

THE ULTRASTRUCTURE OF ADULT VERTEBRATE PERIPHERAL
MYELINATED NERVE FIBERS IN RELATION TO
MYELINOGENESIS*

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This paper presents evidence derived from electron microscope studies of sections of adult reptilian myelinated nerve fibers which supports a new theory of myelinogenesis recently proposed by Geren (7). She observed in sections of chick embryonic nerve that at a very early stage of development the axons are enveloped by Schwann cells. A double membrane¹ was seen in Schwann cytoplasm connecting the outer surface membrane of the Schwann cell with the double membrane (axon-Schwann membrane or axolemma) shared by the axon and Schwann cell. This structural arrangement recalls that described by Gasser (5) in adult C fibers. At a later stage of development this connecting double membrane² (hereafter designated surface connecting membrane, SCM) appeared in cross sections to be wrapped about the axon in a helical fashion with its initial connections maintained. These observations suggest that the lamellae of adult myelin may consist of a closely packed helically wound double membrane. The ends of this membrane should be continuous with the outer surface membrane of the Schwann cell and with the axon-Schwann membrane. Further, the directions of entrance and exit of these double connecting membranes into the myelin should be opposite if the helix is a simple one. Evidence is presented here that such inner and outer connecting membranes exist and that their relations to myelin are consistent with a simple helical arrangement of the myelin lamellae.

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¹ The term "double membrane" as used here means a membrane which in cross section consists of a central zone which is relatively transparent to electrons bordered by thinner edges which are more opaque to electrons. It is used in the same sense as the terms "dense edged" and "double contoured" which have been used previously.

² Called "mesaxon" by Gasser.

Materials and Methods

The preparations studied in thin sections were all of chameleon (*Anolis carolinensis*) peripheral nerve. The sections were taken predominantly near the terminal ramifications of nerve fibers in skeletal muscle. However, one of the sections is from sciatic nerve. The chameleons were decapitated and small muscle fibers were quickly removed and tied to applicator sticks at rest length. The preparations were fixed in 1 per cent osmium tetroxide buffered at about pH 7.6 by the method of Palade (12). In some instances fixation was carried out at room temperature, and in others at 0°C. for times varying from 1½ to 4 hours. The specimens were removed from the fixative, rinsed briefly in distilled water, and placed in either distilled water or 1 per cent phosphotungstic acid (PTA) buffered with phthalate at pH 5.4 for about 12 to 18 hours at room temperature. Staining was often continued in PTA for 24 to 48 hours. The specimens were then dehydrated in alcohol and embedded in a mixture of 15 per cent methyl methacrylate in *n*-butyl methacrylate. Polymerization was catalyzed by benzoyl peroxide and either dry heat or ultraviolet light. Sections were cut with a glass knife and an International rotary (Minot) microtome. An RCA model EMU-2b electron microscope was used.

RESULTS

A cross section of a myelinated nerve fiber in chameleon skeletal muscle is shown in Fig. 1. A portion of the fiber is enlarged in Fig. 2. A double membrane connection (SCM) clearly exists between both the outer surface membrane of the Schwann cell and the outer myelin lamella (arrow 1) and between the axon-Schwann membrane and the inner myelin lamella (arrow 2). The inner SCM enters the myelin sheath in a direction opposite to that of the outer SCM. These opposing directions would be expected if the myelin sheath were formed in the helical fashion postulated by Geren. The axon-Schwann double membrane (arrow 3) is in this case separated from the inner layer of the myelin sheath near the region of attachment of the SCM. Elsewhere it is so closely apposed to the innermost myelin lamella as to be distinguishable as a separate membrane only by an increase in its width. Indeed, this intimate apposition is the usual situation as can be seen in Figs. 3 and 4 and 7 to 9. In Fig. 10, however, it is seen separated. On the other hand the outer surface membrane of the Schwann cell is quite often distinctly separated from the myelin even in sections not containing Schwann nuclei (Figs. 1 to 4, 7, and 11).

Although clearly defined outer and inner connecting membranes such as those seen in Fig. 1 are rarely seen in the same fiber, one or the other of the membranes is not infrequently seen in optimally fixed preparations. For example, the inner SCM is discernible in Figs. 3 and 5 (arrow) and the outer one in Figs. 4 and 6 (arrow). The SCM in Figs. 3 and 5 appears to have been made more easily visible by a wide separation, presumably an artifact, of the axon-Schwann membrane from the myelin at just the right place. In Fig. 7 there are loci on the inner edges of the outer membranes of the Schwann cells

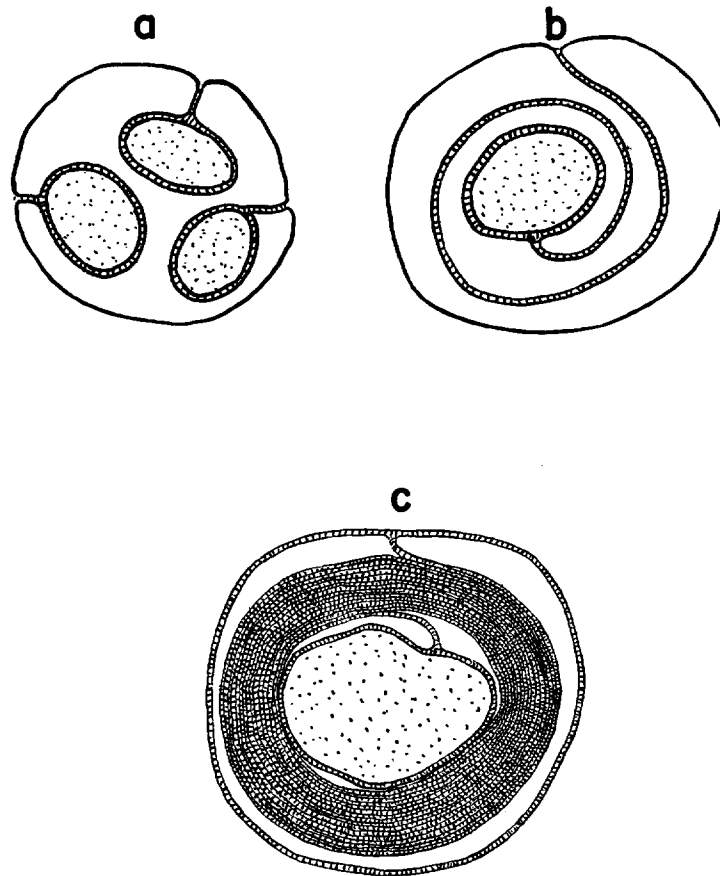
which suggest an invagination of the membranes into Schwann cytoplasm in all three of the fibers shown. However, a connection between these loci and the myelin sheath cannot be traced with certainty.

The light zones of the myelin lamellae in Figs. 1 and 2 average about 70 Å in thickness and the dense zones average about 50 Å in thickness. Thus the repeat period of the myelin lamellae in this case is about 120 Å. The light zones and the dense zones of the lamellae are continuous with the corresponding zones in the outer and inner SCM's and the axon-Schwann membrane. However, it is evident in the micrographs that these zones are individually increased in thickness in the latter situations.

Although in Fig. 1 the outer surface membrane of the Schwann cell does not appear clearly double, in many instances in these preparations it does (Figs. 7 to 11). Hess and Lansing (11) found a double Schwann cell surface membrane in 14 day old guinea pig nerve fibers not stained with PTA. The width of the double membrane observed by them was 300 Å. The over-all thicknesses of the Schwann cell surface membranes observed here are about 500 Å (measured from the beginning of one dense edge to the end of the other). It is quite probable that the lower figure of Hess and Lansing is more nearly correct since these membranes seem quite susceptible to swelling to the point of complete disappearance of the outer dense edge. The unsharpness of the outer edge in Fig. 1 seems most probably due in part to such swelling. It also should be noted in this connection that while the inner edge of this membrane is in contact with cytoplasm the outer edge is in contact with extracellular fluid. This difference of environment may well be related to the behavior of the different components of the membrane toward the fixative.

The sarcoplasmatic membrane³ of the skeletal muscle fiber shown in Fig. 7, although somewhat swollen, appears quite similar to the outer double membranes of the Schwann cells. In addition the surface membranes of the cells of the endoneurial sheath (*endo. m.*) also appear to be of the double type. The outer dense edges of all these cell surface membranes apparently are the sites of deposition of basement membrane material. However, very little of this material is present. The light central zones of all these membranes are considered tentatively to be in some significant ways structurally analogous. It is clearly possible that a portion of the light zones represents a space left by separation of the basement membrane. However, the persistence of the light zone of the sarcoplasmatic membrane within the junctional folds of the subneural apparatus in motor end plates (14, 16, 19) in a reduced and

³ The term "sarcoplasmatic membrane" has been adopted (18) for the surface double membrane of muscle cells after the terminology of Gutman and Young (10). It has been suggested (18) that the term "sarcoplasmic membrane" be used to designate membranes bounded on both sides by sarcoplasm.



TEXT-FIG. 1. (a) Diagram of a chameleon Remak fiber. Three C fibers are seen in Schwann cell cytoplasm. Each fiber is connected to the Schwann cell surface membrane by a double SCM. The light central zones (cross hatched) of the SCM's are continuous with those of the axon-Schwann membranes. The dense edges of the SCM's are continuous with the Schwann cell surface membrane and with the Schwannian dense edge of the axon-Schwann membrane. (b) Diagram of Geren's embryonic intermediate myelinated fiber. The light central zones of the double SCM are continuous with those of the double axon-Schwann membrane as in (a). The dense edges of the SCM are continuous with the Schwann cell surface membrane and the Schwannian dense edge of the axon-Schwann membrane. (c) Diagram of an adult myelinated fiber. The light central zone of the double outer Schwann cell surface membrane is continuous with that of the double outer SCM the dense edges of which are continuous with the inner dense edge of the surface membrane of the Schwann cell. The SCM is apparently helically wrapped about the axon and united by means of the inner SCM with the double axon-Schwann membrane. The dense edges of the inner SCM's are continuous with the Schwannian dense edge of the axon-Schwann membrane. Axoplasm is represented by the dots.

more constant thickness suggests that this light zone represents more than an artifactual space. The ultrastructure of the sarcoplasmic membrane is considered in detail in another paper (18).

Text-fig. 1 includes diagrams of a Remak fiber containing three C fibers⁴ (*a*), Geren's embryonic intermediate myelinated fiber (*b*), and an adult myelinated fiber (*c*). An outer dense edge for the surface membrane of the Schwann cell is included in (*c*) since it has been seen with sufficient frequency to suggest that, although fixed only with difficulty, it is present in adult fibers as noted above. Whether or not its absence in the embryonic forms and Remak fibers so far studied is real or an artifact remains to be determined by further study. The structural relationship of adult Remak fibers to embryonic and adult myelinated fibers is shown in the diagrams. The only obvious difference between the Remak fibers and the earliest embryonic nerve fibers (7) is the frequent appearance in the former of more than one axon in Schwann cytoplasm. Thus it seems that the earliest embryonic form observed by Geren might be a prototype of both Remak fibers and myelinated fibers.

The cytoplasm of the cells of the endoneurial connective tissue sheath (*endo. m.*) in Fig. 7 contains numerous round or oval vesicular appearing bodies which measure about 200 to 400 Å in diameter. Although less clearly shown here, similar bodies are seen in the cytoplasm of the Schwann cells in this section. This appearance of Schwann cytoplasm is not infrequently seen in PTA-stained material. Similar appearing vesicles are seen prominently in the axoplasm of the terminal axons in sections of motor end plates (14, 19), in synaptic axoplasm (3, 17), in vertebrate Schwann cells (2), and in endothelial cells (13).

DISCUSSION

A controversy has existed in the past regarding whether or not the myelin sheath is an integral part of the Schwann cell, the axon, or both. Thus Ranvier compared the Schwann cell to a fat cell rolled around the axis cylinder in the form of a tube, while others regarded the myelin sheath as a part of the axon (15). It now appears that the conception of Ranvier is in some ways correct although crude. The evidence presented here in conjunction with the embryological findings (7) seems sufficient to establish that the myelin sheath is an integral part of the Schwann cell. Another problem is an exact understanding of the mechanism by which the surface membranes of the Schwann cell grow into its cytoplasm to form the layered myelin structure.

The structural relations of the double membranes of embryonic (7) and adult Schwann cells suggest that myelin is laid down helically during em-

⁴This diagram is constructed from observations made in this laboratory on chameleon Remak fibers. Gasser's paper may be consulted for details (6).

bryonic development and retains this helical configuration at maturity. It appears that myelin consists of one double membrane which is wrapped helically about the axon. The development of the relatively straight embryonic SCM into the helical form shown in Geren's intermediate fiber, and later into the adult form with the Schwann cell nucleus located outside the layered myelin structure, could occur simply by a rotation of the locus of union of the SCM with the axon-Schwann membrane or in a more complicated manner by a rotation of the entire Schwann cell or axon during development. If both the inner and outer loci of union remained fixed and the membrane itself merely elongated to form a closed loop which was thrown into helical folds about the axon, Schwann cell nuclei might be expected occasionally to lie between the adult myelin and the axon. This is not observed. Furthermore, the inner and outer SCM's would be more likely to enter the adult myelin in the same direction. That this is not the case is indicated in Figs. 1 and 2 and Text-fig. 1 *c*. Therefore, the evidence in this paper as well as that in Geren's paper seems to suggest rotation during development.

Polarization optical studies (21, 22) have indicated that the myelin lamellae are composed of layers of radially oriented lipide molecules and thinner tangentially oriented layers of protein molecules. The demonstration of a direct continuity of myelin lamellae with cytoplasmic double membranes and axon-Schwann membranes suggests the possibility of a similarity of molecular pattern among these double membranes. The fact that Schwann cell cytoplasmic and surface double membranes structurally similar to those observed here seem to be responsible for the metatropic reaction in invertebrate nerve fibers (9, 20) further suggests that the molecular structure of this type of membrane may be closely similar to that of the myelin lamellae.

The double membranes of myelin are distinctly wider and less constant in width when they lie free in Schwann cytoplasm outside of the compact myelin. This broadening appears to be due to an increase in the thickness of both the light central zones and the dense edges of the membranes. There are even greater widening and variability in the surface membranes of the Schwann cells. It seems quite possible that these differences are merely due to variations in reaction to the fixative depending on the environment of the membranes concerned. On the other hand significant differences in structure cannot be excluded.

The work of Geren (7) and that reported here suggest a direct relation between axon-Schwann membranes, myelin membranes, and cell surface membranes. Thus additional evidence is presented for the concept that the molecular structure of cell surface membranes is related to the structure of myelin lamellae. Axon-Schwann membranes have recently been implicated by Geren and Schmitt (9) in the formation of mitochondria. Obviously the kind of structural pattern present in nerve myelin may be present in various other

kinds of cytoplasmic double membranes with the smectic lipides providing a relatively non-specific structural framework on which specific proteins are oriented. It is then clearly quite important to determine where the lipides and proteins of the myelin double membranes are located with respect to the densities observed in electron micrographs. Sjöstrand (23) has assumed that the light central zones of the double membranes of mitochondria represent lipide and the dense edges protein. Hess and Lansing (11), on the other hand, state that on the basis of the density of staining with OsO_4 the narrow dense lines in nerve myelin are presumably the lipide-containing bands and the lightly staining zones the protein bands. This statement appears to be based on the histological classification of OsO_4 primarily as a fat stain, a conception which is not entirely supported by current chemical evidence (1). Although OsO_4 does indeed react with certain lipides its reaction with some proteins is quite vigorous, and there is evidence which suggests that it does not react or reacts only slightly with the saturated non-polar portions of certain lipide molecules. The hypothesis that the light central zones of the myelin lamellae represent lipide is, therefore, not unreasonable, though unproven. It is possible that more detailed chemical studies of the interaction of cytoplasmic constituents with OsO_4 such as the above and further biophysical studies of myelin (4) and model lipide systems (8) may be of considerable importance in deciding this question.

SUMMARY

Adult chameleon myelinated peripheral nerve fibers have been studied with the electron microscope in thin sections. The outer lamella of the myelin sheath has been found to be connected as a double membrane to the surface membrane of the Schwann cell. The inner lamella is connected as a similar double membrane with the double axon-Schwann membrane. The relations of these double connecting membranes suggest that the layered myelin structure is composed of a double membrane which is closely wound about the axon as a helix. These findings support the new theory of myelinogenesis proposed recently by Geren. The possible significance of these results with respect to cell surface membranes and cytoplasmic double membranes is discussed.

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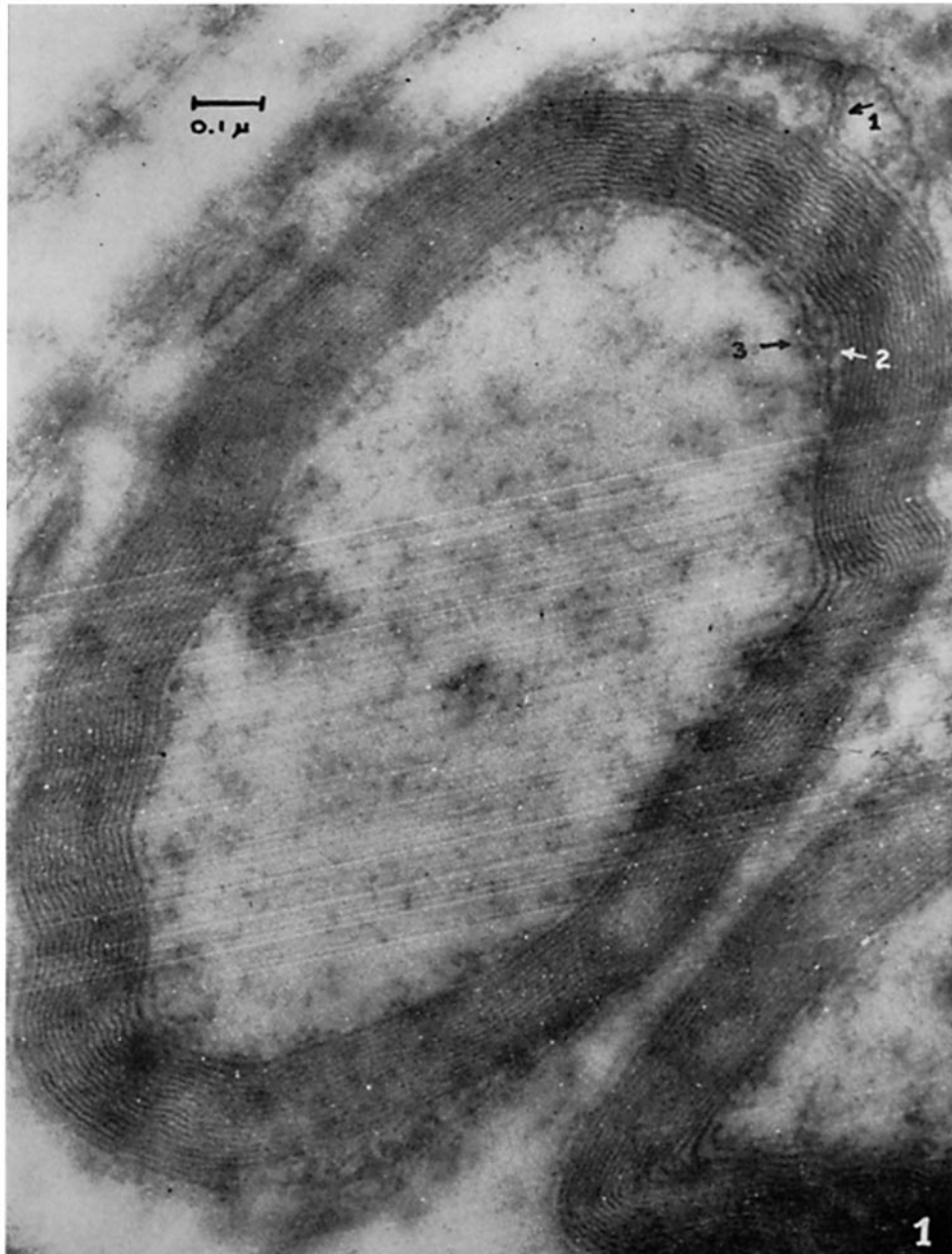
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EXPLANATION OF PLATES

PLATE 76

FIG. 1. Transverse section of a small myelinated nerve fiber between skeletal muscle fibers in the chameleon. The outer SCM connecting the Schwann cell surface membrane and the outer myelin lamella is indicated by arrow 1. The inner SCM connecting the axon-Schwann membrane with the inner myelin lamella is indicated by arrow 2. The axon-Schwann membrane is indicated by arrow 3. The outer dense edge of the surface membrane of the Schwann cell cannot be seen clearly in this preparation. The axon-Schwann membrane is difficult to trace with certainty beyond its junction with the inner SCM because its dense edge next to axoplasm is frequently broken. OsO₄ 3 hours. PTA 24 hours. × 91,000.



(Robertson: Ultrastructure of myelinated nerve fibers)

PLATE 77

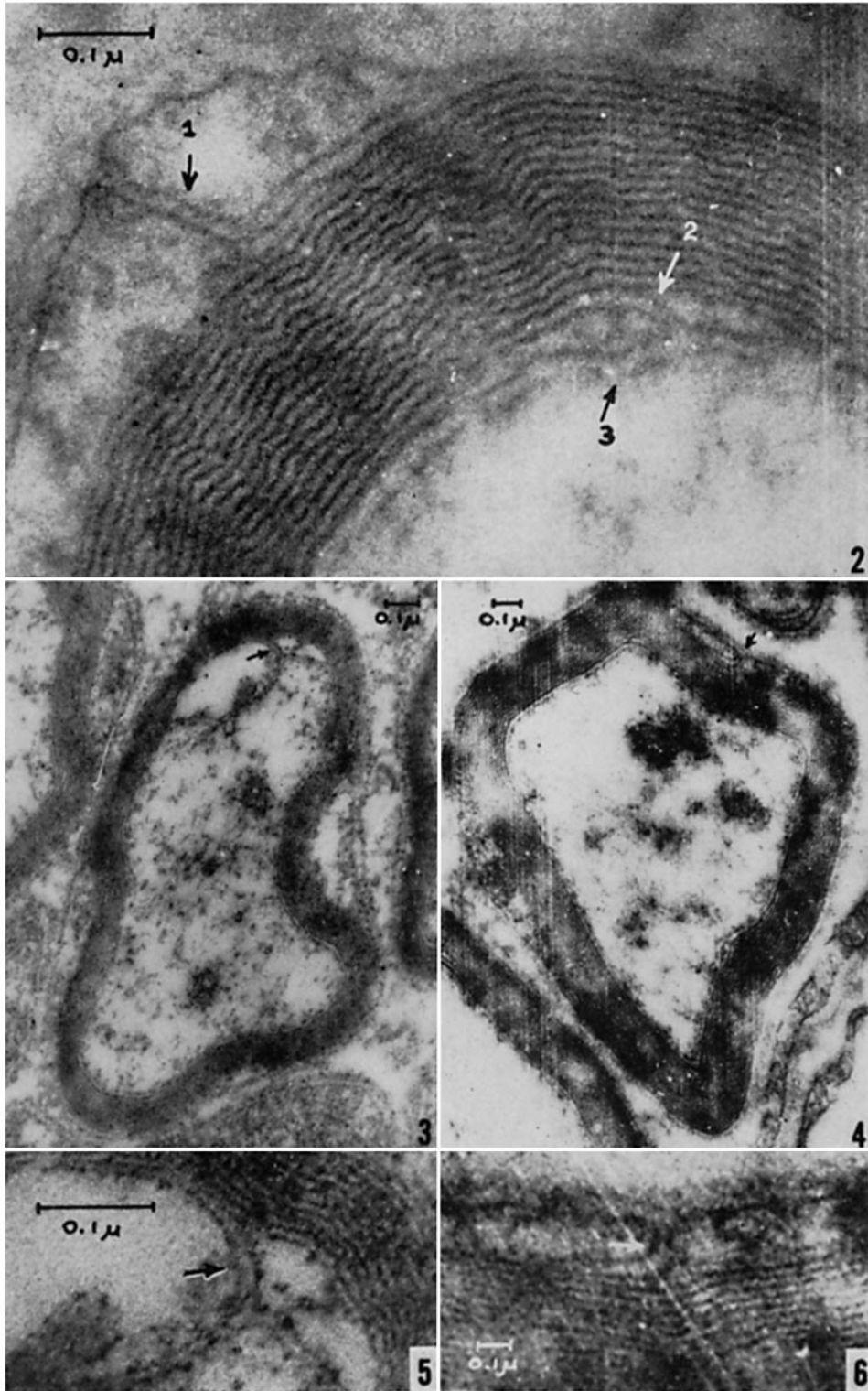
FIG. 2. Enlargement of the portion of Fig. 1 containing the inner and outer SCM's. OsO₄ 3 hours. PTA 24 hours. × 160,000.

FIG. 3. A small myelinated nerve fiber in a cross section of chameleon sciatic nerve. The inner SCM is indicated by the arrow. The axon-Schwann membrane in the region of the SCM is separated from the myelin presumably by an artifact. The outer surface membrane of the Schwann cell is swollen and indistinct. A somewhat less swollen Schwann cell surface membrane can be seen on the fiber to the upper left. OsO₄ 2 hours. PTA 48 hours. × 47,000.

FIG. 4. Cross section of a small nerve fiber near the one shown in Fig. 1. The outer SCM can be seen at the arrow. OsO₄ 3 hours. PTA 24 hours. × 44,000.

FIG. 5. Enlargement showing the SCM designated by the arrow in Fig. 3. OsO₄ 2 hours. PTA 48 hours. × 165,000.

FIG. 6. Enlargement showing the SCM designated by the arrow in Fig. 4. OsO₄ 3 hours. PTA 24 hours. × 165,000.



(Robertson: Ultrastructure of myelinated nerve fibers)

PLATE 78

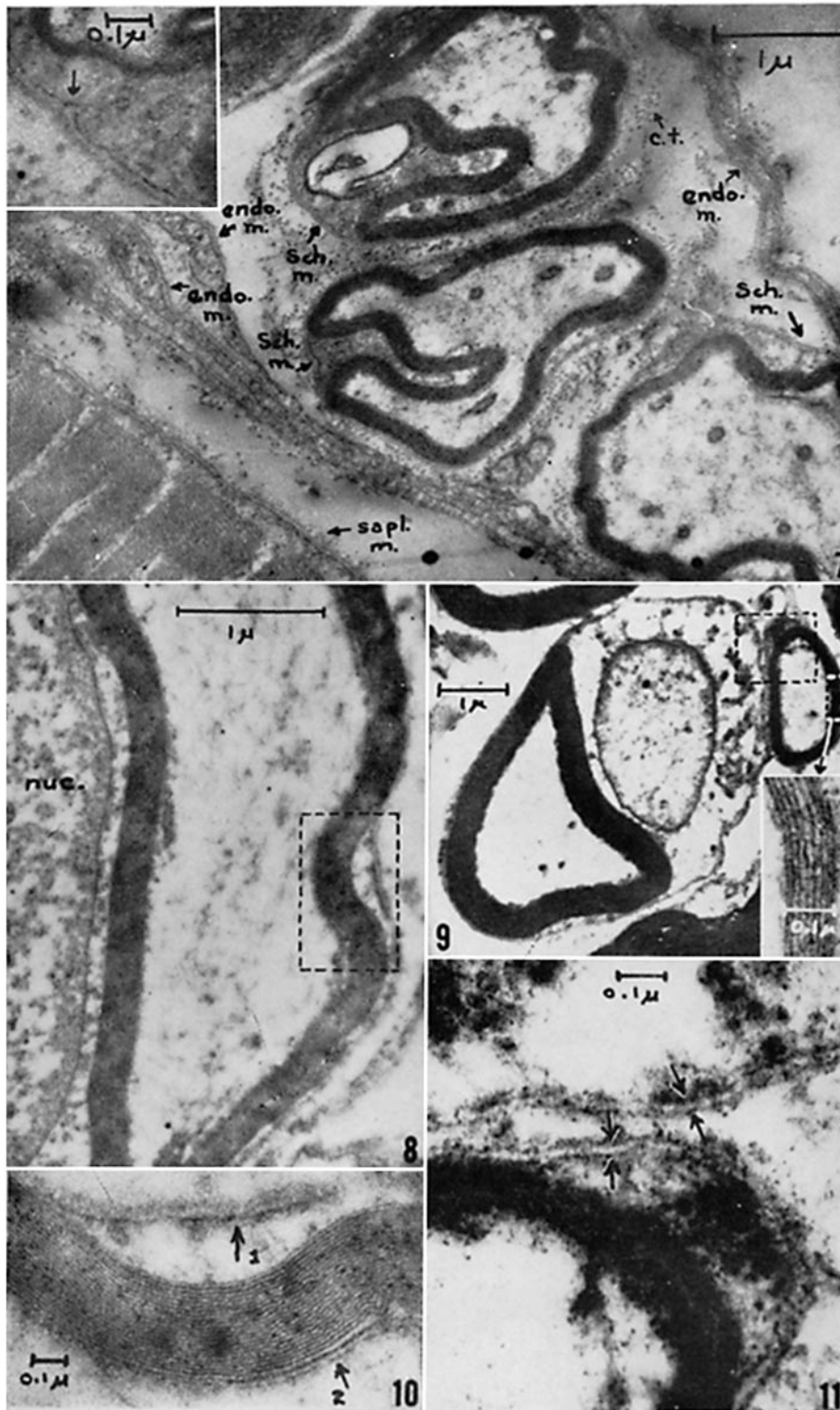
FIG. 7. A bundle of small myelinated nerve fibers surrounded by the endoneurial connective tissue sheath (*endo. m.*). A cross section of a skeletal muscle fiber with myofilaments is seen to the lower left. The double membranes (*m.*) at the surfaces of the Schwann cells (*Sch. m.*), endoneurial sheath cells (*endo. m.*), and muscle cell (*sapl. m.*) are shown. Slight invaginations into Schwann cytoplasm of the inner dense edges of the surface membranes of the Schwann cells may be seen adjacent to the arrows. One such region is shown in the inset. Small transversely cut connective tissue fibrils (*c. t.*) are seen both in relation to the cellular layers of the connective tissue sheath and the nerve fibers. OsO₄ 4 hours. PTA 48 hours. $\times 20,000$. Inset $\times 60,000$.

FIG. 8. A longitudinal section of a chameleon myelinated nerve fiber between skeletal muscle fibers. The edge of a Schwann cell nucleus (*nuc.*) can be seen to the left. The dotted area, which is enlarged in Fig. 10, shows the double axon-Schwann membrane separated from the myelin to the left and the double Schwann cell surface membrane to the right. $\times 21,000$.

FIG. 9. A cross section of a chameleon myelinated fiber in a large bundle of nerve fibers between skeletal muscle bundles. This section is rather thick and printed for high contrast so that the myelin period does not show clearly; however, the double nature of the outer surface membranes of the Schwann cells may be seen. A Schwann cell nucleus is shown in the larger fiber. Although the Schwann nucleus of the small fiber to the right is not present in this plane of sectioning, a distinct layer of Schwann cytoplasm is seen external to the myelin. The inset is an enlargement of the smaller dotted area printed to show the myelin lamellae. The larger dotted area is enlarged in Fig. 11. OsO₄ 1½ hours. $\times 9,600$. Inset $\times 72,000$.

FIG. 10. Enlargement of the dotted area in Fig. 8. Arrow 1 points to the double Schwann cell surface membrane and arrow 2 to the double axon-Schwann membrane. OsO₄ 4 hours. $\times 48,000$.

FIG. 11. This enlargement of the larger dotted area in Fig. 9 shows the double nature of the surface membranes of the Schwann cells. The arrows point to double Schwann cell surface membranes. OsO₄ 1½ hours. $\times 70,000$.



(Robertson: Ultrastructure of myelinated nerve fibers)