PHASE AND ELECTRON MICROSCOPIC STUDIES
OF EXPERIMENTAL DEMYELINATION

I. VARIATIONS IN MYELIN SHEATH CONTOUR IN NORMAL GUINEA PIG SCIATIC NERVE*

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During an electron microscopic study of experimental demyelination, many variations in the contour of the myelin sheath were observed in normal guinea pig sciatic nerves. These variations consisted of loops and folds of compact myelin which indented the axoplasm or protruded into the Schwann cell cytoplasm. Depending on the plane of section they were seen as isolated ovoids of myelin within axoplasm or Schwann cell cytoplasm. Under the light microscope, these loops and ovoids closely resembled myelin forms seen in early demyelinating lesions and it was necessary to define their structure and distribution before proceeding with the pathological study.

Variations in myelin sheath contour are not described in the classical histological studies of Ranvier (16), Nageotte (14), and Cajal (1). In their figures, the myelin sheath is shown as a smooth, axoplasm filled, cylindrical tube with interruptions at Schmidt-Lantermann incisures and the nodes of Ranvier. A slight indentation is present in the region of the nucleus. Juxtanodal irregularities of the myelin sheath are illustrated as preparative artifacts although other observers (12, 2) described round, osmophilic structures located between the myelin sheath and the Schwann cell membrane near the nucleus and elsewhere along the length of normal peripheral nerve fibers. Ridging of the myelin sheath adjacent to the node was noted by several investigators including Hess and Young (11); one of their figures also shows a large fold of myelin indenting axoplasm near a node shown in longitudinal section, but it was not described or discussed. A few electron micrographs in several subsequent studies of peripheral nerve ultrastructure show similar myelin loops and folds (7, 18). However, their fine structure and distribution were not discussed. Hess and Lansing described an isolated ovoid with the lamellar structure of myelin within the Schwann cell cytoplasm of guinea pig sciatic nerve and tentatively identified it as an Elzholz

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body (10). Thus, previous studies offer little data on the occurrence and significance of variations in myelin sheath contour of normal peripheral nerve fibers.

**MATERIAL AND METHODS**

Guinea pig sciatic nerves were exposed surgically during intraperitoneal nembutal anesthesia and fixed *in situ* with buffered 2 per cent osmium tetroxide at room temperature for 15 to 45 minutes. The nerves were removed prior to death and fixed further in 2 per cent osmium tetroxide for 6 to 8 hours at 4°C. They were then dehydrated in acetone at 4°C, brought to room temperature with several changes of 100 per cent acetone, cut into blocks suitable for cross and longitudinal section and embedded in Araldite using the proportions and technique suggested by Robertson (20). Two micron sections were mounted serially for phase microscopy. Thin sections were cut with a diamond knife (6), mounted on a Porter Blum microtome, and were examined with an RCA EMU 3B electron microscope.

**RESULTS**

*Phase Microscopy:* Phase microscopic study of 2 micron, longitudinal sections through nodes of Ranvier shows many variations in the contour of the normal juxtanodal myelin sheath (figs. 1-3). Isolated loops or ovoids of myelin within the axoplasm are shown by serial section to be continuous with large infoldings of myelin or with a relatively smooth sheath contour. These infoldings can produce marked asymmetry of the sheath (fig. 3), so that study of a thicker section shows apparent focal thickening of the myelin segment to one side of the node. There are also small evaginations of the juxtanodal myelin which are seen serially as isolated ovoids of myelin within Schwann cell cytoplasm (figs. 1, 2, 3). Figure 4 illustrates these changes in size and direction of myelin folds adjacent to the node. The continuity of a juxtanodal intracytoplasmic myelin ovoid with a protruding fold can be readily traced.

The loops and folds shown in Figures 1-4 are present adjacent to the node of Ranvier in more than 75 per cent of the largest fibers (>10 microns in diameter) and are most numerous in this location. They are also found near the node in smaller fibers (2-10 microns in diameter) and, although less frequent, are present in the internodal portion of the sheath in large and small fibers. The study of serial sections has shown that these contour variations are not related to branching of the axon with the accompanying sheath. Myelin invaginations and protrusions are present in fibers of proximal and distal portions of the sciatic nerve and in the nerves making up the brachial plexus.

*Electron Microscopy:* In thin sections cut from the same material examined with the electron microscope, several important features of the fine structure of these variations in the normal myelin sheath contour are immediately apparent. In the first place, the myelin sheath and the loops and folds continuous with it show the lamellar structure of normal compact myelin as defined by the studies of Fernandez-Moran (5), Sjostrand (21), Geren (8), and Robertson (19) (figs. 5, 6, 7). The dense lines alternate with faint, frequently discontinuous intermediate lines and have a fundamental period of 130 to 150 Å. In addition, the ultrastructure of Schwann cell cytoplasmic constituents within the loops and folds is well preserved. For example, the inner mesaxon, which connects the inner layer of compact myelin and the axon-Schwann membrane, is clearly shown in Figure 5. Similarly, the continuity of the Schwann cell cytoplasmic constituents within the loops and folds is well preserved. For example, the inner mesaxon, which connects the inner layer of compact myelin and the axon-Schwann membrane, is clearly shown in Figure 5. Similarly, the continuity of the Schwann cell surface membrane with the outer layer of compact myelin via the outer mesaxon is also illustrated in the upper fiber in Figure 5 and in Figure 6.

In addition to the above loops and folds of myelin, there are isolated ovoids which have the characteristic lamellar structure of normal myelin and are located in the juxtanodal (fig. 11) or internodal (figs. 8, 9, 14) cytoplasm of Schwann cells. These structures are similar to the myelin ovoids that were shown by examination of serial two micron sections to be continuous with protrusions of the sheath into Schwann cell cytoplasm.

Although more difficult to interpret, several other observations seem pertinent. Occasionally, there are ovoids of fairly uniform density that do not clearly show the lamellar
structure of myelin and are located within axoplasm (fig. 13) or Schwann cell cytoplasm (figs. 13, 15). Occasional collections of 50 to 100 Å, dense particles, apparently packed with some degree of regularity, are present at the margin of these structures (fig. 15) as well as at the periphery of the intracytoplasmic myelin ovoids (fig. 14). Finally, formations composed of irregular loosely packed zones of lamellae with a period of 130 to 150 Å are occasionally present within axoplasm adjacent to the axon-Schwann membrane (fig. 7) or appear to be continuous with the inner layer of compact myelin (fig. 10).

DISCUSSION

The variations in normal myelin sheath contour, illustrated in our phase and electron microscopic study of sciatic nerves, are present throughout the guinea pig peripheral nervous system. The myelin sheath of rabbit sciatic nerve fibers has similar loops and folds, and they have been observed by others in the peripheral nervous system of cats (7, 13, 15), rats (15), fish (15), and chameleons (18). These contour variations are not the result of a specific preparative technique. They are present in guinea pig sciatic nerves exposed without stretching during anesthesia and fixed completely prior to removal from the animal. Myelin sheaths of nerves fixed in KMnO₄ or in osmium tetroxide by freeze substitution (4) also show loops and folds. Furthermore, they are found in nerves embedded in methacrylate, paraffin, and also in polyvinyl alcohol, a water soluble embedding medium (3). Changing the direction of sectioning has no effect on the architecture of these loops. In addition, a juxtanodal myelin infolding in a fresh, teased, cat, sciatic nerve fiber is illustrated in a recent study of Lubinska and Lukaszewska (13). Finally, the overall preservation of fine structure in our material indicates clearly that these contour variations are not the result of mechanical or preparative artifacts. The normal lamellar pattern of myelin is shown in all portions of the myelin sheath including the folds. The Schwann cell cytoplasm is abundant in the vicinity of large infoldings; the surrounding Schwann cell membrane is smooth and continuous and the mesaxons traversing Schwann cell cytoplasm are undistorted adjacent to these folds. The presence of myelin folds in fresh material and in that prepared by a variety of techniques in a number of experimental animals indicates that these variations in myelin contour are part of normal peripheral nerve structure.

The data of the present study do not in themselves suggest a mechanism of formation of these loops and folds. It seems reasonable to suggest that irregularities in the sprouting axon, similar to those observed by Speidel in living tadpole tails (22), are subsequently myelinated according to the Geren hypothesis of myelinogenesis in the peripheral nervous system (8). However, this suggestion could only be verified by a phase and electron microscopic study of serial, cross and longitudinal sections prior to and during myelination. Although many isolated juxtanodal or internodal myelin ovoids are continuous in two micron serial sections with loops and folds of the adjacent myelin sheath, our study has not conclusively established this continuity by extensive observations on serial sections utilizing electron microscopy.

The nature of the uniformly less dense intracytoplasmic or axonal ovoids
which do not show the lamellar structure of myelin is not clear. They may be tangential surface sections of loops in which the myelin period is obscured by the plane of the section. The significance of the dense particles illustrated at the margin of these structures and of the intracytoplasmic myelin ovoids is also not apparent at the present time, but it is of interest that their density and size approximate those of the dense particles observed in hemosiderin granules by Richter (17). Formations made up of irregular zones of lamellae with the period of myelin have occasionally been observed within axoplasm or in continuity with the inner layer of compact myelin. The lack of lamellar separation in the adjacent myelin sheath suggests that the relatively loose structure of these formations is not the result of preparative technique. The focal aggregation of lamellae with the period of myelin in the axon-Schwann membrane region indicates that this zone may be important in the synthesis of layered structures as previously postulated by Geren and Schmitt (9).

The presence of variations in contour of the normal myelin sheath is of particular significance to those attempting to utilize phase and electron microscopy in a pathological study because the loops, folds and isolated ovoids closely resemble the myelin forms seen with the light microscope during Wallerian and segmental demyelination. Vial, in a recent electron microscopic study of the axoplasmic changes associated with Wallerian degeneration (23), described and illustrated the formation of myelin sheath infoldings as an important feature of the pathological process. Our results indicate that these loops and folds of myelin are present throughout the normal peripheral nervous system and may be unrelated to the pathological process as he described it. The isolated, intracytoplasmic, myelin ovoids observed to be continuous with the myelin sheath in our study may be similar to the osmophilic bodies observed by Key and Retzius in normal nerves (12). During a controlled study of ischemic necrosis in human nerves and Wallerian degeneration in cats, Elzholz subsequently described and illustrated similar structures that were also present in normal material and were Marchi positive as well as osmophilic (2). The Marchi method was not utilized in the study of our material; however, observations now in progress will attempt to clarify the relationship of these Elzholz bodies to the process of demyelination.

Finally, the increased membrane surface area produced by the large infoldings and outpouchings of myelin in the juxtanodal region of the large fibers may be physiologically significant.

SUMMARY

Phase and electron microscopic study of normal guinea pig sciatic nerves reveals numerous variations in the myelin sheath contour. For the most part, these variations consist of infoldings into axoplasm or protrusions into Schwann cell cytoplasm and can be seen as isolated myelin ovoids in a single plane of section. They are most numerous in the juxtanodal region of the largest fibers and are of particular importance to neuropathologists because of their resemblance to myelin forms seen with the light microscope in early demyelinating
lesions. Several other features of peripheral nerve ultrastructure are briefly described.

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REFERENCES
Key To Plate Abbreviations

A  axoplasm
S  Schwann cell cytoplasm
M  myelin sheath
E  endoneural connective tissue

All illustrations are from Osmium fixed, araldite embedded, guinea pig sciatic nerve. Figures 1–4 are phase photomicrographs of two micron sections, while the remaining plates are electron micrographs of thin sections from similar material.

Fig. 1. Serial two micron longitudinal sections through a node of Ranvier. There is a shallow indentation in the left myelin segment in (a) which is continuous with a large infolding shown in (b) and (c). In (d), the continuity of this loop with the upper margin of the sheath is almost lost, and in subsequent serial sections (not illustrated) this region contains an intra-axoplasmic myelin ovoid which is completely detached from the sheath. The justanodal zone of the right myelin segment in (a) consists of several myelin balls one of which in (b) is continuous with the upper margin of the sheath as a small protrusion of myelin into Schwann cell cytoplasm. In (c) and (d), this outpouching enlarges and forms a large, paranodal ovoid of myelin; × 2400.
FIG. 2. Two micron longitudinal sections through a node of Ranvier. The section between (a) and (b) is not illustrated. In (a), a large infolding of myelin in the left segment crosses the axoplasm obliquely. However, in (b) the contour of the myelin sheath in the corresponding area is relatively smooth. In the right segment of the sheath, there is an isolated ovoid of myelin in (a). The subsequent section shows partial fusion of the ovoid and the myelin sheath (not illustrated). In the next serial section shown in (b), there is only focal thickening of the sheath in this area; X 2400.

FIG. 3. Two micron longitudinal sections through a node of Ranvier. The section between (a) and (b) is not illustrated. In (a) there are two myelin balls above the left myelin segment. One of these balls is continuous with a loop of the tapering myelin sheath in (b). The other ball shown in (a) is larger in (b) and has a less dense central zone. Also, in the left myelin segment, one of the two loops indenting axoplasm in (a) is seen as an isolated ovoid in (b). The upper portion of the right segment is thickened by a large loop of infolded myelin adjacent to the upper layer of the sheath in (a) and (b) as well as in several additional serial sections (not illustrated); X 2400.
Fig. 4. Two micron cross sections selected from 26 serial sections of the same fiber adjacent to the node of Ranvier shown in (a). Two folds of the sheath are present in (b), the lower loop can be followed in (c–h), while the upper one is less apparent in (c) and subsequent sections. In (d) an intraerytoplasmic ovoid of myelin is present above a corresponding area of indentation; it can be followed in (e) and (f) and is fused with the myelin sheath in (g); X 2400.
Fig. 5. Cross section of two adjacent fibers. The myelin sheath of each fiber forms a large fold indenting the axoplasm. The Schwann cell cytoplasm within the loop is undistorted and is surrounded by a smooth Schwann cell membrane; $\times$ 25,000. The lamellar pattern of normal compact myelin is present in adjacent portions of the loop in the upper fiber (insert). Also, in this area, the inner mesaxon (arrow) joins the inner layer of compact myelin and the axon Schwann membrane; $\times$ 45,000.
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Fig. 6. Cross section. A large fold in the myelin sheath indents the axoplasm. Within the Schwann cell cytoplasm at the base of this fold, the continuity of the Schwann cell membrane and the outer layer of compact myelin can be traced via the outer mesaxon; $\times 35,000$. The area between the arrows is enlarged in the insert which shows the lamellar pattern of myelin in a portion of the fold. Also, in this area, several of the outer lamellae are separated by zones of Schwann cell cytoplasm and cross the base of the fold where they continue to form the margin of the loop bordered by Schwann cell cytoplasm; $\times 100,000$. 
Fig. 7. Cross section. A small protrusion of compact myelin is present within Schwann cell cytoplasm. At the base of this fold, several lamellae are separated by zones of Schwann cell cytoplasm; $\times$ 45,000. Within axoplasm, adjacent to the axon-Schwann membrane, there is a large formation made up of lamellar zones some of which show the period of the adjacent compact myelin (see insert); $\times$ 85,000.
Fig. 8. Cross section. An ovoid is shown within Schwann cell cytoplasm; $\times$ 25,000.

Fig. 9. Same as Figure 8. The lamellar pattern of normal myelin is clearly shown in the ovoid and adjacent myelin sheath; $\times$ 75,000.
Fig. 10. Cross section. There is a formation consisting of irregular lamellar zones a few of which are continuous with the inner layer of compact myelin. The period of the lamellae in several of these zones corresponds to that in the adjacent myelin sheath; $\times 100,000$. 
Fig. 11. Longitudinal section, node of Ranvier. The two segments of myelin lie within Schwann cell cytoplasm which is discontinuous at the node (arrow) where there is a small outpouching of the axon membrane. Adjacent to the lower segment of the myelin sheath, there is a large ovoid with the lamellar structure of normal compact myelin (see insert). At one margin of this ovoid there are 2 hexagonal structures with poorly defined central linear densities; × 30,000. Insert; × 90,000.
Fig. 12. Longitudinal section through the internodal region. There is a large fold of the myelin sheath containing Schwann cell cytoplasm which indents axoplasm; X 20,000.

Fig. 13. Internodal longitudinal section. Adjacent to the small myelin sheath indentation into axoplasm, there are two round structures of fairly uniform density (arrows) which do not clearly show the lamellar pattern of the adjacent sheath. One lies within axoplasm and one within Schwann cell cytoplasm; X 11,500.
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Fig. 14. Longitudinal section. An ovoid with the lamellar pattern of normal myelin is shown within internodal Schwann cell cytoplasm. The Schwann cell membrane is to the right (double arrows) and the myelin sheath to the left. At the upper margin of the ovoid there is a collection of dense particles measuring 50 to 100 Å; \( \times 55,000 \).

Fig. 15. Internodal longitudinal section. Two round bodies of fairly uniform density are present within Schwann cell cytoplasm. A faint lamellar pattern can be made out in some areas particularly at the periphery. An aggregation of dense particles measuring 50 to 100 Å (arrow) with some degree of orderly packing lies between these bodies and the Schwann cell membrane (double arrows); \( \times 65,000 \).