry zone (small arrows) and also in the CNS part (large arrows). Epok 812 sections stained with toluidine blue. Bars = 25 μm.

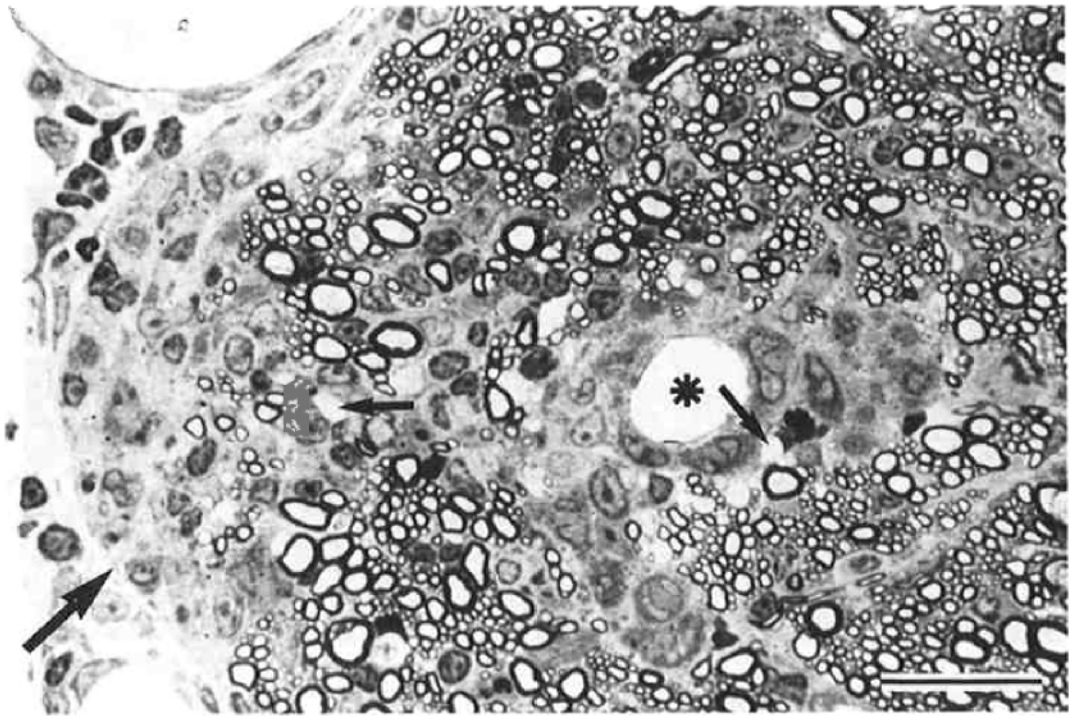

Fig. 2. Transverse section through a lateral funiculus of the L5 spinal cord segment of a rat with MBP-induced EAE, 3 days after the onset of neurological signs. Demyelinated axons (small arrows) are present in perivascular (asterisk in vessel) and subpial regions of mononuclear infiltration (large arrow indicates surface of spinal cord). Epok 812 section stained with toluidine blue. Bar = 25 μm.

Electron microscopy demonstrated primary demyelination of small and large diameter fibres with sparing of axons in rats with either type of EAE (Fig. 5). Macrophages containing myelin debris were often present within myelin sheaths. In the spinal cord and in the spinal roots there were occasional myelinated or demyelinated axons showing degenerative changes. Degenerating unmyelinated fibres were also occasionally seen.

(2) In rabbits. Paraffin sections stained with haematoxylin and eosin showed meningeal and subpial infiltration and perivascular cuffing and infiltration with mononuclear leukocytes in the brainstem and spinal cord. In the PNS, similar perivascular lesions were found in the dorsal and ventral roots and particularly the DRGs. There was minimal if any involvement of the sciatic and spinal nerves. Some polymorphonuclear leukocytes were also present in the CNS and PNS inflammatory infiltrates. Paraffin sections stained with luxol fast blue and cresyl fast violet showed some demyelination in the regions of mononuclear infiltration but semi-thin Araldite sections were far superior for detecting this. Demyelination was most severe in the DRGs but was also present in the dorsal and ventral roots (Fig. 6) and in the spinal cord. In the spinal cord the demyelination was most evident in the subpial region, particularly the ventral root exit zones (Fig. 7) and dorsal root entry zones. In the regions of demyelination, axons were preserved and intracellular myelin debris was present within macrophages (Figs. 6 and 7).

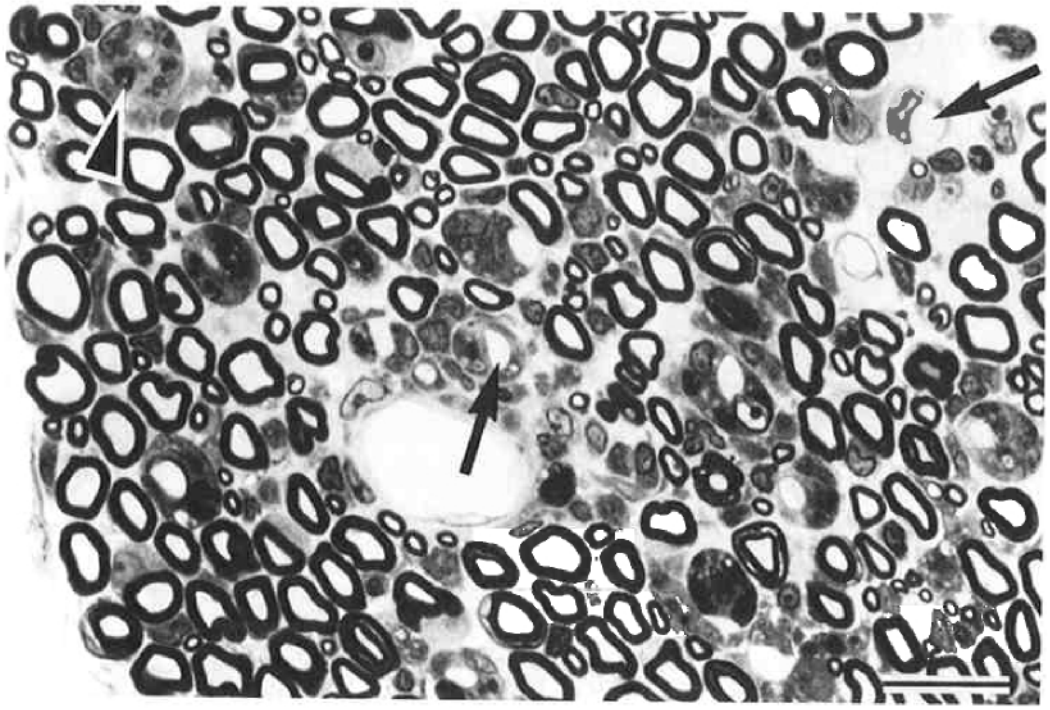

Fig. 3. Transverse section through a L4 ventral root of a rat with MBP-induced EAE, 3 days after the onset of neurological signs. Many demyelinated axons are present (arrows). Myelin debris (arrowhead) can be seen within macrophages. HistoResin section stained with cresyl violet. Bar = 25 µm.

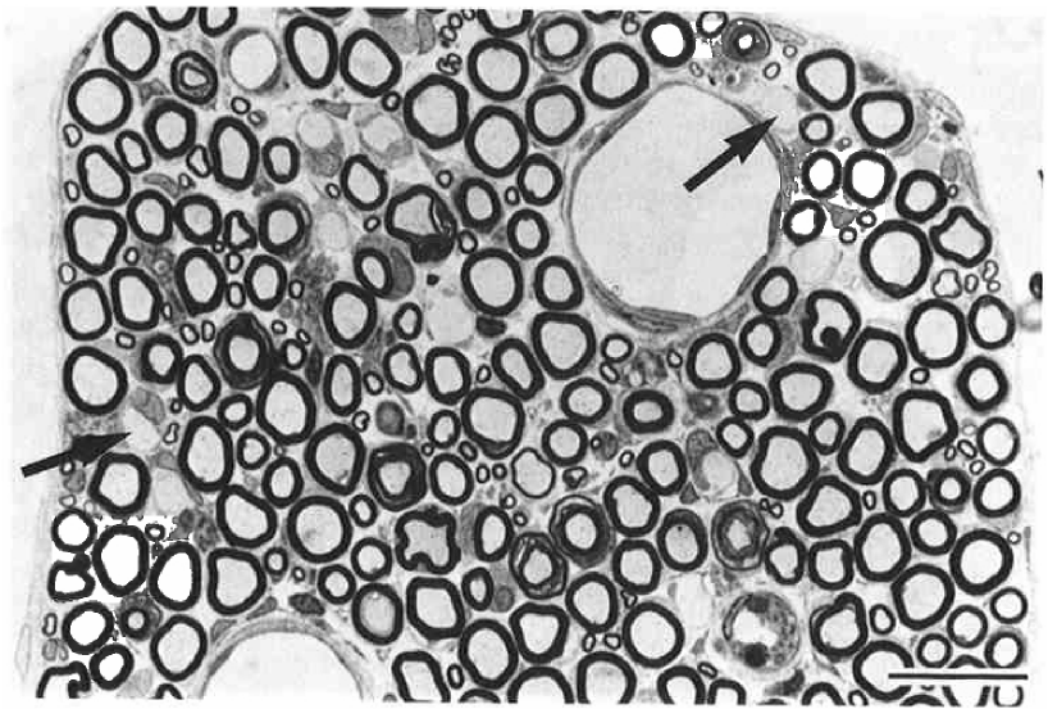

Fig. 4. Transverse section through a S3 ventral root of a rat with MBP-induced EAE, 2 days after the onset of neurological signs. Demyelinated axons can be seen (arrows). Epok 812 section stained with toluidine blue. Bar = 25 µm.

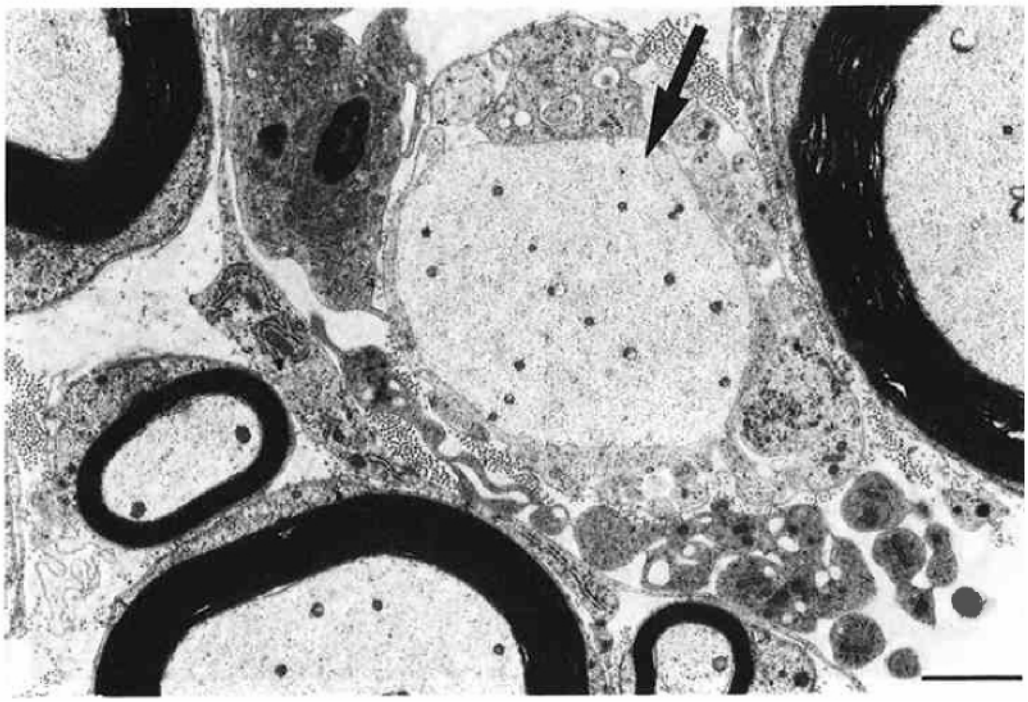

Fig. 5. Electron micrograph of the ventral root in Fig. 4. A demyelinated axon is seen (arrow). Bar = 2 μm.

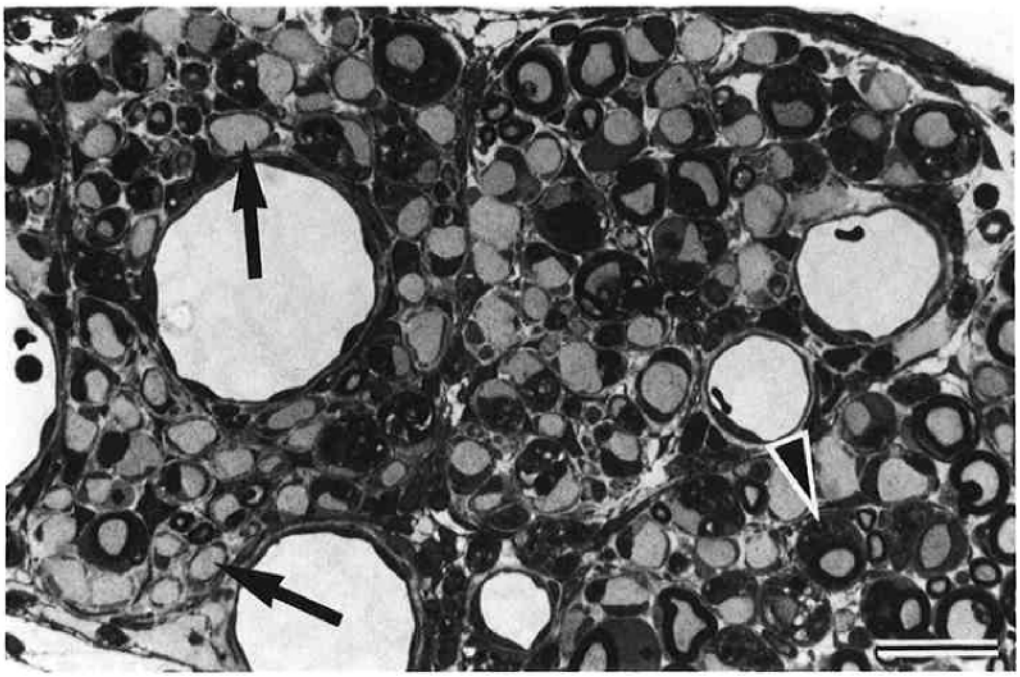

Fig. 6. Transverse section through a S1 ventral root of a rabbit with EAE, 5 days after the onset of neurological signs. There are many demyelinated axons (arrows). Myelin debris (arrowhead) can be seen, within macrophages. Araldite section stained with toluidine blue. Bar = 25 μm.

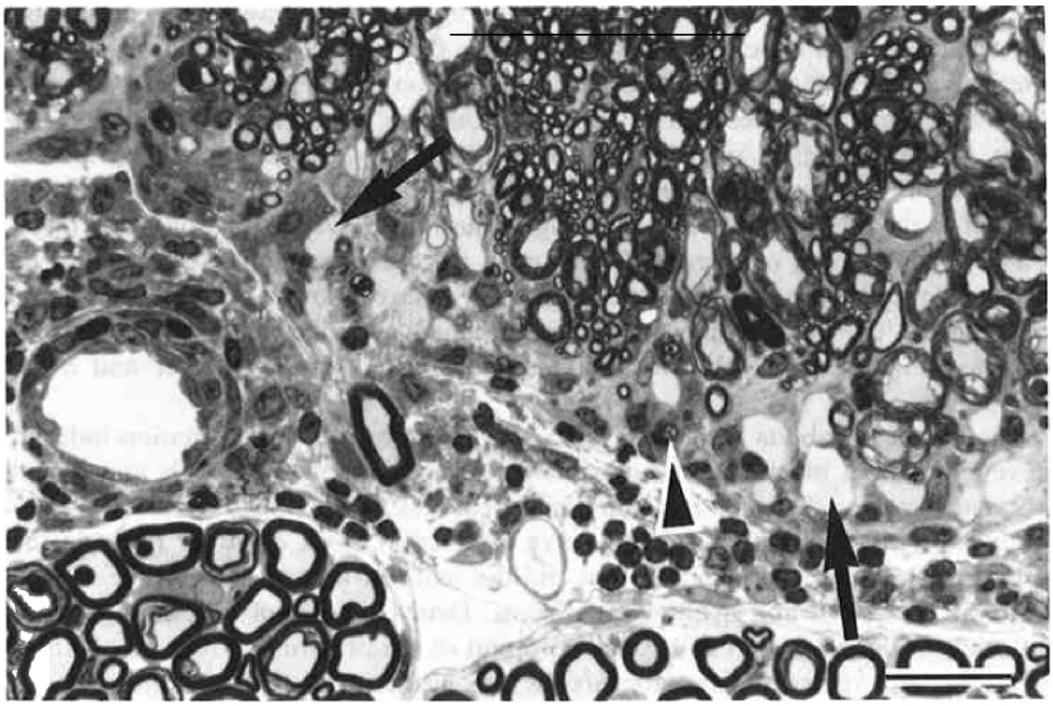

Fig. 7. Transverse section through a ventral root exit zone of the S1 spinal cord segment of a rabbit with EAE, 2 days after the onset of neurological signs. Mononuclear leukocytes infiltrate the meninges and the subpial region where they are associated with demyelination (arrows). Myelin debris (arrowhead) can be seen within macrophages. Araldite section stained with toluidine blue. Bar = 25 µm.

Discussion

The present paper demonstrates that there is ample demyelination in the PNS and CNS to account for the neurological signs of MBP-induced EAE in rats as well as of whole-spinal-cord-induced EAE in rats and rabbits. Other workers have also reported demyelination in these models (Waksman and Adams 1955; Wisniewski et al. 1969; Piliero and Cremonese 1973; McFarlin et al. 1974; Dal Canto et al. 1977). Electrophysiological studies have shown focal nerve conduction abnormalities in the DRGs and at the dorsal root entry zone in rabbits with EAE (Pender and Sears 1982, 1984, 1985), at the ventral root exit zones and in the DRGs of rats with whole-spinal-cordinduced EAE (Pender 1986a; Pender and Sears 1986) and in the sacrococcygeal dorsal roots of rats with MBP-induced EAE (Pender 1986b). As this localization of functional impairment corresponds with the localization of demyelination, it is likely that demyelination is the cause of the nerve conduction abnormalities. This conclusion is supported by the finding of the following characteristic pathophysiologic sequelae of demyelination: conduction slowing; conduction block; and the effects of temperature and the potassium channel blocking agent, 4-aminopyridine, on the conduction block. As inflammation is invariably present in the demyelinated regions, one could argue that an antibody or other product of the inflammatory response might cause the nerve conduction block by an action on the axonal membrane. If this were the case, conduction should also be blocked in unmyelinated axons. However, we have shown that conduction is preserved in the unmyelinated axons of inflamed and demyelinated dorsal root ganglia, while it is severely impaired in demyelinated axons (Pender and Sears 1984).

The ataxia in rabbits with EAE is accounted for by the demyelination-induced nerve conduction block of large diameter afferent fibres in the DRGs and dorsal root entry zones. The tail and hindlimb weakness in rats with EAE is readily explained by demyelination-induced nerve conduction block of motor fibres in the ventral horn, between the ventral horn and the ventral root exit zone, in the ventral root exit zone itself and in the ventral

roots. Demyelination of descending motor pathways in the brainstem and spinal cord and of the preterminal regions of fibres synapsing with ventral horn motoneurones may also contribute. The limb weakness in rabbits with EAE is likely to have a similar explanation. It should be noted that the functional effects of multiple demyelinating lesions scattered along the length of motor fibres from the ventral horn to the distal ventral root are cumulative, provided that different fibres are affected in each lesion. Demyelination can account for the neurological signs even if large demyelinating lesions are not present. As nerve conduction can be rapidly restored in demyelinated fibres before remyelination occurs (see below), the development of neurological signs in EAE is likely to be determined not only by the degree but also by the time course of demyelination. Slowly evolving asynchronous demyelination is much less likely to cause dysfunction than rapid simultaneous demyelination in the same number of fibres.

The ascending progression of weakness, a characteristic feature of EAE in the rat (Williams and Moore 1973; Simmons et al. 1982), is at least partly accounted for by the caudally increasing length of the ventral roots. In a study on male albino rats, Waibl (1973) showed that the lengths of the spinal roots increase progressively from the first thoracic root (2 mm) to the third coccygeal root (59 mm). Thus the probability of many lesions in an entire ventral root increases progressively in a caudal direction. Therefore the probability of a high proportion of ventral root fibres undergoing demyelination-induced conduction block increases caudally. Simmons et al. (1982) have used a similar anatomically based explanation to account for the ascending weakness in rats with EAE but they attributed the weakness to oedema rather than to demyelination. The severe involvement of the sacrococcygeal segments of the spinal cord probably also contributes to the ascending pattern of weakness. In rats with EAE there is also an ascending impairment of tail nociception, which appears to be due to demyelination of small diameter afferent fibres in the sacrococcygeal DRGs, dorsal roots and dorsal root entry zones (Pender 1986b).

Levine et al. (1975) reviewed reports of clinical signs without histological lesions of EAE. They concluded that these reports were invalid because of the nonspecificity of clinical signs, the occurrence of intercurrent diseases, the inadequacy or incorrect timing of histologic evaluations, and the lack of controls for specificity of the signs. The present paper emphasizes that, if clinicopathological correlations are to be made in EAE, one must thoroughly examine the PNS, which is known to be affected in EAE in all species studied (Freund et al. 1947; Ferraro and Roizin 1954; Waksman and Adams 1955, 1956; Levine and Wenk 1963; Wisniewski et al. 1969; Piliero and Cremonese 1973; McFarlin et al. 1974; Dal Canto et al. 1977), as well as the CNS, particularly the lumbar, sacral and coccygeal segments of the spinal cord. The involvement of the PNS when animals are inoculated with whole CNS tissue or purified CNS MBP is explained by the fact that the P1 MBP from the PNS is similar, if not identical to, CNS MBP (Brostoff and Eylar 1972; Greenfield et al. 1973). Demyelination of the PNS may account for the occurrence of neurological signs in the absence of CNS demyelination in guinea pigs with bovine MBP-induced EAE or encephalitogenic peptide-induced EAE (Hoffman et al. 1973; Raine et al. 1981) and in guinea pigs during the acute phase of chronic relapsing EAE (Lassmann and Wisniewski 1979). When assessing the PNS in guinea pigs with EAE, particular attention should be given to the peripheral nerves themselves, as well as to the spinal roots and ganglia, because the peripheral nerves are particularly affected in this species (Waksman and Adams 1956). It should be noted that CNS demyelination does occur in MBP-induced EAE in guinea pigs when guinea pig MBP or higher doses of bovine MBP are used (Lampert and Kies 1967; Colover 1980; Moore et al. 1985). CNS demyelination also occurs in MBP-induced EAE in mice (Raine et al. 1984) and in monkeys (Alvord et al. 1980) as well as in rats (see above). The use of inadequate techniques probably often accounts for the failure to detect demyelination in EAE. To adequately assess demyelination, tissues should be fixed with glutaraldehyde and formaldehyde, post-fixed with osmium and embedded in a glycol methacrylate or epoxy resin. When HistoResin (LKB Bromma), a new glycol methacrylate-based embedding medium, is used, sections can be readily cut on a standard microtome and do not require the time, expertise and ultramicrotome needed to cut epoxy-resin-embedded material (Pender 1985).

It has been stated that the recovery of function in rats with EAE is too rapid to be accounted for by remyelination, and that demyelination is therefore not responsible for the neurological signs (Panitch and Ciccone 1981; Simmons et al. 1981). However, 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 by diphtheria toxin or lysophosphatidyl choline (Bostock and Sears 1978; Smith et al. 1982). Such a mechanism could readily account for the rapid recovery in rats with EAE. Rapid recovery could also be due to repair of structurally minor, yet functionally significant, damage to the myelin sheath, for example loosening of the paranodal axoglial junction (Hirano and Dembitzer 1978). In addition to demyelination, axonal degeneration may occur in EAE (Lampert and Kies 1967; Brown et al. 1982) and probably contributes to the persistent neurological deficits occurring in chronic EAE and to the neurological signs of hyperacute EAE.

Acknowledgements

I am grateful to Ms. Ailsa Rolinson and Miss R. Bashir for excellent technical assistance and to the National Multiple Sclerosis Society of Australia and the National Fund for Research into Crippling Diseases (Great Britain) for support. I also thank Professor T.A. Sears for many helpful discussions.

References

Alvord, Jr., E.C., C.M. Shaw, S. Hruby and M.W. Kies, Encephalitogen-induced inhibition of experimental allergic encephalomyelitis: prevention, suppression and therapy, Ann. N.Y. Acad. Sci., 122 (1965) 333-345.

Alvord, Jr., E.C., C.M. Shaw, S. Hruby and L.R. Sires, Chronic relapsing experimental allergic encephalomyelitis induced in monkeys with myelin basic protein, J. Neuropathol. Exp. Neurol., 39 (1980) 338.

Bostock, H. and T.A. Sears, The internodal axon membrane: electrical excitability and continuous conduction in segmental demyelination, J. Physiol. (London), 280 (1978) 273-301.

Brostoff, S.W. and E.H. Eylar, The proposed amino acid sequence of the P1 protein of rabbit sciatic nerve myelin, Arch. Biochem. Biophys., 153 (1972) 590-598.

Brown, A., D.E. McFarlin and C.S. Raine, Chronologic neuropathology of relapsing experimental allergic encephalomyelitis in the mouse, Lab. Invest., 46 (1982) 171-185.

Carnegie, P.R., Properties, structure and possible neuroreceptor role of the encephalitogenic protein of human brain, Nature, 229 (1971) 25-28.

Colover, J., A new pattern of spinal-cord demyelination in guinea pigs with acute experimental allergic encephalomyelitis mimicking multiple sclerosis, Br. J. Exp. Pathol., 61 (1980) 390-400. Dal Canto, M.C., R.S. Fujinami and P.Y. Paterson, Experimental allergic encephalomyelitis in suckling Lewis rats: comparison with the disease in adult animals, Lab. Invest., 37 (1977) 395-405.

Deibler, G.E., R.E. Martenson and M.W. Kies, Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species, Prep. Biochem., 2 (1972) 139-165.

Ferraro, A. and L. Roizin, Neuropathologic variations in experimental allergic encephalomyelitis (hemorrhagic encephalomyelitis, perivenous encephalomyelitis, diffuse encephalomyelitis, patchy gliosis), J. Neuropathol. Exp. Neurol., 13 (1954) 60-89.

Freund, J., E.R. Stern and T.M. Pisani, Isoallergic encephalomyelitis and radiculitis in guinea pigs after one injection of brain and mycobacteria in water-in-oil emulsion, J. Immunol., 57 (1947) 179-194. Greenfield, S., S. Brostoff, E.H. Eylar and P. Morell, Protein composition of myelin of the peripheral nervous system, J. Neurochem., 20 (1973) 1207-1216.

Hirano, A. and H.M. Dembitzer, Morphology of normal central myelinated axons. In: S.G. Waxman (Ed.), Physiology and Pathobiology of Axons, Raven Press, New York, 1978, pp. 65-82.

Hoffman, P.M., D.D. Gaston and L.E. Spitler, Comparison of experimental allergic encephalomyelitis induced with spinal cord, basic protein, and synthetic encephalitogenic peptide, Clin. Immunol. Immunopathol., 1 (1973) 364-371.

Kerlero de Rosbo, N., C.C.A. Bernard, R.D. Simmons and P.R. Carnegie, Concomitant detection of changes in myelin basic protein and permeability of blood-spinal cord barrier in acute experimental autoimmune encephalomyelitis by electroimmunoblotting, J. Neuroimmunol., 9 (1985) 349-361.

Lampert, P.W. and M.W. Kies, Mechanism of demyelination in allergic encephalomyelitis of guinea pigs. An electron microscopic study, Exp. Neurol., 18 (1967) 210-223.

Lassmann, H. and H.M. Wisniewski, Chronic relapsing experimental allergic encephalomyelitis: effect of age at the time of sensitization on clinical course and pathology, Acta Neuropathol., 47 (1979) 111-116.

Levine, S. and R. Sowinski, Therapy of allergic encephalomyelitis in rats after onset of paralysis. In: A.N. Davison and M.L. Cuzner (Eds.), The Suppression of Experimental Allergic Encephalomyelitis and Multiple Sclerosis, Academic Press, London, 1980, pp. 199-209.

Levine, S. and E.J. Wenk, Allergic neuritis induced in rats without the use of mycobacteria, Proc. Soc. Exp. Biol. Med., 113 (1963) 898-900.

Levine, S., R. Sowinski, C.-M. Shaw and E.C. Alvord, Jr., Do neurological signs occur in experimental allergic encephalomyelitis in the absence of inflammatory lesions of the central nervous system?, J. Neuropathol. Exp. Neurol., 34 (1975) 501-506.

McDonald, W.I., Pathophysiology in multiple sclerosis, Brain, 97 (1974) 179-196.

McFarlin, D.E., S.E. Blank and R.F. Kibler, Recurrent experimental allergic encephalomyelitis in the Lewis rat, J. Immunol., 113 (1974) 712-715.

Moore, G.R.W., U. Traugott, S.H. Stone and C.S. Raine, Dose-dependency of MBP-induced demyelination in the guinea pig, J. Neurol. Sci., 70 (1985) 197-205.

Panitch, H. and C. Ciccone, Induction of recurrent experimental allergic encephalomyelitis with myelin basic protein, Ann. Neurol., 9 (1981) 433-438.
Paterson, P.Y., Experimental allergic encephalomyelitis and multiple sclerosis as immunologic diseases: a critique. In: F. Wolfgram, G.W. Ellison, J.G. Stevens and J.M. Andrews (Eds.), Multiple Sclerosis: Immunology, Virology and Ultrastructure, Academic Press, New York, 1972, pp. 539-567.
Paterson, P.Y., Experimental allergic encephalomyelitis: role of fibrin deposition in immunopathogenesis of inflammation in rats, Fed. Proc., 35 (1976) 2428-2434.
Paterson, P.Y., Molecular and cellular determinants of neuroimmunologic inflammatory disease, Fed. Proc., 41 (1982) 2569-2576.
Pender, M.P., A simple method for high resolution light microscopy of nervous tissue, J. Neurosci. Methods, 15 (1985) 213-218.
Pender, M.P., Conduction block due to demyelination at the ventral root exit zone in experimental allergic encephalomyelitis, Brain Res., 367 (1986a) 398-401.
Pender, M.P., Ascending impairment of nociception in rats with experimental allergic encephalomyelitis, J. Neurol. Sci., 75 (1986b) 317-328.
Pender, M.P. and T.A. Sears, Conduction block in the peripheral nervous system in experimental allergic encephalomyelitis, Nature, 296 (1982) 860-862.
Pender, M.P. and T.A. Sears, The pathophysiology of acute experimental allergic encephalomyelitis in the rabbit, Brain, 107 (1984) 699-726.
Pender, M.P. and T.A. Sears, Vulnerability of the dorsal root ganglion in experimental allergic encephalomyelitis, Clin. Exp. Neurol., 21 (1985) 211-223.
Pender, M.P. and T.A. Sears, Involvement of the dorsal root ganglion in acute experimental allergic encephalomyelitis in the Lewis rat - A histological and electrophysiological study, J. Neurol. Sci., 72 (1986) 231-242.
Piliero, S.J. and P. Cremonese, Acute allergic encephalomyelitis (EAE) in the rat with special reference to immunologic and demyelinization effects, J. Reticuloendothel. Soc., 14 (1973) 100-118.
Raine, C.S., Analysis of autoimmune demyelination: its impact upon multiple sclerosis, Lab. Invest., 50 (1984) 608-635.
Raine, C.S., U. Traugott, M. Farooq, M.B. Bornstein and W.T. Norton, Augmentation of immune-mediated demyelination by lipid haptens, Lab. Invest., 45 (1981) 174-182.
Raine, C.S., F. Mokhtarian and D.E. McFarlin, Adoptively transferred chronic relapsing experimental autoimmune encephalomyelitis in the mouse: neuropathologic analysis, Lab. Invest., 51 (1984) 534-546.
Simmons, R.D., C.C.A. Bernard, K.T. Ng and P.R. Carnegie, Hind-limb motor ability in Lewis rats during the onset and recovery phases of experimental autoimmune encephalomyelitis, Brain Res., 215 (1981) 103-114.
Simmons, R.D., C.C.A. Bernard, G. Singer and P.R. Carnegie, Experimental autoimmune encephalomyelitis: an anatomically-based explanation of clinical progression in rodents, J. Neuroimmunol., 3 (1982) 307-318.
Simmons, R.D., C.C.A. Bernard and P.R. Carnegie, Factors underlying ascending paralysis in rodents during experimental autoimmune encephalomyelitis (EAE). In: A.D. Kidman, J.K. Tomkins, C.A. Morris and N.A. Cooper (Eds.), Molecular Pathology of Nerve and Muscle: Noxious Agents and Genetic Lesions, Humana Press, Clifton, NJ, 1983, pp. 99-111.
Smith, K.J., H. Bostock and S.M. Hall, Saltatory conduction precedes remyelination in axons demyelinated with lysophosphatidyl choline, J. Neurol. Sci., 54 (1982) 13-31.
Waibl, H., Zur Topographic der Medulla spinalis der Albinoratte *(Rattus norvegicus),* Adv. Anat. Embryol. Cell. Biol., 47 (1973) 5-42.
Waksman, B.H. and R.D. Adams, Allergic neuritis: an experimental disease of rabbits induced by the injection of peripheral nervous tissue and adjuvants, J. Exp. Med., 102 (1955) 213-236. Waksman, B.H. and R.D. Adams, A comparative study of experimental allergic neuritis in the rabbit, guinea pig and mouse, J. Neuropathol. Exp. Neurol., 15 (1956) 293-333.
White, S.R., Experimental allergic encephalomyelitis: effects on monoaminergic neurotransmission. In: C.D. Barnes (Ed.), Brainstem Control of Spinal Cord Function, Academic Press, Orlando, FL, 1984, pp. 257-281.
Williams, R.M. and M.J. Moore, Linkage of susceptibility to experimental allergic encephalomyelitis to the major histocompatibility locus in the rat, J. Exp. Med., 138 (1973) 775-783.
Wisniewski, H., J. Prineas and C.S. Raine, An ultrastructural study of experimental demyelination and remyelination. 1. Acute experimental allergic encephalomyelitis in the peripheral nervous system, Lab. Invest., 21 (1969) 105-118.