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An electron microscopic study of central and peripheral nodes of Ranvier*

DWIGHT D. PHILLIPS, RICHARD G. HIBBS, JEFFREY P. ELLISON AND HARVEY SHAPIRO

Department of Anatomy, Tulane University, New Orleans, Louisiana, and the Laboratory of Perinatal Physiology, U.S.P.H.S., San Juan, Puerto Rico

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INTRODUCTION

Many investigators, among whom are Uzman & Villegas (1960), Maturana (1960), Peters (1966), Laatch & Cowan (1966), and Hirano & Dembitzer (1967, 1969) have described nodes of Ranvier of the central nervous system. It has been implied that all central nodes are similar in structure, and that any difference in morphology from one node to another is quantitative, and related to the diameter of the fibre and the thickness of the myelin sheath.

Studies on the nodes of peripheral fibres by Robertson (1959), Uzman & Nogueira-Graf (1957), Rosenbluth (1962) and Harkin (1964) indicate that peripheral nodes have the same basic structure as the central nodes. This oversimplified picture of node structure was summarized by Peters, Palay & Webster (1970), who said, 'The structure of the node is essentially the same in both peripheral and central nervous systems'. In assessing the validity of this viewpoint, one should remember that most previous investigators, because of the greater ease of fixation, have studied only the nodes of relatively small fibres. Only one (Berthold, 1968*b*) thoroughly investigated the nodes of large fibres, and this excellent study was restricted to peripheral nodes.

Further, it has been assumed that the node is a symmetrical structure, and that no feature of its organization reflects the functional polarization of the axon. This assumption has not yet been subjected to a critical evaluation.

This study was designed to test the validity of the above assumptions, by examining nodes of widely different sizes in axons from functionally different regions of the nervous system.

MATERIALS AND METHODS

Tissue was obtained from four normal, juvenile rhesus monkeys (*Macaca mulatta*) weighing 2·0 to 3·4 kg. The monkeys were anaesthetized with Nembutal, and placed on intermittent positive-pressure respiration using room air as the respiratory gas. After thoracotomy, a perfusion cannula was inserted into the left ventricle of the heart, and the animal was fixed by perfusion. Approximately 2 litres fixative were used for each animal. The fixative consisted of 3 % glutaraldehyde in 0·1 M phosphate buffer at pH 7·4, and was used at room temperature.

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Blocks of tissue were taken from the pyramids, inferior colliculus, median nerve, and medial antebrachial cutaneous nerve. These blocks were cut into half-millimetre cubes and immersed in 3% buffered glutaraldehyde for 2 hours, then washed in phosphate buffer and post-fixed for 2 hours in 1% buffered osmium tetroxide. Tissues were dehydrated in ethanol and embedded in Maraglas (Freeman & Spurlock, 1962).

Sections of about 1 μm thickness were cut on an LKB ultratome, stained with toluidine blue, and used for orientation. Thin sections from the same blocks were stained with aqueous uranyl acetate and lead citrate and examined with an RCA EMU 3 G electron microscope.

OBSERVATIONS

The following observations indicate that the nodes of Ranvier of axons in the central nervous system are basically similar in structure to those of peripheral fibres, but that two distinct types of nodes are present in both central and peripheral fibres. In the ensuing description nodes will be designated 'central type I', 'central type II', 'peripheral type I', and 'peripheral type II'. While type I nodes are generally associated with small fibres, and type II nodes with large fibres, there is considerable overlap, so that fibres of intermediate size may possess nodes of either type.

Type I nodes of C.N.S. *axons*

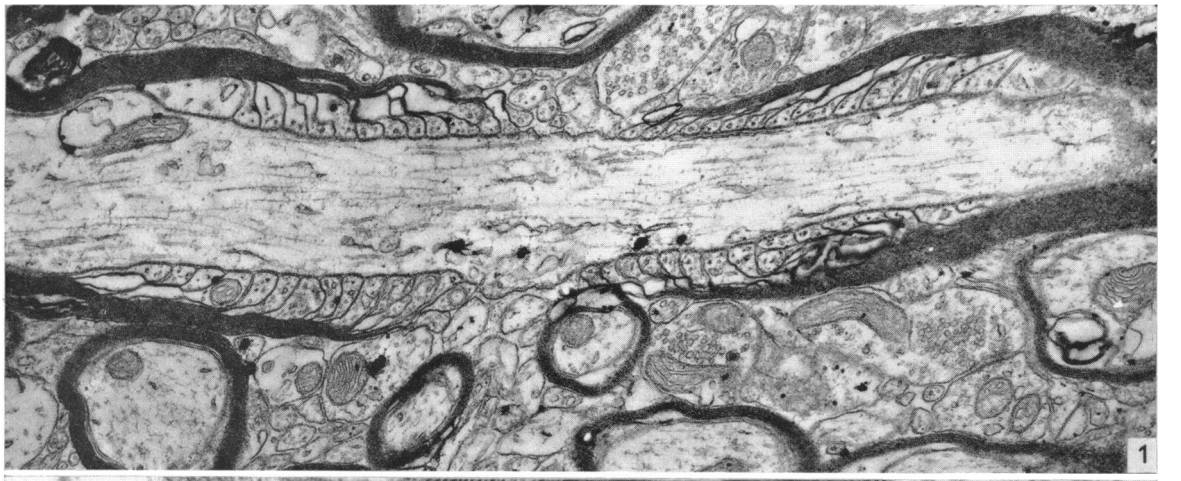
Longitudinal sections through the nodes and paranodes of small central nerve fibres (Fig. 1) showed a series of cytoplasmic loops, whose membranes were continuous with the main period lines of the myelin lamellae. The membranes of some loops were apposed to the axolemma of the paranode. At high magnification (Fig. 2) the trilaminar structure of the sheath cell membrane and of the axolemma was resolved. These membranes were each approximately 7·5 nm thick (the outer laminae approximately 2 nm thick, the inner laminae approximately 3 nm and the intervening electron-lucid layers each approximately 2·5 nm thick). In adequately preserved tissue all leaflets of these trilaminar membranes were free of interruptions.

In longitudinal sections, periodic densities were seen between the sheath cell loops and the axis cylinder, and between the internal sheath cell tongue and the paranodal axon (Fig. 2). These densities were in contact with both the axolemma and the sheath cell membrane. Each density was approximately 10 nm in diameter and the centre to centre spacing of the densities was 20 nm. The number of densities in contact with each cytoplasmic loop was directly proportional to the width of the loop. As few as two, and as many as twenty, densities contacted a single loop. In tangential section, the densities appeared as a series of parallel, diagonal bars (Fig. 3). Recon-

Fig. 1. Central type I node of a small nerve fibre from the inferior colliculus. Note the orderly arrangement of loops in the paranode. × 15000.

Fig. 2. High magnification of a portion of a central type I node of a large fibre of the inferior colliculus. Note the periodic densities between the outer lamina of the axolemma and the outer lamina of the sheath cell loop (arrows), and the cross-sectional profiles of tubules within the loops. × 52000.

Fig. 3. A tangential section of a central type I node. The periodic densities are shown here as parallel bars (arrows). × 36000.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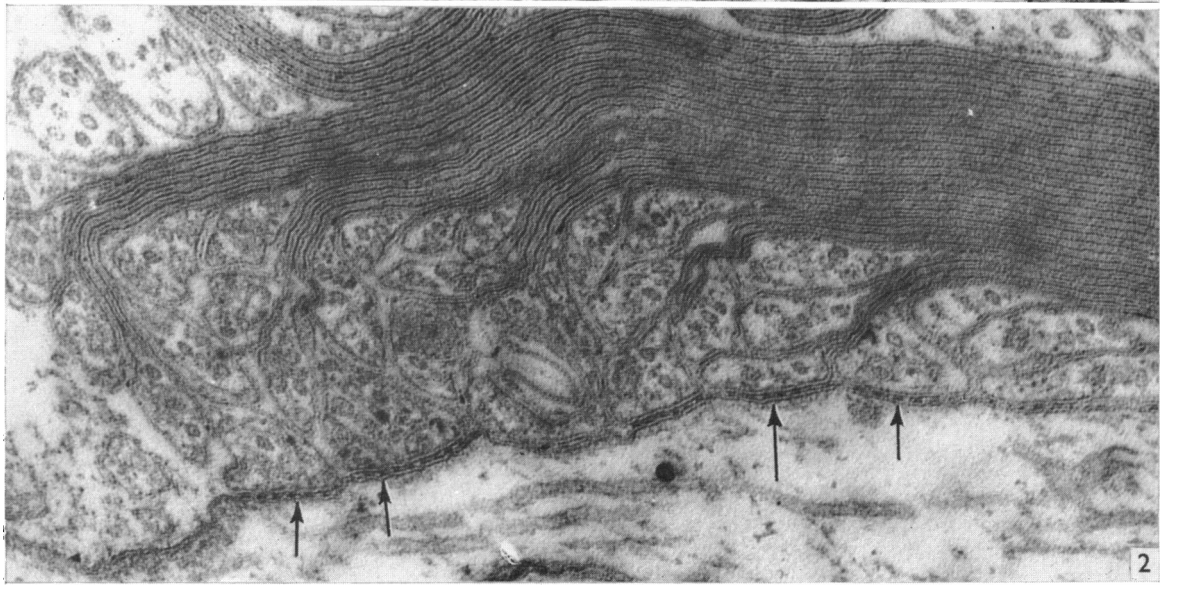
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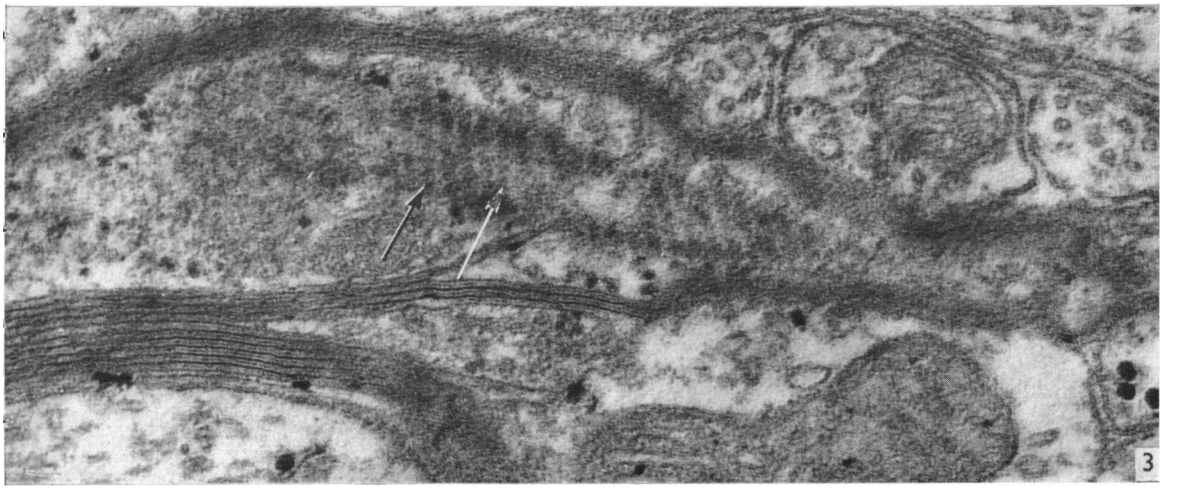
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struction of longitudinal and tangential sections showed that the densities were cross-sections of parallel cord-like structures of various lengths, arranged in a spiral fashion around the axon.

The cytoplasm within the lateral loops was of variable density, and contained small circular profiles and occasional mitochondria (Figs. 1, 2). Tangential sections showed that at least some of the circular profiles were cross-sections of tubules. Evaginations of axons containing synaptic vesicles were seen at some of the node gaps (Fig. 4).

In a few of the more thickly myelinated type I fibres (Fig. 2), all the myelin lamellae terminated in relatively short paranodes, so that some sheath cell loops overlaid others and only a few made contact with the axolemma. The fraction of lateral loops which contacted the axolemma was thus inversely proportional to the thickness of the myelin sheath. In the larger type I fibres there were as many as 4 or 5 layers of loops.

Type I nodes of peripheral nerves

The nodes of small peripheral fibres closely resembled their type I counterparts in the C.N.S. The myelin lamellae of type I peripheral nodes terminated in lateral cytoplasmic pockets that were applied in an orderly fashion to the paranodal axon (Fig. 5). As in central nodes, except for those of the smallest fibres, not all the loops made contact with the axolemma, and the degree of stacking of loops was proportional to the thickness of the myelin sheath. Periodic densities similar to those of central nodes were present between the lateral loops and the axolemma, although they were never visualized as distinctly as the densities of central nodes.

As noted by previous investigators, finger-like Schwann cell processes largely filled the node gap but did not make contact with the bare area of the axon.

Type II nodes of peripheral nerves

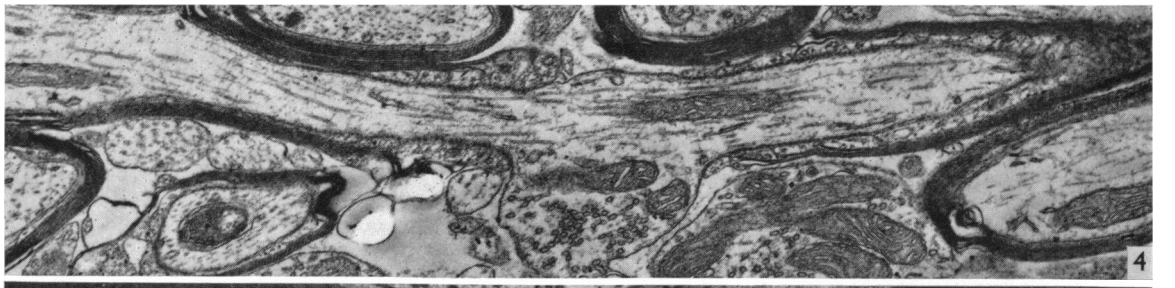
The nodes of large peripheral fibres were radically different from type I nodes. The myelin sheaths terminated in tiny teardrop-shaped loops at the paranodes of large fibres. Few of these loops made contact with the axolemma; most were piled up either randomly, or in herringbone fashion (Figs. 6, 7, 8). No periodic densities were seen between the lateral loops and the axolemma, although those loops which were in contact with the axolemma seemed to be firmly attached to it.

All the loops of many type II nodes were filled with tiny osmiophilic particles, although in some nodes only a proportion of the loops contained such particles. In these nodes the particle-containing loops were asymmetrically distributed, and were often confined to one side of the bare segment of the axon.

Fig. 4. A central type I node of small fibre of the inferior colliculus. Note the evagination of the bare segment of the axon containing synaptic vesicles. × 14000.

Fig. 5. A peripheral type I node. A few simple Schwann cell processes lie over the 'bare' segment of the node (arrow). × 16000.

Fig. 6. A peripheral type II node. Note the small size and extremely dense content of the Schwann cell loops, only a few of which contact the axolemma. The defects in the myelin (arrows) were produced during sectioning because of the presence of tiny pockets of dense particles between the myellin lamellae. × 22000. (*S*); Schwann cell process.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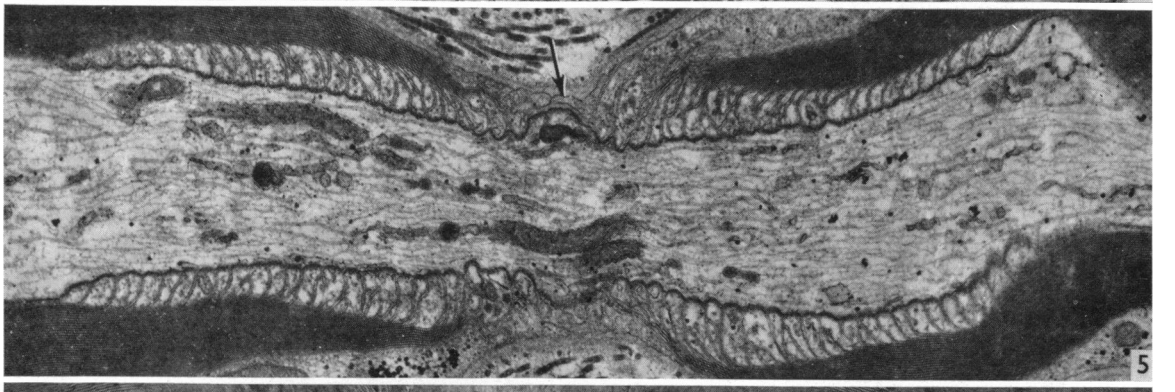
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Dense granules, which closely resembled the glycogen granules of the sheath cells, were usually present within the axons in the regions of such nodes (Fig. 8).

Peripheral type II fibres were commonly attenuated and some were telescoped at the paranodes; the resulting constricted regions of the axons were crowded with neurotubules, neurofibrils and other cytoplasmic elements (Figs. 6, 7, 8).

Fine osmiophilic particles, which resembled those in the sheath cell loops, were often scattered between the myelin lamellae of the internodes. These granules were apparently hard in texture, since they were often shifted in position during sectioning. This resulted in the numerous small defects seen in our thin sections of myelin (Fig. 6).

Type II nodes of C.N.S. *axons*

Although care was taken to obtain the best possible fixation, we were unable to preserve satisfactorily the large fibres of the central nervous system – at least those of the dorsal funiculus, corpus callosum and pyramids – although small diameter fibres and cell bodies in the same blocks were adequately preserved. In spite of the poor tissue preservation, these micrographs do show that the nodes of these large central fibres have at least some of the features of the type II peripheral nodes (Figs. 9, 10); the sheath cell loops terminate in short paranodes, the loops are small, teardrop-shaped structures and contain tiny osmiophilic particles; they are piled up at the paranodes, often in a herringbone fashion, as in the type II peripheral nodes. In the central type II nodes, however, the terminal loops which are in contact with the axolemma are expanded and flattened, and periodic densities are present between these loops and the axolemma (Fig. 9). Infoldings of the axolemma, lying parallel with it, are frequently present at the periphery of the nodal regions of such axons (Fig. 10).

DISCUSSION

Descriptions of nodes of Ranvier, as they appear in the current literature, are mainly of those of small mammalian and amphibian myelinated fibres. Only Berthold (1968*b*) studied the large myelinated elements, and he confined his attentions to peripheral nerves. This lack of emphasis on the fine structure of large myelinated fibres, especially of the central nervous system, may have resulted from problems encountered in preservation. Fixation problems are especially severe in the white matter of the central nervous system, and may well account for our ignorance of the structure of the large central node.

The small nodes (type I) examined in this study present many of the characteristic

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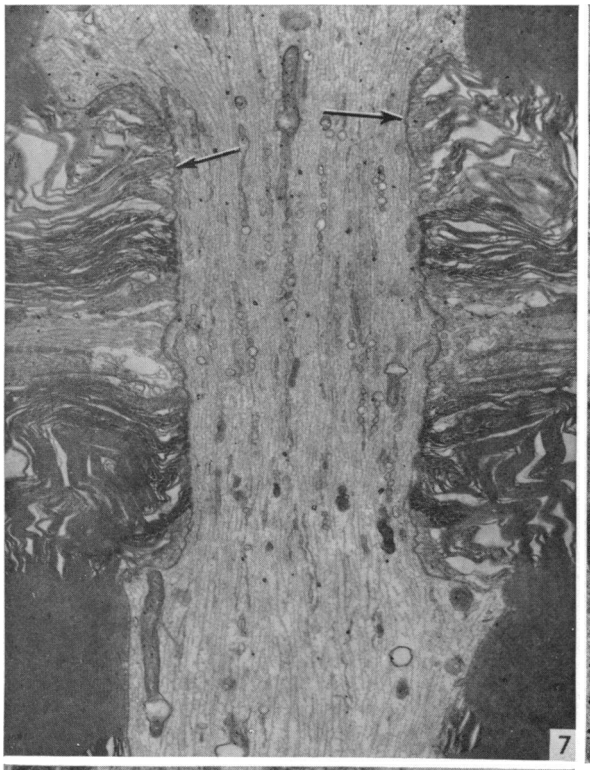
Fig 7. A peripheral type II node. Many of the tiny Schwann cell loops, at one side of the node (arrows), are devoid of dense particles. × 12000.

Fig. 8. Peripheral type II node. Note the presence of dense particles within the loops on one side of the node, and their absence on the other side. Note also the resemblance of the dense granules (arrow) in the Schwann cell. × 18000.

Fig. 9. A central type II node from a fibre of the pyramid. Note the flattening and spreading out of those loops which contact the axolemma. Periodic densities can be seen at the tips of the arrows. × 30000.

Fig. 10. A central type II node from a fibre of the pyramid. Note the valve-like infoldings of the axolemma (arrows). × 26000.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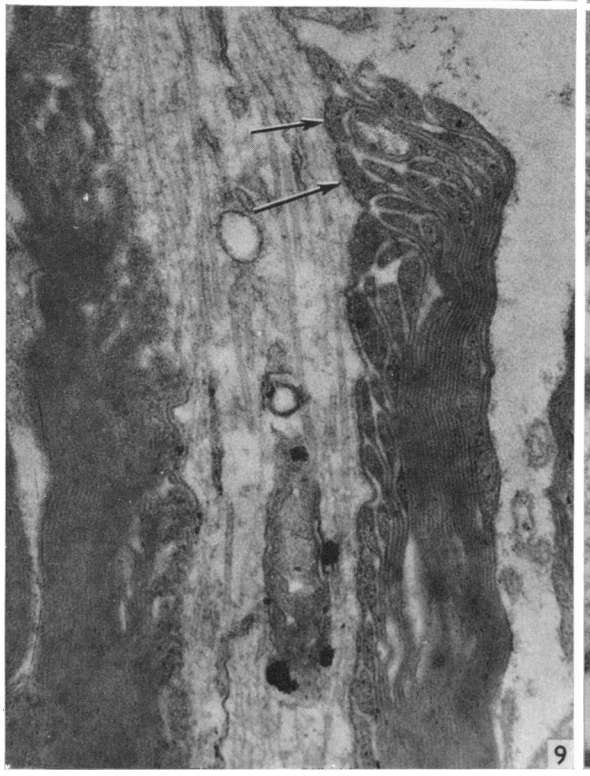
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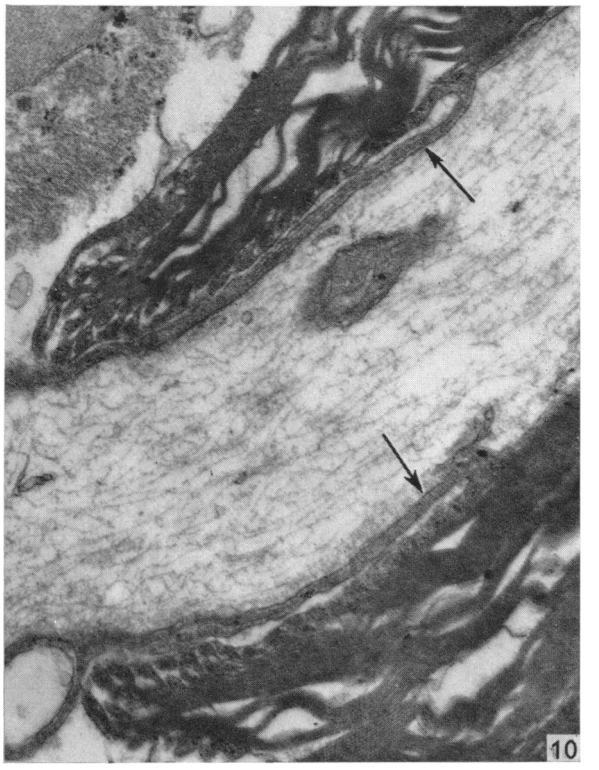
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features assigned to central nodes depicted in the literature. Typically, in such nodes the lateral loops of the myelin sheath are supposed to end in sequential contact with the paranodal axon. However, even in relatively simple nodes we often saw loops that made no contact with the axolemma. Between the attached loops and the outer leaflet of the axolemma lie periodic densities, which we feel are sections of parallel, spirally arranged periaxonal bands. Hirano & Dembitzer (1969) postulated that a spiral channel between the bands connects the node gap with the periaxonal space. Previously it had been thought by some workers (Peters *et al.* 1970) that the bands formed a barrier between these two spaces. Unlike Hirano & Dembitzer, we feel that the densities are sections of several bands of different lengths, because they are restricted to regions of loop/axon contact, and because loops of different widths make contact with different numbers of densities.

If the densities were multiple sections of a single spiral band, this band would have to cross the regions underlying the points where adjacent loops are in contact, and densities would occasionally be encountered in these positions.

If the densities were sections through a number of parallel cords, all of which were continuous through the paranode, some of these cords would also have to cross from one loop to another. Densities have never been seen under the regions where adjacent loops are in contact; in fact there is usually a rather wide space devoid of densities at these points.

We also believe that the densities are interposed between the outer membranes of the oligodendrocyte and the axon. They do not appear to be thickenings in the outer leaflet of the axolemma, as was suggested by Peters *et al.* (1970).

The central type II nodes studied were of the large fibres of the pyramids. Most of the small lateral loops seen in these nodes do not contact the axolemma, but those that do are flattened against the axolemma even in imperfectly fixed tissue.

Myelination in type II nodes is hard to explain on the basis of Hirano & Dembitzer's hypothesis (1967) that paranode length is proportional to the number of lateral loops ending at the node, and presumably also to the thickness of the myelin sheath. Type II nodes and the larger type I nodes do not conform to this pattern, since thick fibres which have many lateral loops also have short paranodes. Also, in such nodes, few of the lateral loops contact the axolemma, and 'contact' and 'free' loops are arranged in a highly irregular fashion. Figures 6 to 10 show how far the type II node deviates from the simple geometric model of Hirano. Certainly this often-quoted model is of little value in explaining the complex sheath cell/axon relationship of the type II node; a relationship that cannot be reduced to conveniently simple geometric terms.

Large and small peripheral fibres are similar in many ways to their counterparts in the C.N.S., but in a few respects they differ significantly. Most authors (see review by Peters, 1970, for references) have paid little attention to these differences, being content to emphasize the more obvious similarities between central and peripheral nodes. At least the simpler small peripheral nodes, like the central nodes, have periodic densities between the axolemma and the lateral loops. However, these densities are more difficult to preserve in peripheral nerves, and have rarely been reported. It is also possible that they are not universally present in peripheral nodes. For example, although clearly demonstrated in the myelinated nerves of the adrenal medulla by

Bargmann & Lindner (1964), they were not seen in equally satisfactory micrographs of the sympathetic trunk (Elfvin, 1961).

Type II peripheral nodes typically have small dense lateral loops, few of which contact the axolemma. Nodes of similar morphology were shown by Berthold (1968*b*) and by Allt (1969). Telescoping of the paranodal myelin sheath results in marked axon constriction at the node itself. Berthold (1968*a*) noted this phenomenon in nodes of the sural nerve, but not in ventral nerve roots. Correlating this finding with the relative absence of collagen from the former situation, Berthold postulated that nodal crowding might be an artefact due to fixation shrinkage of perineural collagen. Since we have not observed marked nodal constriction in the central nervous system, which is devoid of collagen, but have frequently encountered it in peripheral nerves, we tend to agree with Berthold's suggestion.

Some large peripheral type II nodes have asymmetric paranodes with dense Schwann cell lateral loops on one side of the node and translucent ones of the same size on the other. In many such nodes the intranodal axon contains a dense granular material, similar in appearance to the glycogen in Schwann cell processes. We have observed these polarized nodes in sensory nerves, specifically in the antebrachial cutaneous nerve and in the dorsal root. The presence of the 'polarized nodes' suggests to us a possible functional correlation, perhaps with conduction direction or functional modality. Stimulation studies are planned to test this hypothesis.

SUMMARY

Nodes of Ranvier from various regions of the central nervous system and from peripheral nerves were studied by electron microscopy. Two distinct types of nodes were described in both the central and peripheral fibres. Nodes of one type (type I) were found in fibres of small diameter, while those of the other type (type II) were found predominantly in the large fibres. Many type II nodes were asymmetrical.

REFERENCES

ALLT, G. (1969). Ultrastructural features of the immature peripheral nerve. *Journal of Anatomy* **105**, 283–293.

BARGMANN, W. & LINDNER, E. (1964). Uber den Feinbau des Nebennierenmarkes des Igels. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **64**, 868–912.

BERTHOLD, C. (1968*a*). Postnatal development of feline paranodal myelin-sheath segments. *Acta Societatis medicorum upsaliensis* **73**, 127–144.

BERTHOLD, C. (1968*b*). Ultrastructure of the node–paranode region of mature feline ventral lumbar spinal root fibers. *Acta Societatis medicorum upsaliensis* **73**, 37–73.

ELFVIN, L. G. (1961). Electron microscopic investigation of the plasma membrane and myelin sheath of autonomic nerve fibers in the cat. *Journal of Ultrastructure Research* **5**, 388–407.

FREEMAN, J. & SPURLOCK, B. (1962). A new epoxy embedment for electron microscopy. *Journal of Cell Biology* **13**, 437–443.

HARKIN, J. C. (1964). A series of desmosomal attachments in the Schwann sheath of myelinated mammalian nerves. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **64**, 189–195.

HIRANO, A. & DEMBITZER, H. M. (1967). A structural analysis of the myelin sheath in the central nervous system. *Journal of Cell Biology* **34**, 555–567.

HIRANO, A. & DEMBITZER, H. M. (1969). The transverse bands as a means of access to the periaxonal space of the central myelinated nerve fibre. *Journal of Ultrastructure Research* **28**, 141–149.

LAATCH, R. H. & COWAN, W. M. (1966). A structural specialization at the nodes of Ranvier in the central nervous system. *Nature, Lond.* **210**, 757–758.

MATURANA, H. R. (1960). The fine anatomy of the optic nerve of Anurans: an electron microscope study. *Journal of Biophysical and Biochemical Cytology* **7**, 107–120.

PETERS, A. (1966). The node of Ranvier in the central nervous system. *Quarterly Journal of Experimental Physiology* **51**, 229–236.

PETERS, A., PALAY, S. L. & WEBSTER, HENRY de F. (1970). *The Fine Structure of the Nervous System.* New York: Harper and Row.

ROBERTSON, J. D. (1959). Preliminary observations on the ultrastructure of nodes of Ranvier. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **50**, 553–560.

ROSENBLUTH, J. (1962). Fine structure of the acoustic ganglion in the rat. *Journal of Cell Biology* **12**, 329–359.

UZMAN, B. G. & NOGUEIRA-GRAF, G. (1957). Electron microscopic studies of the formation of nodes of Ranvier in mouse sciatic nerve. *Journal of Biophysical and Biochemical Cytology* **3**, 589–598.

UZMAN, B. G. & VILLEGAS, G. M. (1960). A companion of the nodes of Ranvier in sciatic nerves with node-like structures in optic nerves of the mouse. *Journal of Biophysical and Biochemical Cytology* **7**, 761–762.