ELECTRON MICROSCOPE STUDIES OF THE FORMATION OF NODES OF RANVIER IN MOUSE SCIATIC NERVES*

By BETTY GEREN UZMAN‡, M.D., and GENEVIEVE NOGUEIRA-GRAF

(From The Children's Cancer Research Foundation and the Department of Pathology, Harvard Medical School, Boston)

Plates 187 to 194

(Received for publication, February 21, 1957)

The node of Ranvier has been the object of particular attention from students of the nerve fiber primarily for two reasons. One of these, of special concern to anatomists, is due to the prolonged controversy ensuing from Ranvier's initial concept of the Schwann cell as a structural unit giving rise to, as well as providing natural limits to, the internodal lengths of the myelin sheath. Another has been the urge to provide anatomical loci for the measurable electrical properties of the nerve fiber, both at rest and during impulse propagation. Such anatomical-physiological correlations have hinged on the continuities and discontinuities thought to exist both structurally and functionally throughout the extent of the fiber.

Our own studies (1, 2) on the formation of myelin in peripheral nerves of infant mice and embryonic chicks have led to the conclusion that the myelin sheath is formed by a process of infolding and spiral wrapping of the surface of the Schwann cell. This conclusion was based on an electron microscope study of transverse sections of nerve fibers in the early stages of myelination. The spiral wrapping of the myelin sheath provided a plausible mode of increasing the thickness of myelin, but the manner in which the myelin sheath is elongated to cover the entire internodal region of the axon was not apparent, nor were there any hints in the transverse sections described in these earlier studies concerning the formation or appearance of discontinuities such as Schmidt-Lantermann clefts or nodes of Ranvier. The present report deals with a study of longitudinal sections of infant mouse sciatic nerves, with particular emphasis on the length and forms of the early segments of myelin, as well as the relationship between the Schwann cell, the myelin sheath, and the axon at regions of discontinuity of the myelin.

*This investigation was supported in part by a research grant (B-190) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service, and in part by a grant from the United Cerebral Palsy Association, Inc.

‡From The Children's Cancer Research Foundation, and the Department of Pathology, Harvard Medical School, Boston, Massachusetts.

Among the earlier workers, Nageotte (3) strongly supported both the view that the myelin sheath is an axonic appendage and the concept that the cells of Schwann form a continuous syncytial network covering the nodes. DeRenyi's (4) interpretation of his own microdissections of nerve fibers gave further credence to the syncytial nature of the Schwann cell sheath. Another concept of the myelin sheath is that fostered by Young (5, 6) and his coworkers, who have considered an internodal segment of myelin as a liquid droplet whose cylindrical form is maintained by outward pressure of the axon and resistance to stretch of the neurilemma. The internodal segment, according to their ideas, would represent a stable length of the liquid droplet.

The results of the investigations described here strongly support the original concept of Ranvier that the Schwann cells form the basic units of structure that determine the internodal segments of the myelin sheath. Our evidence illustrates the apposition of Schwann cells at the node during myelin formation and suggests that it is only by way of the separateness of the Schwann cells during myelin formation that such a structure as the node can arise.

The terminology adopted here with reference to the fine structure of myelin deserves explanation in order to avoid confusion with similar terms used previously by earlier investigators, such as Nageotte (3). The myelin sheath is seen in electron micrographs of both cross- and longitudinal sections as a series of alternating dense and less dense bands or lines (Fig. 1 *a*). In the process of formation of the myelin sheath, the Schwann cell surface is infolded and spirally wrapped (Fig. 1 *b*). The apposition of the infolded faces of the Schwann cell surface is seen as two dense lines separated by a less dense region, and this "double membrane" is referred to here as a *myelin lamella* (arrow, Fig. 1 *b*). One can observe the spiral course of this lamella in cross-sections of myelinating nerve fibers. Early in the course of myelination, the turns of the spiralled lamella are separated from each other by Schwann cell cytoplasm (1, 2). In later stages, the turns of the spiralled lamella are so closely packed that the dense edges of adjacent turns of the lamella fuse and are seen as a single dense line (arrow, Fig. 1 *c*). Regions of the myelin sheath, so formed of closely packed layers, are referred to here as "compact myelin." For brevity and clarity we shall refer to the compact myelin in terms of layers, but to regions of the myelin sheath in which the turns of the spiral are not closely packed, in terms of individual myelin lamellae (realizing that adjacent lamellae are, in reality, continuous with each other in the spiralling of the infolded Schwann cell surface).

Materials and Methods

The mice used in this study were Swiss albino mice, Wistar strain, 7 to 14 days of age. In the previous studies of transverse sections it was observed that during this period of development, many of the peripheral nerve fibers showed only a few (1 to 15) lamellae or layers in their myelin sheath.

Under intraperitoneal nembutal anesthesia (0.6 to 0.9 mg./mouse) the sciatic nerves were exposed and flooded with ice-cold fixative. The fixative used was that described by Palade (7), the pH's of the fixing fluids before addition to tissue ranging between 7.4 and 7.7.

The nerves were dehydrated in an ethyl alcohol series and imbedded in *n*-butyl methacrylate containing 0 to 15 per cent methyl methacrylate, catalyzed with 0.15 per cent benzoyl peroxide. Polymerization was achieved under ultraviolet illumination at room temperature. Sections were cut with glass knives in a modified Minot rotary microtome, and examined in an RCA type EMU-2D electron microscope equipped with an objective aperture.

All the material here presented deals with longitudinal or near longitudinal sections of myelinating nerve fibers (*i.e.*, one or more lamellae and/or layers of myelin surround the axon in some region of its observed length). The areas selected for particular study and photographic recording were those in which the myelin sheath appeared to undergo partial or complete interruption in its course along the fiber. Major interruptions of the myelin sheath due to the sectioning procedure or to "explosion" of fibers during imbedding when encountered were easily identified, and are not included in the data. In some of the figures (Figs. 3, 6, 7, and 9), one can note that either the myelin sheath or the cell surfaces or both show minor discontinuities due to fixation or sectioning. Such discontinuities have been consistently encountered, but with some experience on the part of the investigator, do not limit the interpretation of the micrographs.

RESULTS

The identification and differentiation of the two well known discontinuities of the myelin sheath, the nodes of Ranvier and the Schmidt-Lantermann incisures have been based on the histologically well known general forms and dimensions of these regions in the myelin sheaths of adult fibers. In this study, the funnel shaped incisures have appeared in longitudinal sections as roughly linear, symmetrical clefts in the myelin on either side of the axon. The characteristic V-shape of the clefts has made their identification easy. The individual layers of the myelin sheath have most frequently been observed to terminate abruptly on either side of the clefts, although across the narrowest clefts observed (presumably the best fixed), some of the layers are continuous, either as such or as separated lamellae, (Figs. 10 and 11). In such instances, the separated lamellae are quite tortuous in their path across the incisures. Within the incisures, irregularly shaped granules of high electron density, together with short lamellar fragments, were frequently observed. The layer of Schwann cell cytoplasm outside the myelin was always continuous across the incisural regions. These clefts have not been observed by us in mice younger than 10 days, or in fibers with only a few (5 to 10) lamellae or layers of myelin.

Clearly distinct from the incisures is the type of myelin discontinuity at what are presumably nodal regions. In the early stages of myelin formation, such identifying criteria of nodes as inward curvature of the myelin sheath with the concomitant narrowing of the axon so characteristic of adult nodes were not observed. After careful examination of more than a thousand fibers, we have identified early nodes of Ranvier as regions in which the following two features are observed: (*1*) the overhanging pattern of the lamellae of myelin on either

side of the node, and (*2*) the usually closely apposed terminations of adjacent Schwann cell cytoplasm at some point between adjacent internodal lengths of the myelin sheath. In a few instances, as can be seen in Fig. 2, the terminations of adjacent Schwann cells are separated by fairly long gaps.

The pattern of lamellar endings at nodes is distinct and is characteristic of all fibers, since, in those in which the myelin sheath is of the compact type, the myelin layers separate at the node into the component lamellae. Each lamella terminates at the axon surface just short of the termination of the next outermost lamella, forming an overhanging pattern which appears in mirror image on the other side of the node (Figs. 2 and 3), although the number of layers of myelin involved is not always the same on both sides of the node (Fig. 2). Between the separated lamellar endings the Schwann cell cytoplasm can be identified including occasionally such formed elements as mitochondria (Fig. 2). Text-fig. 1 *a* is a diagrammatic representation of a cutaway view of a node of Ranvier in a myelinating fiber, illustrating in detail the lamellar and Schwann cell cytoplasmic relationships.

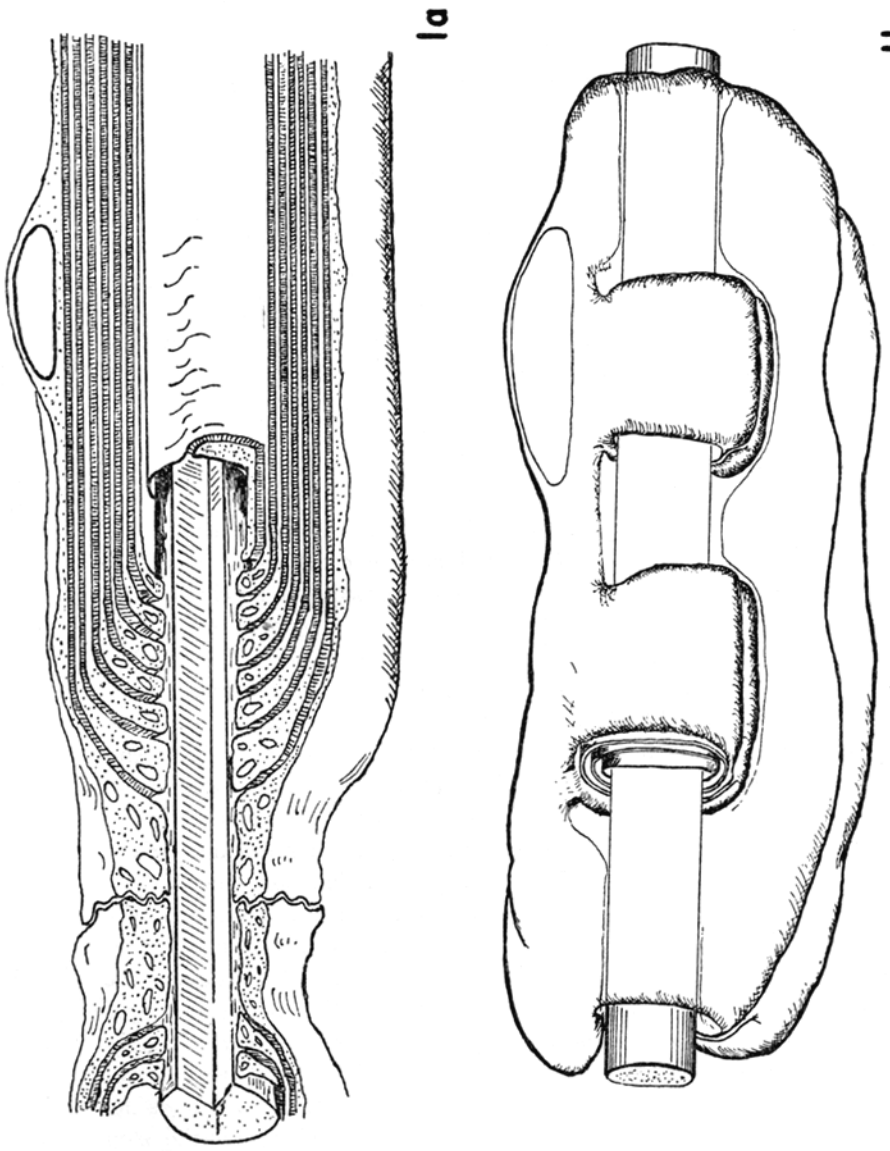
The axon at the node is, in every case, continuous, and its surface represented by an electron-dense line, unbroken except for faults due to sectioning (Figs. 3 and 4).

The terminations of the adjacent Schwann cell cytoplasm are usually closely apposed and sometimes quite irregularly interdigitated (Figs. 2 to 4).

A poorly demarcated dense line of varying thickness was observed around each nerve fiber. This is continuous across the node and does not follow the terminal interdigitations of the Schwann cells at the node. We have previously (2) considered this to be a component of the connective tissue and have so designated it in the figures (*CT*). Outside this hazy layer (Figs. 3 and 4) the fibrous elements of the connective tissue are cut in various degrees of obliqueness.

Turning now from the clearly identifiable nodal regions, we shall consider interruptions of the myelin sheath in which the overhanging or nodal pattern of myelin lamellar endings was observed, but which lacked the other distinguishing features of nodes of Ranvier. The simplest variation is illustrated in Fig. 5 in which both the Schwann cell cytoplasm and myelin sheath terminate as at a node, but the axon beyond the ending of the Schwann cell is not covered by Schwann cell cytoplasm. Presumably this could represent a developing node in which the adjacent Schwann cell cytoplasms are separated by a distance considerably greater than that usually observed.

In a single micrograph (Fig. 8 *b*), the myelin sheath is seen as three separate segments within the confines of one Schwann cell. In spite of the distortions apparent in the myelin due to fixation, it is clear that the segments at either end are composed of many layers, and the central segment of only four lamellae. This isolated observation is significant when considerd in the light of all of the results presented, and will be discussed later.



TEXT-FIG. 1 *a*. Diagrammatic representation of cutaway view of early node of Ranvier illustrating relationship between myelin lamellar endings, Schwann cell cytoplasm, and axon. The myelin lamellae are cross-hatched. Cut surfaces of the Schwann cell cytoplasm are stippled and include outlines of formed cytoplasmic organelles. Filamentous structures in the axoplasm indicated only at cut-end of axon.

TEXT-FIG. 1 *b*. Schematic diagram of two pseudopodia of the Schwann cell separately infolded and spirally wrapped about the axon. These are covered by the "transparent" total extent of the Schwann cell infolded and spirally wrapped about the axon.

Another type of discontinuity in the myelin sheath, illustrated in Fig. 9 is that of a fiber with 34 to 35 layers in its compact myelin, of which the inner 13 or 14 layers are separated into individual lamellae that end, as at nodes, at the axon surface with each inner lamella being shorter than the one just outermost. The outer 20 layers of myelin, as well as the Schwann cell cytoplasm, are continuous. This, too, is an isolated observation.

We have frequently recorded, in overlapping sequences of micrographs, long portions of a single fiber. Up to 10 days of age, when the Schmidt-Lantermann clefts are first observed, the myelin sheath, in single internode portions, was observed to be continuous over distances of 20 to 30 micra. In a few instances, however, as illustrated in Figs. 6 and 7, the entire longitudinal extent (not just short, obliquely cut bits of myelin as judged by the continuity of the axon) of a myelin segment is only 5 to 8 micra. The Schwann cell cytoplasm, although not traceable for more than a few micra from either end of these short segments, appears to be continuous, and this despite the fact that at either end of the segments the lamellar endings are of the same form as at nodes.

DISCUSSION

If one considers the results of previous studies of transverse sections (1, 2) in conjunction with the observations presented here, a general picture of the changes that the Schwann cell undergoes in the process of myelination of peripheral nerve fibers can be formulated. The initial envelopment of the axon by the infolded Schwann cell surface occurs without interruption along the entire extent of the Schwann cell. This process is followed by the continued infolding of the Schwann cell surface so that a "double membrane" consisting of the apposed faces of the infolded Schwann cell surface is formed. This membrane, which constitutes the basic, repetitive, structural feature of the myelin sheath, extends and spirally wraps itself over and over as the infolding of the Schwann cell surface continues. In this manner, the increase in thickness of the myelin sheath in its cross-sectional diameter is easily understood. One mode of extension of the myelin sheath along the length of the axon is revealed in the present studies of longitudinal sections. It is apparent that each of the consecutive turns of the spiral increases in size along the length of the axon as the number of turns increases. This is evidenced by the fact that each spiral layer (or lamella as already denoted) overhangs the previous or just innermost layer at each end. These successive overhangs of myelin lamellae present two features of structural interest: (*1*) with each additional turn of the spiral, not only is the radial thickness of the myelin sheath increased, but also the length of the axon covered by the myelin segment in each Schwann cell domain; and (*2*) each turn of the spiralling membrane, because of the overhang, is still, at each end, in close contact with the axon surface.

It is not possible to state whether or not the tremendous increase in length

of the myelin sheath in the confines of a single Schwann cell (*i.e.*, internodal segment) that occurs during growth of the animal (5, 8) can be accounted for solely by the increased length of axon covered by each successive turn of the spiral as just described. Although the apparent average difference between adjacent layers in their length along the fiber axis is a small fraction of a micron, the outermost layer or lamella frequently is observed to be ½ to 1 micron longer than the one just innermost. This suggests that the innermost lamellae may, in some manner, either by stretching or actual addition of material, be elongated to approximate more closely to the outermost lamella with each turn of the spiral. If such a process does occur, it could account for the magnitude of increase in length of internodal segments during growth as indicated for other species (5, 8).

At the junction of two Schwann cells along an axon, the directions of the lamellar overhang of the myelin endings are of opposite sense. It is this junction of adjacent Schwann cells with the termination of the myelin sheath within the confine of each, that constitutes the region designated the node of Ranvier. Nageotte (3) referred to the shape of the endings of the myelin sheath on either side of the node in adult fibers as cupolas or domes of inverse senses of direction, and it is now clear that both the shape and the direction stem from the overhanging of the spiralled layers of the myelin sheath.

From the above, an internodal segment of myelin sheath would be considered as that length of myelin sheath formed within a single Schwann cell and a node of Ranvier would refer to the structure complex at the junction of two adjacent Schwann cells. Some of the nodal structures that have been described with the use of special stains may now find explanation. The appearance of the spiny bracelet of Nageotte, (3) *e.g.* could result from the penetration of stain, not between adjacent lamellae of myelin (as defined here) but between adjacent groups of lamellae. This cleavage of the myelin sheath into thicknesses composed of several lamellae could result fortuitously from the trauma of preparation, or from the occasional retention of Schwann cell cytoplasm between lamellae. The possibility of the latter occurring is illustrated in Fig. 2.

The discussion, so far, has been based on the appearance of the majority of fibers we have observed in the early stages of myelin formation. In our results, we have also described certain isolated observations that can now be treated as exceptions to the usual mode of formation of myelin. In a few fibers, myelination may occur in several separate segments within a single Schwann cell. This leads us to postulate that the initial envelopment of the axon by the Schwann cell occurs in separate pseudopodia, two of which are diagrammatically illustrated in Text-fig. 1 *b*. In the diagram, the outermost wrapping of the axon by the full length of the Schwann cell is presumed to be transparent so that the pseudopodia within are visible. Lending support to this hypothesis are the observations of very short myelin segments (5 to 8 micra in length) illustrated

in Figs. 6 and 7. These segments are very much shorter than the extent of the axons covered by a Schwann cell in most of the micrographs, and could hardly, therefore, represent the total length of even an early internode. According to this hypothesis, the endings of the separate initial segments within a single Schwann cell would be subsequently obscured at the time that a greater continuous extent of the Schwann cell is involved in the spiral wrapping of the myelin sheath. This is also diagrammatically illustrated in Text-fig. 1 *b* and the continuity of the outer layers of myelin shown in Fig. 9 is interpreted here as resulting from such a process. These three observations—(*1*) the occurrence of very short initial segments of myelin; (*2*) the occurrence of several such segments in a single Schwann cell; and (*3*) the observation of continuous outer layers of the myelin sheath across discontinuities in the inner layers of the myelin sheath (these discontinuities having the overhanging pattern of lamellar endings)—may all be examples, then, of an unusual or exceptional manner of formation of the myelin sheath resulting from the initial encounter of separate pseudopodia of a single Schwann cell with an axon. Previously, from observations of myelinating fibers with the light microscope, Larsell (9) has concluded that myelin is formed in droplets along the axon, an internodal length of myelin resulting from end-to-end merger of such droplets. The isolated observations just discussed here are in keeping with these earlier findings but view the initial "droplets" as separate initial segments of lamellar myelin, and the merger of droplets as resulting from the spiral wrapping of outer layers of myelin from greater extents of the Schwann cell on top of the initial segments. In our experience, this represents an unusual way of myelination.

Finally, the appearance of Schmidt-Lantermann clefts in longitudinal sections of myelinating fibers has led to the following tentative conclusions. First, the occasional persistence of the myelin lamellae across narrow clefts suggests that with more perfect fixation, all lamellae might be continuous. Second, the absence of Schmidt-Lantermann clefts in the very early stages of myelin formation suggests that their origin is unrelated to the manner in which the Schwann cell surface is infolded to form the myelin lamellae, but that more probably they represent specialized regions of weakness in the thicker compact myelin, although their funnel shape may reflect the spiral mode of wrapping of the myelin sheath.

SUMMARY

Observations with the electron microscope of longitudinal sections of the sciatic nerves of infant mice during the period of early myelin formation are described.

These observations are interpreted in relation to previous studies of transverse sections, and a general picture of the formation of an internodal length of the myelin sheath in three dimensions is formulated.

In general, an internodal length of myelin sheath is attained by the spiral wrapping of the infolded Schwann cell surface; the increase in length of the internode during maturation is at least partially explained by the increased length of axon covered by the overlapping of successive layers during the wrapping of the infolded Schwann cell surface; and the nodes of Ranvier refer to the structure complex at the junctions of adjacent non-syncytial Schwann cells. The fact that the mode of formation of myelin brings each of its layers into intimate contact with the axon surface at the nodes is emphasized because of the possible functional significance of this arrangement. The manner of origin of Schmidt-Lantermann clefts remains obscure.

Certain isolated observations provide evidence for the possibility that occasional internodes of myelin may form from several small segments of myelin within a single Schwann cell.

The valuable assistance of Mrs. Phyllis Hubbard and Mr. Sumner Roper is gratefully acknowledged.

REFERENCES

1. Geren, B. B., *Exp. Cell Research*, 1954, **7**, 558.
2. Geren, B. B., Cellular Mechanisms in Differentiation and Growth, Princeton, Princeton University Press, 1956, chap. X, 213.
3. Nageotte, J., L'Organisation de la Matière, Paris, Librairie Félix Alcan, 1922, pt. 2, sect. 1, 217.
4. de Renyi, G. S., *J. Comp. Neurol. and Psychol.*, 1929, **48**, 293.
5. Vizoso, A. D., and Young, J. Z., *J. Anat.*, 1948, **82**, 110.
6. Hess, A., and Young, J. Z., *Proc. Roy. Soc. London, Series B*, 1952, **140**, 301.
7. Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
8. Hiscoe, H. B., *Anat. Rec.*, 1947, **99**, 447.
9. Larsell, O., *in* Morris' Human Anatomy, (J. P. Schaeffer, editor), Philadelphia, P. Blakiston's Son & Co., 10th edition, 1942, section 8, 863.

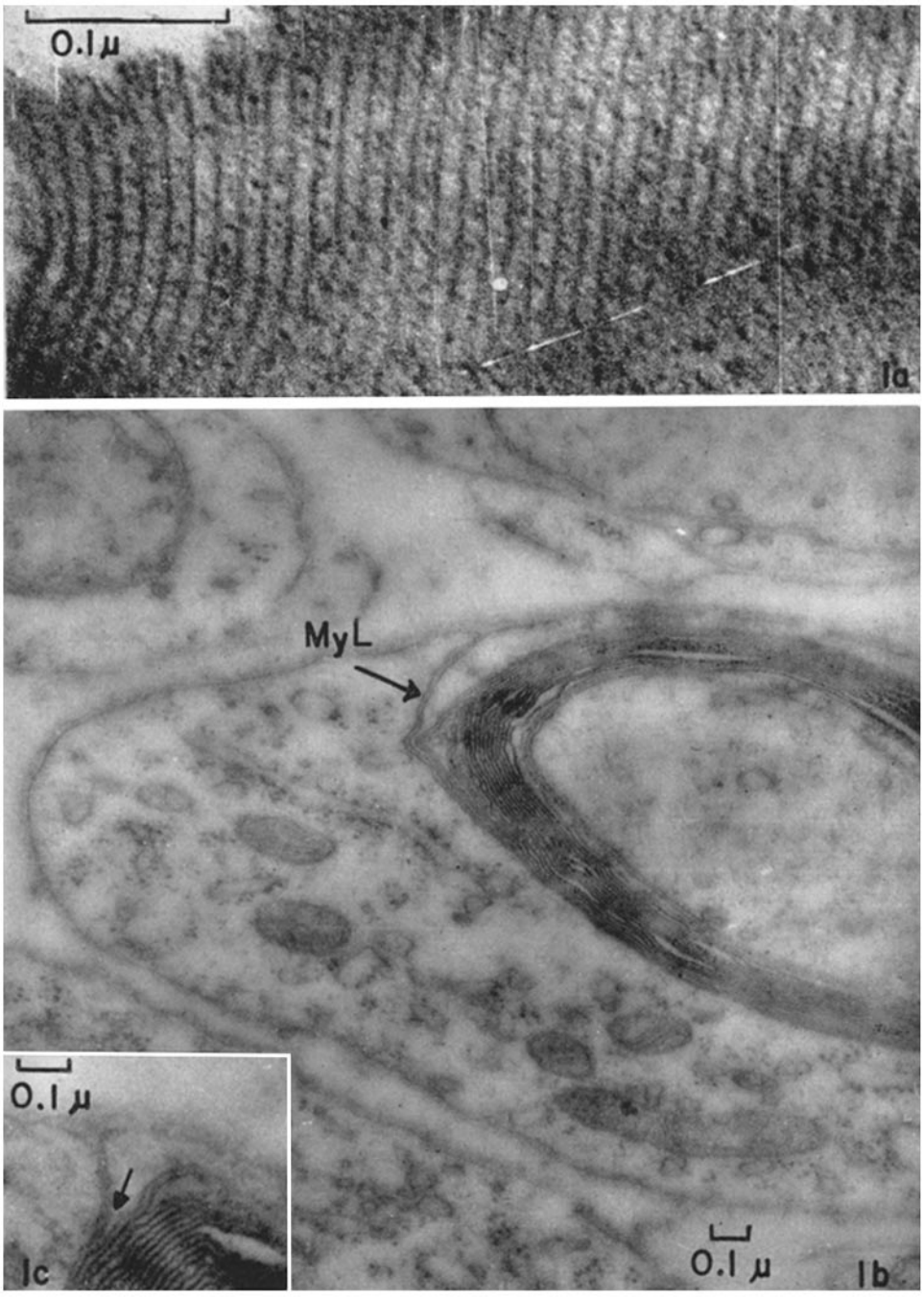
EXPLANATION OF PLATES

PLATE 187

FIG. 1 *a*. Transverse section through "compact" myelin showing alternation of electron-dense and less dense bands.

FIG. 1 *b*. Transverse section. Outermost layer of compact myelin is continuous with the myelin lamella (*MyL*) formed by infolded Schwann cell surface.

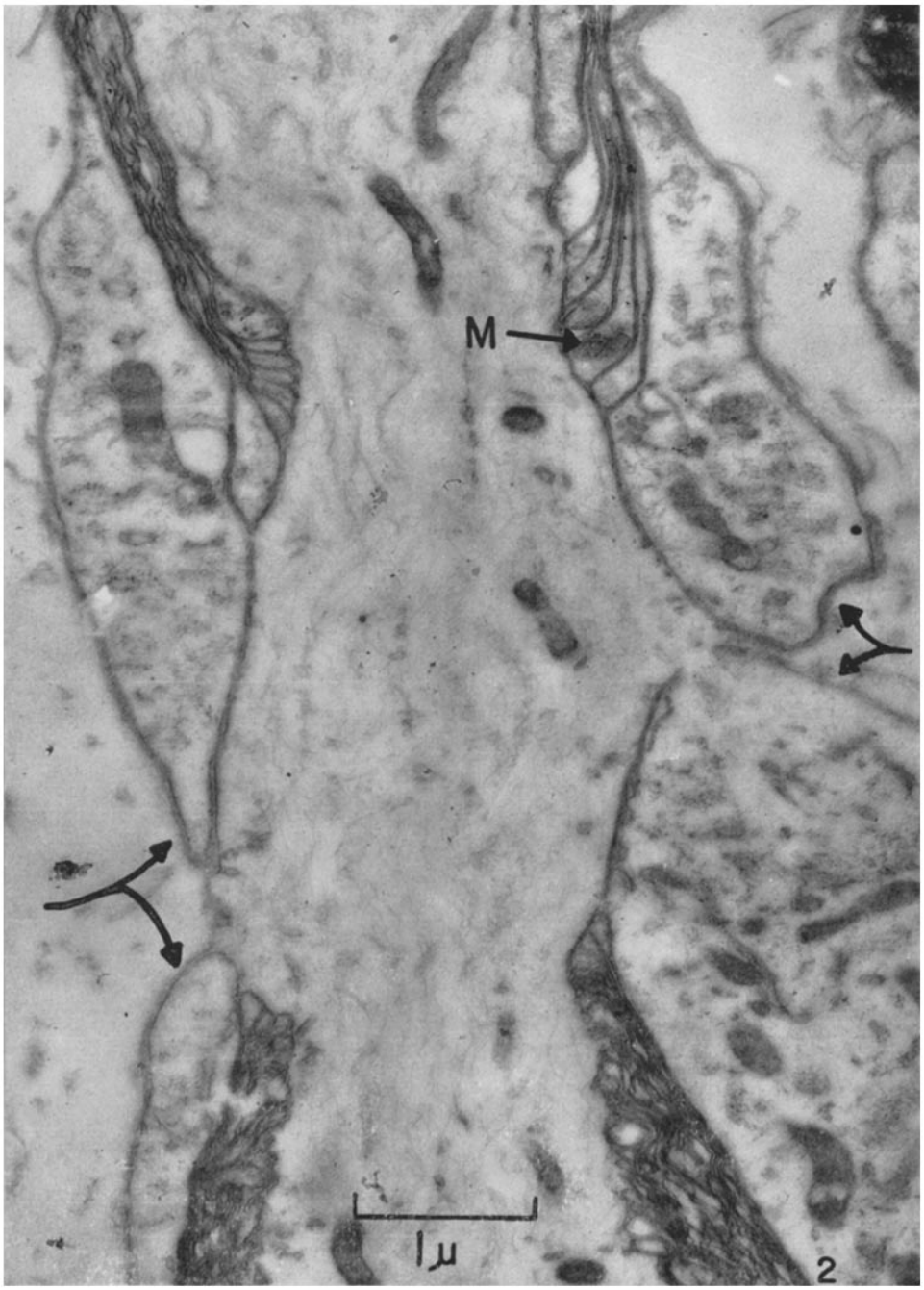
FIG. 1 *c*. Transverse section. Arrow indicates region in which the electron-dense edge of the myelin lamella fuses with the outermost electron-dense band of the compact myelin.



(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)

PLATE 188

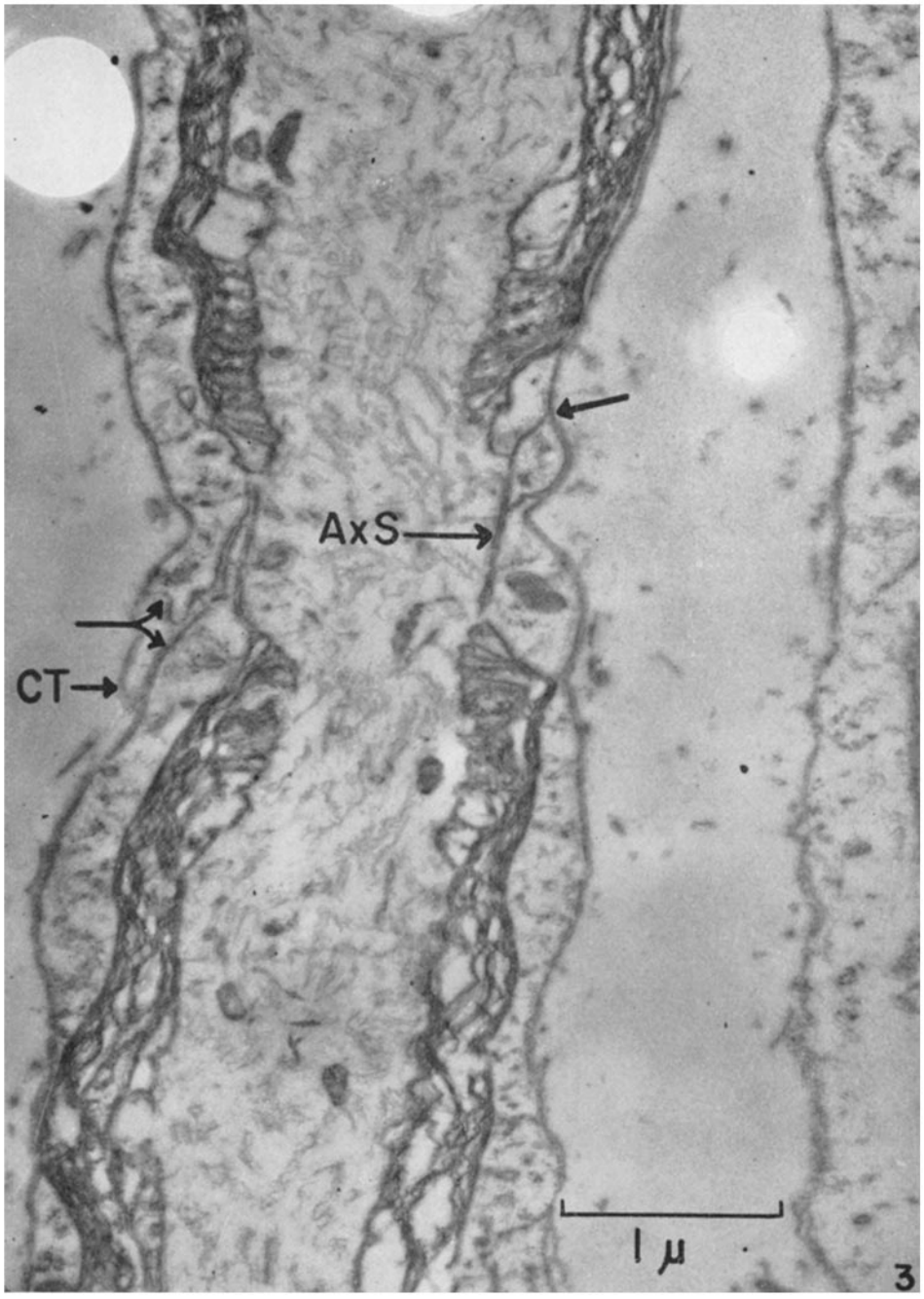
FIG. 2. Longitudinal section through region of node of Ranvier. Unmarked arrows indicate apposition of edges of adjacent Schwann cells. Note mitochondria (*M*) between overhanging lamellae.



(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)

PLATE 189

FIG. 3. Longitudinal section through region of node of Ranvier. Unmarked arrows indicate apposition of adjacent Schwann cells. The ill defined dense layer presumably of the connective tissue sheath (*CT*) is indicated on one side of the node. Arrow, *AxS*, indicates electron-dense line at axon surface.

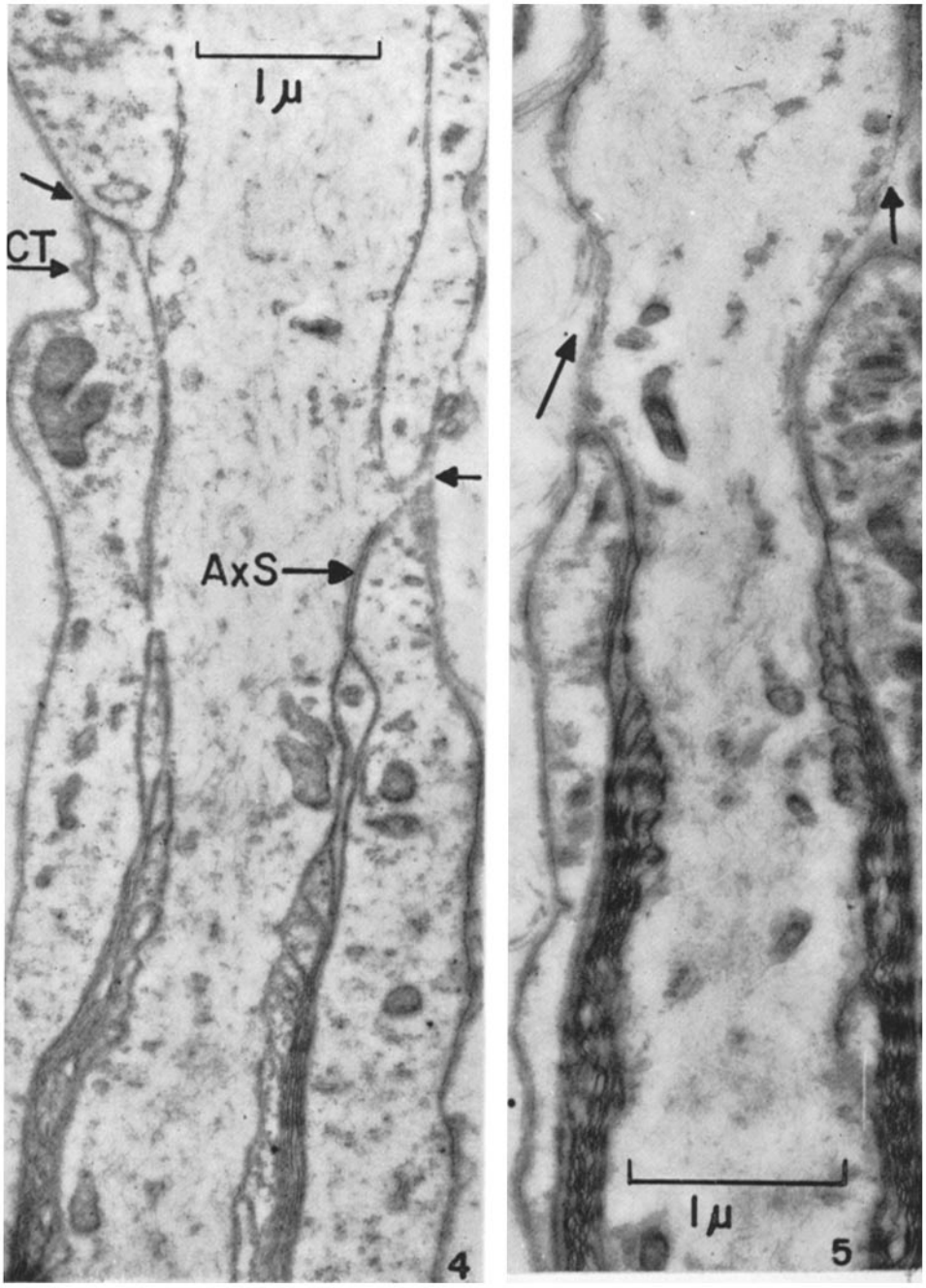


(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)

PLATE 190

FIG. 4. Longitudinal section. Overhanging lamellar endings of myelin are visible on one side of node, only. Unmarked arrows indicate regions of apposition of adjacent Schwann cells. The electron-dense line indicating the axon surface (*AxS*) is indicated. Arrow labelled *CT* again indicates ill defined dense layer, presumably of connective tissue origin.

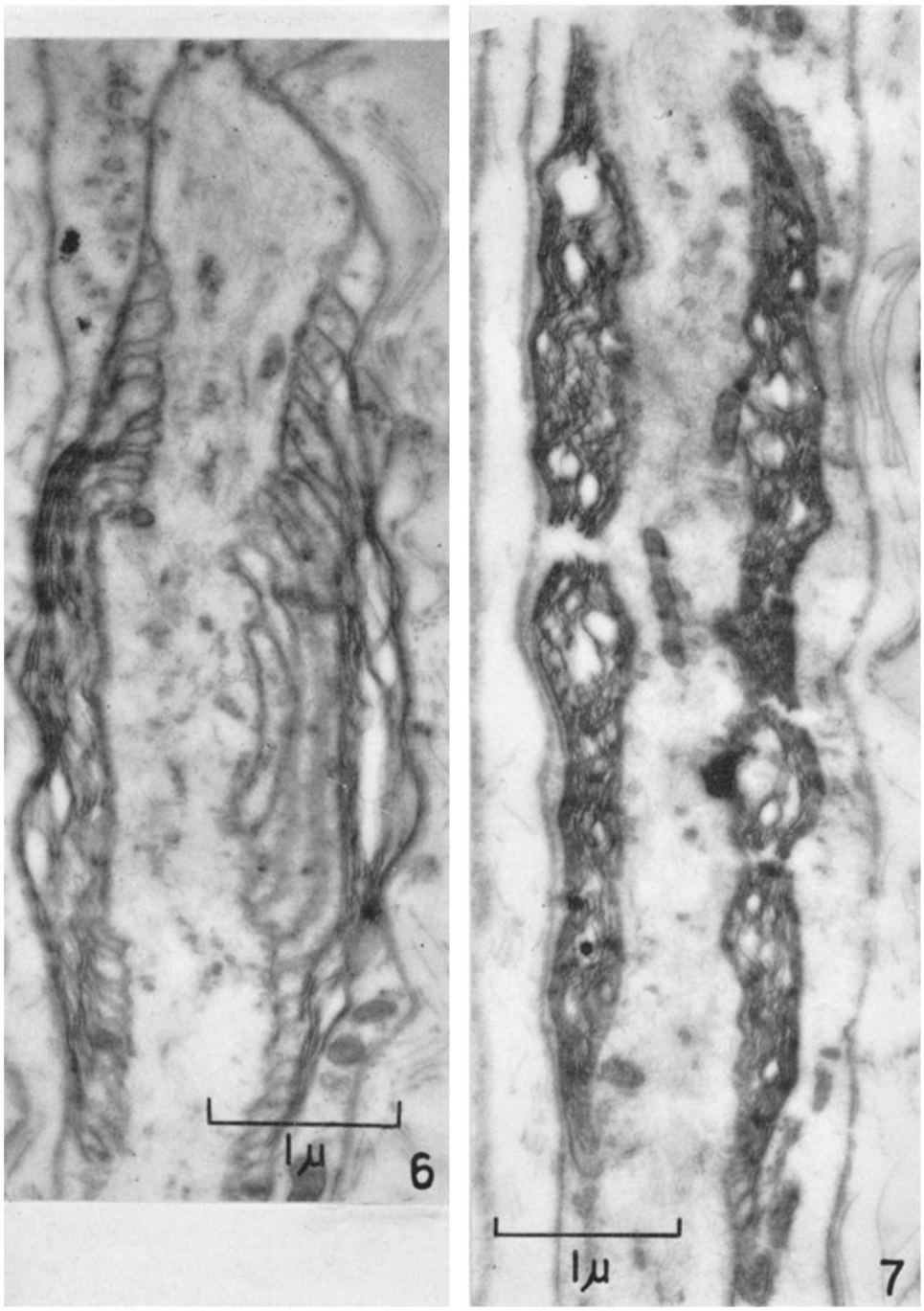
FIG. 5. Longitudinal section. Both the myelin sheath and Schwann cell cytoplasm terminate. Axon uncovered by Schwann cell layer beyond arrows.



(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)

PLATE 191

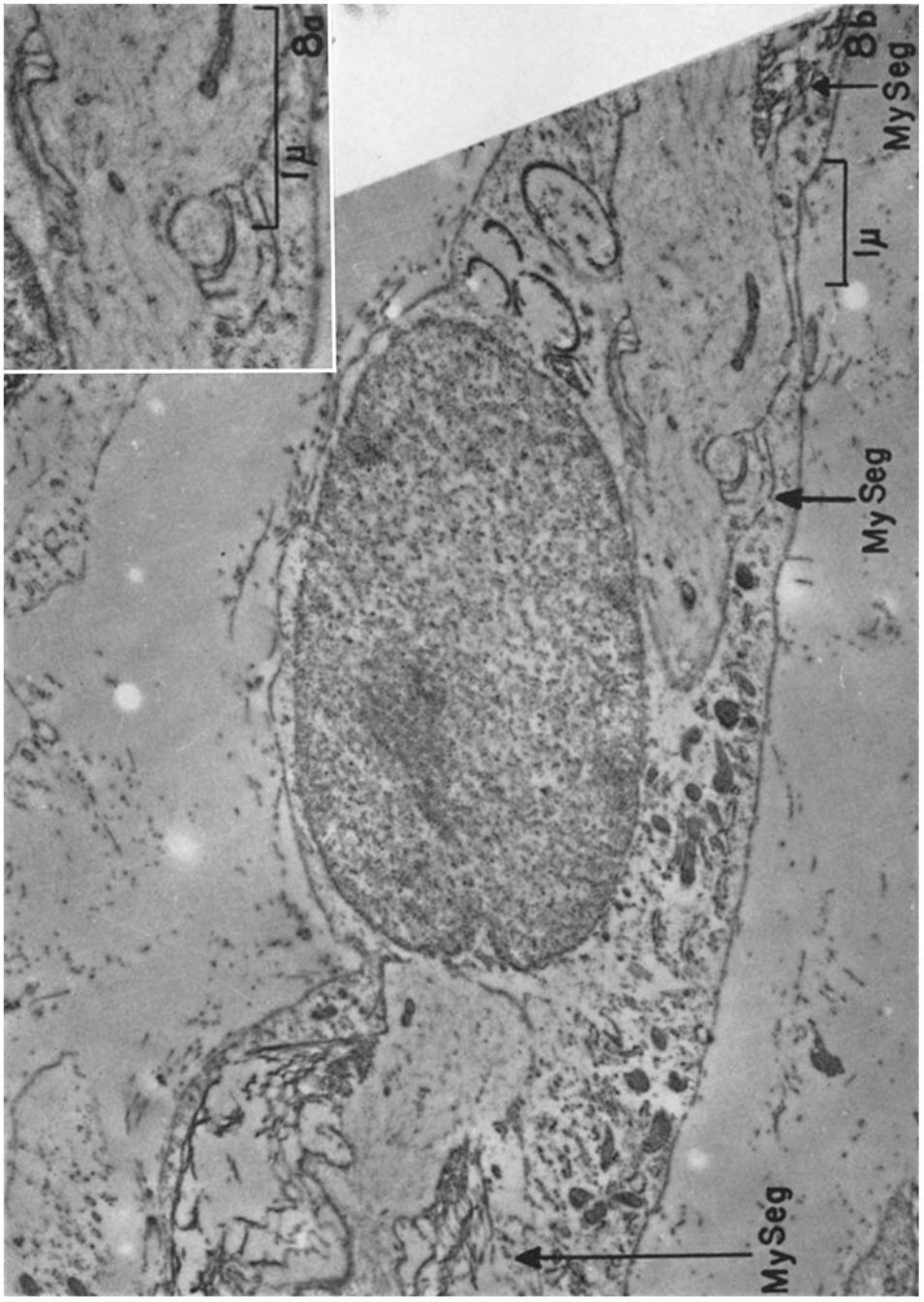
FIGS. 6 and 7. Short initial segments of myelin cut in their entire longitudinal extent. The axon is continuous through both. The myelin sheath terminates at either end of each segment in the overhanging lamellar fashion characteristic of nodal endings of myelin. The Schwann cytoplasm does not terminate at either end of either segment.



(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)

PLATE 192

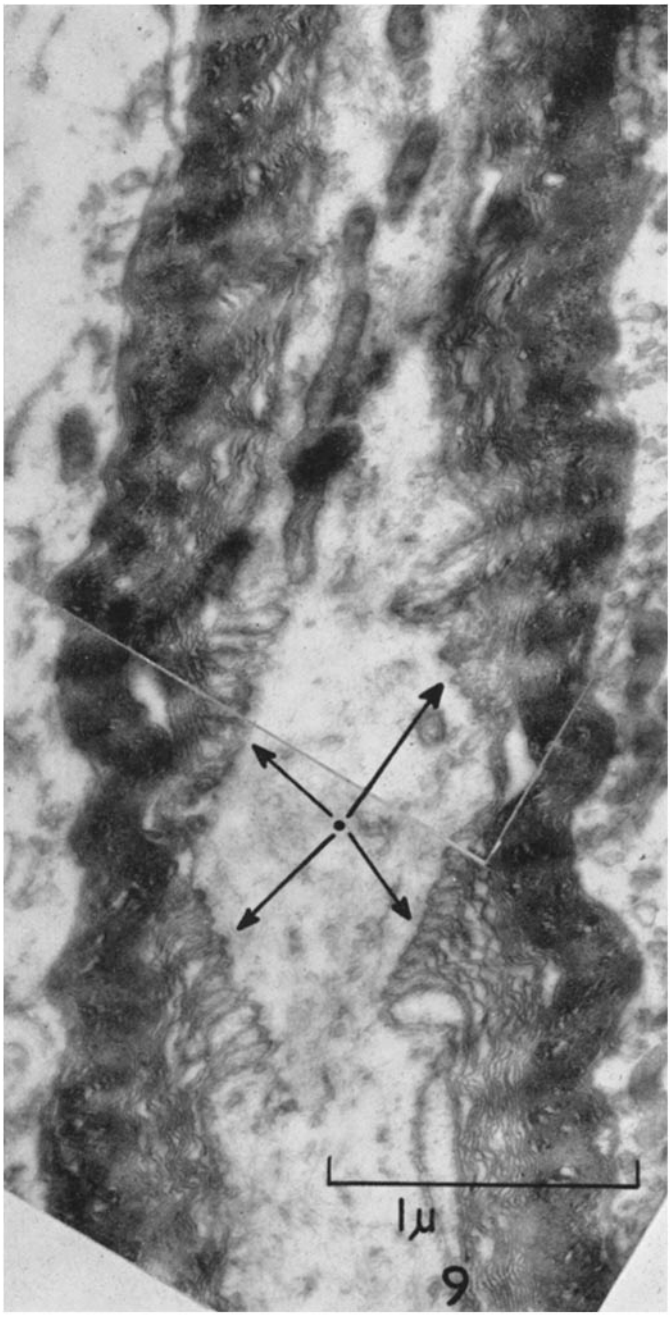
FIG. 8 *b*. Longitudinal section through region of Schwann cell nucleus. The axon is out of the plane of section near the nucleus. Three separate segments of myelin are indicated by *My Seg*. Note that the segments at either end consist of many layers, whereas the central segment (enlarged in inset, Fig. 8 *a*) consists of only four lamellae.



(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)

PLATE 193

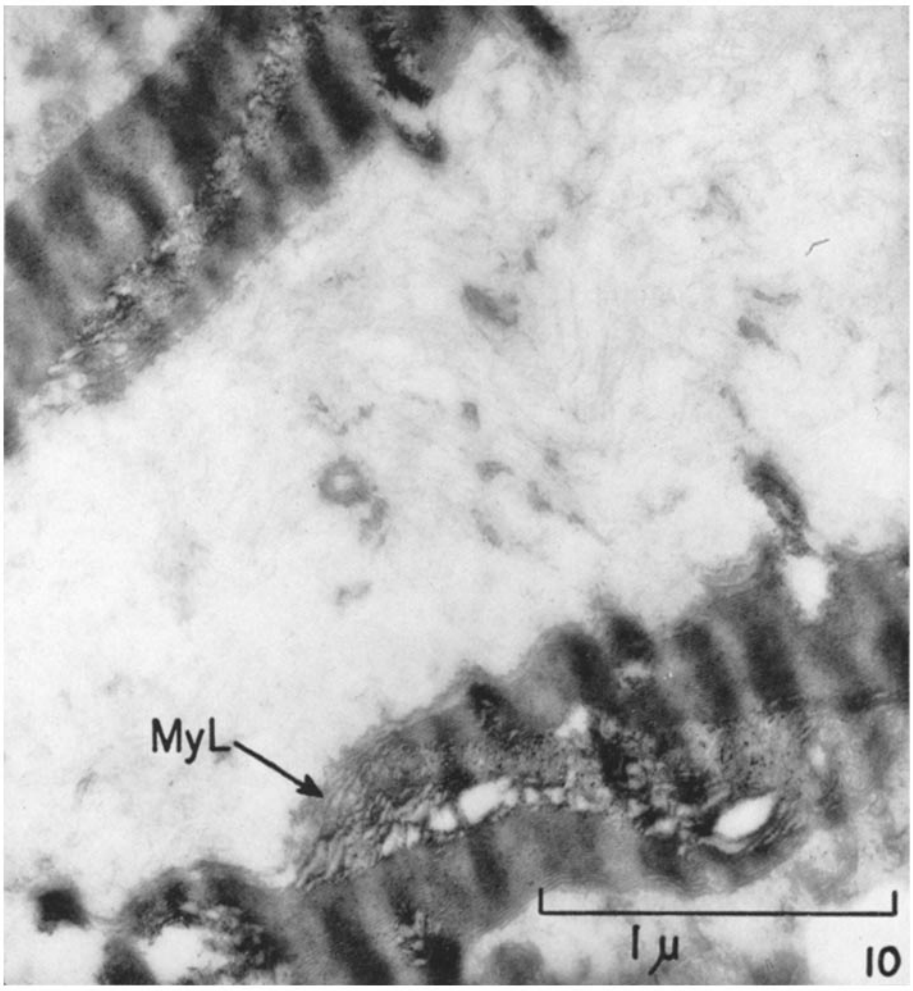
FIG. 9. Longitudinal section. The inner layers of the myelin sheath terminate in overhanging lamellar fashion, in regions indicated by arrows. The outer layers of the myelin sheath, as well as the Schwann cell cytoplasm are continuous.

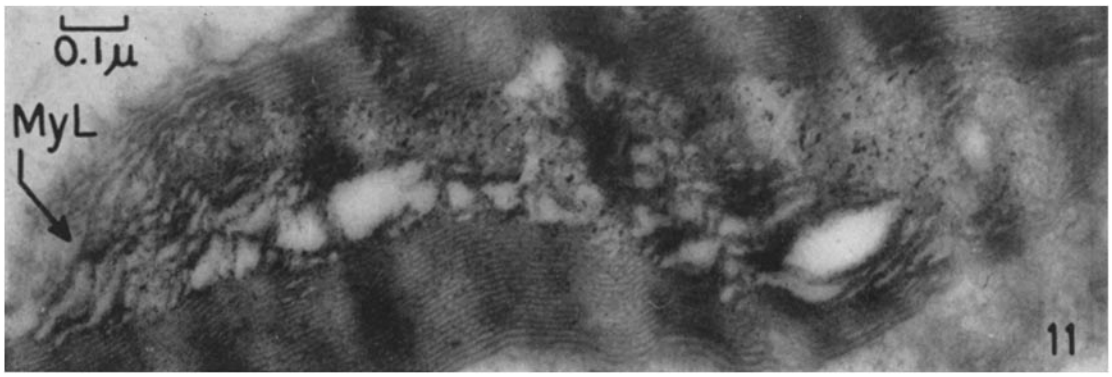


(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)

PLATE 194

FIG. 10. (Fig. 11 is an enlargement of the side of the cleft seen in the lower half of Fig. 10). Longitudinal section through region of Schmidt-Lantermann incisure. Note V-shaped symmetry of cleft. The continuity of some of the myelin layers as lamellae extending across the cleft is indicated by arrows (*MyL*).





(Uzman and Nogueira-Graf: Formation of nodes of Ranvier)