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The Pathophysiology of Acute Experimental Allergic Encephalomyelitis in the Rabbit

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Abstract

Clinical, histological and electrophysiological studies were performed on rabbits with acute experimental allergic encephalomyelitis (EAE). The clinical features were similar to those previously described, with the notable exception of the new findings of areflexia, respiratory slowing and hypothermia. The histological findings were also similar to those previously reported, with inflammatory demyelinating lesions both in the central and peripheral nervous system, especially the dorsal root ganglia.

Electrophysiological studies performed one to nine days after the onset of neurological signs demonstrated conduction block in a high proportion of the large diameter afferents in the lumbosacral and thoracic dorsal root ganglia. Single fibre studies with spike-triggered averaging confirmed the conduction block in the dorsal root ganglia. That the conduction block was due to demyelination was indicated by slowing of conduction in large diameter fibres, normal conduction in unmyelinated fibres and the specific effects of temperature and of the potassium channel blocking agent, 4-aminopyridine. These conduction abnormalities in the peripheral nervous system, focused on the dorsal root ganglia, account for the postural disturbance, hypotonia, ataxia and areflexia in rabbits with EAE. Such conduction block is likely to mask the expression of any lesions of the central nervous system that alone could produce similar signs. The implications of these findings for the human demyelinating diseases are discussed.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE), an autoimmune demyelinating disease, has been widely studied as a possible model of multiple sclerosis (MS). This approach has been criticized as EAE is typically a monophasic illness, in contrast to MS which usually has a chronic relapsing or chronic progressive course (Raine, 1976). This criticism has been overcome by the development of chronic relapsing EAE (Wiśniewski and Keith, 1977; Lassmann and Wiśniewski, 1979). Nevertheless there is one aspect of EAE that has been almost entirely neglected, namely, its pathophysiology. Although it is widely assumed that the neurological signs of EAE are due to CNS lesions, two lines of experimental evidence raise strong doubts about this assumption. First, histological involvement of the PNS has been described in all species examined, including the rabbit (Waksman and Adams, 1955; Wiśniewski *et al*., 1969), guinea pig (Freund et al., 1947; Waksman and Adams, 1956), mouse (Waksman and Adams, 1956) and monkey (Ferraro and Roizin, 1954). Involvement of the PNS also occurs in rabbits with chronic EAE (Raine *et al*., 1969) and guinea pigs, rats and mice with chronic relapsing EAE (Madrid and Wiśniewski, 1978; Lassmann et al., 1980; Brown *et al*., 1982). Surprisingly, the functional consequences of these lesions in the PNS have been totally ignored, and the belief that the neurological signs are due to CNS lesions has remained unchallenged. Secondly, there have been many reports of clinical signs of EAE occurring in the absence of CNS lesions, although after a comprehensive review of the literature Levine *et al*. (1975) regarded these observations as invalid.

The above considerations lead inevitably to the question as to how far the neurological signs in these animals are due to PNS lesions. Very few electrophysiological studies have been directed towards this question. Whereas Lumsden *et al*. (1975) found sciatic nerve conduction velocity to be normal in guinea pigs with EAE, Kaeser (1962) and Kaeser and Lambert (1962) reported it to be reduced. The latter workers attributed their findings to the known histological involvement of peripheral nerve in EAE in this species (Waksman and Adams, 1956).

The importance of determining the site and nature of the lesions responsible for the clinical signs in EAE is threefold. First, if the PNS lesions contribute significantly to the clinical signs, then remyelination of such lesions could be responsible for recovery, and hence the validity of chronic relapsing EAE as a model of MS would need to be questioned. Secondly, the suppression of, or improvement in, the clinical signs of EAE by such agents as myelin basic protein (Alvord *et al*., 1965) and immunosuppressants (Levine and Sowinski, 1980) provides the basis for their use in the treatment of MS. Thirdly, a knowledge of the site and nature of the lesions responsible for the neurological signs of EAE would add an important dimension to the study of EAE as an autoimmune neurological disease in its own right.

We have therefore undertaken a clinical, histological and electrophysiological study of acute EAE in the rabbit. Acute EAE was chosen because this stage of the disease is common to both acute and chronic relapsing EAE and because an understanding of its pathophysiology would allow the relapses of chronic relapsing EAE to be viewed in perspective. The specific aims of the study were (1) to establish that the disease we induced was typical of acute EAE in the rabbit; (2) to analyse critically the clinical and histological features, in order to determine to what extent such analyses per se could elucidate the site and nature of the lesions responsible for the neurological signs; (3) to study nerve conduction in the PNS, including the roots and dorsal root ganglia, in order to determine the functional consequences of the PNS lesions; and (4) to correlate the clinical, histological and electrophysiological observations. A preliminary report has already been published (Pender and Sears, 1982).

MATERIALS AND METHODS

Rabbits

New Zealand white male rabbits, obtained from one source, were aged between 4 and 6 months on arrival and weighed 2 to 3 kg. They were kept individually and fed water and rabbit-guinea pig pellets *ad libitum*.

Preparation of Inoculum

Each batch of inoculum was prepared by mixing 5 g of rabbit spinal cord (after removal of the nerve roots) with 7 ml of incomplete Freund's adjuvant (Difco), 125 mg of H37RA heat-killed *Mycobacterium tuberculosis* (Difco) and 3 ml of 0.9 per cent saline.

Inoculation Procedure and Management of Inoculated Animals

An interval of about seven days elapsed between arrival and inoculation. All except 2 animals weighed between 2.5 and 3.5 kg at the time of inoculation, which was by the intradermal injection of 0.1 ml of inoculum into each of four sites in the nuchal region, chosen in preference to the footpad in order to avoid the possibility of foot pain interfering with the assessment of motor function. Inoculated animals were examined and weighed every second day for the first ten days, and daily thereafter. *Ataxia* was graded as (1) mild, when noticeable on turning or on stopping suddenly after hopping, but with little interference with hopping in a straight line; (2) moderate, with impairment of hopping in a straight line; and (3) severe, with inability to hop. *Weakness* was graded as (1) mild with slight dragging of limbs when hopping; (2) moderate, with weakness of intermediate grade between mild and severe; and (3) severe, with severe weakness and virtually no movement.

During the first few days of the illness, rabbits were often given small amounts of cabbage to promote their appetites. As the illness progressed, more severely affected animals were given daily intraperitoneal or subcutaneous injections of 50 ml of a dextrose-saline solution (4 per cent dextrose;

0.18 per cent saline) and 25 ml of 0.9 per cent saline to minimize dehydration from reduced drinking. Of those animals that developed clinical signs, 25 were anaesthetized and underwent electrophysiological studies in terminal experiments one to nine days after the onset of signs. At the end of the electrophysiological recordings 3 rabbits were perfused with fixative for histological examination. In 2 others subjected to electrophysiological experiments the ganglia studied were removed at the end of the experiment and immersed in fixative. Of the remaining affected animals, 5 were anaesthetized with 25 per cent urethane i.v. and perfused with fixative for histological examination. Six were anaesthetized and underwent lumbosacral laminectomy but died without an electrophysiological study being performed. The remainder were sacrificed by the i.v. injection of urethane or pentobarbitone sodium, either at the height of the illness or after a period of spontaneous recovery.

Controls

Five normal rabbits, observed and weighed for periods of at least one month, served as clinical controls, 2 of these also serving as controls for electrophysiological experiments and 1 for histology. An additional 5 normal animals served as controls for electrophysiological experiments and 1 other normal rabbit as a second control for histology.

Histological Studies

The animals were perfused through the left ventricle with 100 to 200 ml of 0.9 per cent saline followed by 1.2 1 of fixative. In 1 control animal and 4 clinically affected animals, modified Karnovsky fixative (2.5 per cent glutaraldehyde and 2 per cent paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4) was used; in 1 normal animal and 1 animal with EAE the fixative was 10 per cent formol calcium and in the remaining 3 animals with EAE, 10 per cent formol saline. The brain, optic nerves, spinal cord with attached roots and dorsal root ganglia (DRG), lumbosacral spinal nerves, left lumbosacral plexus and left sciatic nerve were removed and immersed in fixative. The tissues perfused with formol saline or formol calcium were embedded in paraffin wax and sections stained with haematoxylin and eosin. Tissue perfused with Karnovsky fixative was immersed in this fixative, postfixed with 1 per cent osmium tetroxide, and semithin sections (0.5 μm, Araldite embedding) stained with toluidine blue.

Electrophysiological Studies

Preparation of animals for recording. Anaesthesia was induced by urethane (25 per cent in 0.9 per cent saline, i.v., 7 ml/kg) and supplemented by 25 per cent urethane (0.5 ml/kg i.v.) or pentobarbitone sodium (4 mg/kg). Animals breathed spontaneously through a tracheostomy. During the experiment 100 to 200 ml of either 0.9 per cent saline or Hartmann's solution (compound sodium lactate BP, Travenol) were given slowly by parenteral injection.

Experiments on the lumbosacral region. The animal was mounted in an animal frame (Transvertex). A laminectomy was performed from the fifth or sixth lumbar to the second sacral vertebra, and the left S1, and sometimes L7 and S2, dorsal root ganglia and spinal nerves were exposed. A pool was made with the skin flaps and the dura opened. The left hindlimb was extended and supported in a horizontal position, the left sciatic nerve and gastrocnemii exposed, and a pool formed with skin flaps. The sciatic nerve in the midthigh was dissected free, care being taken to avoid damage to its blood supply. The peroneal nerve was cut in the popliteal fossa. The tissues were covered in paraffin oil. The sciatic nerve and laminectomy pool temperatures were maintained at 37° C by a D.C. heating element in the sciatic nerve pool and a controlled radiant heat lamp. The sciatic nerve in the midthigh was lifted away from the volume conductor and placed across a pair of silver stimulating electrodes 5 mm apart. Since the rabbit sciatic nerve is very susceptible to ischaemia, its continuity was usually maintained. For the C and A delta fibre studies, however, which were performed after the large diameter fibre studies, the nerve was cut and the proximal end stimulated. Stimuli were 0.1 ms square-wave voltage pulses except for the C and delta fibre studies when 1 ms pulses were used. The stimulation rate was 1.0 Hz

except for the studies on C and delta fibres when it was reduced to 0.1 Hz to provide good stability of the responses.

All recording electrodes were connected by driven shielded leads to FET source-followers, thence to a preamplifier (band width limited to 1.6-3000 Hz) and then for display on an oscilloscope. Oscilloscope traces were photographed for measurements. Conduction velocities were calculated after allowing for a utilization time of 0.1 ms (Blair and Erlanger, 1936). The area under the curve of the compound action potential was derived by tracing the photographed curve on a digitizer tablet linked to a MOP AM-03 image analyzer (Kontron), coupled to a microcomputer (Research Machines 380Z).

The compound action potential of the left medial gastrocnemius muscle was recorded with a `belly tendon lead' through two 21-gauge hypodermic needles, one in the centre of the muscle belly and the other near the Achilles tendon.

For monophasic recording from the distal cut end of the left S 1 dorsal or ventral root, the filaments of the root were carefully identified, tied together close to the root entry or exit zone and cut proximal to the tie. The distal cut end was lifted away from the volume conductor into oil and placed on a pair of platinum wire electrodes 4 mm apart. Care was taken not to stretch or kink the root. For all recordings in the present study, negativity at the active electrode gave an upward deflection on the oscilloscope. Volume conductor recordings were made in turn over the left S l spinal nerve or ventral primary ramus (subsequently referred to as the spinal nerve) 3 to 6 mm from the midpoint of the DRG, over the left Sl DRG and over the left S1 dorsal root entry zone (*see* diagram in fig. 6). The active electrode was a length of 0.5 mm diameter platinum wire insulated to its tip. A platinum reference electrode was placed on nearby bone. Before all recordings any accumulated CSF or other body fluid was aspirated.

At the end of the experiment the dissection was extended to expose the left lumbosacral plexus and the entire length of the conduction pathway from the sciatic nerve to the relevant dorsal root ganglia. The contribution of the spinal nerve, of the segment or segments studied, to the sciatic nerve was confirmed. The L7 and S1 spinal nerves always contributed to the sciatic nerve; the S2 spinal nerve usually but not always contributed. The L6 spinal nerve was not exposed routinely but was noted to give no contribution in some animals and a small or moderate contribution in others. The contribution of S2, when present, and that of L7 varied in size whereas the S1 contribution was always large. The S1 segment was therefore chosen as the standard one for recordings. Conduction distance was measured as the length of a thread placed along the conduction pathway.

Experiments on the thoracic region. A lower thoracic laminectomy was performed and three to five of the T6 to T10 dorsal root ganglia and spinal nerves were exposed. One of the respective internal intercostal nerves was exposed, freed, tied and cut just distal to the tie (*see* Sears, 1964, for anatomical details). For recording the compound action potentials the stimulating and recording procedures were the same as those used in the lumbosacral experiments.

Single fibre studies. Conduction in single afferent fibres was studied by spike-triggered averaging as described by Kirkwood and Sears (1975). Afferent discharges were recorded from the external intercostal nerve or an internal intercostal nerve filament ‘in-continuity’. The discharges of a single afferent were selected with a spike discriminator (Digitimer D130) to provide a trigger for averaging the volume conductor recordings over the respective spinal nerve (2 to 4 mm from the midpoint of the DRG), DRG, dorsal root and dorsal root entry zone. The recording arrangements are illustrated schematically in fig. 11. To obtain the trigger spike, the intercostal nerve or filament was freed and lifted away from the volume conductor by two small pieces of polyethylene tubing inserted underneath the nerve. The active electrode was a platinum wire touching the nerve midway between the pieces of tubing. A platinum wire placed nearby on non-nervous tissue served as the reference electrode. The signal was amplified (band width 50-10 000 Hz) and fed to a spike discriminator through a second high pass filter (time constant 2 ms), which was needed to provide good baseline stability of the trigger pulses. The volume conductor recordings over the spinal nerve, DRG, dorsal root and dorsal root entry zone were amplified (band width 50-10 000 Hz) and averaged with a transient recorder (Datalab DL905) and signal averager (Datalab DL 4000B).

RESULTS

Clinical Findings

An animal was only considered to have EAE if it had neurological signs; weight loss alone was not regarded as sufficient evidence. The day of onset therefore refers to that of the neurological signs. Of the 51 animals inoculated, 41 developed EAE. Two others died from intercurrent infection in the first seven days after inoculation. The remaining 8 did not develop neurological signs during postinoculation periods ranging from 32 to 84 days. The mean day of onset after inoculation was 20 ± 6.4 (± SD) with a range of 12 to 53 days. Only in 1 rabbit did the day of onset exceed 30, and this was one whose age (18 months) and weight (4.75 kg) were much greater than those of the others at the time of inoculation.

Usually the neurological signs evolved over three to four days, were symmetrical and associated with weight loss. Lateral splaying and ataxia of the hindlimbs usually developed first. Hypotonia, loss of the knee jerks and forelimb involvement followed. As the disease progressed, impaired nociception, weakness of the limbs, perineal soiling, paradoxical breathing and slowing of respiration then developed. Thus the clinical findings were generally similar to those previously reported (Morrison 1947; Waksman and Adams 1955, 1956) with, however, the addition of the three previously unreported features of areflexia, hypothermia and slowing of respiration.

Arejlexia. In 33 affected animals the knee jerks were absent bilaterally. In 4 others, at least one knee jerk was depressed or absent; in the other 4 the knee jerks remained normal.

Hypothermia. In the normal controls the rectal temperature ranged from 39.0° C to 40.0° C with a mean of 39.4 ± 0.3° C and in 22 rabbits with EAE it ranged from 36.5° C to 41.5° C with a mean of 39.2 ± 1.2° C. The lower and upper limits of normal were taken as the mean ± 2 SD (38.8° C and 40.0° C). Of the rabbits with EAE, 11 had temperatures within these limits, 7 had temperatures below 38.8° C and 4 had temperatures above 40.0° C. All 7 rabbits with temperatures below 38.8° C had severe hindlimb ataxia or weakness.

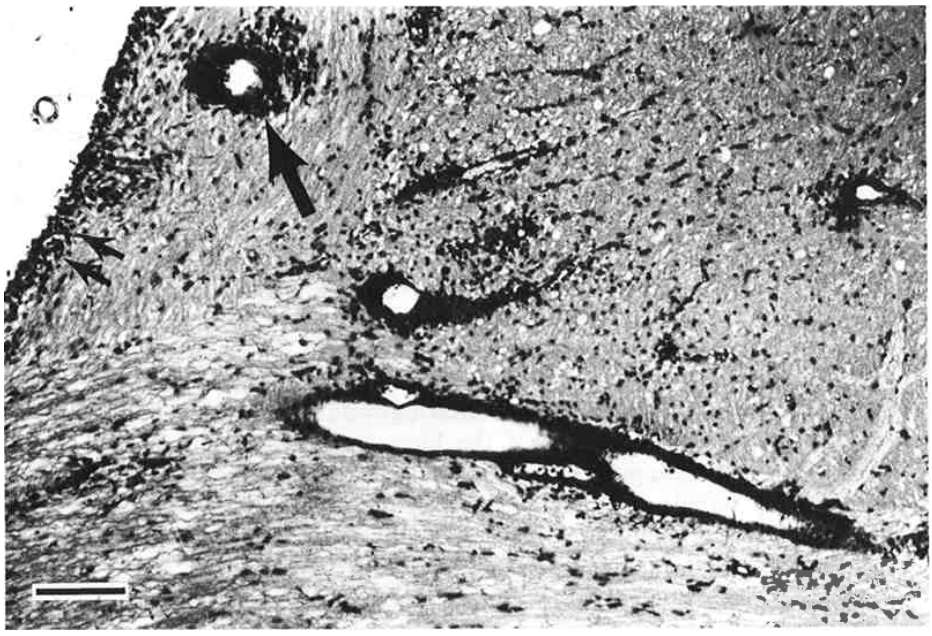

Fig. 1. Transverse section through the brainstem of a rabbit with EAE, four days after the onset of neurological signs. There is extensive perivascular cuffing and para-adventitial infiltration with mononuclear cells (large arrow) as well as meningeal and subpial infiltration with mononuclear cells (double arrows). Paraffin section, H and E stain. Bar = 100 μm.

Slowing of respiration. In 9 normal awake rabbits the resting respiratory rate ranged from 94/min to 170/min, with a mean of 144 ± 23/min. The lower limit of the normal respiratory rate was taken as the mean -2 SD (namely, 98/min). The respiratory rates were counted in 38 animals with EAE, and in 26

of these the rate fell below 98/min during the clinical course. Almost invariably in these animals the rate was normal on the day of onset and fell progressively as the neurological signs increased. The range of the lowest respiratory rate recorded in these animals was 24 to 88/min (mean 61/min).

All animals remained alert throughout the illness and no epileptic seizures were observed. The pupillary and blink responses to light were normal in all.

Spontaneous improvement. Fifteen affected animals showed definite spontaneous neurological improvement. This was first evident two to five days after the onset of EAE. The degree of improvement was usually mild or moderate. Only 2 animals recovered completely, and neither of these had been severely affected.

Histological Findings

Controls. Sections stained with haematoxylin and eosin from normal rabbits showed occasional scattered foci of mononuclear cells in the CNS, particularly the cerebral cortex. Such lesions may occur in normal rabbits, and are attributed to infection with *Encephalitozoon cuniculi* (Goodpasture, 1924; Waksman and Adams, 1956). Semithin sections stained with toluidine blue showed no evidence of demyelination and no other abnormality.

Animals with EAE. Sections stained with haematoxylin and eosin from rabbits with EAE showed widespread meningeal and subpial infiltration with mononuclear cells and some eosinophils, and widespread perivascular cuffing and para-adventitial infiltration with such cells throughout the CNS, especially in the brainstem (fig. 1) and spinal cord. These lesions were most pronounced in the dorsal root entry and ventral root exit zones. The anterior horn cells and other neurons in the CNS were normal. Semithin sections of the spinal cord stained with toluidine blue showed mild demyelination strictly confined to the regions of subpial and perivascular mono-nuclear infiltration (fig. 2), including the dorsal root entry and ventral root exit zones. Myelin debris was prominent within macrophages in the lesions, and plasma cells were occasionally seen among the infiltrating cells. Axons were preserved.

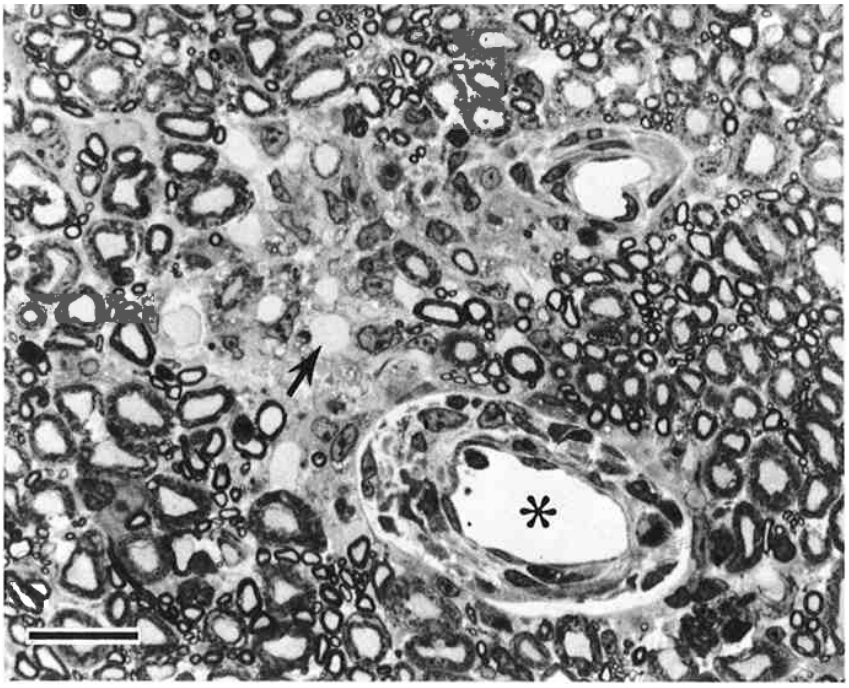

Fig. 2. Transverse section through the dorsal column of the lumbar spinal cord of a rabbit with EAE, four days after the onset of neurological signs. Two perivascular lesions (asterisk in one vessel) have become confluent. Mononuclear cells are present in the Virchow-Robin spaces and in the adjacent neural parenchyma. Demyelinated axons (arrow) can be seen within the region of mononuclear infiltration. Araldite section, toluidine blue stain. Bar = 25 μm.

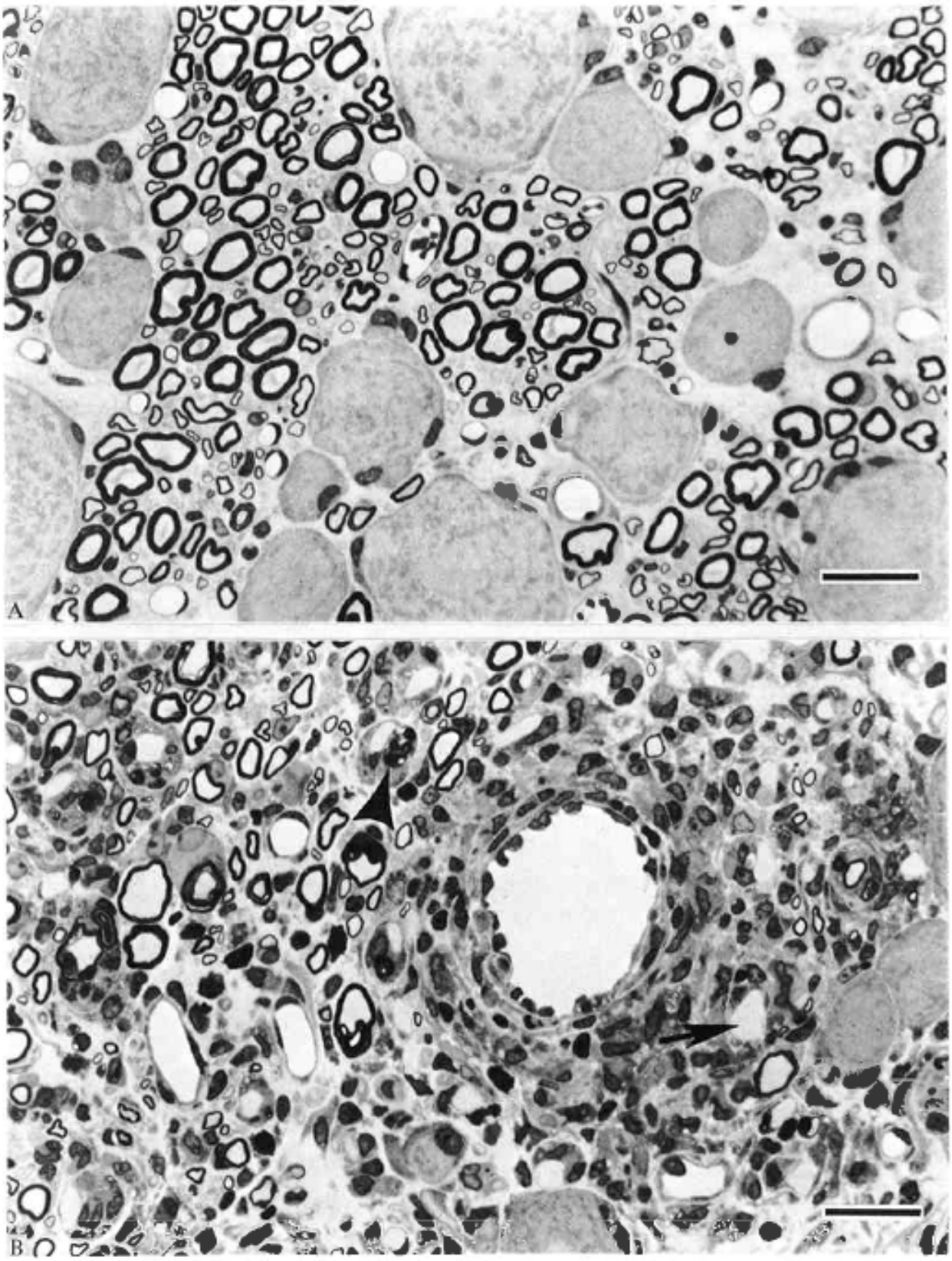

Fig 3. A, Transverse sections through the Si dorsal root ganglion of a normal rabbit and B, through that of a rabbit with EAE, two days after the onset of neurological signs. B shows a blood vessel surrounded by a large cuff of mononuclear cells which are invading the parenchyma. There are many demyelinated axons (arrow) within the region of mononuclear infiltration, and intracellular myelin debris (arrowhead) is visible. Araldite sections, toluidine blue stain. Bars = 25 μm.

The region most consistently and severely involved was the DRG. Cervical, thoracic and lumbosacral DRG were all affected and lesions were present in every ganglion examined. The lesions again consisted of perivascular cuffing and paraadventitial infiltration with mononuclear cells and eosinophils. Semithin sections showed demyelination in all the ganglia examined (*see* fig. 3). This was often extensive but was always limited to the regions of mononuclear infiltration. There was abundant myelin debris within macrophages, and plasma cells were occasionally present among the infiltrating cells. Axons were normal. The DRG neurons were usually normal. In contrast to the DRG, the spinal nerves were only mildly involved (*see* fig. 7).

Sections of the dorsal and ventral roots showed perivascular lesions similar to those in the DRG, but usually less frequent and less extensive. Sciatic nerve sections were either normal or showed minimal involvement.

Electrophysiological Findings

Conduction in motor nerve fibres. To assess motor nerve conduction and neuromuscular transmission in animals with EAE, the compound action potential evoked in the left medial gastrocnemius muscle by stimulation of the sciatic nerve in the midthigh was examined. In all 16 animals with EAE that were studied, motor nerve conduction and neuromuscular transmission were normal in every respect. To assess motor conduction more proximally, a total of 8 monophasic ventral root recordings were made in 6 animals with EAE. In 5 of these animals the recordings were normal in every respect, both for alpha and gamma fibres (e.g. fig. 4). In the other animal the responses were normal in configuration but severely reduced in amplitude. The most likely explanation for this reduction in amplitude was acute damage to the ventral roots during preparation because, as shown below, when EA E affects nerve conduction, the configuration of the compound action potential is altered.

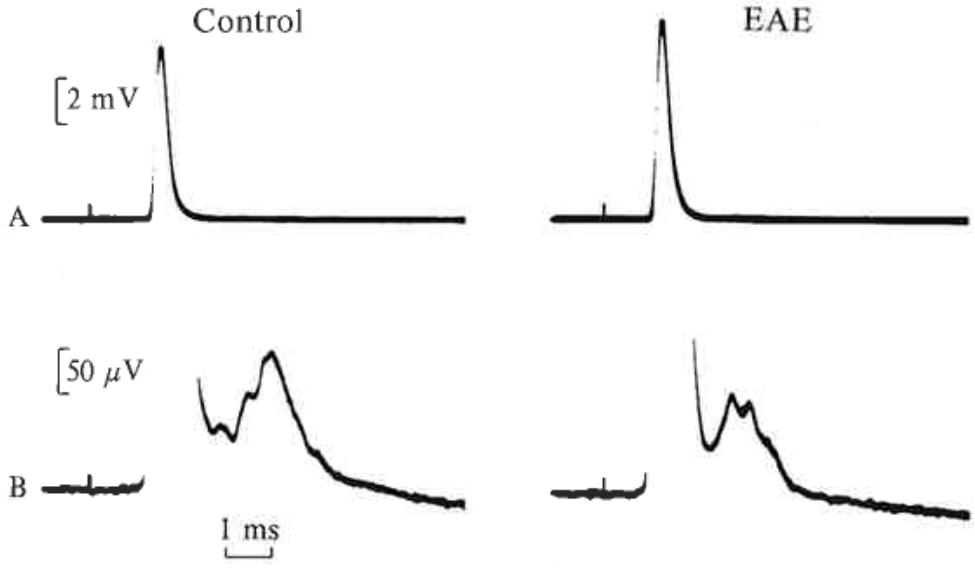

Fig. 4. Monophasic compound action potentials recorded from the distal cut end of the Si ventral root in a normal control rabbit and in a rabbit with EAE, in response to stimulation of the sciatic nerve. A, maximum A alpha fibre compound action potential. B, maximum A gamma fibre compound action potential. For these and all other recordings, negativity at the active electrode is represented by an upward deflection.

Conduction in sensory nerve fibres. In contrast to the normal conduction in motor nerve fibres, conduction in sensory nerve fibres from the sciatic nerve to the dorsal root was severely abnormal. All of the 13 dorsal root monophasic compound action potentials from the 9 animals with EAE showed similar abnormalities. Fig. 5A shows typical maximum amplitude responses from a normal control and from an animal with EAE. The response from the latter is greatly diminished in amplitude and has a

markedly abnormal irregular configuration replacing the smooth triangular form of the normal response. Compared to a mean of 2.4 mV (range 1.2 to 3.1 mV) for the peak amplitude in the normal controls, the mean in the animals with EAE was 0.4 mV (range 0.08 to 1.1 mV). This reduction would be due to conduction block, temporal dispersion (due to unequal slowing of conduction) or a combination of both. Conduction block was strongly suggested by the areas of the responses and also by measurements of threshold.

The area of the maximum amplitude response in the animals with EAE had a mean of 0.6 mV ms (range 0.1 to 1.3 mV ms) compared with a mean of 1.3 mV ms (range 0.7 to 1.7 mV) in the normal controls. Activation of the same fibres in the sciatic nerve was ensured by using the stimulus giving the maximum amplitude of the negativity in the spinal nerve response, which was normal in the animals with EAE (*see below*). At this stimulus intensity the area of the dorsal root response in the animals with EAE had a mean of 0.4 mV ms (range 0.08 to 0.72 mV ms) compared to a mean of 1.1 mV ms (range 0.9 to 1.33 mV ms) in the normal controls, indicating conduction block in a high proportion of the large diameter afferents in animals with EAE. For simplicity, the term `large diameter' will be used for fibres whose normal conduction velocity is greater than 25 m/s.

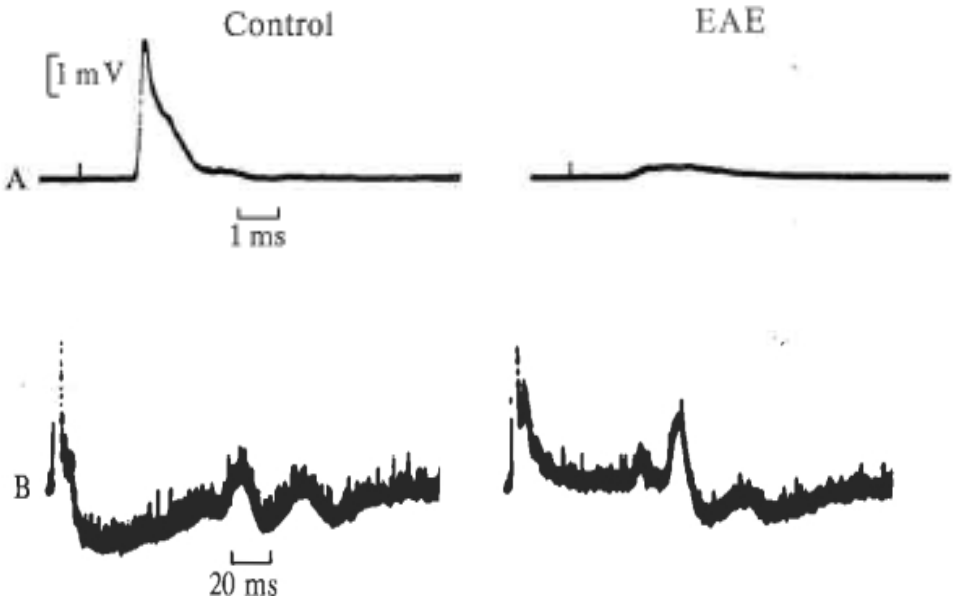

Fig. 5. Compound action potentials recorded from the distal cut end of the Si dorsal root in a normal control rabbit and in a rabbit with EAE, in response to stimulation of the sciatic nerve. A, maximum A alpha-beta (referred to as `large diameter' in the present study) fibre compound action potential. B, maximum C fibre compound action potential. Note the different amplitude and time scales.

In the normal controls the threshold for the dorsal root response (monophasic recording) was the same as that for the respective spinal nerve response (volume conductor recording). However, in 3 of 5 animals with EAE the threshold for the dorsal root response was increased to as much as 1.5 times threshold for the respective spinal nerve response, indicating conduction block in low threshold large diameter afferents.

At 37° C the conduction velocities of the foot and peak of the dorsal root compound action potential evoked by the stimulus giving the maximum spinal nerve response had means of 64.6 m/s (range 48.9 to 84.3 m/s) and 44.1 m/s (range 32.7 to 55.2 m/s), respectively, in the animals with EAE, compared to means of 89.4 m/s (range 83.2 to 97.9 m/s) and 68.3 m/s (range 63.9 to 75.2 m/s), respectively, in the normal controls. This reduced conduction velocity in EAE could be due to conduction block in the fastest fibres, slowing of conduction, or a combination of both. In some

animals with EAE, slowing of conduction was evidenced by the occurrence of delayed components in the dorsal root response at a stimulus intensity giving a normal spinal nerve response.

To determine whether conduction in unmyelinated afferent fibres (C fibres) was affected by the disease, monophasic recordings were made from a total of 9 dorsal roots from 6 animals with EAE. In 8 the C fibre responses were normal in every respect (e.g. fig. 5B). The C fibre compound action potential had three negative peaks with mean velocities of 1.3 m/s, 1.0 m/s and 0.8 m/s, respectively, in the controls and 1.5 m/s, 1.2 m/s and 0.8 m/s, respectively, in the animals with EAE. In fig. 5 the normal C fibre response in the animal with EAE contrasts with the greatly reduced maximum amplitude and the abnormal configuration of the large diameter myelinated fibre response in the same dorsal root recording. Conduction in small diameter myelinated afferents (delta fibres) appeared to be normal in the same roots in which the C fibre response was normal; however, the assessment of delta fibre conduction was limited by the proximity of the delta and large diameter fibre responses. In one dorsal root recording from an animal with EAE, no delta or C fibre response could be elicited. The reason for this is unclear. The large diameter fibre response from this dorsal root showed the typical abnormal configuration and was greatly reduced in amplitude and area.

Localization of the site o f conduction abnormalities in afferent fibres. To determine whether the site of the block and slowing of conduction was in the DRG, as anticipated from the histological studies, volume conductor recordings were made over the spinal nerve and DRG in response to stimulation of the sciatic nerve.

In the normal control the spinal nerve response recorded 3 to 6 mm distal to the DRG consisted of a triphasic wave (positive-negative-positive), the amplitude of the negativity being greater than that of the initial positivity (fig. 6). The initial positivity is due to passive outward current driven by the approaching impulses, while the negativity represents inward current during the rising phase of the action potential under the recording electrode. Recordings were made from a total of 14 lumbosacral spinal nerves in 12 animals with EAE. All recordings were normal in threshold, peak-to-peak amplitude and the conduction velocities of the positive and negative waves (e.g. fig. 6). Also, the ratio of the amplitude of the negativity to that of the initial positivity in the maximum spinal nerve response was normal, with a mean of 1.5 (range 1.0 to 2.3) compared to a mean of 1.4 (range 0.9 to 2.0) in the normal controls. Therefore the normal spinal nerve response in EAE could be used as a reliable index of the population of fibres activated in the sciatic nerve.

The recording over the DRG in the normal control animals was the same as that over the spinal nerve (fig. 6). Recordings were made from a total of 18 dorsal root ganglia from 13 animals with EAE. There was usually a marked decline in the amplitude of the negativity with respect to that of the initial positivity *(see* fig. 6), the mean ratio of the former to the latter at the stimulus intensity giving the maximum spinal nerve response being 0.5 (range 0.2 to 1.0) compared to 1.5 (range 1.0 to 1.8) in the normal controls. The reduction in the negativity indicates that the dorsal root ganglion is the site of the conduction block in the large diameter afferents. This localization corresponds well with the site of the lesions, as illustrated in fig. 7. A normal response was recorded over the S l spinal nerve where a longitudinal section showed normal myelin sheaths, except for a few small foci of demyelination. In contrast, the recording over the DRG, only 3 mm proximally, shows a markedly abnormal response typical of that described above for EAE, and the corresponding longitudinal section shows extensive demyelination affecting the majority of large diameter fibres.

The proportion of fibres with conduction block in the DRG is likely to be even greater than indicated by the volume conductor recordings over the DRG, because the residual negative wave in these recordings is probably due in part to activity in the subjacent normally conducting ventral root fibres. A large response can be recorded over the DRG when the distal cut end of the respective ventral root is stimulated.

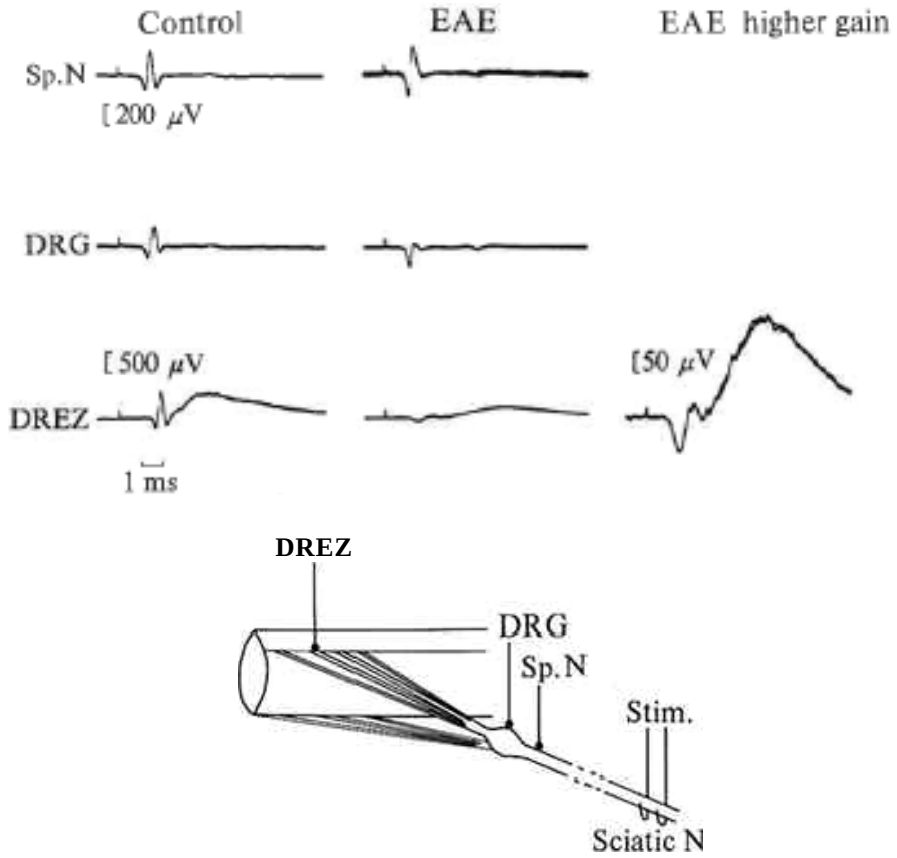

Fig. 6. Compound action potentials recorded, in the volume conductor, over the S 1 spinal nerve (Sp.N), dorsal root ganglion (DRG) and dorsal root entry zone (DREZ) in a normal control rabbit and in a rabbit with EAE, in response to stimulation of the sciatic nerve at the intensity giving the maximum spinal nerve response. Note the different gains. The 200 μV calibration bar applies to the spinal nerve recordings and also the dorsal root ganglion recordings. The recording arrangements are illustrated diagrammatically below. Stim. = stimulating electrodes on the sciatic nerve (Sciatic N).

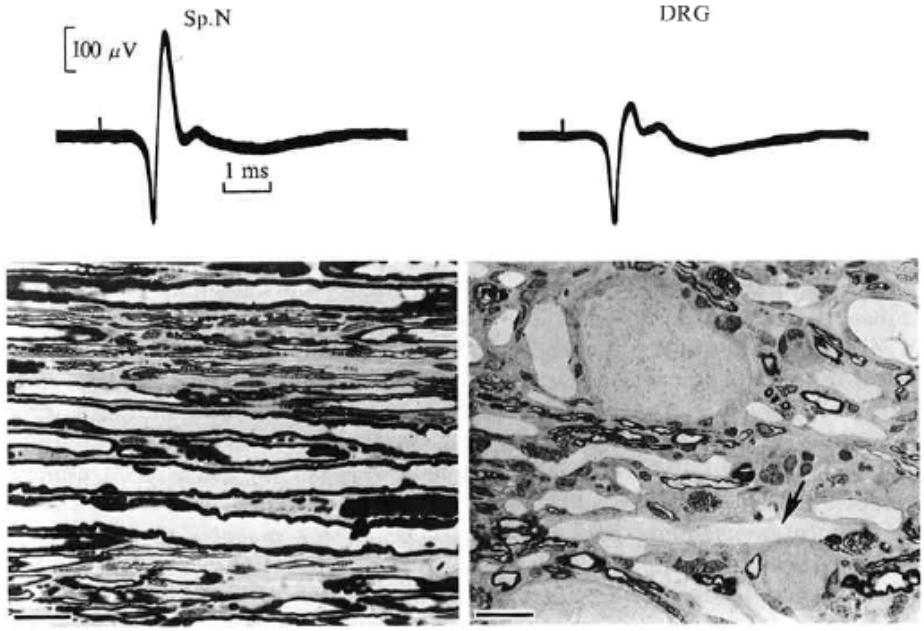

Fig. 7. Above. Compound action potentials recorded, in the volume conductor, over the Si spinal nerve (Sp.N) and dorsal root ganglion (DRG) of a rabbit with EAE, in response to stimulation of the sciatic nerve at the intensity giving the maximum spinal nerve response. The recording sites were 3 mm apart. The experiment was performed five days after the onset of neurological signs. Below. Longitudinal sections through the same spinal nerve and the dorsal root ganglion at the sites of the

above recordings. The myelin is largely preserved in the spinal nerve, but only 3 mm away in the dorsal root ganglion there is severe demyelination with long stretches of demyelinated axons (arrow) associated with mononuclear infiltration. Araldite sections, toluidine blue stain. Bars = 25 μm.

The lumbosacral DRG was shown also to be the site of slowing of conduction by the demonstration of prolongation of the negative wave in the recording over the DRG (illustrated for a thoracic DRG in fig. 10).

Effects of the conduction abnormalities in the DRG on the afferent volley arriving at the dorsal root entry zone. Conduction from the sciatic nerve to one or more of the lumbosacral dorsal root entry zones was studied by volume conductor recording. The normal response (fig. 6) consisted of an initially positive triphasic wave representing the afferent volley, and a late slow negative wave, the N wave, which is a field potential due to the synaptic currents in the second order dorsal horn neurons excited mainly by low threshold cutaneous afferents. Dorsal root entry zone recordings were made in 14 animals with EAE (24 recordings). The following abnormalities were present (*see* fig. 6). (1) There was a marked reduction in the peak-to-peak amplitude of the triphasic wave, the mean amplitude being 120 μV (range 50 to 400 μV) compared with 740 μV (range 430 to 1000 μV) in the normal controls. This reduction is accounted for by the conduction block in the DRG. (2) There was a gross reduction in the amplitude of the negative component of the triphasic wave, the mean amplitude being 40 μV (range 0 to 190 μV) compared with 550 μV (range 300 to 750 μV) in the normal controls. This reduction is largely accounted for by the conduction block in the DRG, but in part may also be due to conduction block at the dorsal root entry zone in large diameter afferents still conducting through the ganglion. In one animal with EAE, conduction block was demonstrated at the dorsal root entry zone in large diameter afferents that were still conducting, albeit slowly, through the DRG. (3) The conduction velocity of the negative component of the triphasic wave was reduced owing to conduction block and slowing of the fastest fibres in the DRG. (4) The amplitude of the peak of the maximum N wave was reduced, the mean being 320 μV (range 80 to 520 μV) compared to 730 μV (range 630 to 940 μV) in the normal controls. This is most likely to be due to conduction block in low threshold cutaneous afferents in the DRG and possibly at the dorsal root entry zone. (5) The latency to the peak of the N wave was prolonged, the mean being 5.1 ms (range 4.4 to 5.9 ms) compared with 3.3 ms (range 3.1 to 3.4 ms) in the normal controls. In general, the prolongation of the N wave latency was associated more with conduction block than with slowing of conduction in large diameter fibres.

Effects of temperature and 4-aminopyridine on the monophasic dorsal root compound action potential. To determine whether demyelination was responsible for the conduction block in the DRG, the effects of temperature (*see* Rasminsky, 1973) and of the potassium channel blocking agent, 4-aminopyridine (4-AP) (*see* Sherratt *et al*., 1980; Bostock *et al.,* 1981), on the dorsal root monophasic compound action potential were studied.

The effect of temperature was assessed in 6 animals with EAE. These effects were variable. In 3 animals, there was no effect beyond that seen in the normal control, in 1 a mild effect on amplitude but not on area, in another a mild effect on both amplitude and area and in the sixth animal a marked effect on amplitude and area. The recordings from the last animal were illustrated in a preliminary communication (Pender and Sears, 1982). When the temperature of the laminectomy pool in this animal was increased from 37° C to 40° C, the amplitude and area declined to 48 and 50 per cent of their original values, respectively (*cf* 88 and 74 per cent in the normal control) and when the temperature was reduced to 34° C they increased to 204 and 218 per cent (*cf* 108 and 125 per cent in the normal control). This animal exhibited a reversible increase in the degree of limb splaying and ataxia when its temperature was increased by 1.5° C on the day before the terminal experiment. These physiological and clinical effects of temperature can be accounted for by conduction block occurring in a greater number of demyelinated afferent fibres when the temperature is increased and by restoration of conduction by cooling. Davis and Jacobson (1971) observed similar effects of temperature on the compound action potentials of the peripheral nerves of guinea pigs with experimental allergic neuritis (EAN).

The effects of 4-AP on the compound action potentials recorded monophasically from the dorsal root and in the volume conductor over the spinal nerve were assessed in 2 animals with EAE. Fig. 8 shows the recordings from one. The amplitude and area of the dorsal root response had already increased one minute after the intravenous administration of 4-AP and continued to increase with further administration. After 1.25 mg had been given, the amplitude and area of the dorsal root response had increased to 112 and 124 per cent of their original values, respectively, compared with corresponding increases to 103 and 105 per cent for the negative wave of the spinal nerve response (*middle traces*). After the administration of another 1.25 mg of 4-AP, the amplitude and area of the dorsal root response increased to 117 and 136 per cent (not shown). When the temperature was then lowered to 34° C, the amplitude and area of the dorsal root response increased to 138 and 158 per cent of their original values, compared with the corresponding increases to 107 and 123 per cent for the negative wave of the spinal nerve response (*lower traces*). The combined effect of 4-AP and lowering of temperature on the amplitude and area of the dorsal root response was considerably greater than the effect of lowering temperature alone, whereas the combined effect on the amplitude and area of the negative wave of the spinal nerve response was similar to the effect of lowering temperature alone. 4-AP was observed to have similar effects in the other animal with EAE. These effects could be due either to restoration of conduction through the DRG in some fibres or to prolongation of the single unit action potentials at the dorsal root recording site. In either case, paranodal demyelination would have to be present to expose potassium channels, which are absent in the normal mammalian node of Ranvier (Sherratt *et al*., 1980; Bostock *et al*., 1981). In view of the histological findings, the effects of 4-AP are more likely to be due to restoration of conduction through the DRG than to an action on fibres at the dorsal root recording site.

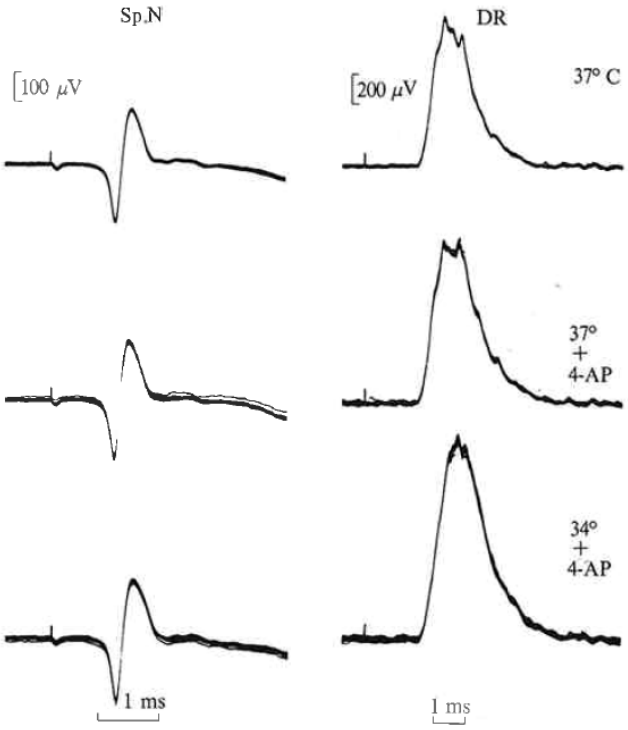

Fig. 8. The effect of 4-aminopyridine (4-AP) and of 4-AP combined with lowering of temperature on the compound action potential recorded, in the volume conductor, over the Si spinal nerve (Sp.N) and monophasically from the distal cut end of the Si dorsal root (DR) of a rabbit with EAE. For all recordings the sciatic nerve was stimulated at an intensity supramaximal for the amplitude of the dorsal root response.

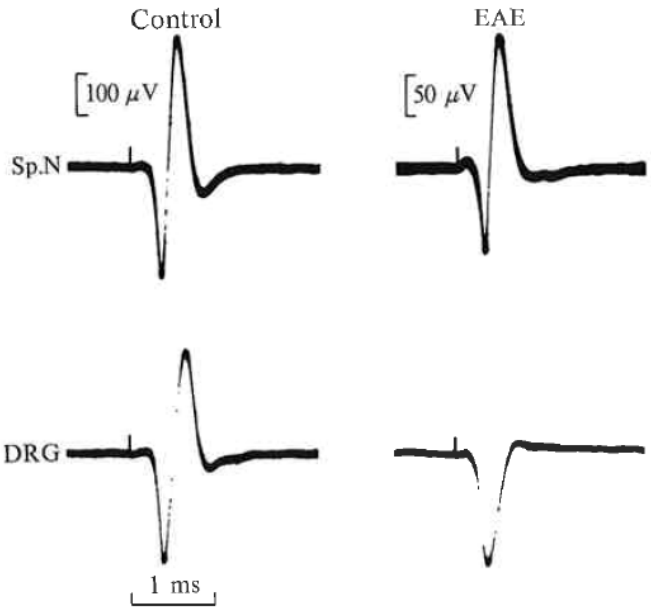

Fig. 9. Compound action potentials recorded, in the volume conductor, over the T10 spinal nerve (Sp.N) and dorsal root ganglion (DRG) in a normal control rabbit and in a rabbit with EAE, in response to stimulation of the respective internal intercostal nerve at the intensity giving the maximum spinal nerve response.

Conduction through the thoracic root ganglia. To determine whether conduction was impaired in the dorsal root ganglia of other segments, conduction through the lower thoracic ganglia was also studied. Volume conductor recordings were made over a lower thoracic spinal nerve, DRG and dorsal root entry zone in response to stimulation of the respective internal intercostal nerve in 5 animals with EAE. These studies demonstrated normal conduction from the internal intercostal nerve to the spinal nerve, but conduction block in a high proportion of the large diameter afferents in the DRG (*see* fig. 9). At the stimulus intensity giving the maximum spinal nerve response, the ratio of the amplitude of the negativity to that of the initial positivity in the DRG recording had a mean of 0.25 in the animals with EAE compared with 0.94 in the normal controls. The recordings over the thoracic dorsal root entry zone showed similar abnormalities to those in the lumbosacral recordings. One animal was studied later in the clinical course (six days after onset as opposed to three days or earlier for the other 4 animals) and after it had shown spontaneous neurological improvement. Its recordings (fig. 10), provide clear evidence of slowing of conduction in the DRG. They were made at the stimulus intensity giving the maximum spinal nerve response. In contrast to this response, which is normal, the DRG recording shows a reduction in the amplitude, and also a prolongation, of the negativity. The difference in the latencies to the ends of the negative waves in the spinal and DRG recordings is 0.6 ms. From this and the distance of 3.5 mm between the recording sites a velocity of 6 m/s was calculated. It is likely that the slowest conduction velocity of the large diameter fibres in the DRG was even lower than this, for two reasons. First, the conduction time for some fibres may have been greater than 0.6 ms, as the large diameter fibres with the slowest velocity in the DRG were not necessarily the slowest large diameter fibres contributing to the spinal nerve response. Secondly, the actual distance over which demyelination had occurred and thus conduction had been slowed in individual fibres was probably less than 3.5 mm. It is likely therefore that the real velocity was in the range of continuously conducting unmyelinated fibres, implying that the demyelinated internodal axonal membrane was now electrically excitable. The dorsal root entry zone recording from the animal with EAE shows an isolated low amplitude positivity and a delayed, predominantly negative, triphasic wave representing conduction through the dorsal root entry zone in fibres conducting slowly through the DRG. Further confirmation of slowing of conduction in the DRG in this animal was provided by the recording of delayed, predominantly negative triphasic waves over the DRG and dorsal root entry zone at threshold for the spinal nerve response.

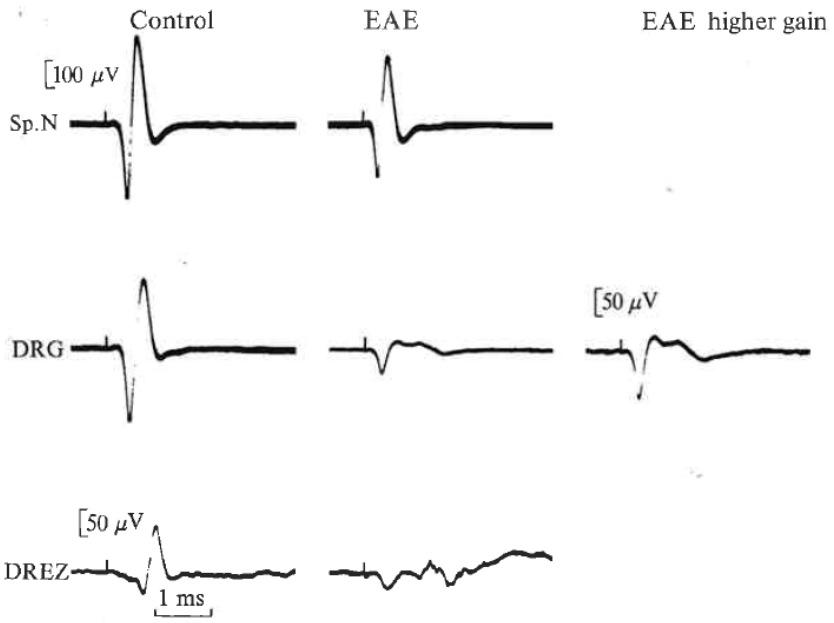

Fig. 10. Compound action potentials recorded, in the volume conductor, over the T10 spinal nerve (Sp.N), dorsal root ganglion (DRG) and dorsal root entry zone (DREZ) in a normal control rabbit and in a rabbit with EAE, in response to stimulation of the respective internal intercostal nerve at the intensity giving the maximum spinal nerve response. The 100 μV calibration bar applies to the DRG recordings in the left and middle column, as well as to the spinal nerve recordings.

Single fibre studies. The compound action potential recordings in both the lumbosacral and the thoracic regions have strongly indicated conduction block in a high proportion of the large diameter afferents in the DRG. Nevertheless, they have not provided absolute proof of conduction block, as the reduction in the amplitude and area of the compound action potential could be due to such severe temporal dispersion (due to unequal slowing of conduction) that the slowed responses were hidden in the background noise.

To provide absolute proof of conduction block in the DRG, conduction in single afferent fibres in the thoracic region was studied with spike triggered averaging as described by Kirkwood and Sears (1975). These studies were performed in 2 normal control animals and in 2 animals with EAE. In the normal controls the single fibre potentials recorded over the spinal nerve, DRG, dorsal root and dorsal root entry zone were biphasic (positive-negative) or triphasic (positive-negative-positive) signals, with the negativity always having the greater (greatest) amplitude. The initial positivity is due to the passive outward current generated by activity at the last activated nodes of Ranvier, and the negativity is due to the active inward current occurring during the rising phase of the action potential under the recording electrode. Fig. 11 shows the averaged recordings over the T8 spinal nerve and DRG of an animal with EAE, the trigger spike being provided by a spontaneously firing muscle afferent in the respective external intercostal nerve. The spinal nerve response is a normal biphasic wave, but the response recorded over the DRG 2 mm away is a positive monophasic wave, proving that conduction block has occurred in the DRG. The response over the dorsal root (not shown) was a positive monophasic wave of lower amplitude than that recorded over the DRG, the decline in amplitude being due to the electrotonic decrement in the absence of impulse propagation past the DRG. The single fibre study in the other animal with EAE also showed normal conduction from the external intercostal nerve to the spinal nerve, and conduction block in the DRG.

Correlation between the clinical and electrophysiological findings. There was a good correlation between the severity of hindlimb ataxia on the day of the terminal experiment and the electrophysiological findings, both in the lumbosacral and thoracic regions. Most rabbits with moderate or severe hindlimb ataxia on the day of the terminal experiment had evidence of conduction block in a high proportion of the large diameter fibres in the DRG and at least moderately prolonged N

wave latencies. The 3 animals with only mild hindlimb ataxia on the day of the terminal experiment had conduction block in only a small proportion, but slowing of conduction in many of the large diameter fibres in the DRG, and had normal or only mildly prolonged N wave latencies.

There was an even better correlation between the electrophysiological findings and the time interval from the onset of signs to the electrophysiological study. All animals studied one to three days after the onset of signs had evidence of conduction block in a high proportion of the large diameter fibres in the DRG and at least moderately prolonged N wave latencies. The 4 animals studied six or more days after the onset of signs had conduction block in a small proportion, but slowing of conduction in many, of the large diameter fibres in the DRG and normal or only mildly prolonged N wave latencies. Three of these 4 animals had shown considerable neurological improvement. Animals studied four or five days after the onset of signs had electrophysiological findings intermediate between those of the animals studied before four days and those of the animals studied after five days.

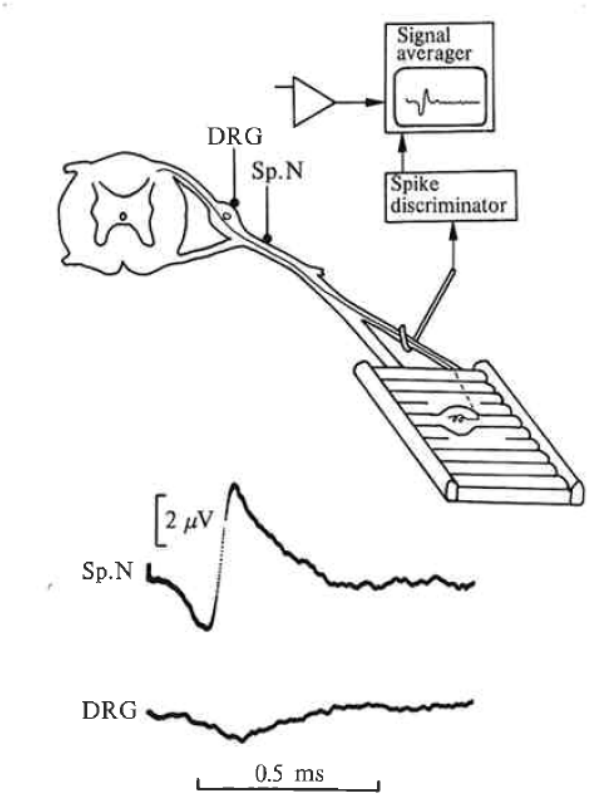

Fig.11. Above. Diagram illustrating the arrangements used for single fibre studies with spike-triggered averaging. Recordings were made from the external intercostal nerve (or from an internal intercostal nerve filament) in continuity', and a spike discriminator was used to select spikes arising from a single receptor nerve fibre. These spikes triggered the signal averager, which received its input from volume conductor recordings over the respective spinal nerve (Sp.N) or dorsal root ganglion (DRG). Below. Averaged single fibre potentials recorded, in the volume conductor, over the left T8 spinal nerve (Sp.N) and dorsal root ganglion (DRG) of a rabbit with EAE. Each recording is the average of 512 sweeps.

DISCUSSION

Clinical Findings

EAE developed in 80 per cent of the inoculated animals. The mean day of onset was 20 compared to the mean of 14 found by Waksman and Adams (1955). This difference is probably due to the fact that they induced the disease by intradermal injections into the four footpads simultaneously, which method is much more effective than intradermal injections in the neck (Levine and Wenk, 1961).

The clinical features of weight loss, and of splaying, ataxia and weakness of the limbs are the same as those previously described for EAE in the rabbit. Our findings of areflexia, hypothermia and

slowing of respiration are new, areflexia, in particular, being strikingly consistent. Curiously, other workers, with the exception of Morrison (1947), have not mentioned the state of the tendon reflexes in rabbits with EAE. Morrison found that these reflexes were usually present, even sometimes increased, but in one animal they were sluggish, and in another, sometimes difficult to elicit; he also observed clonus in several animals. In the present study the conspicuous and consistent absence of the tendon jerks focuses attention on the functional consequences of the PNS lesions. The hypothermia noted in 7 animals in the present study was probably due to an impairment of thermoregulation caused by autonomic involvement. The increase in body temperature observed in 4 animals may have been due to the autoimmune and tuberculin inflammatory reactions or to intercurrent infection. The slowing of respiration was not due solely to hypothermia, as 8 animals with slowed respiration had normal or increased body temperatures; it may have been due to a lesion affecting the afferent limb of the Hering-Breuer reflexes, perhaps in the nodose ganglion, but this was not investigated. The paradoxical movement of the chest wall observed during respiration indicates intercostal muscle weakness, but physiological studies are required to establish whether its primary cause is a functional deafferentation *(see* Nathan and Sears, 1960; Sears, 1964) or involvement of thoracic motor nerve fibres either in their ventral roots or within the spinal cord (*see below*).

Histological Findings

Our histological findings, including the PNS lesions, are similar to those previously described in rabbits with EAE (Morrison, 1947; Waksman and Adams, 1955, 1956; Wisniewski *et al*., 1969). The consistent and severe involvement of the DRG was a striking feature in the present study. In contrast to the paucity of demyelination in the CNS, there was usually extensive demyelination in the dorsal root ganglia. The vulnerability of the rabbit to the lesions of EAE and also to those of experimental allergic neuritis (EAN) was described by Waksman and Adams in 1955, but its functional implications in EAE seem to have been totally ignored.

Functional Deafferentation Due to Conduction Block in the DRG

The compound action potential recordings strongly indicate conduction block in a high proportion of the large diameter fibres in the lumbosacral and thoracic dorsal root ganglia of rabbits with EAE. Absolute proof of conduction block was provided by spike-triggered averaging from single muscle spindle afferents in the respective external intercostal nerve. That the conduction block in the DRG is due to the observed demyelination is indicated by the slowing of conduction in large diameter fibres in the DRG, the normal conduction in unmyelinated fibres and the effects of temperature and the potassium channel blocking agent, 4 amino-pyridine, on conduction. The normal conduction in unmyelinated fibres indicates that axonal damage *per se* is not responsible for the conduction block. The limited effectiveness of 4-AP is accounted for by the requirement that, for 4-AP to restore conduction, potassium channels must be exposed by demyelination of the paranodal region penultimate to the site of conduction block (*see* Sears and Bostock, 1981).

As demyelination causes an impaired ability to transmit a rapid train of impulses (Lehmann and Tackmann, 1970; McDonald and Sears, 1970), it is highly likely that, at physiological firing rates, failure of conduction would occur in those fibres already conducting slowly at the comparatively low rate of stimulation (1.0 Hz) used in the present study. This would contribute to the functional deafferentation.

It is likely that the particular morphology of the pseudomonopolar ganglion neuron confers, on the DRG, vulnerability to the pathophysiological consequences of demyelination. The stem process of the larger ganglion cells consists of a proximal unmyelinated segment followed by short thinly myelinated internodes leading to the branch point (Spencer *et al.*, 1973; Lieberman, 1976). Although a load is presented at the branch point by the stem process and the cell body, it would seem from work on the toad DRG that the safety factor for centripetal conduction through the normal ganglion is maintained high because of the very short internodes of the peripheral process immediately adjacent to the bifurcation (Ito and Takahashi, 1960). It would be expected that demyelination within the ganglion would affect not only the myelin of peripheral and central processes but also that of the stem process. Hence the branch point is likely to be particularly susceptible to conduction block, which perhaps may result from only a minor degree of demyelination.

Because of the conduction block in a high proportion of the large diameter fibres in the DRG, it was usually not possible to determine by orthodromic conduction studies whether the dorsal root and root entry zone were also sites of conduction block for afferent impulses. However, in one animal, conduction block was demonstrated at the dorsal root entry zone in large diameter fibres that were still conducting, albeit slowly, through the DRG.

The severe functional deafferentation resulting from the conduction block in the large diameter fibres in the DRG and to a lesser extent at the dorsal root entry zone accounts for the postural disturbance, hypotonia, ataxia and areflexia in rabbits with EAE. Furthermore, the conduction block in the DRG is likely to mask the expression of any CNS lesions that alone could produce these signs.

The finding of slowed conduction in many large diameter fibres in the DRG at the time of clinical improvement suggests that the improvement is due to restoration of conduction through the DRG. Such restoration could be due either to the development of internodal membrane excitability and continuous conduction *(see* Bostock and Sears, 1976, 1978) or to remyelination. Evidence, suggesting the development of internodal membrane excitability and continuous conduction, was provided in one animal with EAE by the finding of a conduction velocity so slowed as to be compatible with continuous conduction. Remyelination following segmental demyelination is not likely to occur within seven days, as judged by the lysolecithin lesion studied by Smith and Hall (1980), so that the earlier recovery seen in some animals is unlikely to be due to this. On the other hand, lesser degrees of myelin or Schwann cell damage may be repaired more quickly, so recovery by the reconstitution of the myelin sheath remains an open question.

Neurological improvement could also be due to restoration of conduction through the DRG as a result of a fall in body temperature from either a normal or high level, but this possibility was not examined. The electrophysiological studies throw no light on the possible role of CNS functional adaptation in the recovery process.

Weakness

The cause of the limb weakness was not established. The electrophysiological studies exclude a disorder of neuromuscular transmission and any significant lesions in the spinal and peripheral nerves. Furthermore, in two animals with hindlimb weakness, conduction in the distal ventral root was normal, thus excluding significant lesions at this site. These results suggest lesions in a more proximal site as the basis of the weakness. Our finding of demyelination in the region of the ventral root exit zone involving central and peripheral myelin, which confirms the observations by Prineas *et al.* (1969), provides the most likely explanation for the weakness. Such lesions were not assessed electrophysiologically. The paucity of demyelination and of the other parenchymal damage elsewhere in the CNS of these rabbits makes it unlikely that the weakness was of the upper motor neuron type. Finally, the extensive functional peripheral deafferentation due to the lesions in the DRG could also contribute to the weakness (*see* Mott and Sherrington, 1895; Nathan and Sears, 1960).

Impairment of Nociception

The impairment of nociception noted by us is difficult to explain in the light of the findings of normal C fibre conduction and apparently normal delta fibre conduction through the DRG. The most likely explanation is delta fibre conduction block that has been undetected by the electrophysiological studies because of the limited quantitative assessment.

Cause of the PNS Lesions in EAE

Why do PNS lesions occur when animals are inoculated with CNS tissue? When first describing PNS involvement in rabbits with EAE, Waksman and Adams (1955) considered the possibility that the PNS lesions were due to sensitization to PNS antigens from spinal roots contaminating the inoculum. This was not the explanation, as they found PNS lesions when tissue derived solely from the CNS, for example optic nerve, was used. Furthermore, inflammatory demyelinating lesions have since been produced after inoculation with myelin basic protein (Whitaker, 1981). The PNS involvement in EAE has been explained by the finding that the amino acid sequences of the myelin basic protein from the rabbit CNS and of the P, myelin basic protein (P1 protein) from the rabbit PNS are identical (Brostoff

and Eylar, 1972), this similarity having also been found in other species including man (Greenfield *et al.*, 1973).

Possible Role of PNS Lesions in Acute EAE in Other Species, in Chronic Relapsing EAE and in the Human Demyelinating Diseases

EAE. It is highly likely that PNS lesions play an important role in the production of neurological signs in acute EAE in other species and in chronic relapsing EAE because (1) PNS lesions have been described in acute and chronic relapsing EAE in all species examined (*see* Introduction); (2) the neurological signs are such as could be accounted for by PNS lesions.

It is important to note that the distribution of PNS lesions in EAE varies with the species. In contrast to rabbits, guinea pigs show greater involvement of the peripheral nerves than of the dorsal root ganglia and spinal roots (Waksman and Adams, 1956). Involvement of the PNS, particularly the peripheral nerves, could account for the severe paraparesis or tetraparesis in guinea pigs with EAE with only slight or undetectable morphological changes in the CNS (Wiśniewski *et al*., 1976; Wiśniewski and Keith, 1977).

In chronic relapsing EAE the neurological improvement occurring during the remissions may be due not to restoration of conduction in the CNS, as would appear to occur in MS, but to restoration of conduction in the PNS either by rcmyelination or by the development of continuous conduction.

Human demyelinating disease. The similarity, if not identity, in humans of the P1 protein of the PNS to the CNS myelin basic protein (*see above*) has a number of implications for the human demyelinating disease. First, it may explain the PNS involvement in the human analogue of EAE, namely the `neuroparalytic accidents' following rabies vaccination (McIntyre and Krouse, 1949; Appelbaum *et al*., 1953). Secondly, it has implications for acute disseminated encephalomyelitis. There is evidence that cell-mediated immunity to encephalitogenic myelin basic protein is a concomitant of this disease and may be important in its pathogenesis (Lisak *et al*., 1974). If this is the case, PNS involvement would be expected which could possibly account for many of the neurological signs, for example cranial nerve palsies, hypotonia, weakness, ataxia, sensory loss and areflexia, described in this disease (Miller *et al*., 1956). Thirdly, it has implications for the aetiology of MS. There have been several recent reports of PNS involvement in otherwise typical MS (Hopf, 1965; Calder and Pollock, 1976; Schoene *et al*., 1976). Further research is needed to establish the frequency and extent of this. If PNS involvement were shown to be common in MS, the implication would be that the target of the immune response, whether a neural antigen, viral antigen, or otherwise, is located in both the CNS and PNS; the target could therefore be myelin basic protein. On the other hand, if PNS involvement were shown to be rare in MS, the implication would be that the antigen is confined to the CNS and therefore unlikely to be myelin basic protein.

In the assessment of nervous system diseases in both humans and experimental animals, CNS lesions should not be regarded as being responsible for the clinical signs unless it has been established that PNS lesions either are absent or could not be responsible for the signs.

Vulnerability of the DRG

In the present study there was morphological and physiological evidence of severe involvement of the DRG. The vulnerability of the rabbit DRG to diphtheritic neuropathy (Waksman *et al*., 1957), EAN and EAE (Waksman and Adams, 1955) was attributed by Waksman (1961) to the reduction that he observed in the blood-nerve barrier in the DRG. The DRG of other mammalian species also have a reduced blood-nerve barrier to proteins, explained by the fenestrae in, and the open junctions between, the endothelial cells (Olsson, 1971; Jacobs *et al*., 1976). In the cat with diphtheritic neuropathy the DRG is the major site of demyelination and conduction abnormalities (McDonald, 1963*a, b*).

In EAE, access to the DRG not only of circulating protein, in particular antibody and complement, but also cells, in particular lymphocytes and monocytes, must be considered. The actual mechanisms whereby demyelination is produced in animals developing EAE after active immunization are unclear. In the present study the absence of any definite acute (within four hours) effect, on nerve conduction, of EAE sera injected into the peripheral nerve or spinal cord (Pender, 1983) excludes any significant contribution of complement-dependent antibody-mediated immunity,

as this mechanism results in acute conduction block (Saida *et al*., 1979; Sumner *et al*., 1979). By exclusion, the demyelination in the present study is likely to be caused by antibody-dependent cell-mediated immunity *(see* Brosnan *et al*., 1977) and/or antibody-independent cell-mediated immunity. As lymphocytes appear to pass through, rather than between, endothelial cells (Marchesi and Gowans, 1964; Astrom *et al*., 1968), the endothelial fenestrae rather than the open intercellular junctions may be the critical factor determining the access of lymphocytes to the DRG.

Thus the DRG has a dual vulnerability: one to the development of demyelinating lesions due to the ineffectiveness of the blood-nerve barrier; the other to conduction block due to demyelinating lesions in the vicinity of the branch point.

The vulnerability of the rabbit DRG to EAE and EAN as well as to diphtheritic neuropathy raises the question as to whether the human spinal ganglion, which is particularly vulnerable to the lesions of diphtheritic neuropathy (Fisher and Adams, 1956), is also vulnerable to inflammatory lesions. The spinal ganglia and roots are certainly involved in acute idiopathic polyneuritis (Asbury *et al*., 1969), but it is unclear as to whether the ganglia (and roots) show greater histological involvement than peripheral nerve. Involvement of the spinal ganglia and adjacent spinal roots could account for the observations that some patients with diphtheritic neuropathy and some with idiopathic polyneuritis have severe neurological signs but normal peripheral nerve conduction studies (Kurdi and Abdul-Kader, 1979; McLeod, 1981).

Conclusion

It is clear from these results and our survey of the literature that the functional consequences of the well-known PNS lesions in EAE have either been ignored or underestimated. PNS involvement in EAE in general and the dual vulnerability of the dorsal root ganglia described in this work have important implications for the human demyelinating diseases.

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