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Node-like Axonal Specialisations Along Demyelinated Central Nerve Fibres: Ultrastructural Observations*

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Summary. Ultrastructural examination of long-term (1 month to 1 year) demyelinated axons of the central nervous system (CNS) has revealed the presence of certain features typical of nerve fibres at the node of Ranvier. Focal regions of dense undercoating of the axolemma were observed and these always extended along regions which were intimately associated with the processes of gial cells. In many cases the glial cells were astrocytes, but in some cases the cells resembled Schwann cells. The astrocytic and Schwann cell processes were sometimes finger-like and they thus resembled the normal projections of these cells onto the nodal axolemma of central and peripheral nerve fibres, respectively. Since the undercoated regions which were associated with astrocytic processes were also often remote from any oligodendrocyte or Schwann cell processes, it seems that certain node-like specialisations may form or be sustained in the absence of myelin-forming cells.

Key words: CNS – Node of Ranvier – Demyelination – Astrocytes

Introduction

At the nodes of Ranvier of normal myelinated axons, the inner side of the axolemma is coated with an electron-dense material about 20–30 nm thick (Robertson 1959; Elfvin 1961; Peters 1966), and the outer surface is commonly contacted by the finger-like processes or either astrocytes or Schwann cells depending upon whether the nodes are in the central or the peripheral nervous system (PNS). During demyelination, the nodal undercoating and its associated extracellular components disappear and detailed studies of acutely demyelinated axons, with conventional stains, have failed to reveal the development of any new undercoating prior to remyelination. However, certain specialisations of axon and glial membranes have been described in some chronically demyelinated and amyelinated preparations. In experimental allergic encephalomyelitis (EAE), Raine (1978) has reported the presence of regional densifications of demyelinated axolemma, which often apposed a similar region of densification on the investing astroglial membrane. These membrane specialisations occurred in more than 60% of the affected axons and the occasional presence of desmosomes, gap and punctate junctions was also reported. The presence of small desmosome-like junctions between demyelinated axons and astroglia has also been noted in 30% of affected axons in a plaque of multiple sclerosis (Raine 1978). Raine (1978) also reported that many naked axons in MS plaques showed extensive subaxolemmal thickening, typical of that at nodes. However, these were not illustrated in his paper. A thickening of apposing axonal and astroglial membranes, similar to that described by Raine in EAE, was occasionally found by Ludwin (1980) in regions of chronic demyelination induced by cuprizone. In the PNS, Rosenbluth (1979) has described regions along the amyelinated fibres of the dystrophic mouse where Schwann cells extended finger-like projections towards undercoated regions of axolemma. Rosenbluth noted that these regions resembled, in some respects, the node of Ranvier and supposed that such structural differentiation of the axolemma occurred only where axons were in intimate contact with myelinogenic cells.

On examining chronically demyelinated axons in the CNS, produced as a result of intraspinal injections of ethidium bromide or of lysolecithin, we could not

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confirm the presence of membrane specialisations similar to those frequently seen by Raine (1978) in EAE. However, we wish to report the presence of a new structure found on chronically demyelinated central axons. These specialisations were found only infrequently and consisted of a node-like axolemma undercoating subjacent to the terminating finger-like processes of astroglial cells. No myelin-forming cells were observed associated with these regions. The specialisations thus differed from those reported previously, and were reminiscent of the nodal structures at normal central nodes of Ranvier.

Methods

The observations were made on each of four rats and five cats. Immediately prior to induction of the demyelinating lesions, the spinal cords of all the rats and three of the cats were irradiated (400 gy) to prevent repair by remyelination (Blakemore and Patterson 1978). During irradiation the animals were anaesthetised with Nembutal (Abbott), laid on their sides and partly covered with thick lead plates to restrict the radiation to the low thoracic/high lumbar spinal cord (Blakemore and Patterson 1978). The remaining two cats were not irradiated. In both species the demyelinating lesion was induced under anaesthesia by direct injection into the dorsal columns of either ethidium bromide (one to four injections, each of 0.5 – 1.5 µl, 5 – 10 mg/ml) or, in one of the non-irradiated cats, by the intraspinal injection of lysolecithin (lysophosphatidyl choline, LPC; Blakemore et al. 1977). The injections were made using micropipettes inserted into the spinal cord via small holes made in the dura. Penicillin was sprinkled into the wounds of rats (taking care to avoid the exposed spinal cord), and in all animals the wound was closed in layers. At various periods, from 1 month to 1 year, the animals were again anaesthetised and perfused via the descending aorta with glutaraldehyde (4%, 0.1 M phosphate buffer), post-fixed with osmium tetroxide (1%, 0.1 M phosphate buffer), dehydrated in ethanols and embedded for ultrastructual study. Sections were stained with uranyl and lead salts prior to examination. Some lesions were also stained "en bloc" in uranyl acetate prior to dehydration.

Results

Intraspinal injections of ethidium bromide induced demyelination by destroying oligodendrocytes and in non-irradiated animals nearly all the demyelinated axons were remyelinated by Schwann cells (Blakemore 1982). However, if the animals received prior X-irradiation (400 Gy) of the spinal cord, remyelination by Schwann cells and oligodendrocytes was inhibited and a large population of chronically demyelinated axons resulted. In the centre of such lesions axons clumped together, while around the edges, next to normal tissue, they were separated by astrocytic processes (Fig. 1). The structures we wish to report were found around the edges of the lesions and were most frequently observed at the dorsal margin, in areas where astrocytic association with axons was merging into the glial-free centre of the lesions. In such regions some axons showed lengths of electron density beneath the axon membrane (Figs. 2 – 6) similar to that observed at normal nodes of Ranvier.

The regions of axonal undercoating were focal, in axons cut in either the longitudinal or transverse planes of section (Figs. 2, 3). In longitudinal section they varied in length from 0.7 – 14 µm and were always restricted to a single side of the axon (Fig. 5). In transverse sections they were resricted to short lengths of the circumference and never extended around the whole axon (Figs. 2, 6). The regions of undercoating therefore took the form of patches of variable size. Astrocyte processes were associated with these patches, and in many cases this took the form of fine finger-like processes, similar in appearance to those sometimes found associated with nodes of Ranvier in the central nervous system (Fig. 2). In no instance, in any animal, were oligodendrocyte processes associated with these patches, nor were periodic densities seen between the associating astrocyte membranes and the axons.

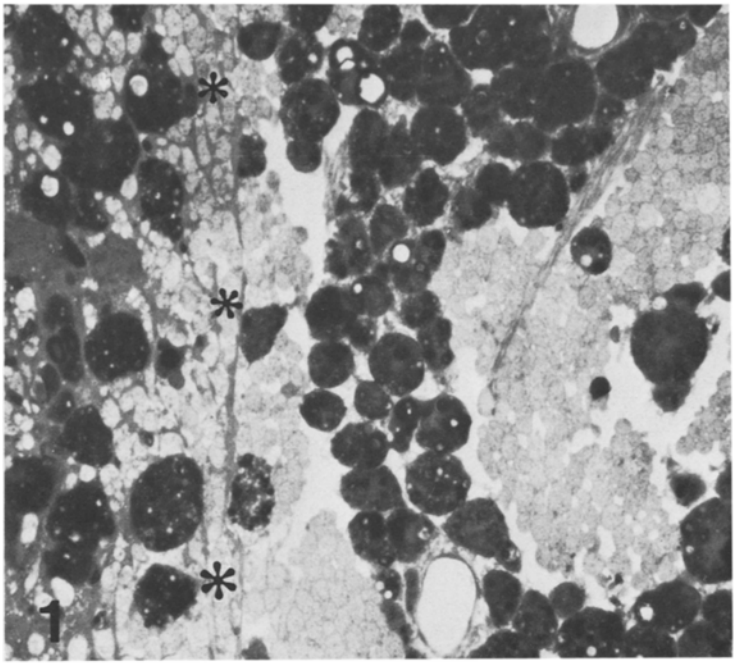
In some animals, especially the rats, cells presumptively identified as Schwann cells were sometimes associated with regions of axonal undercoating. It must be appreciated, however, that, in the absence of myelin formation, difficulty can be experienced in distinguishing astrocytes from Schwann cells in regions of pathological change where both cells may be present; since both cells can be surrounded by basement membrane and contain 8 nm filaments. Therefore, although we can state with certainty that, in the cats, most of the axolemmal densities were associated with astrocytes (Figs. 3, 5), in the rats some of the cells associated with the patches of undercoating could have been of either type of cell. However, in no case were periodic densities, terminal loops, or structures associated with the paranodes of myelin sheaths, seen.

Fig. 1. The appearance of the dorsal columns 1 month after injection of ethidium bromide into a previously X-irradiated area of spinal cord. The centre of the lesion contains demyelinated axons unassociated with glial cells. The axons showing undercoating are found in the area in which astrocytes are present (*asterisks*). × 625

Fig. 2. A demyelinated axon showing focal membrane undercoating (*arrow*) and related fine astrocyte process. × 32,000

Fig. 3. A small area of axonal undercoating in a demyelinated axon. Astrocyte processes (*a*) relate to the area but no oligodendrocyte processes are present. × 32,000

Fig. 4. Axonal undercoating on a demyelinated axon. Note how astrocytic processes relate to this area. × 32,000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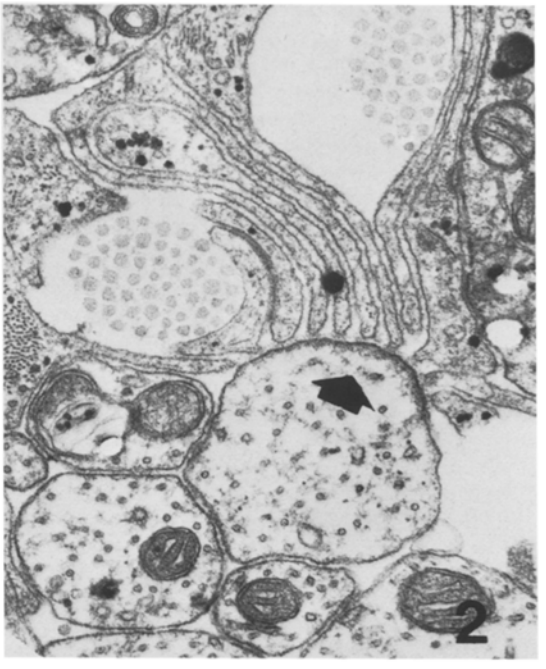
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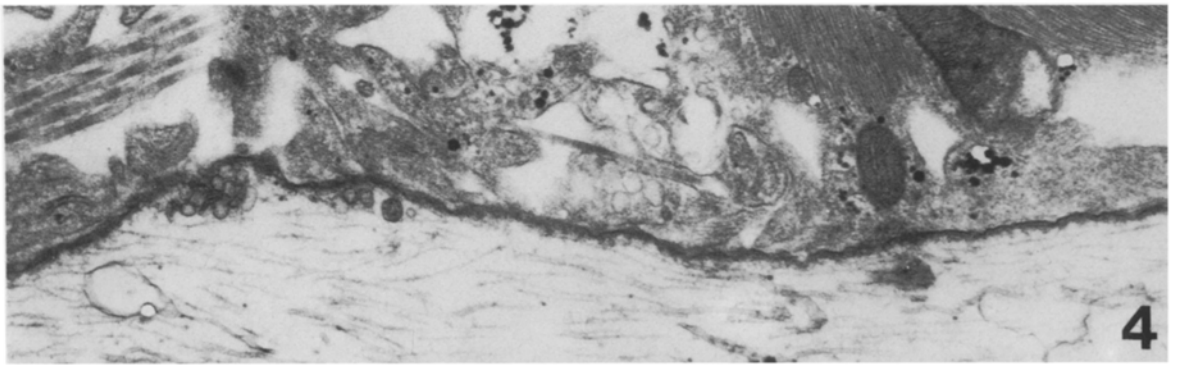
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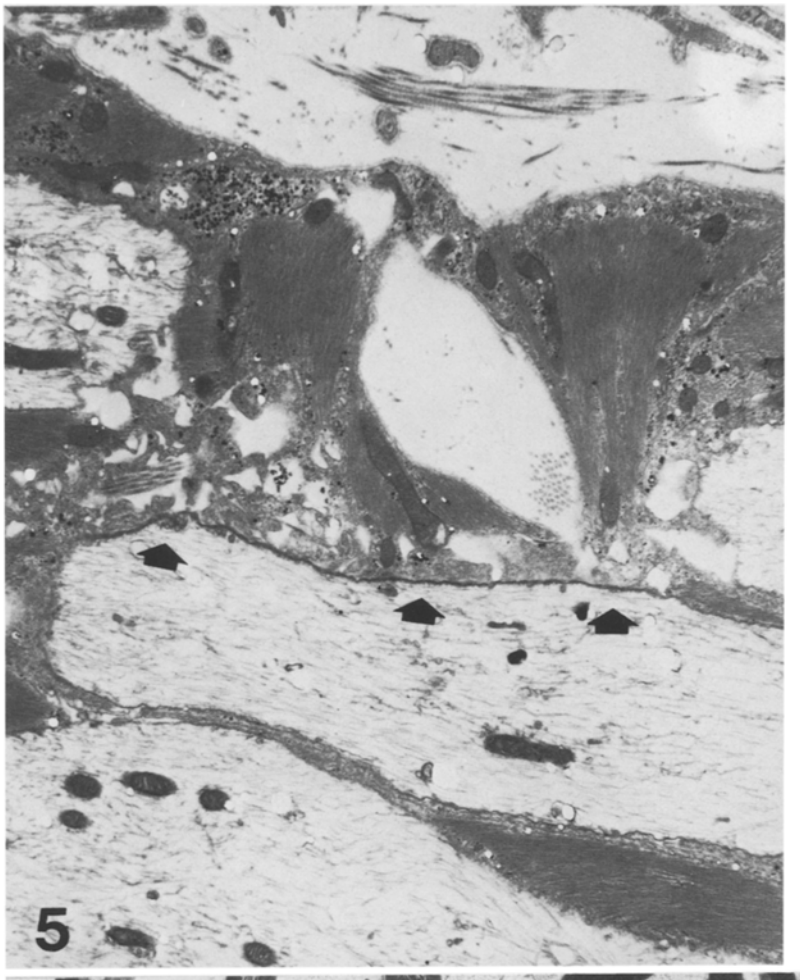
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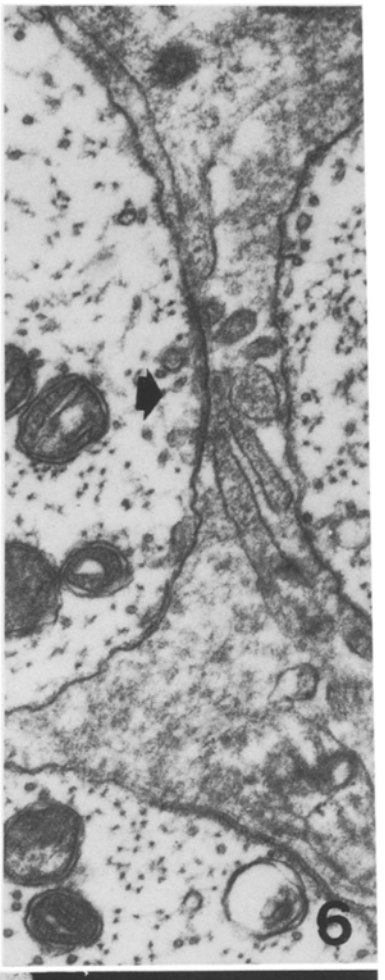
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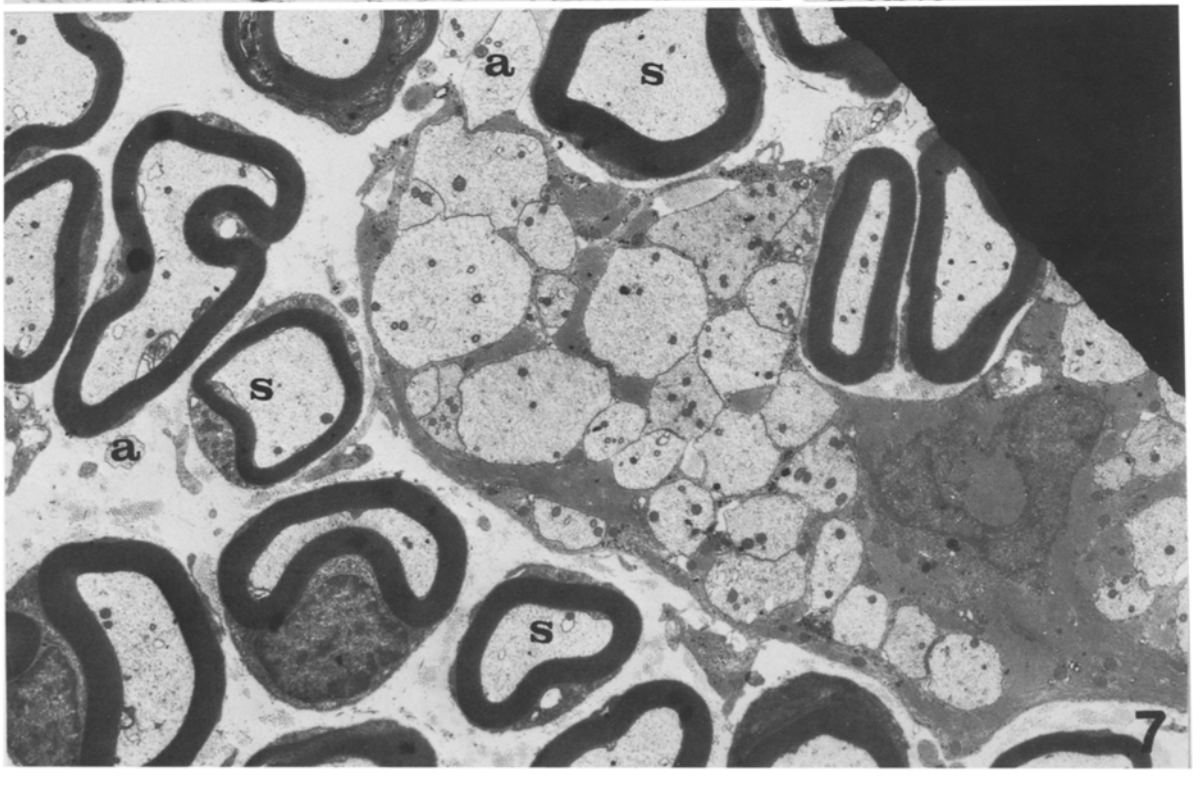
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The regions of axonal undercoating were found only infrequently, so that no more than ten were found in any one section of the dorsal column. The regions occurred most often in axons lying with some portion of their border exposed to the extracellular fluid and, as yet, they have not been seen in lesions under 1 month old.

As seven of the nine animals in which these structures were seen had received X-irradiation to suppress remyelination, it is pertinent to describe the low power morphology of the ethidium bromide lesion in the one animal which did not receive X-irradiation. This lesion was examined 1 year after injection with ethidium bromide and differed from all other similar lesions so far examined in that not all the demyelinated axons became remyelinated (Fig. 7). The non-myelinated axons occurred in groups surrounded by astrocytic processes. In these groups some axons were remyelinated by oligodendrocytes, but the majority remained demyelinated. The axons showing undercoating occurred at the periphery of these groups and often had one surface, usually that showing undercoating, exposed to regions containing Schwann cell remyelinated axons. The astrocytic relationships to the patches of undercoating were similar to those already described for the other animals. In this animal Schwann cells were never associated with the undercoated regions.

Discussion

The observations show that focal regions of axonal undercoating, similar to those found at nodes of Ranvier and the initial segment, can occur on chronically demyelinated central axons. These regions were only seen on demyelinated axons which showed cell association. The cells involved were usually astrocytes and sometimes Schwann cells. The patches were never seen associated with oligodendroglial cell processes and were never bounded on any side by normal paranodal structures, such as periodic densities. In some cases finger-like processes of astrocytes, similar to those sometimes observed at central nodes of Ranvier, were related to these regions. These observations indicate that node-like specialisations can form in the absence of myelination and even in the absence of myelin-forming cells.

Membrane specialisations have previously been described between demyelinated axons and astrocytic processes by Raine (1978) and Ludwin (1980). The structures described by Raine differ from those seen in the present investigation in that the axonal undercoating associated with his specialisations was not as prominent and no finger-like processes were observed. In Raine's material the astrocyte membrane apposed to the axonal surface was enhanced, due to the presence of material of medium electron density in the extracellular space. We did not see this material. Furthermore, Raine's specialisations were frequently observed while our structures were not common. Although Ludwin (1980) found structures similar to those described by Raine, they occurred infrequently. Rosenbluth (1979) described relationships similar to those observed in the present study between Schwann cells and amyelinated axons in the spinal roots of dystrophic mice.

The finding of node-like specialisations, in the absence of normal paranodal structures, is of interest, for it indicates that these regions can form in the absence of myelin-forming cells. This observation can be interpreted in two ways; either that node-like axonal membrane specialisations can form under the influence of non-myelin-forming cells, or that they can form under axonal control alone – it being argued that the specific glial cell relationships observed represent a normal response of astrocytes, or Schwann cells, to an excitable region. However, as axonal undercoating has not been seen on demyelinated axons in the absence of cell association it seems that glial cells play a role in the formation of these structures.

It is of interest that the axonal undercoating only occurred in patches and never completely surrounded the axon, as would normally be the case at nodes of Ranvier in the CNS. However, in the PNS, nodes have been described in which axonal undercoating does not extend around the whole axolemma (Berthold 1978); such nodes occur along the smallest diameter nerve fibres of mature cats and in certain respects resemble the specialisations we have observed.

Since undercoating of the axolemma occurs at nodes of Ranvier and the initial segment, it is believed to indicate regions of special electrical excitability. We suspect that the node-like specialisations we have observed on the demyelinated nerve fibres may be related to regions of relatively high sodium channel density. In the light of this supposition it may seem that the axonal specialisations may be a morphological correlate of the node-like foci of inward current (termed phi-nodes) detected physiologically along axons seg-

Fig. 5. A demyelinated axon cut longitudinally. There is a long length of axonal undercoating on one side of the axon (*arrows*). × 8,000

Fig. 6. An area of axonal undercoating (*arrow*) to which processes from an unidentifiable cell relate. × 42,000

Fig. 7. A group of demyelinated axons surrounded by astrocyte processes, from a cat injected with ethidium bromide one year previously. Several axons (*a*) lie free in the extracellular space next to axons remyelinated by Schwann cells (*s*). × 4,000

mentally demyelinated with LPC (Smith et al. 1982). However, while we would not be surprised if the structures we have described would resemble phi-nodes in electrical recordings, the available evidence argues against the possibility that the phi-nodes recorded by Smith et al. (1982) had this appearance. Thus careful examination of LPC-demyelinated axons (Smith et al. 1982), processed using a similar protocol to that employed in the present study, failed to reveal any undercoating of the demyelinated axolemma.

While node-like specialisations of the type described in this study have not been noted previously, some sub-axolemmal marking has been revealed in demyelinated axons stained by the ferric ion-ferrocyanide technique (Waxman and Foster 1980; Bostock et al. 1980). However, while the two types of undercoating (those revealed with conventional stains and those with ferric ion-ferrocyanide) have some similarities in their distribution at nodes of Ranvier and the initial segment, this appears not to be so in demyelinated fibres. Thus, while the present undercoating was restricted to short (0.7 to 14 μm) lengths of axolemma always opposite terminating glial cell processes, the ferrocyanide marking is relatively continuously distributed and seemingly unrelated to the surrounding glial cell distribution.

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Note Added in Proof

Since submission of this manuscript we have become aware of a paper by Carlstedt (1977) which describes structures associated with unmyelinated axons in the PNS-CNS transition zone which are similar to those we have described. Also Hildebrand and Waxman (1983) describe similar structures associated with non-myelinated axons in the rat retinal nerve fibre layer.