

New Observations on the Ultrastructure of the Membranes of Frog Peripheral Nerve Fibers. BY J. DAVID ROBERTSON. (*From the Department of Anatomy, University College, London.*)*

Axon-Schwann membranes and mesaxons have hitherto been seen in electron micrographs as two single dense lines <100 Å wide separated by a light interzone about 150 Å wide, the whole making a double membrane 250 to 300 Å wide (4) (Fig. 7). It will be shown in the present preliminary note that each of the supposedly single dense lines now appears as a pair of dense lines making a unit about 75 Å in over-all thickness, and that this unit probably consists of a single bimolecular leaflet of lipid with associated protein.

Fig. 1 shows a frog sciatic Remak fiber fixed in permanganate (7). Two axons (*ax.*) are seen and each is partially surrounded by a Schwann cell. All of the structures included between the arrows *1* make up the axon-Schwann membrane. These structures consist of two pairs of parallel dense lines separated from one another by a light space about 25 Å wide. Each dense line is about 25 Å thick so that each of the pairs makes a unit about 75 Å across.¹ The two units are separated from one another by a light space about 150 Å wide. At arrow 2 a similar set of paired dense lines are seen separating the two Schwann cells. In Fig. 6 a mesaxon (*m.*) is seen to have the same structure.

Text-fig. 1 is a semidiagrammatic tracing of Fig. 1. Measurements of the

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¹ The possibility that these ≈75 Å pairs of lines could represent an out of focus Fresnel fringe phenomenon has been considered. However, this has been checked by through focus series repeatedly and eliminated.

structures between the arrows *1* designated *a*, *b*, and *c* are given below:

<i>a</i> , 284 Å (27)	[230 to 410 Å]
<i>b</i> , 145 Å (32)	[95 to 250 Å]
<i>c</i> , 74 Å (42)	[70 to 85 Å]

Each figure is the average of measurements made in representative areas of micrographs of ten different specimens. The actual number of measurements is given in parentheses and the range in brackets. Only the order of magnitude is considered significant.² The light zone between the component paired lines of *c* is about 25 Å wide. No consistent difference in the density or width of the two bordering dense lines was detected. But this is not considered significant, particularly since the micrographs are slightly underfocused. The relatively small range in the measurements of *c* may, however, be significant since it suggests a paracrystalline underlying structure.

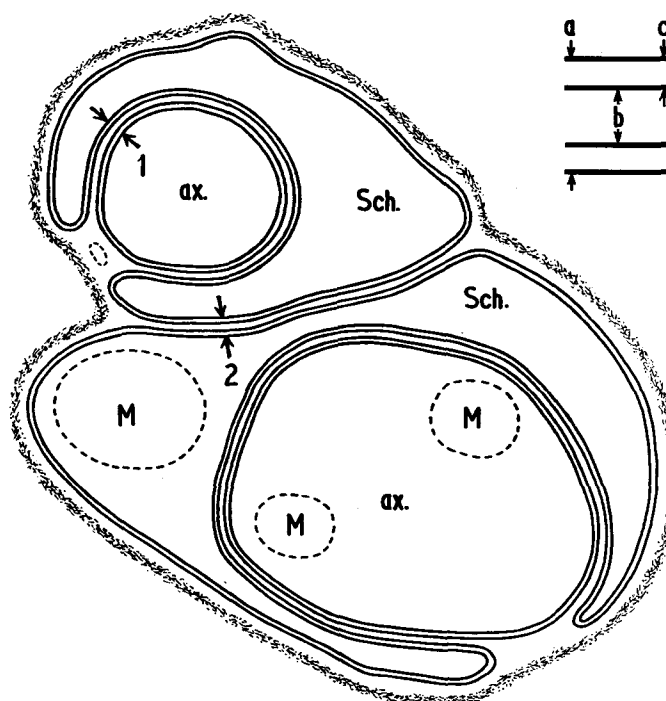
The axon-Schwann membranes and mesaxons of myelinated nerve fibers also consist of two paired lines sometimes separated by a gap ≈150 Å wide (Figs. 3 to 5). In some regions this gap is narrowed and obliterated (Fig. 2, unlabelled arrow; Text-fig. 2, arrows). The axon-Schwann membrane is seen in such regions as three dense lines with its over-all width reduced to ≈150 Å as would be expected from a closure of the gap.

At the outer surface of medullated fibers it can be seen that each myelin lamella is composed of two of the ≈75

² The calibration of the Siemens Elmiskop 1b electron microscope used is considered good to an accuracy of about 15 per cent.

A pairs of lines seen in mesaxons with the intervening gap obliterated (Fig. 5) (Text-fig. 3). The central dense line then is the intraperiod line (1, 12). This unit,

is the fundamental repeating unit deduced by indirect analysis (2, 11). If this is so, each ≈ 75 A membrane probably contains one bimolecular leaflet of lipide.



TEXT-FIG. 1. Interpretative tracing of Fig. 1. Two Schwann cells (*Sch.*) are seen partially enveloping an axon (*ax.*), included in each. Three mitochondria (*M*) are shown in outline. These do not have internal cristae and the details of their membrane structure are not well shown. Note that both the Schwann cell and axon membranes consist of two paired closely spaced lines and that each of these paired lines is separated by a relatively wide gap where the axons and Schwann cells (arrows 1) or the two Schwann cells (arrows 2) are in apposition. The portion of the axon-Schwann membrane between arrows (1) is enlarged to the upper right and the distances *a*, *b*, and *c* designated by apposed arrows. The basement membrane layer is shown by stippling, though it is somewhat indistinct in Fig. 1. The lines here are for simplicity not drawn to scale.

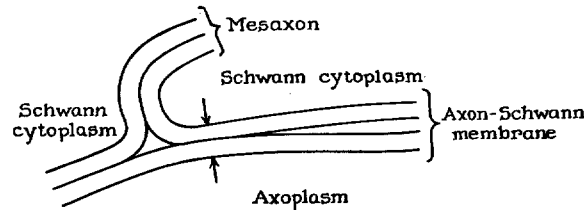
in apposition with the next in the spiral, (5, 8) produces the major dense line. Thus, each of the ≈ 75 A double membranes contains half of the constituents present in each myelin repeating unit. There is reason to believe (10) that this

It remains to be decided exactly what other material is concerned in the production of the observed dense lines, but it seems reasonable to speculate that protein is involved.

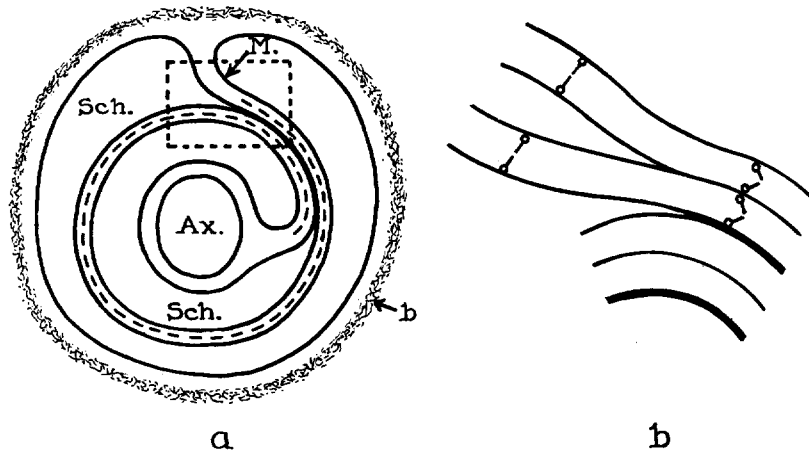
Both the intraperiod and the major

dense lines of myelin result from the contact of two apparently symmetrical ≈ 75 Å cell membranes. In the former case, the outside surfaces of the membranes come together, and in the latter,

the inner (cytoplasmic) surfaces join. It is interesting that the resulting lines of contact in myelin appear different, despite the fact that the pairs of lines making the individual ≈ 75 Å mem-



TEXT-FIG. 2. Drawing of axon-Schwann membrane of a myelinated fiber showing one of the closely spaced mesaxons of the preterminal myelin at a node of Ranvier (9). The lines here are for simplicity not drawn to scale. This is composed from Figs. 5 and 6 which show two related sections of the same nerve fiber. The Schwann membrane is shown above as two paired lines. To the left, two sets of these paired lines are reflected as a mesaxon. Below the axon membrane is shown as another set of paired lines. A gap is present between the axon and Schwann membranes to the right, as in Fig. 3. But at the arrows they come together, obliterating the gap. Here the axon-Schwann membrane consists of three parallel dense lines, as shown in Fig. 2.



TEXT-FIG. 3. *a*, Diagram of a myelinated nerve fiber (*ax.*, axon; *Sch.*, Schwann cytoplasm; *m*, mesaxon; *b*, basement membrane), showing the intraperiod line as dashes. The lines drawn here are for simplicity not drawn to scale. Note the narrowing of the mesaxon of the Schwann cell as the myelin spiral is entered. The dotted region is enlarged in *b*. The mesaxon here is shown as two units each containing one bimolecular leaflet of lipid. These come together as the outermost myelin lamella. This in contact with the next in the spiral produces the major dense line. The polar ends of phospholipide molecules are indicated by the circles and the non-polar carbon chains by the lines attached to the circles.

branes look symmetrical in the micrographs.³ The difference between the intraperiod and the major dense lines after 3 hours treatment with permanganate is one of density. The lower density of the intraperiod line probably reflects an underlying chemical difference between the inside and outside surfaces of the Schwann cell membrane.

The nature of the substance present in the ≈ 150 Å gaps of the compound membranes poses a problem of considerable importance. A question of prime interest concerns the relative volume of free water here in which ionic diffusion can occur. Thus, if some macromolecular constituent is present, it is most important to know whether its concentration is great enough to restrict ionic diffusion. It appears possible that material is present in sufficient concentration for this in the basement membrane layer at the outer Schwann cell surface, but whether or not some such material is present in the gap itself remains unclear.

³ Actually, two questions arise here. First, why do the lines making up the Schwann surface membrane outside myelin look the same in the micrographs if they are different? Second, why do these lines outside myelin not measure much less than 25 Å, since two of them in apposition make a line ≈ 25 Å wide in myelin? It is difficult to give a precise answer to these questions on present evidence, but it seems likely that it involves, first, questions of Fresnel fringe distributions in slightly out of focus images of objects of dimensions near the resolution limit, and second, considerations of section thickness, degrees of specimen tilt, and other factors of specimen preparation. For the moment, it seems best to lay aside these questions for later consideration when more evidence is available. In the meantime, the presence or absence of dimensions below 25 Å in the micrographs presented here must be interpreted with caution.

While this paper provides no answer to the precise nature of the gap substance, it does demonstrate for the first time the presence of definite layers within the Schwann cell membrane⁴ and establishes a direct continuity of each of these layers with layers of comparable dimensions in the myelin sheath. It is shown that no such continuity exists between the ≈ 150 Å central gap in mesaxons with any layer in myelin of comparable dimensions. Indeed, the gap is, to the resolution limits of the micrographs presented here, obliterated in the myelin sheath. Hence it appears that the organized lipid layers in myelin are not continued into the central mesaxon gap. This conclusion bears importantly on the controversy begun several years ago by Sjöstrand and Rhodin (13), with their hypothesis that organized lipid layers are present in the gaps between the dense layers of intercellular membranes and other membranes of this kind. It is considered that this hypothesis is disproven by the findings presented here.

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⁴ It is claimed that the dense lines of the double membranes of mitochondria have been seen in isolated regions in osmic-fixed material as double lines < 100 Å wide (3, 6). In the brush border of mouse intestine, epithelial cell membranes have been seen as double membranes 120 Å wide (14).

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EXPLANATION OF PLATES

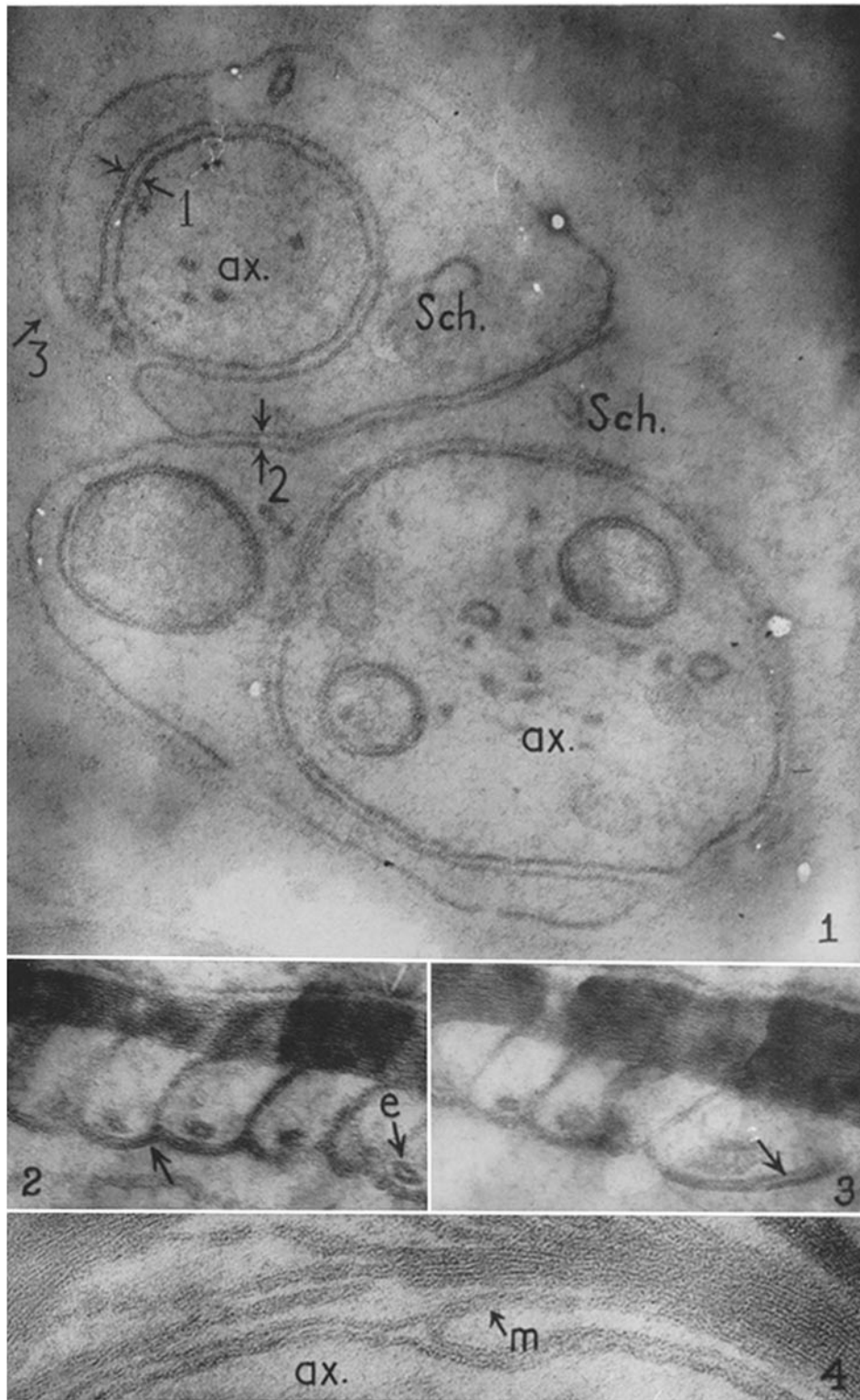
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FIG. 1. Two axons (*ax.*) embedded in two apposed Schwann cells (*Sch.*) are shown. In neither instance are the axons completely surrounded. Therefore, no mesaxons are present. The apposed Schwann cell- and axon-membranes are shown as paired dense lines, each making a unit about 75 Å across. These units are separated from each other by a gap about 150 Å wide (arrow 1). A similar relationship exists between the two Schwann cell membranes (arrow 2). Note the delicate granular appearing basement membrane material (arrow 3). In some areas the cell membranes are not seen because of artifact or oblique sectioning. Text-Fig. 1 is an interpretative tracing of this figure. KMnO_4 -fixed. $\times 87,000$.

FIG. 2. Longitudinal section of myelin near a node of Ranvier (9), showing the closely spaced mesaxons formed as the myelin lamellae peel off from the axonal side of the myelin. Some of the membranes are fragmented or obliquely sectioned. The axon-Schwann membrane shown by the unlabelled arrow is ≈ 170 Å wide, and appears as three dense parallel lines. The central line is produced by contact of the two pairs of lines making the axon-Schwann membrane. Here the gap is eliminated (see Text-fig. 2). Some structures probably representing components of the endoplasmic reticulum, *e*, are shown in the Schwann cytoplasm between the mesaxons. KMnO_4 -fixed. $\times 106,000$.

FIG. 3. Another section of part of the same region shown in Fig. 2 but slightly further away from the node. Here again the membranes are largely fragmented or obliquely sectioned. For these reasons the triple layered regions of the mesaxons and axon-Schwann membrane do not show as clearly as in Fig. 2, but the axon-Schwann membrane to the right is widened to ≈ 200 Å and both its component ≈ 75 Å paired lines are shown with a small gap between them. KMnO_4 -fixed. $\times 106,000$.

FIG. 4. Portion of cross-section of a myelinated fiber with an inner mesaxon (*m*). The two pairs of dense lines of the mesaxon diverge and extend out over the similar paired dense lines of the axon (*ax.*) to form the axon-Schwann membrane. This is about 250 Å across. Each of the units formed by the pairs of lines is ≈ 75 Å across, and the two units are separated by a gap ≈ 100 Å wide. The myelin lamellae are separated to the upper left in a manner analogous to that seen in Schmidt-Lanterman clefts. KMnO_4 -fixed. $\times 130,000$.



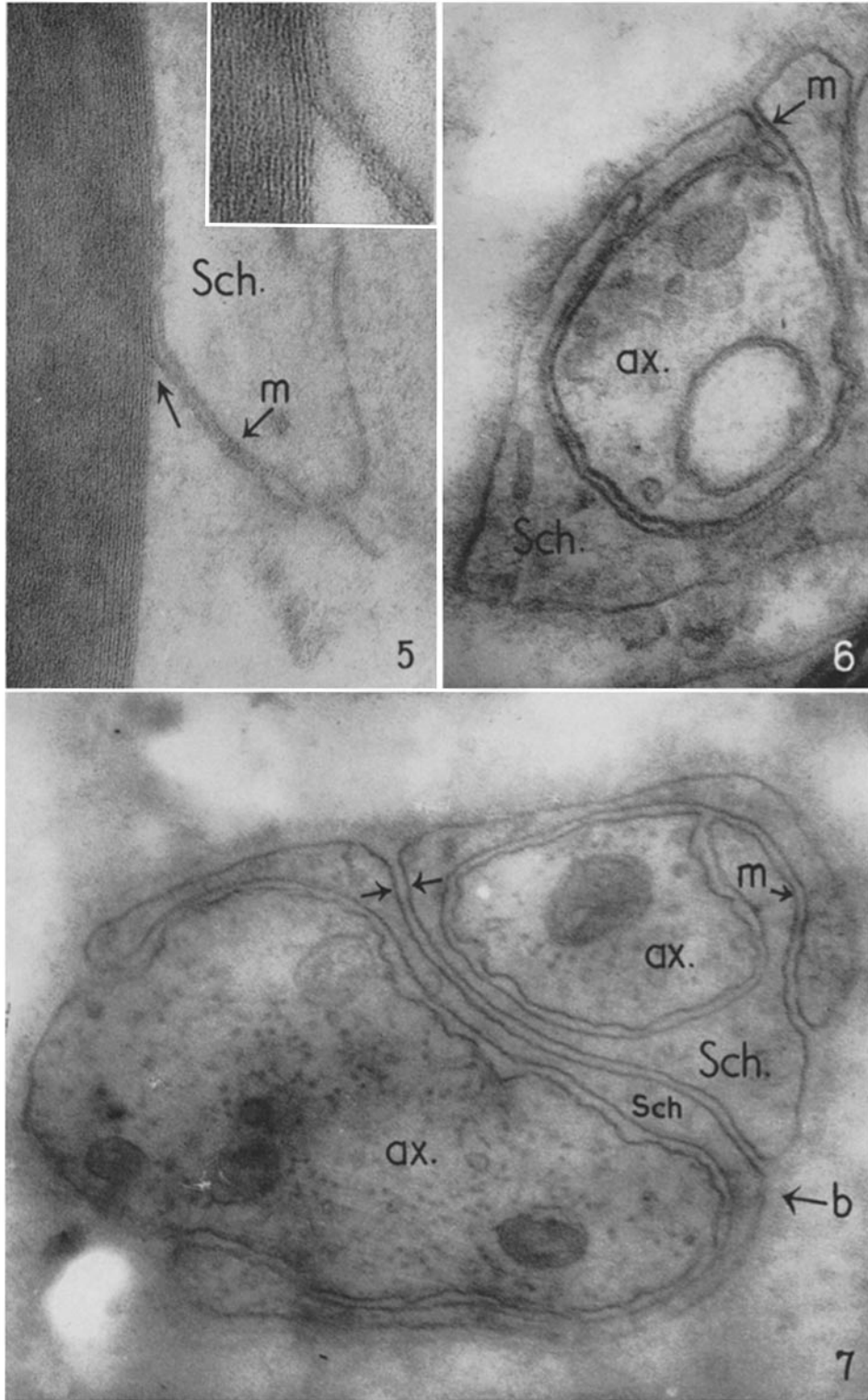
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FIG. 5. Portion of the myelin sheath of a frog myelinated fiber with the outer mesaxon and Schwann cytoplasm (*Sch.*). Note that the mesaxon consists of two sets of paired dense lines making units ≈ 75 A wide. These units are separated from one another by a gap of variable thickness outside the myelin, but come together in the myelin to form the outermost lamella. This, in contact with the next in the spiral, produces the major dense line (unlabelled arrow and inset enlargement). KMnO_4 -fixed. $\times 115,000$. Inset $\times 194,000$.

FIG. 6. Portion of a Schwann cell (*Sch.*), with an embedded axon (*ax.*). A mesaxon (*m*) is present. Both the mesaxon and the axon-Schwann membranes consist of two apposed sets of paired dense lines, making two ≈ 75 A units. A gap ≈ 150 A wide is present between the units. A halo of granular material surrounds the Schwann cell and this is not differentiated into a discrete basement membrane as in Fig. 1. KMnO_4 -fixed. $\times 82,000$.

FIG. 7. OsO_4 -fixed Remak fiber. Two axons (*ax.*) embedded in two apposed Schwann cells (*Sch.*) similar to Fig. 1 are shown. A mesaxon (*m*) appears to the upper right. The axon and Schwann cell membranes appear as single dense lines <100 A thick. The axon-Schwann membranes and the mesaxon each consist of two dense lines separated by a light interzone about 150 A wide, making double membranes 250 to 300 A across. Where the two Schwann cells are in apposition (unlabelled arrows), a similar double membrane is present. The basement membrane layer (*b*), since the preparation was not stained with phosphotungstic acid, appears as a delicate halo around the Schwann cell, resembling that seen in Fig. 6 after KMnO_4 fixation. The axons contain large dense bodies and small dense granules. $\times 77,500$.



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