

# Neuropathological findings in chronic relapsing experimental allergic neuritis induced in the Lewis rat by inoculation with intradural root myelin and treatment with low dose cyclosporin A

P. A. MCCOMBE, S. A. VAN DER KREEK AND M. P. PENDER  
*Department of Medicine, The University of Queensland, Australia*

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## **Neuropathological findings in chronic relapsing experimental allergic neuritis induced in the Lewis rat by inoculation with intradural root myelin and treatment with low dose cyclosporin A**

Experimental allergic neuritis (EAN) was induced in Lewis rats by inoculation with bovine intradural root myelin and adjuvants. Rats treated with subcutaneous cyclosporin A (CsA) (4 mg/kg on 3 days per week from the day of inoculation until day 29) developed a chronic relapsing course. Tissues from the spinal cord, nerve roots, dorsal root ganglia and sciatic nerve of CsA-treated rats sampled during relapses and remissions were studied by light and electron microscopy. Control rats that were not treated with CsA were studied during or after episodes of acute EAN. Both control and CsA-treated animals studied in the first episode of EAN had evidence of inflammation and primary demyelination of the nerve roots and dorsal root ganglia. In control and CsA-treated animals that had recovered from the first episode there was evidence of remyelination. In CsA-treated animals in the second episode there was severe inflammation and demyelination and remyelination of the nerve roots and dorsal root ganglia, and in addition there was significant demyelination and remyelination in the spinal nerves and sciatic nerves and dorsal columns of the spinal cord, particularly in later stages of disease. In later episodes there was less inflammation, but there was continuing demyelination and onion bulbs were present. In animals sampled after recovery from chronic relapsing EAN onion bulbs were present. Occasional small onion bulbs were also observed in control animals that were inoculated with higher doses of myelin. Plasma cells were present in the inflammatory lesions of later episodes. Mast cells were also observed at different stages of the disease. We conclude that the CsA form of chronic relapsing EAN has clinical and pathological similarities with the human disease, chronic inflammatory demyelinating polyradiculoneuropathy.

**Keywords:** experimental allergic neuritis, demyelination, remyelination, cyclosporin A, relapses

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## INTRODUCTION

Acute experimental allergic neuritis (EAN) was first described by Waksman and Adams (1955) and is characterized pathologically by inflammation and demyelination of the peripheral nerves and nerve roots (Ballin & Thomas, 1969). EAN has been induced in many species including the rabbit, guinea pig and rat. Acute EAN is regarded as a good model of the human disease, acute inflammatory demyelinating polyradiculoneuropathy (AIDP) (Arnason, 1984). It is desirable to have a chronic relapsing form of EAN as a model of the human disease, chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) (Dyck *et al.*, 1975; Prineas & McLeod, 1976; McCombe *et al.*, 1987). Relapsing EAN has been induced by repeated injections of antigens into rabbits (Sherwin, 1966) and guinea pigs (Pollard *et al.*, 1975). Chronic EAN can also be induced in juvenile guinea pigs (Suzumura *et al.*, 1985) and in guinea pigs treated with cyclophosphamide (Madrid & Wisniewski, 1982). Harvey *et al.* (1987) produced chronic EAN in rabbits by using a higher than usual dose of inoculum. In the Lewis rat, Brosnan, Lyman and Neighbour (1984) found that 30% of rats inoculated with bovine intradural root myelin developed chronic disease lasting for months, and Craggs *et al.* (1986) produced late relapses of disease in rats inoculated with whole dorsal roots. Adam *et al.* (1989) described the pathological changes of chronic EAN developed in Lewis rats inoculated with bovine peripheral myelin and adjuvants; these rats were reported to have mild tail weakness but no clear relapses. We have previously reported that, while prophylactic treatment with cyclosporin A (CsA) at a dose of 30 mg/kg three times per week prevents the development of EAN during the time of treatment, a dose of 4 mg/kg three times per week until day 29 after inoculation causes relapsing EAN in Lewis rats inoculated with bovine intradural root myelin plus adjuvants (McCombe *et al.*, 1990). In that report it was suggested that this occurs because the low dose CsA spares the effector cells causing EAN, but blocks the normal immunoregulation of the disease. The present paper describes the detailed pathology of chronic relapsing EAN induced in this manner, and compares the pathological features with those of other forms of chronic relapsing EAN and with those of CIDP (Dyck *et al.*, 1975; Prineas & McLeod, 1976).

## MATERIALS AND METHODS

### Animals

Male and female Lewis rats were obtained from the animal breeding facility of the University of Queensland. The rats were housed in cages at the Pathology Animal House at Royal Brisbane Hospital, and fed on rat cubes and water, *ad libitum*.

### Induction of chronic relapsing EAN

Under anaesthesia with ketamine and xylazine, rats were inoculated with a total of 0.1 ml of emulsion (containing 2 mg bovine intradural root (BIR) myelin, 0.05 ml of complete Freund's adjuvant (Difco), 0.5 mg *Mycobacterium tuberculosis* H37RA (Difco) and 0.05 ml saline) per animal. The inoculum was given in divided doses in the medial footpads of both hindlimbs. The myelin was prepared by sucrose density gradient centrifugation from bovine intradural roots obtained within 1 h of death and dissected immediately. The rats were given subcutaneous

injections of 4 mg/kg of CsA three times per week until day 29 after inoculation to produce chronic relapsing EAN.

Ten rats were given a similar inoculum which contained 4 mg myelin, and were treated with low-dose CsA, to determine whether increased numbers of relapses would occur with an increased dose of antigen.

### Controls

Control animals were inoculated as above but were given no treatment or were treated with subcutaneous saline injections three times per week until day 29 after inoculation.

### Clinical assessment

Animals were examined daily for general condition and for neurological abnormalities including weakness of the limbs and tail and ataxia. Neurological signs were graded according to the scale devised by Pender (1986) in which tail weakness, hindlimb weakness and forelimb weakness are graded separately on a scale of 0 (no weakness) to 4 (total paralysis). The scores for each region were then added together to give a total disability score with a maximum possible disability of 12.

### Histological methods

At different times after inoculation animals were anaesthetized with ketamine and xylazine and perfused with Karnovsky's fixative. The animals were dissected and specimens from the spinal cord at three levels, the lumbar nerve roots, lumbar dorsal root ganglia, cauda equina at three levels, lumbar spinal nerves and sciatic nerve were obtained and further immersed in fixative. For light microscopy, specimens were post-fixed in osmium tetroxide and either embedded in HistoResin (LKB Bromma), sectioned and stained with 0.05% cresyl fast violet as previously described (Nguyen & Pender, 1989) or embedded in Epox 812, sectioned and stained with toluidine blue. For electron microscopy, ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined with an Hitachi-300 electron microscope.

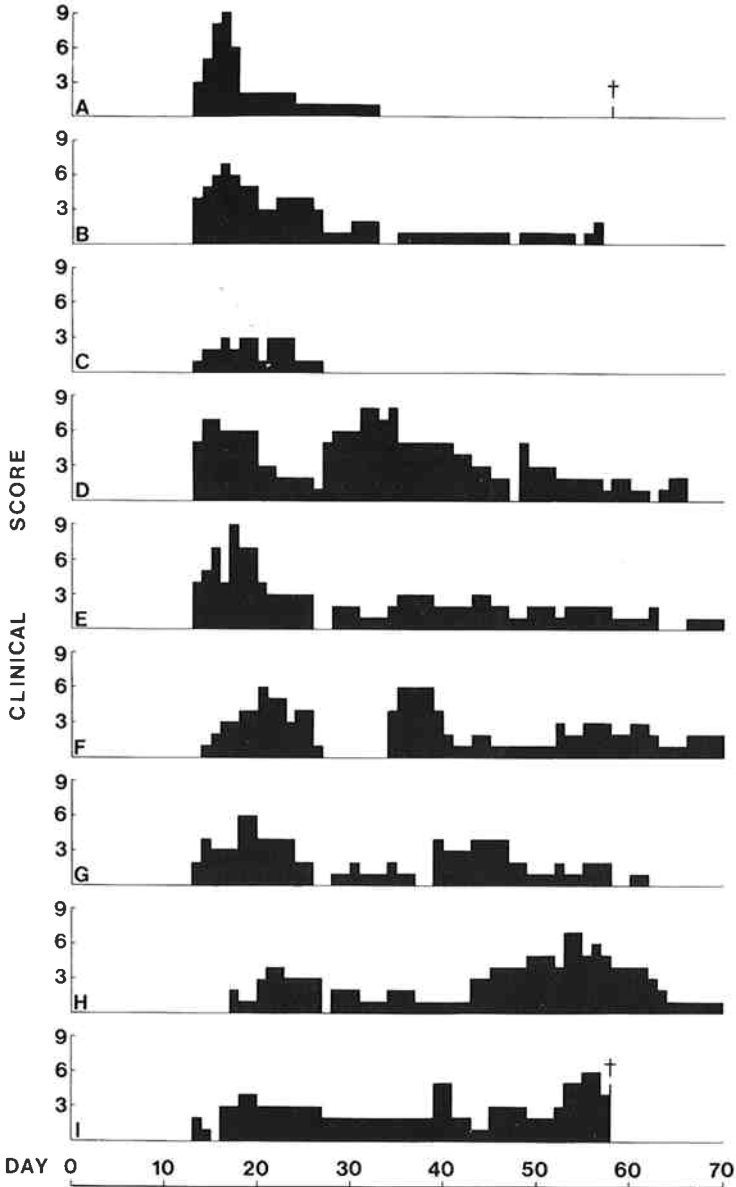
## RESULTS

### Clinical assessment

#### *Controls*

As previously described (McCombe *et al.*, 1990), control animals inoculated with 2 mg BIR myelin developed neurological signs of EAN (tail weakness, limb ataxia and limb weakness) 11–15 days after inoculation, and recovered completely by day 21–24. Approximately one-third of these animals had a mild exacerbation of tail weakness about 30 days after inoculation.

Control animals inoculated with 4 mg BIR myelin developed neurological signs of EAN commencing 10 days after inoculation. One of these animals had persistent mild tail weakness and one developed a chronic relapsing course (Figure 1). The clinical episodes were more severe and protracted in animals given 4 mg BIR myelin than in those given 2 mg BIR myelin.



**Figure 1.** The clinical courses of individual rats inoculated with 4 mg of BIR myelin and either given no treatment (panels A–D) or treated with 4 mg/kg of cyclosporin A three times per week until day 29 (panels E–I). The x-axis shows days after inoculation, The y-axis shows the clinical score, which was obtained by separately grading tail, hindlimb and forelimb weakness on a scale of 0 (no weakness) to 4 (complete paralysis) and then adding together the three numbers.

*CsA-treated animals*

As previously described (McCombe *et al.*, 1990), CsA-treated animals inoculated with 2 mg BIR myelin developed neurological signs of EAN 11–15 days after inoculation. All CsA-treated animals developed further episodes of EAN, which continued for up to day 70 after inoculation. Occasional animals had chronic tail weakness without clear remissions. All animals inoculated with 4 mg BIR myelin and treated with low dose CsA developed a chronic relapsing course with relapses occurring up to day 70 (Figure 1). These animals had more disability than those inoculated with 2 mg BIR myelin and had less recovery between the relapses.

**Pathological findings***Light microscopy*

These observations were made on 25 animals inoculated with 2 mg BIR myelin and four animals inoculated with 4 mg BIR myelin.

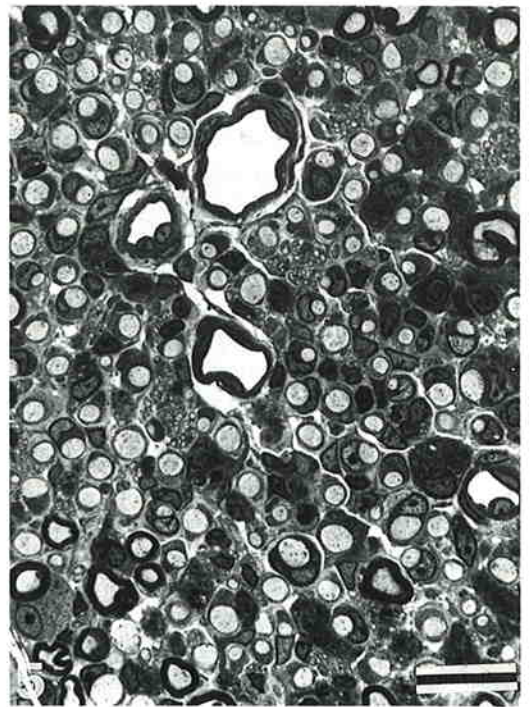
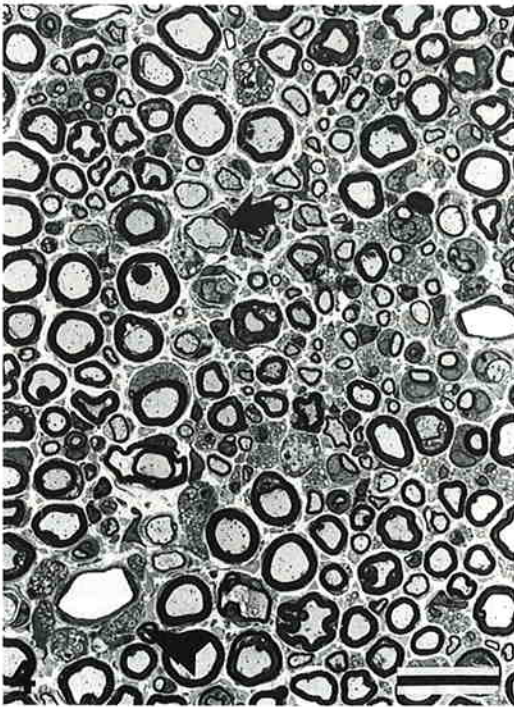
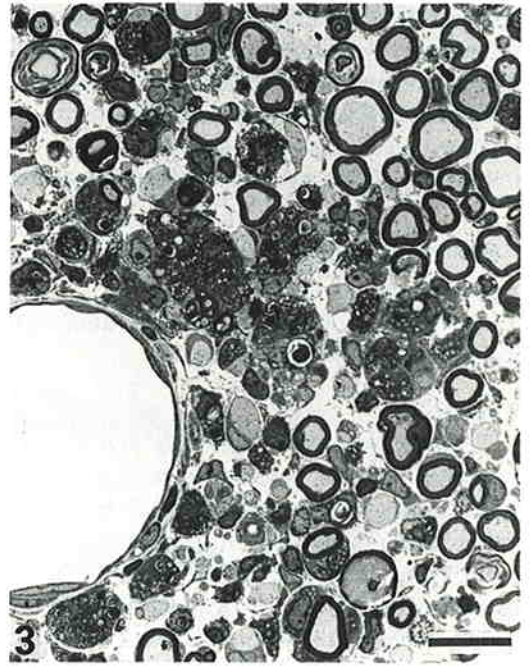
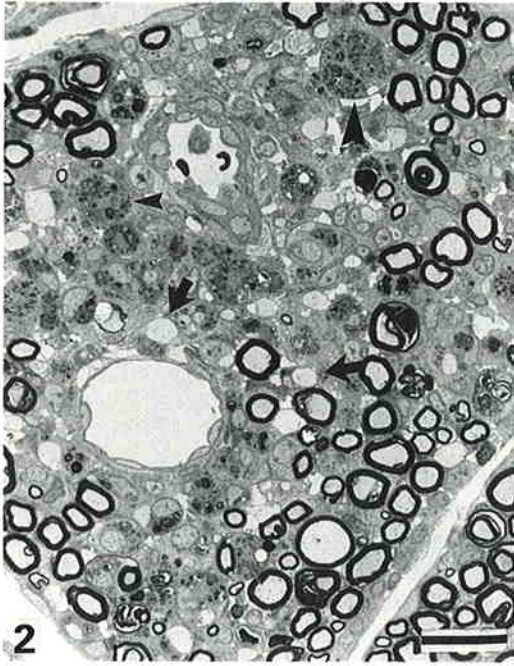
*Controls**(1) Animals inoculated with 2 mg BIR myelin*

*1st episode of EAN:* specimens were taken from five control animals at days 14–17 after inoculation during the first episode of disease. Four animals were aged 4 months and one was aged 7 months at inoculation. In all animals there was perivascular and parenchymal infiltration with mononuclear cells and primary demyelination of the nerve roots and dorsal root ganglia. In two of the five animals there was inflammation of the spinal nerves. In all animals the sciatic nerves were normal. In three animals there was inflammation and some demyelination at the junction of the nerve root with the spinal cord which was otherwise normal. Macrophages containing myelin debris were prominent in areas of demyelination. Occasional Schwann cell mitosis was observed. Endothelial cells were enlarged and projected into the lumina of blood vessels. Figure 2 shows the typical appearance of the nerve roots from a control rat in the first episode of EAN.

*After recovery from 1st episode:* an animal which had recovered completely from EAN was studied at day 43 after inoculation. In the nerve roots and dorsal root ganglia there was remyelination as indicated by inappropriately thin but otherwise normal myelin sheaths. There was no continuing demyelination. Macrophages were still present and Schwann cell mitosis was occasionally observed. Some remyelination and some macrophages with myelin debris were observed in the spinal nerves. Two other control animals were studied at days 154 and 365 after inoculation. In these animals there was well advanced remyelination in the nerve roots and dorsal root ganglia but no evidence of demyelination. Onion bulbs were not observed.

*(2) Animals inoculated with 4 mg BIR myelin*

Two control animals were inoculated with 4 mg BIR myelin and were studied at a time when CsA-treated EAN animals had developed chronic relapsing EAN. In one of these animals, studied at day 58 after recovery from a single clinical episode of EAN, there was evidence in the nerve roots and dorsal root ganglia of well advanced remyelination and occasional small onion bulbs. Macrophages containing myelin debris were present, especially in perivascular locations. Collapsed myelin figures, indicative of axonal degeneration, were seen in the sciatic nerve. Another animal, studied at day 79 after recovery from a single clinical episode of EAN, showed well-advanced remyelination in the nerve roots. Lipid-laden macrophages were present. Occasional onion bulbs were observed in the dorsal root ganglia.



*CsA-treated animals**(1) Animals inoculated with 2 mg BIR myelin*

*1st episode of EAN:* specimens were taken from two CsA-treated animals (each aged 4 months at inoculation) during the first episode of EAN at days 14–19. In the nerve roots and dorsal root ganglia there was perivascular inflammation and infiltration of the parenchyma by mononuclear inflammatory cells. Sub-perineurial oedema and endoneurial oedema were prominent. The most caudal roots were most severely affected. There was primary demyelination and macrophages containing myelin debris were present. Some inflammatory cells were noted in a subperineurial distribution. There was some inflammation and demyelination of the spinal nerves but the sciatic nerves were normal. In one animal there was a zone of inflammatory cells and demyelinated fibres in the central nervous system at the dorsal root entry and ventral root exit zones but there was no other involvement of the spinal cord. In areas of demyelination, blood vessels with enlarged and prominent endothelial cells were observed. Figure 3 illustrates the appearances in a nerve root from one of these animals.

*1st remission of EAN:* specimens were taken from two CsA-treated animals at days 25 and 36 after inoculation. In the dorsal root ganglia and nerve roots there was evidence of extensive remyelination by Schwann cells. Macrophages containing myelin debris were present in the parenchyma, around blood vessels and also beneath the perineurium. Some macrophages had formed giant lipid-filled cells. Around the nerve roots in the cauda equina there was still an inflammatory infiltrate. The spinal nerves showed evidence of remyelination. The sciatic nerves were normal. Figure 4 shows the appearances in the spinal nerve of a typical animal.

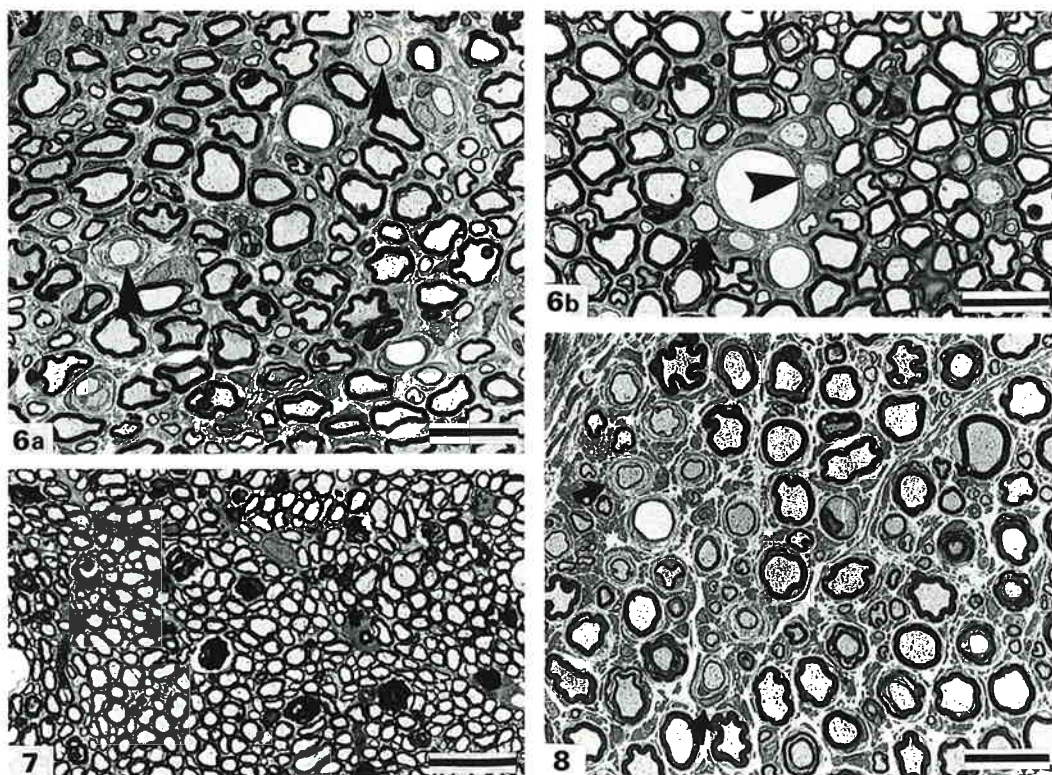
*2nd episode of EAN:* specimens from four CsA-treated animals were studied during a second episode of EAN at days 38–44. One animal was inoculated at age 2 months, two at age 4 months and one at 12 months. In the nerve roots and dorsal root ganglia there was severe inflammation and demyelination. In addition there was remyelination. Demyelination and inflammation were present in the spinal nerves. The sciatic nerves were normal. Plasma cells were observed in most areas of inflammation and mast cells were occasionally present. Macrophages containing myelin debris were present. Very early onion bulbs were seen in some sections of the cauda equina. Collapsed myelin figures indicative of axonal degeneration were observed in some nerve roots. Figure 5 indicates the typical appearances.

**Figure 2.** Section of a nerve root from the cauda equina from a control rat with acute EAN. There is perivascular inflammation. Demyelinated axons (arrows) and many macrophages containing myelin debris (arrowheads) are present. Toluidine blue, epoxy section. Bar represents 25 µm.

**Figure 3.** Sacral nerve root from a low dose-cyclosporin treated rat in the first episode of EAN at day 19 after inoculation. There is perivascular inflammation and demyelination and oedema. Large macrophages containing myelin debris are present. Toluidine blue, epoxy section. Bar represents 25 µm.

**Figure 4.** Lumbar spinal nerve from a low dose CsA-treated rat at day 36 after inoculation after recovery from the first episode of EAN. There are many inappropriately thinly myelinated fibres indicating remyelination (arrow). Lipid-filled macrophages are seen around a blood vessel (arrowhead). Toluidine blue, epoxy section. Bar represents 25 µm.

**Figure 5.** Sections from a sacral nerve root animal in the second episode of chronic relapsing EAN. There are many demyelinated fibres. Inflammatory cells are present, around blood vessels. HistoResin, cresyl fast violet. Bar represents 25 µm.



**Figure 6.** Rats with chronic persistent disease. **a**, Section of the L4 ventral root of a low dose cyclosporin-treated rat at day 65 after inoculation. Onion bulb formations (arrowheads) are seen around demyelinated remyelinated axons. Toluidine blue, epoxy section. Bar represents 25  $\mu$ m. **b**, Section of the L5 dorsal root from a low dose cyclosporin-treated rat at day 57 after inoculation. Demyelinated fibres (arrowhead) and remyelinated fibres (arrow) are seen in a perivascular location. Toluidine blue, epoxy section. Bar represents 25  $\mu$ m.

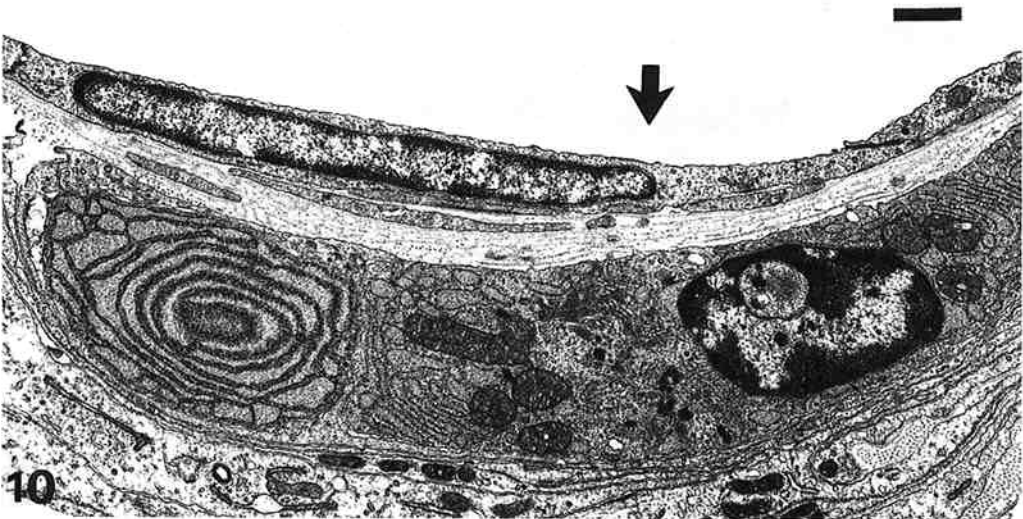
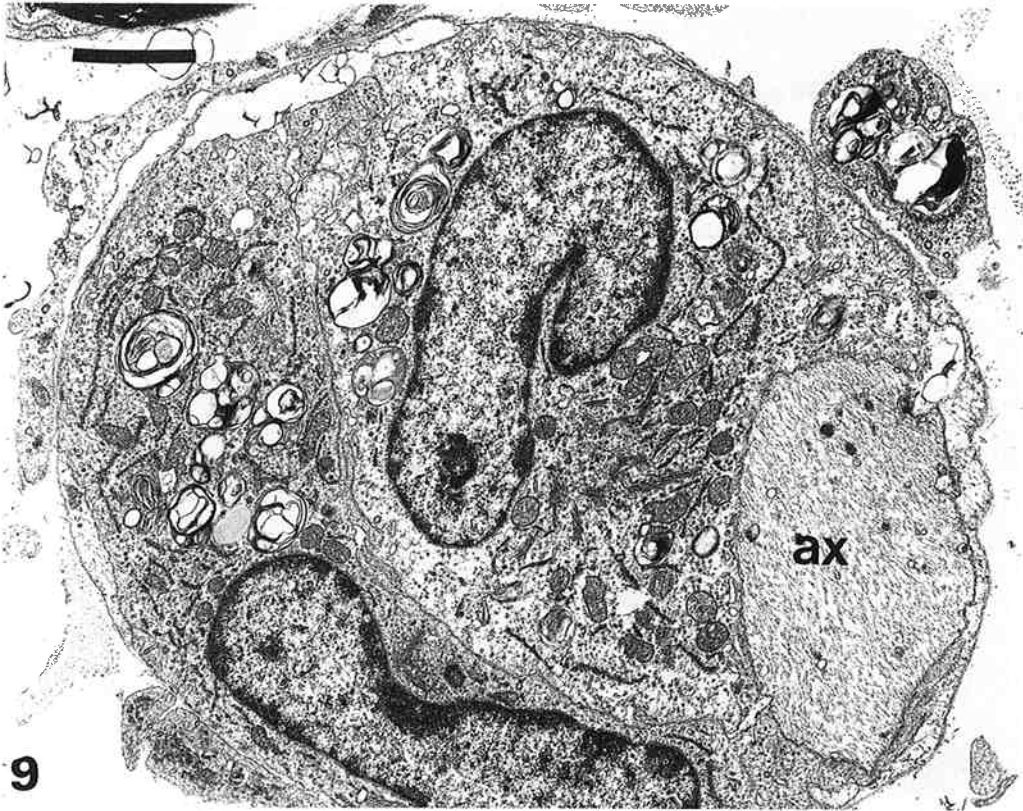
**Figure 7.** Section of the dorsal column of the T6 spinal cord from a low dose cyclosporin-treated animal with chronic tail weakness at day 57 after inoculation. There is prominent axonal degeneration. Toluidine blue, epoxy section. Bar represents 25  $\mu$ m.

**Figure 8.** Specimen from the L3 nerve root of a rat in late remission from chronic relapsing EAN at day 85 after inoculation after four episodes of weakness. There are onion bulb formations containing remyelinated fibres (arrows). Toluidine blue, epoxy section. Bar represents 25  $\mu$ m.

*3rd episode:* one animal, inoculated at age 5 months, was studied at day 65 after inoculation after a third clinical episode of EAN. There was evidence of recent demyelination in the nerve roots and dorsal root ganglia. Some demyelinated fibres were within onion bulbs. The major finding was of remyelination which varied in the stage of progression. Very thinly myelinated fibres and fibres with well-advanced remyelination were observed in the nerve roots, dorsal root ganglia and the spinal nerves. Onion bulbs were seen in these areas. Mast cells and macrophages also were observed.

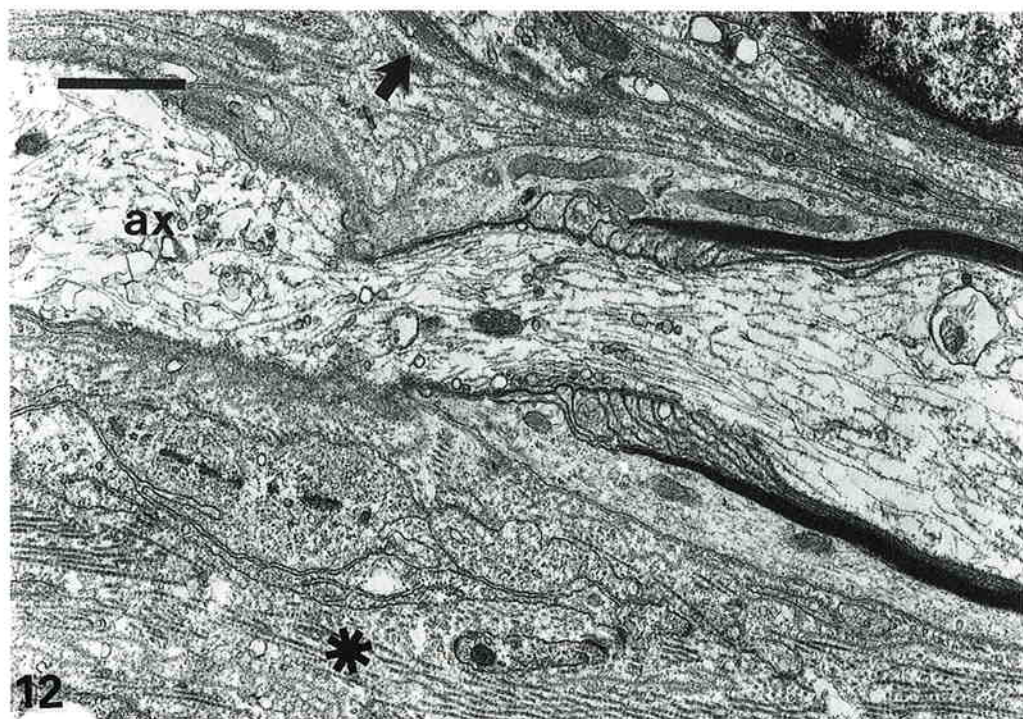
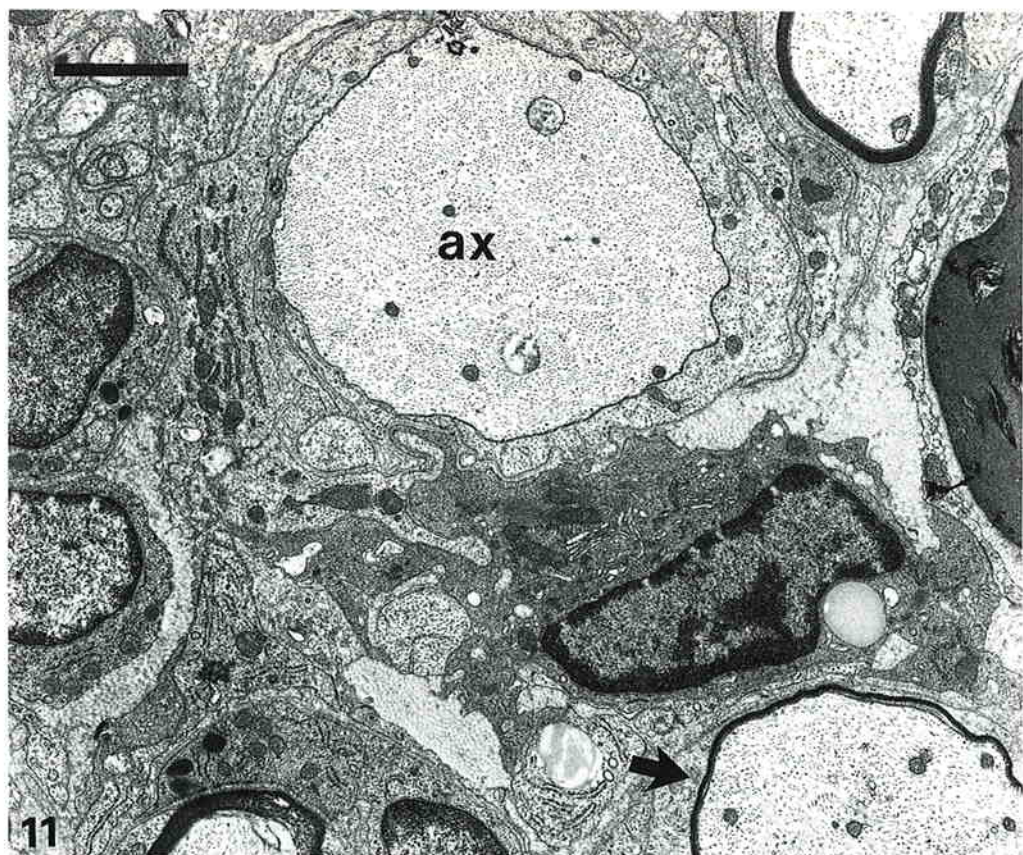
*Chronic persistent disease:* two animals with chronic persistent mild tail weakness without clear remissions were studied at days 57 and 65 after inoculation. There was evidence of both





**Figure 9.** Electron micrograph from a lumbar nerve root of a CsA treated animal in the first episode of EAN at day 19 after inoculation. A demyelinated axon (ax) is seen, associated with macrophages which have entered the Schwann cell basement membrane. Bar represents 2  $\mu$ m.

**Figure 10.** Electron micrograph from the nerve root of an animal in the first remission of CR-EAN. A plasma cell is seen adjacent to a layer of endothelium (arrow). Bar represents 1  $\mu$ m.



recent demyelination and remyelination. Frequent onion bulbs were seen in nerve roots, dorsal root ganglia, and spinal nerves. Macrophages filled with lipid were present, and perivascular inflammation was observed. In the dorsal columns of the spinal cord, collapsed myelin figures indicative of axonal degeneration were observed. Figures 6 and 7 illustrate the typical appearance.

*Late remission (after three or four episodes of disease):* in four animals, studied at days 82–105 after inoculation and after recovery from three or four episodes of clinical signs, there was extensive evidence of remyelination. Onion bulbs were present in roots, ganglia and spinal nerves. Figure 8 shows the typical appearances.

### (3) Animals inoculated with 4 mg BIR myelin

In one animal, studied at day 58 after inoculation during a third episode of EAN, there was severe inflammation and demyelination of the nerve roots, dorsal root ganglia, spinal nerves and sciatic nerves. There were areas of severe demyelination with no remaining myelin sheaths. Another animal, studied at day 79 after recovery from three episodes of EAN, showed remyelination and onion bulb formation. There was also evidence of axonal degeneration in the sciatic nerve and in the dorsal columns of the spinal cord.

### Electron microscopy

Specimens of single nerve roots or blocks of cauda equina containing several roots from 21 animals were studied at electron microscopy.

*Non-CsA-treated controls:* in a saline-treated control animal, studied at day 15 after inoculation, there was evidence of primary demyelination and macrophages containing myelin debris. Lymphocytes were also observed. In some fibres, vesicular dissolution of myelin was observed.

### CsA-treated animals

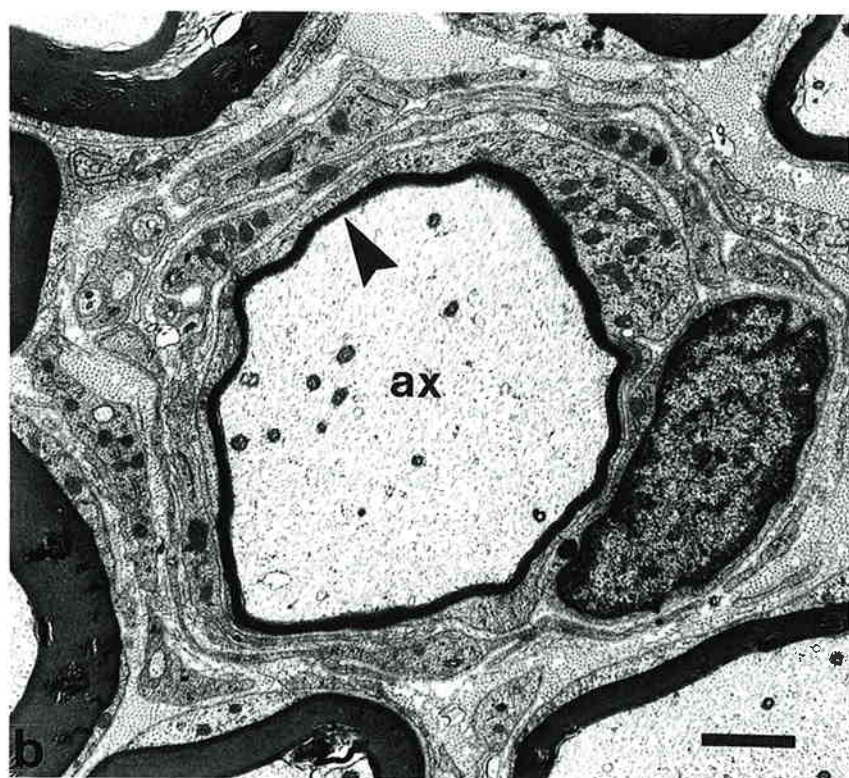
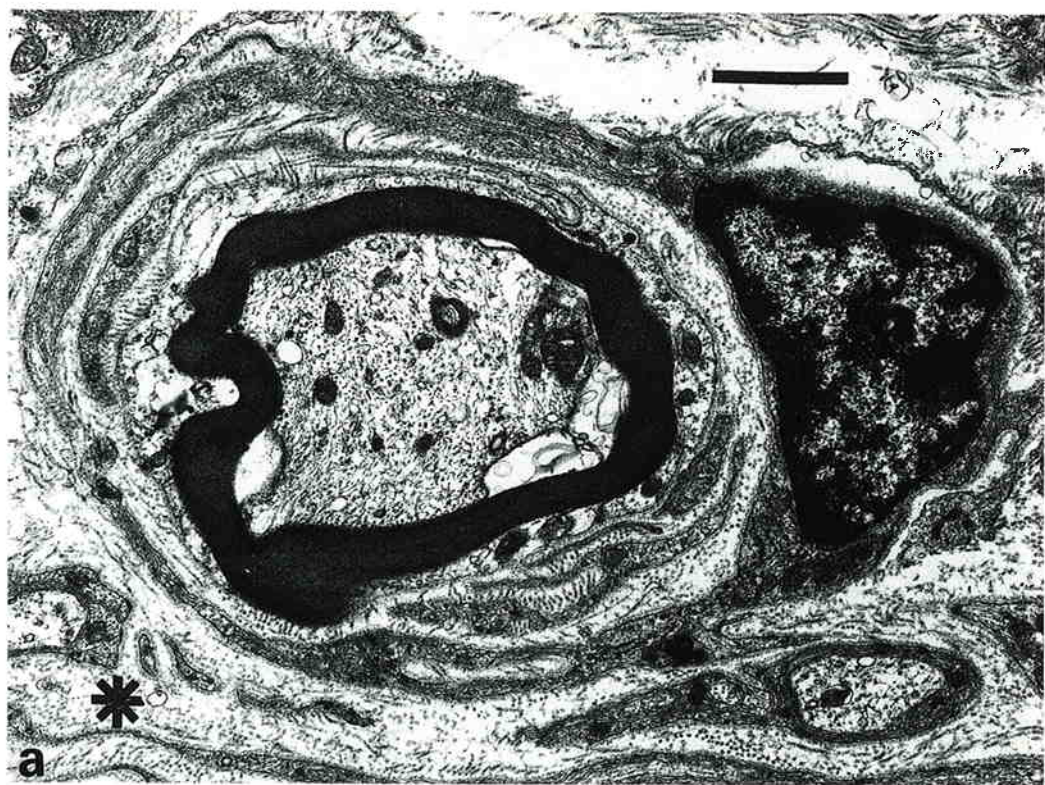
*1st episode of EAN:* nerve roots were examined from two animals at this stage of disease. In these specimens oedema was present. Inflammatory cells typical of lymphocytes were seen and many demyelinated axons were present. Macrophages containing myelin debris were seen, and macrophages were observed within Schwann cell basement membranes. Figure 9 shows a typical appearance.

*1st remission of EAN:* in specimens from two animals examined at this stage of disease, there was evidence of Schwann cell investment (pro-myelin stage) of some axons and formation of thin layers of compact myelin around others. Macrophages containing myelin debris were seen, especially around blood vessels. Figure 10 shows a plasma cell from an animal in the first remission.

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**Figure 11.** Section of a nerve root from a CsA-treated rat in the second clinical episode of EAN at day 44 after inoculation. A remyelinated fibre (arrow) and an axon (ax) invested by Schwann cell cytoplasm as a prelude to remyelination can be seen. A mononuclear inflammatory cell is present. Bar represents 2  $\mu\text{m}$ .

**Figure 12.** Longitudinal section of a coccygeal nerve root from a rat in the second clinical episode of EAN at day 44 after inoculation. A segment of demyelinated axon (ax) is seen next to a node of Ranvier. Excessive collagen (asterisk) and Schwann cell processes (arrow) are seen parallel to the nerve fibre. Bar represents 1  $\mu\text{m}$ .



*2nd episode of EAN:* specimens were examined from four animals at this stage of disease. Many mononuclear inflammatory cells were present including plasma cells. Perivascular cuffs of inflammatory cells were seen. Active demyelination was present and vesicular dissolution, myelin stripping and periaxonal dilatation of the sheaths were observed. Some remyelinated fibres were seen. Lipid-containing macrophages were located near blood vessels. Axonal degeneration was occasionally seen and some axons contained dense bodies. The perineurium was observed to contain many pinocytotic vesicles. The appearances are illustrated in Figures 11–13.

*3rd episode of EAN:* inflammatory cells were seen in the nerve roots. Blood vessels lined by plump endothelial cells were also observed. Onion bulbs were prominent in the dorsal root ganglia, and among the onion bulbs and remyelinated fibres there were many collagen fibres.

*Chronic persistent EAN:* specimens were examined from two animals at this stage of disease. Remyelination was still observed. Occasional large lipid-filled macrophages were seen. Onion bulbs were prominent and there were large deposits of collagen among the nerve fibres in the nerve roots.

*Late remission:* specimens were taken from three animals at this stage of disease. The prominent finding was extensive onion bulb formation associated with prominent collagen deposition. Axonal degeneration was observed and macrophages containing lipid droplets were present. Thinly myelinated fibres indicating remyelination were seen.

## DISCUSSION

The light and electron microscope findings of chronic relapsing EAN induced in the Lewis rat by inoculation with bovine intradural root myelin and treatment with low dose CsA are reported here. CsA is an immunosuppressive cyclic undecapeptide which, in higher doses, can suppress EAN (King *et al.*, 1983; Hartung *et al.*, 1987; McCombe *et al.*, 1990). Treatment with low dose CsA produces relapses of experimental allergic encephalomyelitis (Polman *et al.*, 1988; Pender *et al.*, 1990) and in a previous study we have shown that it can also convert acute EAN into chronic relapsing EAN (McCombe *et al.*, 1990). Different lymphocyte functions are inhibited by different concentrations of CsA (Hess, Esa & Colombani, 1988) and it has been suggested that low dose CsA treatment leads to chronic relapsing EAN by interference with the immunoregulatory cells which normally prevent relapses (McCombe *et al.*, 1990). The aim of the present study was to describe the pathological findings at different stages of this model of relapsing EAN, using animals with acute EAN as controls.

In the first episode of EAN, in CsA-treated animals and controls, there was evidence of perivascular and interstitial inflammation in the nerve roots and dorsal root ganglia with occasional inflammation of the spinal nerves. Plasma cells were not observed. Primary

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**Figure 13.** An onion bulb formation seen in the dorsal root of a rat at day 65 after the third episode of chronic relapsing EAN. Deposits of collagen (asterisk), which are not normally present in nerve roots, are seen. Bar represents 1  $\mu\text{m}$ . **b**, An onion bulb formation in the dorsal root of a rat with chronic mild tail weakness at day 57 after inoculation. The axon (ax) is surrounded by thin myelin (arrowhead) indicating remyelination and is surrounded by Schwann cell processes. Bar represents 2  $\mu\text{m}$ .

demyelination was seen at light microscopy, and at electron microscopy the demyelination was found to be associated with macrophages. The changes of inflammation and demyelination are similar to those described in acute inflammatory demyelinating polyradiculoneuropathy (AIDP) in man (Asbury, Arnason & Adams, 1969).

In this model of EAN, the pathology in the first episode was confined to the intrathecal parts of the peripheral nervous system. The dorsal root ganglia and nerve roots are prominently affected in EAN in the rabbit and mouse (Waksman & Adams, 1956). Hahn *et al.* (1988) have reported that, with lower doses of myelin, the pathological changes of EAN in the Lewis rat are prominent in the roots and ganglia, but that with higher doses the pathological changes are widespread in the peripheral nervous system. The selective involvement of the intrathecal parts of the peripheral nervous system probably occurs because the blood-nerve barrier is relatively permeable in these areas (Jacobs, Macfarlane & Cavanagh 1976; Petterson & Olsson, 1989) and because the spinal roots contain about three times more P2 protein which is a major target antigen in EAN (Kadlubowski, Hughes & Gregson, 1980) than does the sciatic nerve (Greenfield *et al.*, 1973). Similar involvement of the peripheral nervous system restricted to the spinal roots and ganglia occurs in acute experimental allergic encephalomyelitis in the rabbit and the rat (Pender & Sears 1984, 1986; Pender 1987; Pender *et al.*, 1989). King, Thomas and Pollard (1977) have previously described abnormalities in the dorsal root ganglion in relapsing EAN in the guinea pig. In AIDP, for which acute EAN is a model, the entire peripheral nervous system may be involved, but autopsy studies have shown that pathological changes are severe in the nerve roots and dorsal root ganglia (Asbury *et al.*, 1969; Honavar *et al.*, 1991). In the present study, the severe inflammation and demyelination in the dorsal root ganglia and the dorsal roots would account for the ataxia which was a prominent clinical sign. Neurophysiological study of acute EAN induced by this method, has revealed focal conduction block in the dorsal root ganglia (Stanley, McCombe and Pender, in press).

In the later episodes of chronic relapsing EAN, which are comparable to later episodes of chronic inflammatory demyelinating polyneuropathy (CIDP) in man, the pathological changes affected the spinal nerves and the peripheral nerves as well as the nerve roots and dorsal root ganglia. This was particularly evident in the animals inoculated with 4 mg BIR myelin. However, in the animals given 2 mg BIR myelin, the pathological changes, although widespread, were most severe in the nerve roots and dorsal root ganglia; this is also the case in cases of CIDP studied at autopsy (Thomas *et al.*, 1969; Asbury *et al.*, 1969; Matthews, Howell & Hughes, 1970; Borit & Altrocchi, 1971; Sibley, 1972). In later episodes of the CsA model of EAN, plasma cells were occasionally observed in the nerve roots. Plasma cells are not found in sural nerve biopsies from CIDP patients (Prineas & McLeod, 1976). Mast cells were prominent in the nerve roots in later episodes of disease in the present study; Brosnan *et al.* (1984) also found an increase in the number of mast cells in Lewis rats with chronic EAN.

One of the best known pathological features of CIDP is the presence of onion bulbs, whorls which are thought to arise after repeated episodes of demyelination and remyelination (Thomas & Lascelles, 1967; McLeod, Prineas and Walsh, 1971). However, these are not observed in all patients with CIDP (McCombe *et al.*, 1987) and it has been suggested that large multi-layered onion bulbs are most typical of genetically determined neuropathies (Thomas & Lascelles, 1967; Raine, 1977). Onion bulbs were observed in the nerve roots, dorsal root ganglia and spinal nerves in animals studied late after several episodes of EAN in the present study. The onion bulbs consisted of layers of Schwann cell processes and were associated with increased collagen which was very prominent in the nerve roots where collagen is not normally found. Occasional small onion bulbs were also observed in two control animals which had been inoculated with

4 mg myelin. This is in agreement with the observation of Adam *et al.* (1989) that onion bulbs are found in the dorsal root ganglia of Lewis rats studied late after EAN. Raine (1977) found abortive onion bulbs in the nerve roots of rabbits with chronic relapsing EAE, and Harvey *et al.* (1987) found onion bulbs in chronic EAN in the rabbit.

In the present study, at stages of active disease, endothelial cells of blood vessels appeared to be enlarged and to project into the lumen. This appearance was observed in the first and later episodes of EAN. Ballin and Thomas (1969) noted that the cytoplasm of endothelial cells was 'more bulky' than usual in guinea pigs with acute EAN. These appearances are consistent with the views of Burger and Vetto (1982) that endothelial cells have an active role in cell mediated immunity, and of Jalkanen *et al.* (1986) and of Duijvestijn, Schreiber and Butcher (1986) that endothelial cells differentiate into cuboidal cells, equivalent to high endothelial cells of venules in lymph nodes, at sites of immune activity. Cannella, Cross and Raine (1990) have shown that alterations of endothelial expression of cell adhesion molecules correlate with exacerbations of relapsing experimental allergic encephalomyelitis in the mouse.

Axonal degeneration was prominent in the later stages of disease. It was best seen in the sciatic nerves and in the dorsal columns of the spinal cord. Axonal degeneration in the dorsal columns is explained by interruption of the axonal continuity of the central processes of the dorsal root ganglion cells either in the dorsal root ganglia or dorsal roots. It was more obvious in animals inoculated with a higher dose of myelin, which is consistent with the observations of Hahn *et al.* (1988) in EAN induced by different doses of BIR myelin. King *et al.* (1977) described axonal degeneration in the later stages of chronic relapsing EAN in the guinea pig. The presence of dense, condensed bodies in degenerating axons was described by Lampert (1967) in rats with experimental allergic encephalomyelitis. This was a prominent finding in some animals in the present study and may represent early axonal damage. Axonal degeneration is a recognized feature of CIDP, and was found on teased fibre examination by Dyck *et al.* (1975) and at electron microscopy by Prineas and McLeod (1976).

In conclusion, the CsA model of EAN is characterized by relapsing episodes of demyelination. Later episodes are associated with an increase in the extent of involvement of the peripheral nervous system and the presence of plasma cells in the inflammatory infiltrate. Onion bulbs are also observed. Thus CsA EAN provides a good model for the study of the mechanisms of recurrent demyelination of the peripheral nervous system.

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