THE FINE STRUCTURE OF NERVE CELL BODIES AND THEIR MYELIN SHEATHS IN THE EIGHTH NERVE GANGLION OF THE GOLDFISH

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ABSTRACT

The eighth cranial nerve ganglion consists of bipolar nerve cell bodies each occupying part of an internodal segment. The perikaryal sheaths range from a single layer of Schwann cell cytoplasm on the smallest cells to typical thick compact myelin on the largest. On most perikarya, the sheath displays an intermediate form, consisting of multiple layers of Schwann cell cytoplasm (loose myelin), or of loose and compact myelin continuous with each other. Internodes beyond the one containing the cell body bear only compact myelin. In loose myelin the thickness of each layer of Schwann cell cytoplasm is about 100 A. It may be much greater ($\sim$ 3000 A) particularly in the outermost layers of the sheath, or the cytoplasm may thin and even disappear with formation of a major dense line. The cytoplasmic layers are separated from each other by a light zone, 40 to 200 A wide, which in its broader portions may contain an intermediate line. Desmosomes sometimes occur between lamellae. In addition to the usual organelles, the perikaryal cytoplasm contains granular and membranous inclusions. Large cells covered by compact myelin have a consistently higher concentration of neurofilaments, and some of the largest cells, in addition, show a reduced concentration of ribosomes. The functional significance and possible origins of perikaryal myelin sheaths are discussed.

INTRODUCTION

It is a commonplace of neurohistology that in the peripheral nervous system of vertebrates all axons except the smallest are encased in an envelope of myelin, which is derived from and included within the sheath of Schwann cells. It is less generally appreciated, however, that in certain sensory ganglia the nerve cell bodies themselves are also enclosed in myelin. Such myelinated neurons occur preeminently in the acoustic and vestibular ganglia of animals in all classes of vertebrates from elasmobranchs to man, and also sporadically in the other cranial and spinal nerve ganglia of bony fishes (38, 39). According to Münzer (24), 20 per cent of the perikarya in the cochlear ganglion of the frog are myelinated, and in the guinea pig this ganglion consists almost entirely of myelinated neurons.

Although Bidder (1) and Wagner (45) were the first to describe (in 1847) bipolar ganglion cells, perusal of their papers and study of their illustrations indicate that they did not recognize the perikaryal myelin sheath and that they were not, in fact, aware of the myelin sheath of nerve fibers. Myelinated nerve cell bodies were discovered in the trigeminal ganglion by Leydig in 1851 and were first illustrated and clearly described in his paper (19) on the organs of the elasmobranch *Chimaera monstrosa*. He later men-

tioned (20) that he had also found myelinated perikarya in the acoustic ganglia of teleost fishes and in reptiles. Max Schultze (41) confirmed Leydig's discovery, and he, instead of Leydig, has frequently been cited as the first to notice myelinated ganglion cells. During the past hundred years numerous authors have studied these cells not only in fishes, but also in amphibia, reptiles, and mammals (*e.g.*, 18, 30, 39). Münzer (24) gives a complete and detailed historical account of the subject in the introduction to his own paper. The most recent light microscopic studies, using polarized light and special stains, have been reported by Scharf (39).

Despite the general occurrence of these cells in the vertebrates and the persistent interest in the fine structure of the myelin sheath, no x-ray diffraction studies and very little electron microscopy of perikaryal myelin have yet been carried out. Engström and Wersäll (9, 10) have published two electron micrographs of myelinated cells in the spiral ganglion of the guinea pig, and Luse and Naumann (personal communication) have studied eighth nerve ganglia in several mammals. An electron micrograph by S. A. Luse showing a myelinated perikaryon in the vestibular ganglion has been published in a textbook (29, Fig. 91). Luse (21) also refers to this structure in an abstract. These are the only electron microscopic investigations of this topic that have come to our notice. Furthermore, nothing is known about the electrophysiological effects of perikaryal myelin, ts chem istry, or its embryology.

Detailed information concerning the fine structure of these neurons and their sheaths would be of distinct interest. Not only might it extend our morphological knowledge of the cells of Schwann, but also it would direct attention to more general questions respecting the influence of satellite cells upon the metabolism and electrophysiological activity of neurons. The interaction between the Schwann cell sheath and the neuron has been a largely neglected area of cellular physiology (40), except as concerns the electrical activity of the nerve fiber. A detailed description of the special case, presented by the eighth nerve ganglion, of a highly developed sheath which may be expected to interfere with such activities as the exchange of metabolites between the neuron and the pericapillary space may stimulate more serious investigation into what is essentially a common relationship between parenchymal cell and satellite in the nervous system. The present paper reports an attempt to ascertain whether the fine structure of perikaryal myelin differs from that of axonal myelin and whether there are any cytoplasmic structural differences between myelinated and unmyelinated perikarya correlating with the differences in their sheaths.[1]

[1] Some of the observations included in this paper were presented as a demonstration before the Seventh International Congress of Anatomists at New York, April 11-16, 1960 (36).

FIGURES 1 AND 2

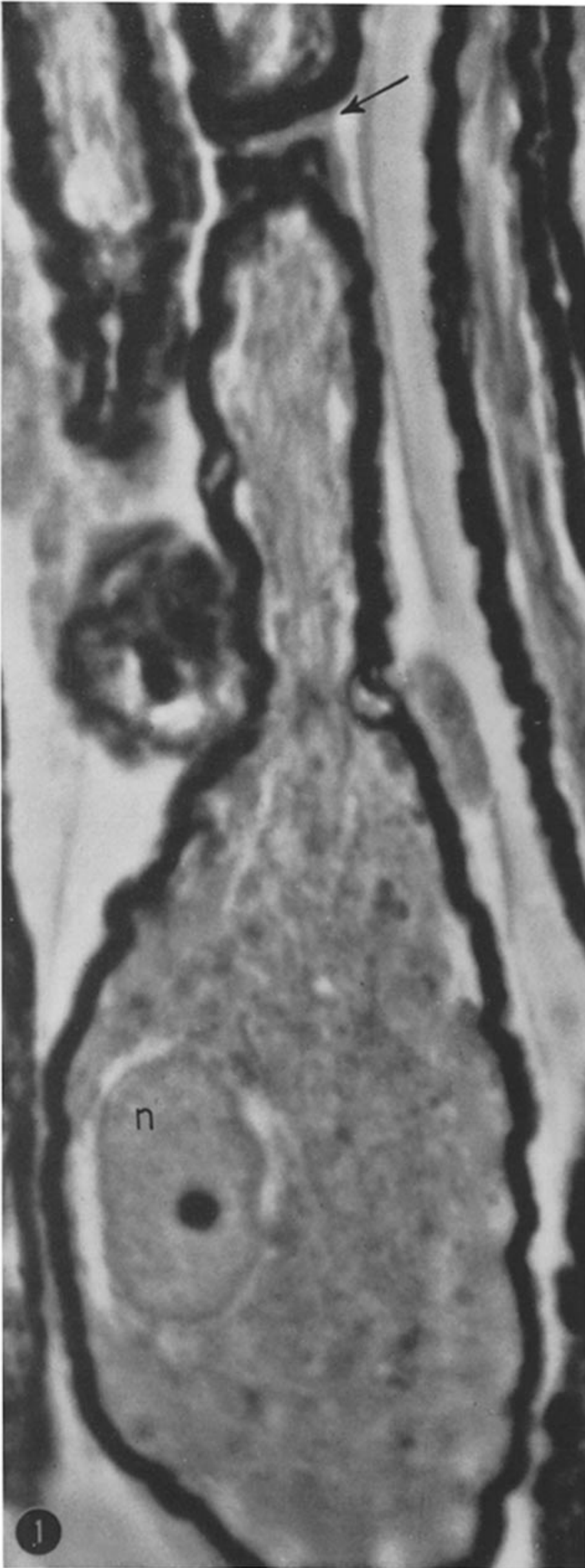
Phase contrast photomicrographs of neurons from the goldfish eighth nerve ganglion showing a range of neuronal types and corresponding variations in their sheaths.

FIGURE 1

A large spindle-shaped neuron whose soma measures 20 by 40 μ. The plane of the section passes just outside the level of the first node (arrow), located 22 μ from the cell body. The nucleus (*n*) is eccentrically located and contains one nucleolus. The perikaryal cytoplasm is granular, in contrast to the axoplasm, which displays filamentous structures coursing through it. The enveloping sheath is $\sim$ 1.0 μ thick and has the density of compact myelin. Araldite embedding. $\times$ 3000.

FIGURE 2

A smaller, more globular perikaryon, which measures 14 by 22 μ. It exhibits granular cytoplasm with several dense inclusion bodies at its poles. The sheath is homogeneous but much less dense than compact myelin. It is $\sim$ 0.5 μ thick and extends up to the first node (arrow), where a compact myelin sheath abruptly begins. A part of a still smaller cell with a relatively large nucleus (*n*) and no apparent sheath is also present. Araldite embedding. $\times$ 3000.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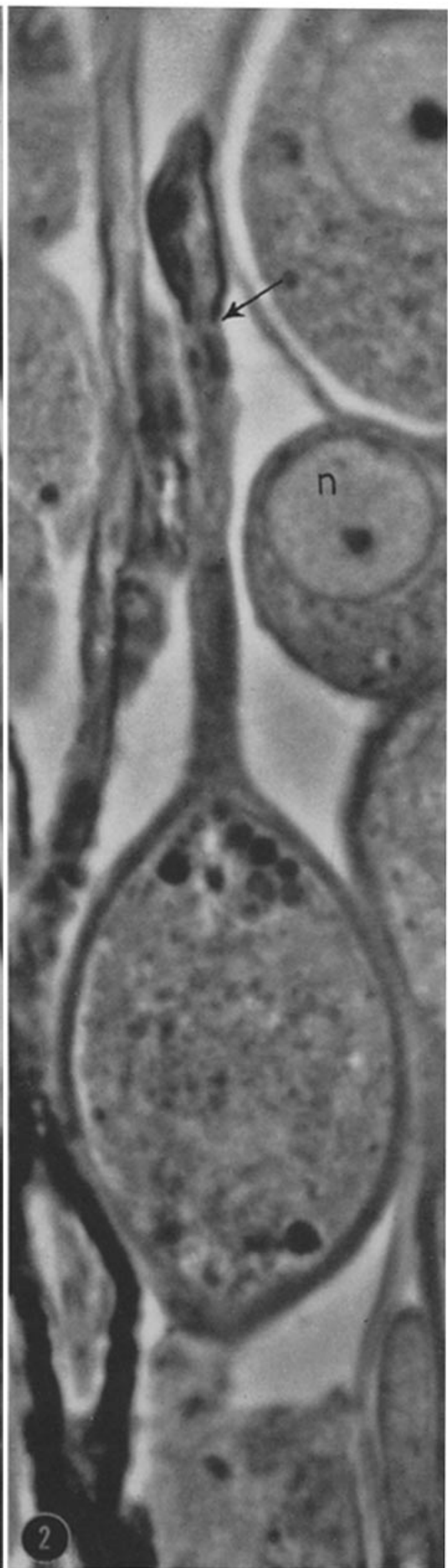
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MATERIALS AND METHODS

Eighth nerve ganglia were obtained from mature goldfish (*Carassius auratus*) 10 to 20 cm. in length. The fish were anesthetized by allowing them to swim in a 0.025 per cent solution of tricain methanesulfonate (MS 222, Sandoz) for several minutes. They were then removed to a dissecting board and fixed in position with the ventral surface upward. Water containing the anesthetic was passed through the mouth and over the gills during the preliminary dissection. The heart was exposed and 0.5 ml. of a 0.7 per cent solution of $NaNO_2$ was slowly injected into the ventricular cavity in order to ensure maximal vasodilation. In the smaller fish a 27 gauge needle attached to a syringe was then inserted through the heart into the conus arteriosus and ligated into position. The sinus venosus was cut and 4 ml. of a warmed isotonic balanced salt solution was perfused through the vascular tree in order to wash out the blood. This solution was immediately followed by 25 to 45 ml. of 1 per cent osmium tetroxide in acetate-veronal buffer (pH 7.4) containing 5.4 mg. calcium chloride per ml. of fixative. The initial 5 ml. of fixative were warmed, and the remainder was chilled to 0–5°C. In the larger fish a glass cannula was used instead of a needle, and approximately 100 ml. of fixative were perfused by gravity flow through the vascular system. The entire perfusion was carried out over a period of about 20 minutes. More complete details of the perfusion technique will be published elsewhere.

Blocks of tissue were removed from both eighth cranial nerve ganglia immediately after the perfusion and were kept in fresh cold fixative for an additional hour. The tissue was dehydrated in methanol or acetone and embedded in methacrylate, Araldite, or Epon 812. Thin sections were cut with glass knives on a Porter-Blum microtome, stained with lead hydroxide or uranyl acetate, and examined in the RCA EMU 3D or 3E electron microscope at magnifications of 5000 to 30,000. Thicker sections (1 to 2 μ) were also prepared from the same blocks, mounted in glycerol, and examined in a phase contrast microscope.

OBSERVATIONS

All the neurons in the eighth nerve ganglion of the goldfish are bipolar cells, measuring 10 to 65 μ long and 10 to 30 μ wide. Examination of thick sections in the phase contrast microscope shows that in the part of the ganglion traversed by myelinated nerve fibers of large caliber, the perikarya

FIGURES 3 TO 5

Electron micrographs illustrating three different types of sheath surrounding eighth nerve ganglion cells.

FIGURE 3

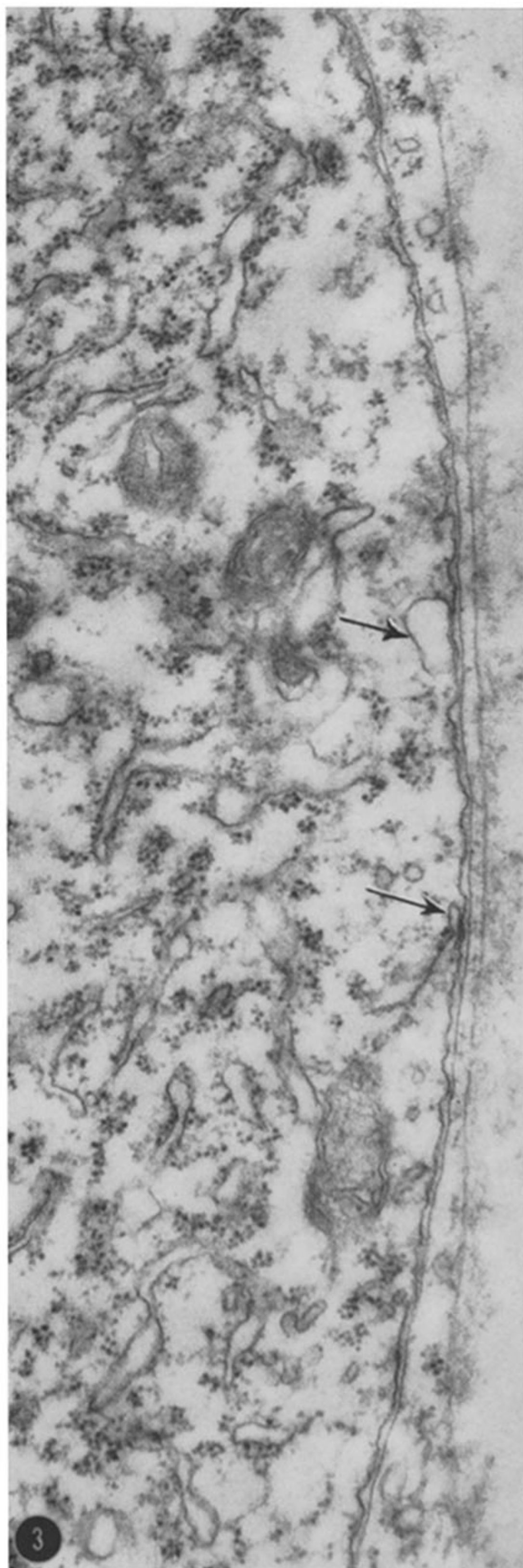
The neuronal cytoplasm is covered by a single layer of Schwann cell cytoplasm which narrows to ~ 400 A. A basement membrane coats its outer surface only. The Schwann cell cytoplasm contains ribonucleoprotein granules and vesicles. The ganglion cell cytoplasm contains abundant Nissl substance, several mitochondria, and two subsurface cisternae (arrows) which are separated from the plasmalemma by less than 100 A. No neurofilaments are apparent. Epon embedding. × 43,000.

FIGURE 4

A part of a compact myelin sheath surrounding a large neuron as illustrated in Fig. 1. A narrow strip of Schwann cell cytoplasm appears at the right. The period of the myelin is ~ 115 A. Intermediate lines (arrow) are irregularly present between the major dense lines. Epon embedding. × 171,000.

FIGURE 5

Five layers of Schwann cell cytoplasm (*s*) lie between two ganglion cells whose limiting membranes are indicated by *p*. In the neuron on the left most of the plasmalemma is tangential to the plane of the section. In a low magnification picture it is evident that two of the Schwann cell lamellae envelop the neuron on the right while the remaining three layers follow that on the left. The connective tissue space between the two sheaths is indicated by *is*. Note that the light zone between successive Schwann cell layers varies from 40 A at *a* to 200 A at *b*. The thickness of the Schwann cell cytoplasm is only 40 A at *c*. Epon embedding. × 171,000.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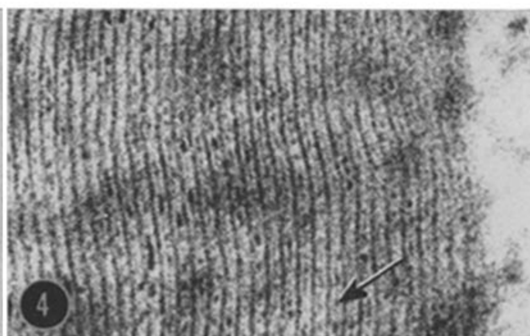
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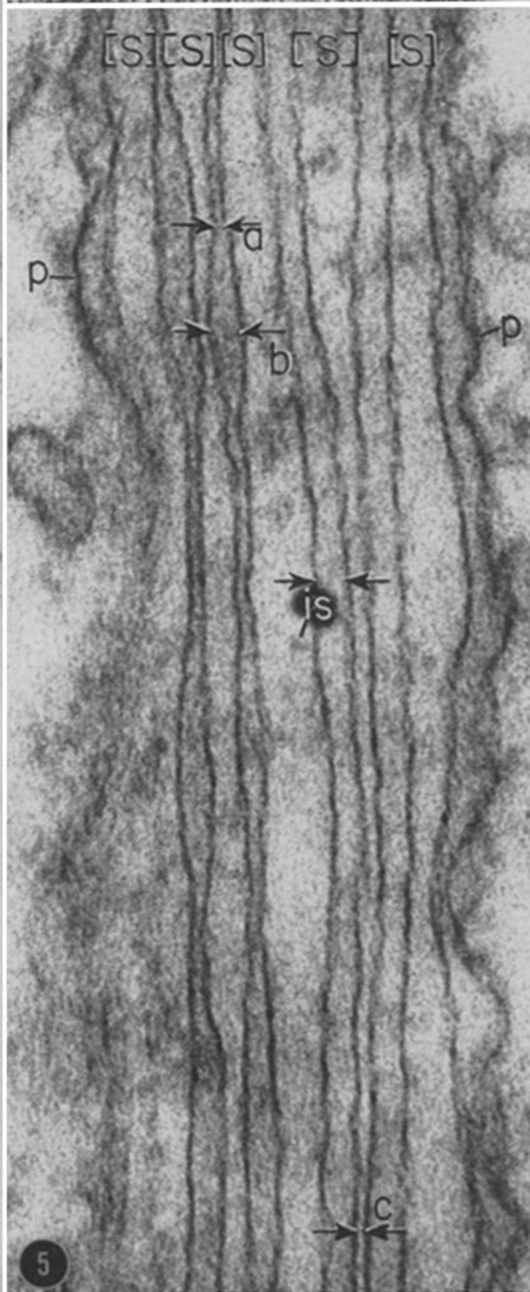
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are large and extremely elongated, appearing, as Leydig (19, 20) remarked, as little more than nucleated, fusiform dilatations of the axons (Fig. 1). In contrast, the perikarya associated with the thinner nerve fibers are smaller and more globular (Figs. 2 and 20). Each cell body contains a single prominent nucleus, which is either round or elongated according to the shape of the cell. The cytoplasm contains small, irregular Nissl bodies, which can be followed at both poles of the cell for variable distances into the processes, so that it is often difficult to decide where the perikaryon ends and where the axons begin. In addition to thin, thread-like or granular mitochondria, the cytoplasm contains large globular inclusions (Fig. 2), which are usually aggregated at the two poles.

Each cell body is surrounded by a capsule of variable thickness, which is continuous with the sheath around the axons. As seen in sections examined with the phase contrast microscope, this capsule apparently consists of only one to three Schwann cells, the nuclei of which usually fit into the angles formed by the axons leaving the perikaryon (Fig. 20). The capsule of the larger cells includes a thick, compact myelin sheath, which has the same density as axonal myelin (Fig. 1). The capsules of the smaller cells appear less dense than the axonal myelin sheath on the one hand, but distinctly denser than ordinary Schwann cell cytoplasm on the other hand (Fig. 2). A few cells appear to be surrounded by only an attenuated sheath of Schwann cell cytoplasm, and some appear to have no sheath at all (at least, by light microscopy). Others have capsules that vary in density and thickness from one region of their circumference to another, as in Fig. 20.

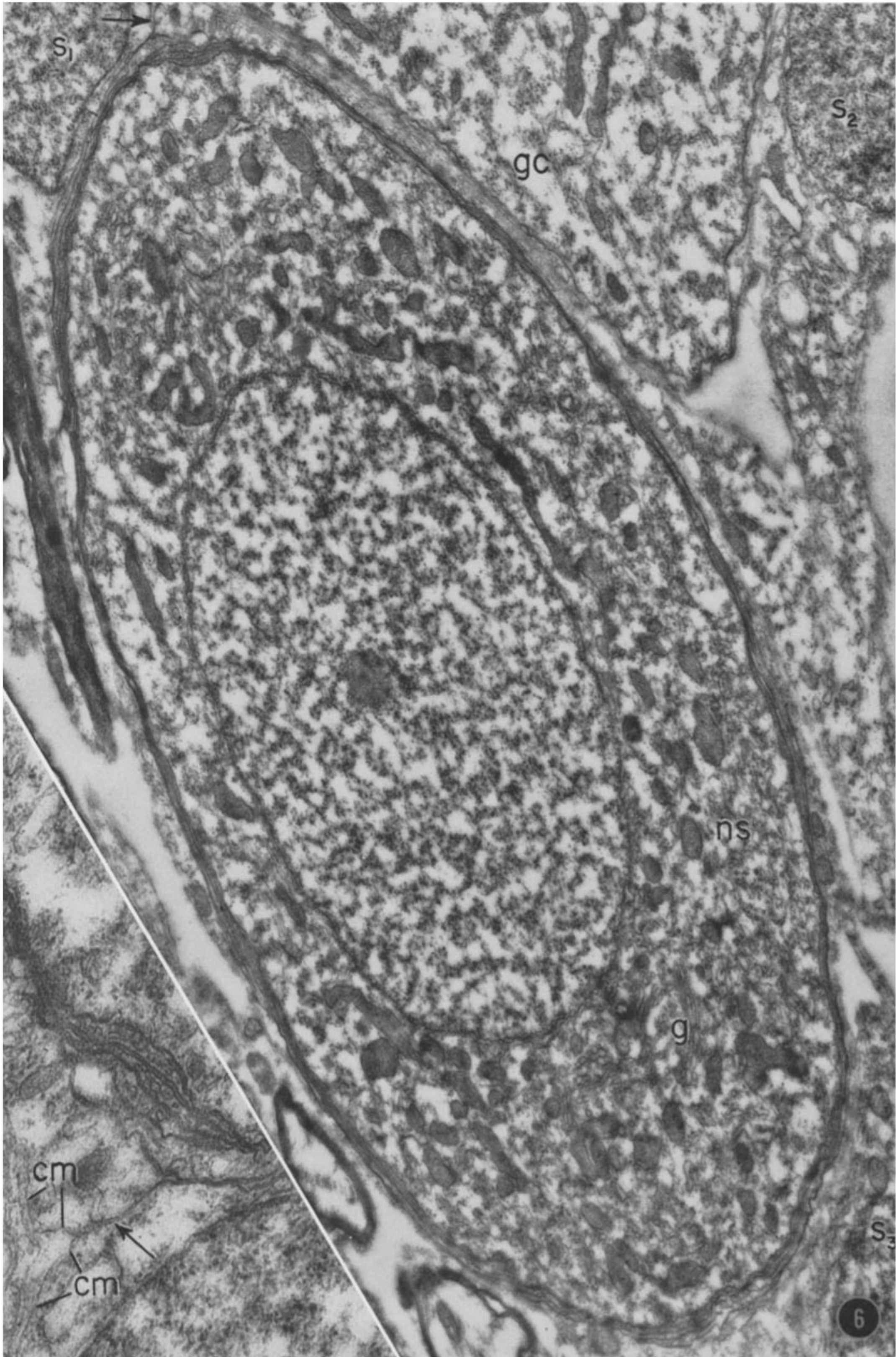
The origin of these differences in density becomes clear upon examination of the electron micrographs. Three types of capsules may be distinguished: those consisting of a single layer of Schwann cell cytoplasm, those composed of loose myelin, and those composed of compact myelin.

1. *Neurons Encapsulated in a Simple Schwann Cell Sheath:* These cells are infrequent. They are completely invested by the Schwann cell layer, which can become much attenuated, as in Figs. 3 and 15, where its smallest width is only about 180 A. The cytoplasm of the Schwann cells contains numerous small vesicles and clusters of ribonucleoprotein particles. Mitochondria and more elaborate portions of the endoplasmic reticulum occur principally in the wider regions of the Schwann cell such as the perinuclear zone. The outer surface of the Schwann cell is invariably coated by a fluffy basement membrane. Between the neuron and the Schwann cell there appears to be only a light, structureless region approximately 200 A wide. As Fig. 3 shows, this interface displays several interesting features. First, the plasmalemma of the neuron is denser and slightly thicker than that of the Schwann cell. Second, the Schwann cell surface is smooth and regular. Third, the neuronal plasmalemma is thrown up into numerous minute and irregular undulations, so that it is nowhere congruent with the inner surface of the Schwann cell ensheathing it. The interstice between the two cells consequently varies considerably in width, from about 120 A to 350 A. Because the two surfaces seem to be morphologically independent, a search was made for specialized structures, such as terminal bars or desmosomes, to account for their close association, but none were found.

Immediately beneath the surface of the ganglion cell lie membrane-limited cisternae similar to those occurring in this position in the dorsal root ganglion cells of the rat (35). In Fig. 3 two examples of these cisternae are shown in which the

FIGURE 6

A small neuron enclosed by a thin sheath of loose myelin. Its nucleus contains one nucleolus, and its cytoplasm contains abundant Nissl substance (*ns*), mitochondria, and Golgi membranes (*g*). Parts of three Schwann cells (s_1, s_2, s_3) and a second neuron (*gc*) are also present. At the upper pole of the perikaryon, shown at a higher magnification in the inset, a channel corresponding to an outer mesaxon is indicated by arrows. The field included in the inset shows that this channel, bounded by Schwann cell plasmalemma (*cm*), opens into a pocket of the interstitial space located between the sheaths of the two neurons present in the figure. Starting from the left side of the inset, 5 layers of Schwann cell cytoplasm (containing vesicles) can be counted. Note that the Schwann cell lamellae are much more variable in thickness than the spaces between lamellae. Methacrylate embedding. × 15,000, inset × 40,000.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interval between the subsurface cisterna and the plasmalemma measures less than 100 A.

The cytoplasm of the ganglion cells is richly supplied with organelles, which were noted by the earliest workers (20), who commented on the granular appearance of these cells in the light microscope. The mitochondria are short, straight or curved, sometimes branched rods which contain either transversely or longitudinally oriented cristae. The Nissl substance, consisting of the endoplasmic reticulum and associated fine granules (25), is diffusely distributed throughout the cytoplasm and is not strikingly massed into Nissl bodies. Its cisternae are broad and tortuous, with many interconnections, and are disposed without any preferential orientation (Fig. 3). The Golgi complex appears as small ribbon-like or elliptical, elongated, and compact masses of agranular cisternae and small vesicles, which are scattered without apparent connection throughout the cytoplasm. A few long, thin neurofilaments and canaliculi interweave among the other organelles, but they are sparse and are much less prominent in these cells than in the heavily myelinated ones.

Many of the perikarya, both myelinated and unmyelinated, contain conspicuous inclusion bodies, 1 μ or more in diameter. Although usually clustered in the axon hillocks at the two opposite poles of the cell body (Fig. 2), they are also distributed singly throughout the cytoplasm. These bodies vary considerably in their fine structure. Some are limited by a single membrane (Fig. 21), some by a double membrane over all or part of their surface (Figs. 7 and 15). Most of these inclusions contain a finely granular, dense material that gives them a homogeneous appearance. But many contain, in addition, delicate membranes in the form of vesicles or tubules, often continuous with one of the surface membranes, in the fashion of the cristae mitochondriales (Figs. 7, 15, and 21). The nature of these bodies is unclear. They are apparently not pigmented, for they display no color when observed in the ordinary light microscope. Their fine structure resembles that of certain lysosomes and peribiliary bodies in the liver (11, 37) or that of microbodies in the kidney (31). Many of them resemble the inclusions described by Duncan and Nall (5, 6) in cultured amnion cells and in dorsal root ganglion cells, and interpreted as derivatives of altered mitochondria.

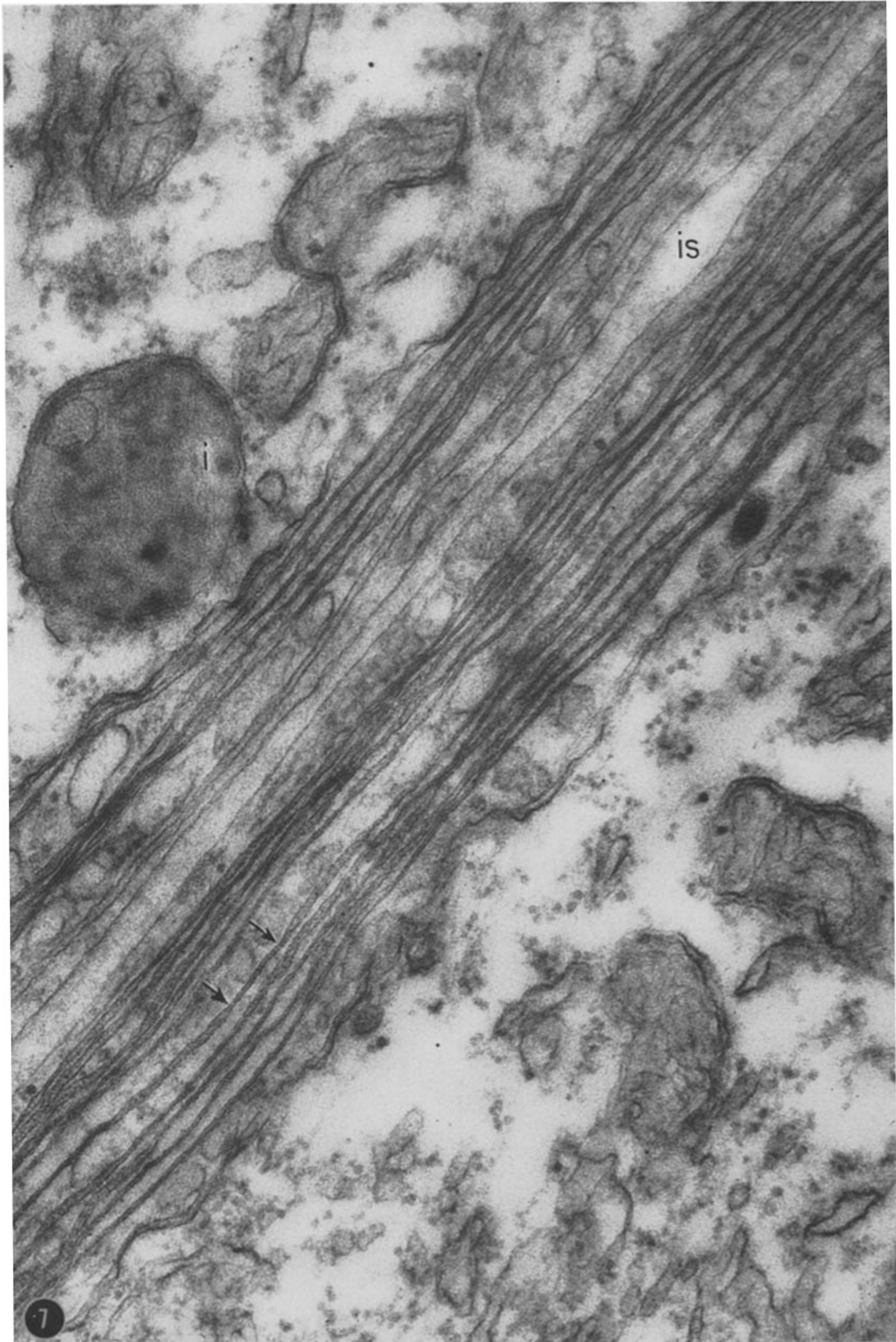
A less common cytoplasmic inclusion found in eighth nerve ganglion cells is illustrated in Fig. 16. These inclusions are whorls of double membranes resembling the early myelin sheaths of growing axons (13, 22, 26, 34). Within the convolutions is an assortment of structures—mitochondria, vesicles, filaments, granules, and small bodies like those described in the previous paragraph. The nature of these whorled inclusions is also unknown. Similar structures have been seen in the neurons of the brain stem in the rat. They are described here in order to call attention to them.

The nuclei of the neurons in the eighth nerve ganglion vary according to the shape of the perikaryon. In the unmyelinated and loosely myelinated cells, which are usually globular, the nucleus is almost perfectly spherical, whereas in the more fusiform, heavily myelinated cells, the nucleus is ellipsoidal and, sometimes, indented (Figs. 1 and 2). In most cells it is eccentrically located. In all cells the nucleoplasm contains, in electron micrographs (Fig. 6), the usual small granules and filaments homogeneously distributed except in the region of the nucleolus, which consists of a dense aggregate of small granules.

2. *Neurons Encapsulated by a Sheath Consisting of Loose Myelin:* These cells are the most common

FIGURE 7

Parts of two adjacent neurons, each with a sheath of loose myelin. A thin interstitial space (*is*) lies between the sheaths. The neuron at the upper left contains a complex inclusion body (*i*) whose limiting membrane is doubled over part of its surface. The sheath covering this cell consists of 6 Schwann cell lamellae containing numerous vesicles. The neuron at the lower right is covered by a sheath of 11 layers. Note that the spaces between lamellae vary in width. Arrows indicate a short segment in which the plasmalemmas covering two cytoplasmic layers have fused to form a complex with a total width of less than 100 A. At either end of this segment the light zone between the cytoplasmic layers widens and exhibits an intermediate line. Araldite embedding. × 84,000.



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in the ganglion. In thick sections examined with the phase contrast microscope, as may be seen in Fig. 2, these perikarya appear to be surrounded by narrow gray husks which vary in thickness from cell to cell. On examination in the electron microscope, the husk proves to be a multilaminate structure consisting of 2 or more circumferentially oriented layers of Schwann cell cytoplasm, each limited by the Schwann cell plasmalemma and separated from its neighbors by a thin cleft (Figs. 5 to 7, 9 to 11, 17 to 19). As the following description will demonstrate, the organization of this sheath bears a close relation to that of typical myelin (13, 34), and it will be designated in this paper as "loose myelin" in order to emphasize this relation.

The two types of myelin may be compared directly by study of Figs. 4 and 5, enlarged to the same final magnification. In Fig. 4 the structure of compact myelin is shown as it appears in a section through the perikaryal sheath of an eighth nerve ganglion cell fixed in osmium tetroxide. It consists of major dense lines, about 30 A thick, having a repeating period of about 115 A, and alternating with lighter stripes, about 85 A wide, each divided down the middle by an interrupted intermediate line. As is well known (32, 34), this pattern is interpreted as arising from the apposition and condensation of the plasmalemmas limiting successive, overlapping Schwann cell sheets from which the cytoplasm has disappeared. In Fig. 5 (see also Fig. 7) the loose myelin sheaths of two neighboring perikarya are shown. In these sheaths, consisting respectively of 3 layers to the left and 2 layers to the right of the intervening connective tissue space (*is*), the cytoplasm of the Schwann cells has not disappeared and consequently the major dense line of compact myelin is absent. That these layers consist of cytoplasm is indicated by the fact that they often contain vesicles (Figs. 6, 7, and 11) and occasionally mitochondria and granules. The continuity of the perinuclear Schwann cell cytoplasm and the outer layers of the loose myelin sheath can be seen in Fig. 6, which shows a small neuronal perikaryon enclosed in a thin sheath. In the upper left corner of the figure (see also inset), a structure corresponding to a mesaxon dips inward toward the neuron, but its further course about the perikaryon cannot be traced with certainty. Nothing corresponding to an inner mesaxon can be distinguished in this micrograph, but such structures have been seen in loose myelin sheaths of other ganglion cells. In the places where the layers can be counted, this sheath consists of 5 layers of Schwann cell cytoplasm. The number of layers can vary from 2 or 3 to 20 or 30 on different cells, but so far as we can ascertain from a study of specimens sectioned in favorable planes, the number of layers appears to be constant in all parts of the sheath surrounding any particular perikaryon. The loose myelin sheath presented in Figs. 9 and 10 consists of approximately 25 layers.

The *thickness* of the cytoplasmic layers, however, is variable within the same sheath (Fig. 7). Usually it is in the range of 100 to 1000 A wide,

FIGURE 8

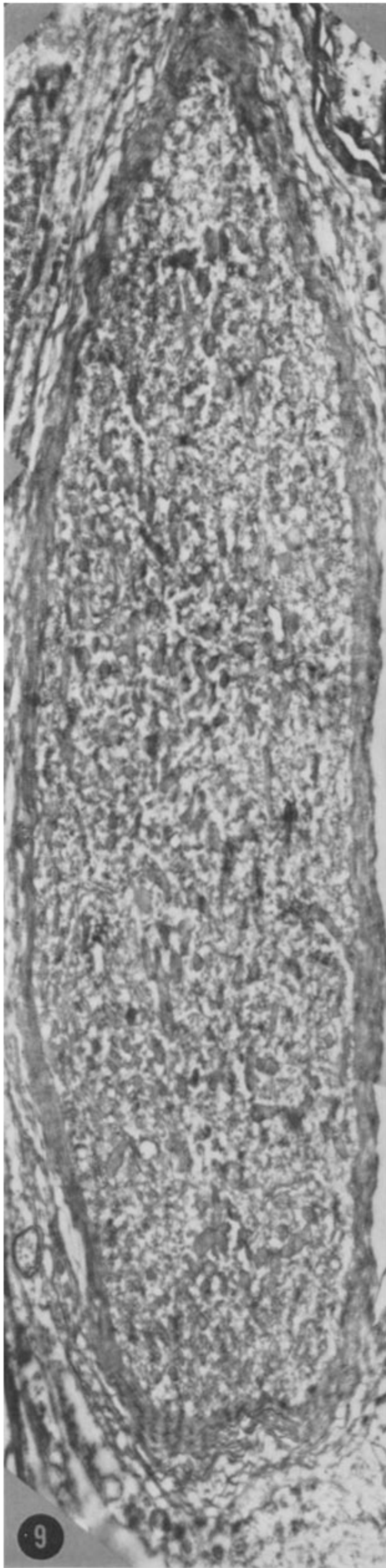
Part of a loose myelin sheath containing a stack of desmosomes (between arrows). There is an accumulation of osmiophilic material on the cytoplasmic sides of each desmosome. The perikaryon lies at the upper right. Additional details of desmosome fine structure appear in Figs. 12 and 13. Epon embedding. × 67,000.

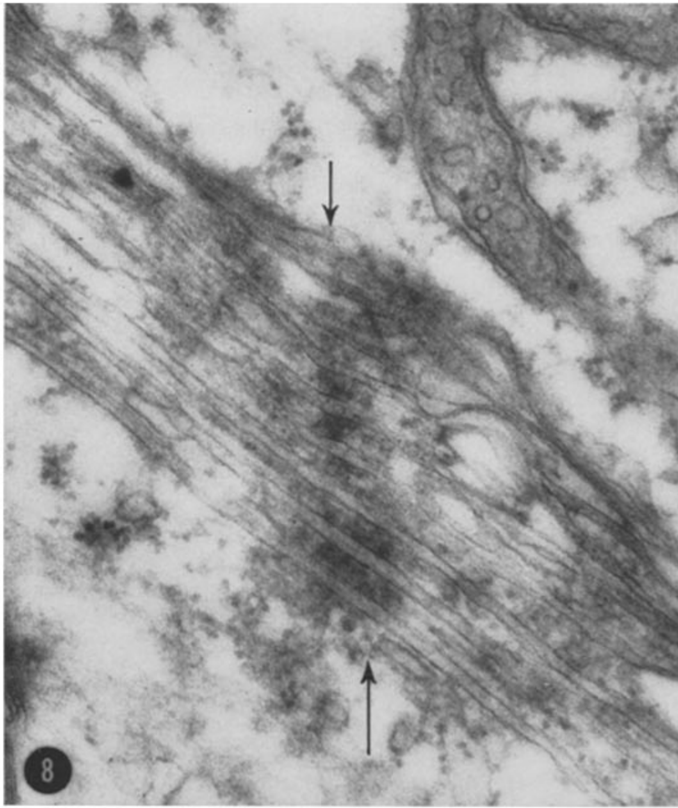
FIGURE 9

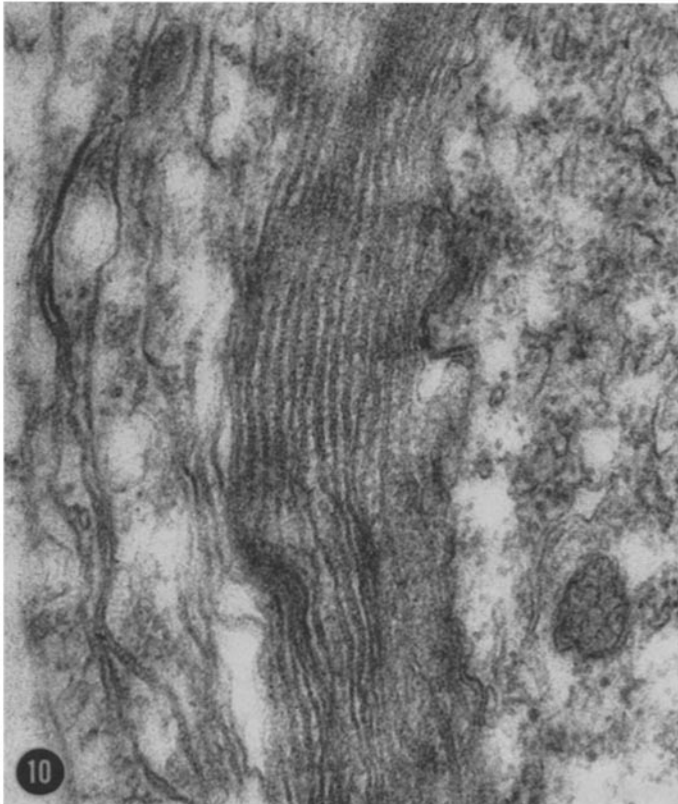
Three-part montage showing a section through a ganglion cell which is completely ensheathed by loose myelin. The neuron contains innumerable mitochondria, Nissl substance, and several clusters of Golgi membranes. Part of the nucleus of a Schwann cell appears at the very bottom of the picture. Methacrylate embedding. × 9000.

FIGURE 10

A higher magnification of part of the sheath in Fig. 9 taken from an adjacent serial section. Although the lamellae cannot be counted accurately, they number approximately 20 to 25. In the region of the sheath showing closest packing of lamellae, the period is approximately 220 A. The perikaryon occupies the right side of the figure. Methacrylate embedding. × 64,000.







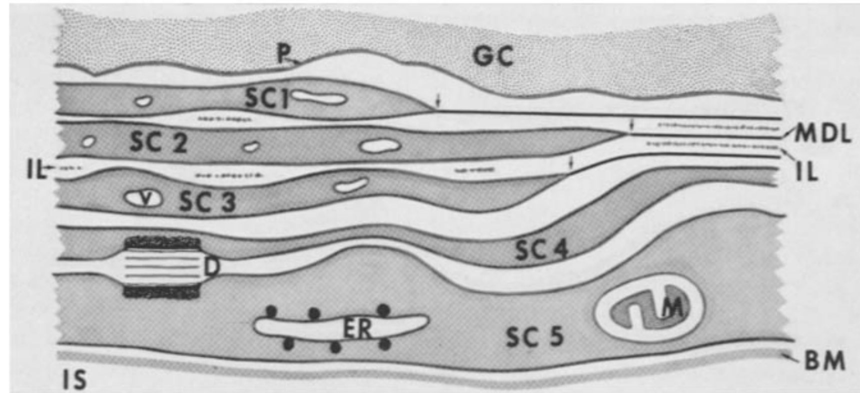


FIGURE 11

Diagram of the perikaryal sheath, showing continuity between loose myelin at left and compact myelin lamellae at right. The ganglion cell (*GC*), with its plasmalemma (*P*), indicated at the top of the figure, is ensheathed by 5 layers of Schwann cell cytoplasm (*SC 1* to *SC 5*). The cytoplasm in the first three of these layers thins and finally disappears so that at each arrow the major dense line (*MDL*) of compact myelin is formed by the apposition of the two internal surfaces of the plasmalemmas. The light zones between the dense lines are relatively constant in width (~ 75 to 100 A) in the region of the compact myelin, but range from 40 to 200 A in the loose myelin. Intermediate lines (*IL*) are often present in these light zones in both loose and compact myelin, except in the markedly narrowed regions in loose myelin, where they never appear. A desmosome (*D*) is indicated between *SC 4* and *SC 5*. *SC 5* contains granular endoplasmic reticulum (*ER*) and a mitochondrion (*M*), whereas the other Schwann cell layers contain only smooth-surfaced vesicles (*V*). The outer surface of the sheath is coated by a basement membrane (*BM*). (Compare with Figs. 5 and 7.)

but it is often much wider, particularly in the outermost layers of the sheath. Moreover, in places the cytoplasm disappears altogether, leaving the apposed plasmalemmas to form a major dense line, as in Figs. 11 and 19. The thin cleft, or light stripe, between the cytoplasmic layers is more restricted in width, ranging from 40 to 200 A. As in compact myelin, it is sometimes divided down the middle by an intermediate line (Figs. 7, 11, and 19). As a result of these features, the repeat period of loose myelin not only is much greater than that of compact myelin, but is also much more variable from place to place in the same sheath. Thus it appears as if the layers of loose myelin want only to be tightened up, compressed, and rectified in order to display the typical pattern of compact myelin.

Loose myelin displays some other noteworthy peculiarities. The first of these is illustrated in Figs. 5, 7, 11, and 19. In Fig. 5 it may be seen that although the two cytoplasmic layers in the sheath on the right are separated by a fairly uniform space about 200 A wide, the three layers on the left are separated by light stripes of varying width. The variation in itself might be considered inconsequential were it not for the fact that the minimal interval between adjacent layers measures about 40 A, or, as may be seen at the very top of the figure, about half the interval between the major dense lines of compact myelin. This distance is smaller than the interval separating adjacent cells in other tissues consisting of apposed cells, such as epithelia. The possible significance of this observation in terms of structure will be discussed below.

Another peculiarity of loose myelin is illustrated in Figs. 8, 12, and 13. These demonstrate that between the layers of Schwann cell cytoplasm structures resembling the terminal bars or desmosomes of epithelia can be found. They occur frequently, either individually or in stacks between successive layers (Fig. 8). They are characterized by widening of the interstitial space, splitting of the apposed plasmalemmas, and an accumulation of amorphous, dense material on the cytoplasmic sides of the limiting membranes. Often, three faint lines parallel to the plasmalemmas appear in the interstitial space. It is of interest that certain invertebrate nerve sheaths also display stacks of desmosomes between adjacent layers

(14). In vertebrates desmosomes have been described between glial cell processes during the earliest stages of myelination in the central nervous system (22), but have not been reported in peripheral nerves.

When a loose myelin sheath is followed around the perikaryon, areas are encountered in which the sheath has become compact myelin throughout all or part of its thickness. The change can be detected in the phase contrast microscope because of the shift in the appearance of the sheath from the dark gray of loose myelin to the black of compact myelin, as is shown in Fig. 20. Electron micrographs of the transition from loose to compact myelin demonstrate that at these sites the layers of Schwann cell cytoplasm, each bounded by its plasmalemma, continue into the lamellae of compact myelin. Thus, as seen in sections, the individual layers of loose myelin are not interrupted as they pass into the corresponding major dense lines of compact myelin; only the Schwann cell cytoplasm disappears. Figs. 17 and 18 show two regions in the sheath of the same ganglion cell. As may be seen in Fig. 17, the sheath contains a total of 14 layers. In Fig. 18, 10 of the layers are compact myelin; in Fig. 19 (which is an enlarged detail from Fig. 17), all the layers are loose, and an intermediate line is visible between some of them in several places. In the more extensive view offered in Fig. 17 the sheath undergoes a change in composition from loose myelin at the bottom of the figure to compact myelin at the top. The number of layers or lamellae remains constant at 14 throughout.

As the phase contrast micrograph in Fig. 2 shows, the loose myelin sheath is restricted to the perikaryon and the portion of its neurite up to the first node of Ranvier. The next and successive internodes are ensheathed in compact myelin. The number of lamellae in the myelin of these latter internodes does not correspond to the number of cytoplasmic layers in the sheath of the perikaryal internode.

The cytoplasm of loosely myelinated perikarya does not differ from that of unmyelinated ganglion cells as described in the preceding section.

3. *Neurons Encapsulated by a Sheath of Compact Myelin:* These cells are the most striking in the eighth nerve ganglion. They are usually large and fusiform, sometimes only slightly wider than the heavy axons leaving their opposite poles. The compact myelin of their sheaths is in every way comparable to the axonal myelin. It consists of multiple dense layers with a period of approximately 115 A, often with an intermediate line (Fig. 4). The outer surface of the myelin is covered by a thin layer of Schwann cell cytoplasm, which differs from that of the Schwann cells covering unmyelinated perikarya in not displaying large numbers of cytoplasmic vesicles. It is, however, covered by a basement membrane. The sheath varies in thickness from 0.1 to 1.0 μ and contains from 10 to 90 (mean about 60) lamellae. In some instances the innermost few lamellae of a thick compact myelin sheath are of the loose variety with layers of Schwann cell cytoplasm replacing the major dense lines. Loose myelin, however, is not found elsewhere in the thickness of these sheaths.

Scrutiny of both loose and compact myelin sheaths in the eighth nerve ganglion has not revealed nodes of Ranvier, Schmidt-Lantermann clefts, or other discontinuities over the surface of the perikaryon. Nodes are usually found at some distance from the perikaryon along the neurites and only occasionally at axon hillocks (Fig. 14). In Figs. 1 and 2, the first node occurs about 20 μ from the origin of the axon. Sometimes the length of the internode containing the perikaryon is remarkable. The longest internode with a cell body at its center that has been observed in this study is 200 μ long. The maximum is undoubtedly even greater, but it has not been seen because of the low probability that such a long axon will remain for any considerable distance in the plane of the 2 μ sections used for phase contrast microscopy. The compact myelin sheath over the perikaryal internode is frequently thinner by several fold than the myelin sheath of the next and successive internodal segments of the axons. There is thus no constant relation between the thickness of the myelin ensheathing the cell body and that surrounding the neurites beyond the internode containing the perikaryon.

Two types of neuronal cytoplasm can be found in cells encapsulated by compact myelin. The first type, shown in Fig. 21, resembles that of unmyelinated perikarya. The Nissl substance is well developed, consisting of broad cisternae of the endoplasmic reticulum and abundant ribonucleoprotein granules. The Golgi complex is relatively inconspicuous, and the mitochondrial profiles in sections are numerous. The cytoplasm is densely populated with formed elements.

The only appreciable distinction between the cytoplasm of these cells and that of unmyelinated perikarya is in the greater concentration and prominence of the long neurofilaments in the myelinated cells. In contrast, the second type of cytoplasm, illustrated in Fig. 22, appears relatively clear and less densely populated. The mitochondria are longer and less numerous. In the Nissl substance tubular and vesicular elements of the endoplasmic reticulum are more common than broad cisternae, and there is a striking reduction in the quantity of ribonucleoprotein particles coating their outer surfaces and lying between them. The Golgi complex is more prominent. As shown in Fig. 22, it consists of closely packed, overlapping, and highly fenestrated cisternae, which give it a reticular appearance resembling the type of agranular endoplasmic reticulum recently described by Manton (23) in dictyosomes of *Anthoceros* meristem cells, and by Porter and Yamada (28) in the pigment epithelium of the frog's eye. The most striking feature of the cytoplasm in these heavily myelinated perikarya is the large number of fine neurofilaments, which course everywhere through the interstices between the other organelles.

DISCUSSION

In summary, the significant features of the eighth nerve ganglion in the goldfish are the following: (*a*) Each bipolar ganglion cell is part of an inter-

FIGURE 12

A desmosome (*d*) lying between the outermost two layers of the sheath around a ganglion cell. A small portion of neuronal cytoplasm appears at the left. The sheath consists of compact myelin with several layers of Schwann cell cytoplasm on either side. At the desmosome the width of the interstice between layers increases to ~ 300 A, compared with the usual intercellular distance of less than 200 A. Three osmiophilic parallel lines lie within this space. On the cytoplasmic sides of the desmosome there is an aggregation of osmiophilic material. Araldite embedding. × 70,000.

FIGURE 13

A desmosome at a higher magnification. The apposed plasmalemmas (arrows) split, and the interlamellar space widens to ~ 400 A. Three osmiophilic lines are present in this space. Part of a second desmosome is visible in the right lower corner. Epon embedding. × 107,000.

FIGURE 14

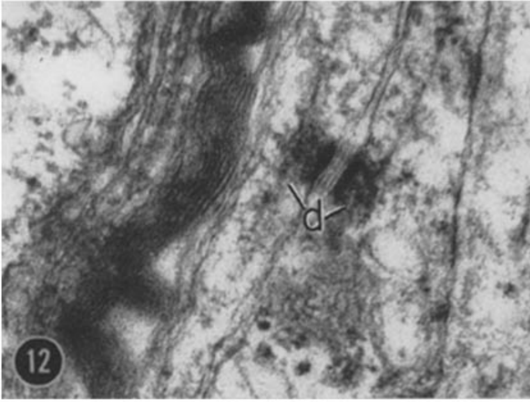
Axon hillock of a ganglion cell showing myelin lamellae terminating as they approach a node, which lies beyond the top of the figure. Neuronal cytoplasm is at the left. Lamellae of compact myelin terminate in five overlapping blind loops (arrows) against the neuronal plasmalemma. Approximately three times as many lamellae cover the next internode (not shown in this figure). Araldite embedding. × 127,000.

FIGURE 15

Dense inclusion bodies in an unmyelinated ganglion cell. These measure approximately 0.8 μ in greatest diameter and are comparable to those shown in Fig. 2. The uppermost body has a finely granular texture and possesses a doubled limiting membrane, which at two points appears to be continuous with internal membranous structures (arrows). The single Schwann cell layer covering this cell (top of figure) is only ~ 180 A wide in this region. Araldite embedding. × 45,000.

FIGURE 16

Membranous inclusion bodies in a ganglion cell. These consist of several layers of paired osmiophilic lines. The members of each pair are separated by approximately 100 to 300 A. Several small dense bodies (arrows) are associated with these membranes. Methacrylate embedding. × 26,000.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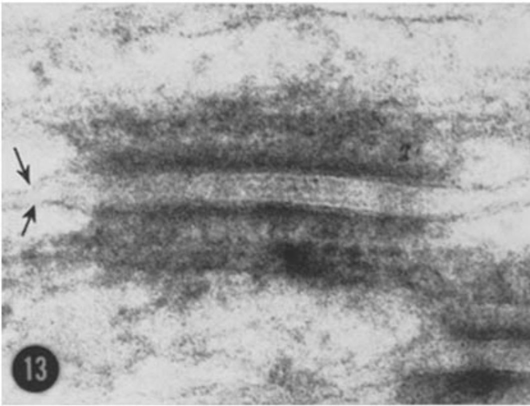
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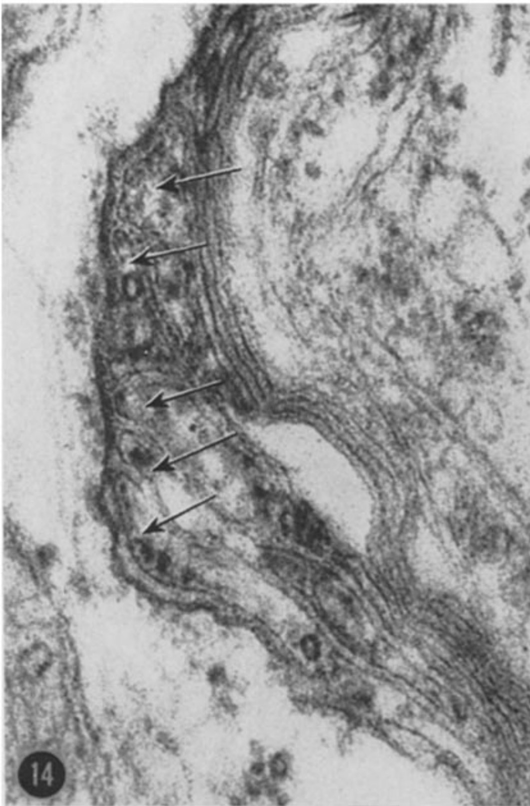
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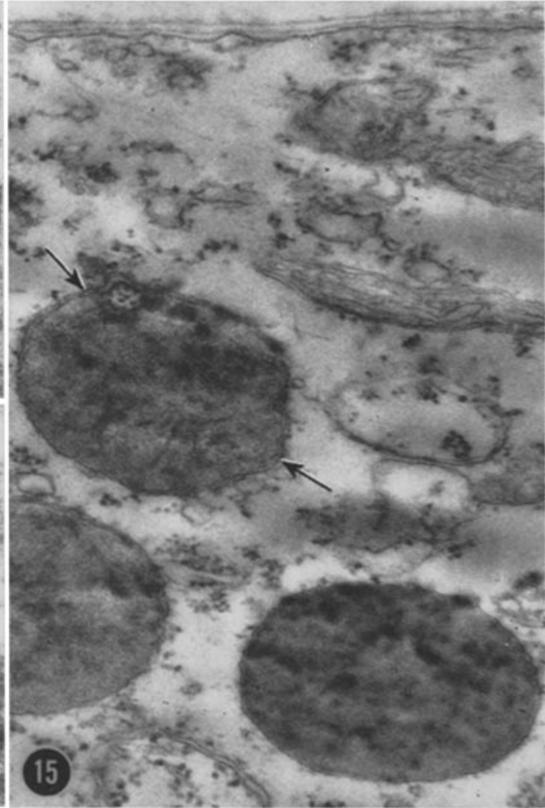
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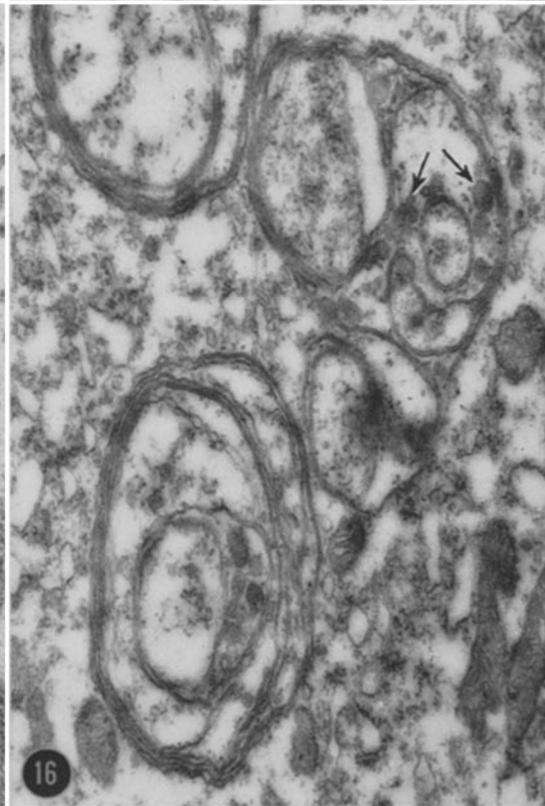
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nodal segment, as Ranvier (30) recognized more than seventy years ago. (*b*) In most cases the internode containing the perikaryon is surrounded by a myelin sheath. (*c*) The number of lamellae in the sheath of this internode is usually less than that in the sheath of adjacent segments. (*d*) The myelin surrounding this internode alone can be entirely or partly loose, *i.e.*, the major dense line has not formed and is represented instead by a layer of Schwann cell cytoplasm. This configuration is comparable to that found in invertebrate nerves (7, 8, 14, 15, 42) and in developing myelinated nerves of vertebrates (13, 22, 26, 34). (*e*) When compact and loose myelin appear together in the same perikaryal sheath, their lamellae are continuous with each other.

These observations lead to four principal questions, none of which can be answered from the information at hand. How do perikaryal myelin sheaths develop? What factors account for the structural peculiarities of these myelin sheaths? How does the presence of loose or compact myelin sheaths around cell bodies affect their neurophysiological behavior? What is the effect of these sheaths on the nutrition and metabolism of the ganglion cells?

The Origin of Perikaryal Myelin Sheaths: According to the currently most popular theory (13, 34), myelin in the peripheral nervous system is formed by the spiral wrapping of Schwann cell cytoplasm about an axis cylinder with subsequent fusion of the cytoplasmic surfaces of the Schwann cell membranes to form major dense lines. Nothing in the structure of the perikaryal myelin sheaths described here suggests that they are formed by a different process. The ganglion cells, however, are so large that it has not yet been possible to trace the lamellae of a perikaryal myelin sheath completely from beginning to end around the cell body in order to ascertain whether or not they actually form a *continuous* spiral. All that can be said now is that structures corresponding to inner and outer mesaxons have been identified and that discontinuities in the lamellae of the sheaths have not been seen. Furthermore, up to the first node, the neurites issuing from any one

FIGURES 17 TO 20

Light and electron micrographs showing the transition from loose to compact myelin in the same sheath.

FIGURE 17

The neuronal cytoplasm (*gc*) at the left contains Nissl material and mitochondria. The sheath of this cell consists of 14 lamellae which in the lower part of the figure are all loose. At the top of the figure most of these layers have formed compact myelin. The interstitial space is indicated by *is*. Araldite embedding. × 54,000.

FIGURE 18

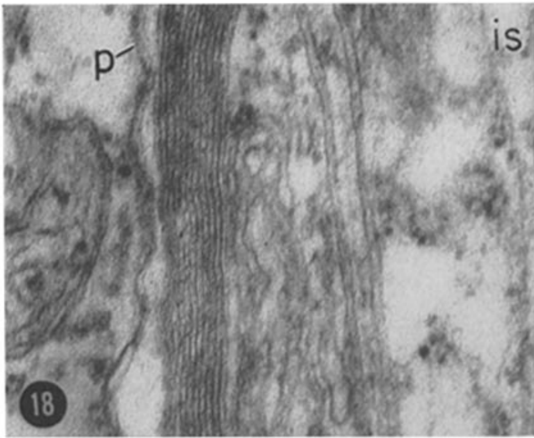
Detail from a near-by part of the same sheath shown in Fig. 17. Neuronal cytoplasm is again on the left. The sheath consists of 1 layer of Schwann cell cytoplasm immediately adjacent to the ganglion cell, 10 lamellae of compact myelin, and 3 more layers of Schwann cell cytoplasm. *p*, neuronal plasmalemma; *is*, interstitial space. Araldite embedding. × 102,000.

FIGURE 19

Enlargement of a part of Fig. 17. Note the variation in width of the spaces between layers and the presence of intermediate lines (*il*) in some of the wider spaces. At several points (arrows) a Schwann cell layer condenses to form a major dense line. *p*, neuronal plasmalemma; *is*, interstitial space. Araldite embedding. × 102,000.

FIGURE 20

Phase contrast photomicrograph of a small bipolar neuron from the eighth nerve ganglion, showing the eccentrically located nucleus and granular cytoplasm. The sheath has the density of compact myelin between arrows and of loose myelin elsewhere. The nucleus of a Schwann cell is indicated by *s*. Araldite embedding. × 2800.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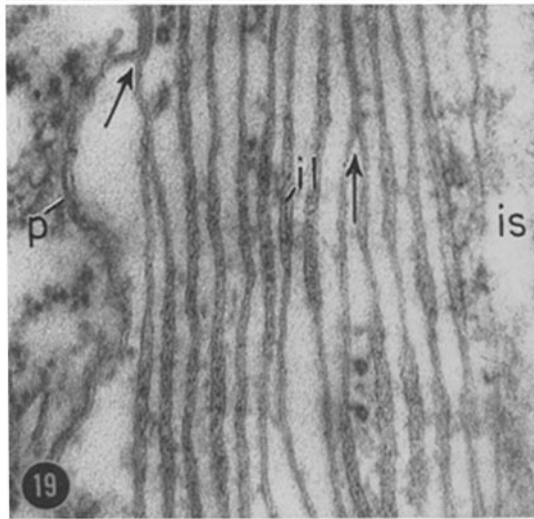
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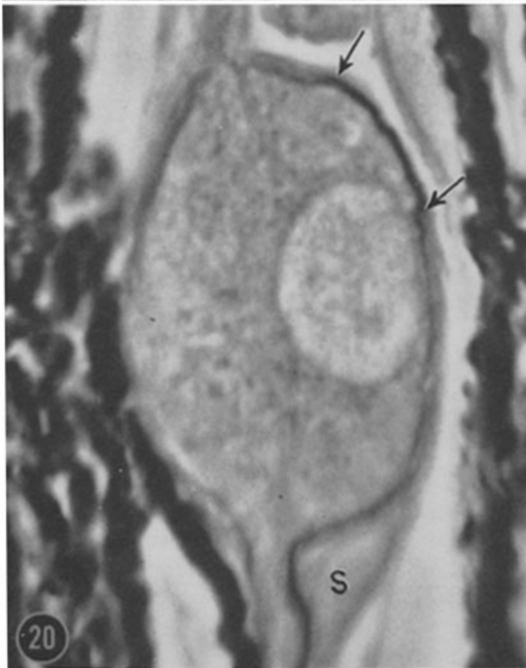
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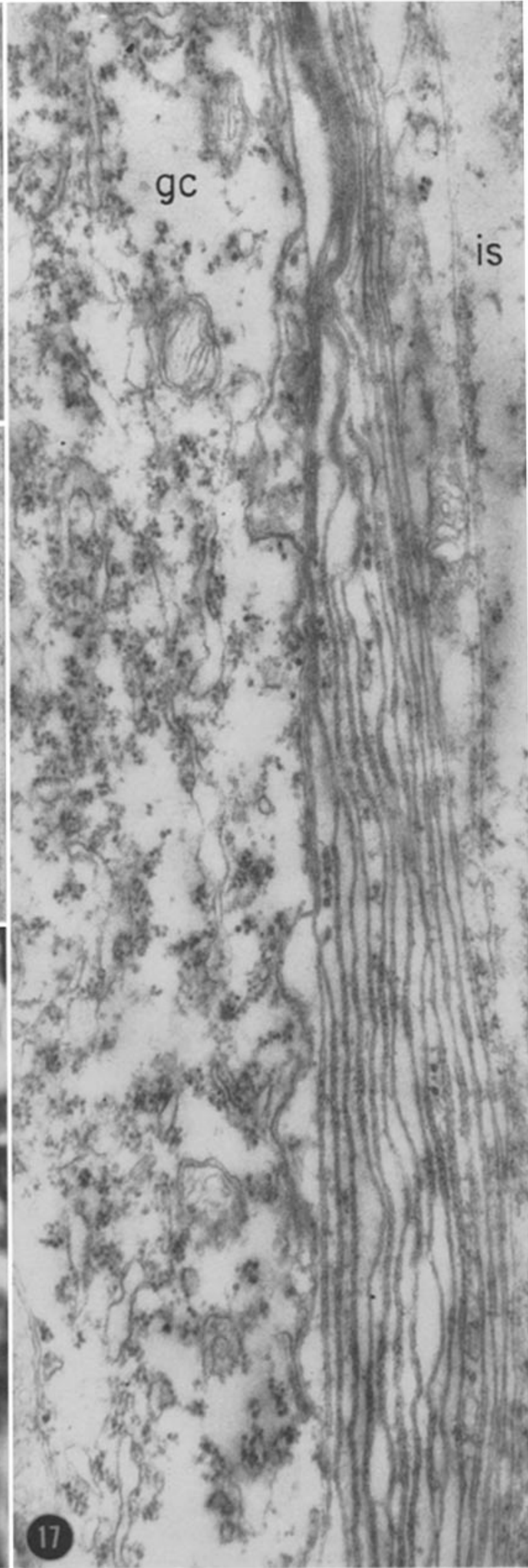
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perikaryon bear the same kind of myelin, apparently with the same number of layers, as does the perikaryon. When the perikaryon is loosely myelinated, so are the neurites; when the perikaryon is compactly myelinated, so are the neurites. Successive internodal segments of the neurites always exhibit typical compact myelin regardless of the type or thickness of the myelin ensheathing the perikaryon. This configuration suggests that the whole cell body plus the proximal parts of its neurites were ensheathed together during the development of the ganglion. We cannot assume, however, that myelination of the internode containing the perikaryon was accomplished by a *single* Schwann cell. It should be noted that sometimes more than one Schwann cell is topographically related to the sheath of a single perikaryon, as in Fig. 6. In these cases it was impossible to ascertain how many of the Schwann cells contribute to the myelin sheath because their extensions could not be traced completely.

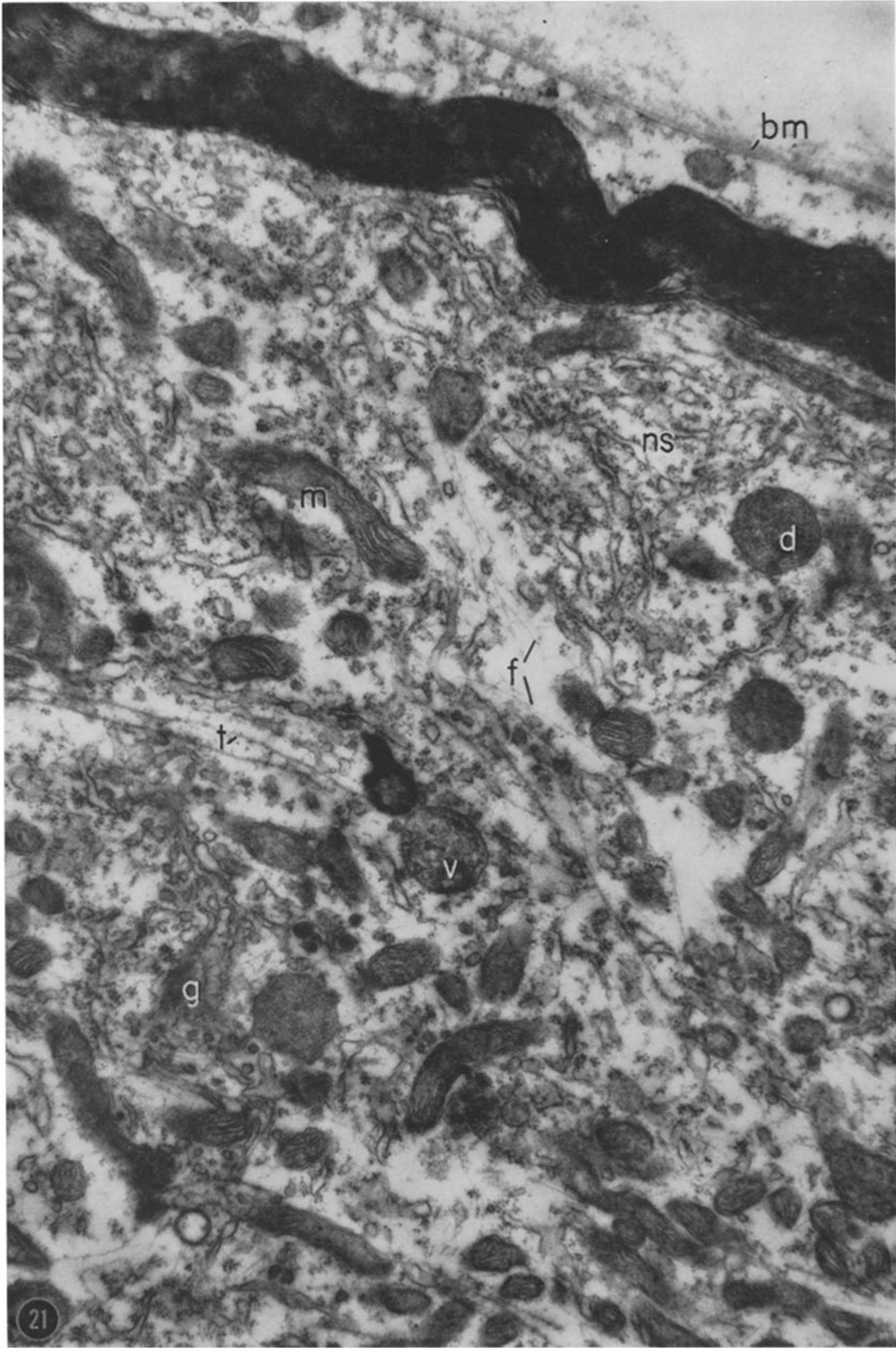
Perikaryal sheaths in the eighth nerve ganglion range in type from a simple Schwann cell layer, through multiple layers of loose myelin, then compact and loose myelin together in the same sheath, to, finally, compact myelin. These forms, which all coexist in any single ganglion, encompass all the stages which would be required to construct a developmental sequence leading to the formation of compact myelin. It is, therefore, reasonable to wonder whether such a progression actually occurs in the adult animal leading ultimately to the formation of compact myelin exclusively, throughout the ganglion. We can say only that the eighth nerve ganglion has the same appearance in old fish as in young ones acquired from the same source. Since the complete spectrum of sheaths exists in both young and old adults, we have no evidence that in the adult loose myelin progresses to form compact myelin.

This observation, however, does not exclude the possibility that such a progression does occur during embryologic development of compact perikaryal myelin. In this case we could explain the appearance of the adult ganglion if myelination began later or proceeded more slowly on the smaller cells than on the larger cells, and if the process ceased simultaneously throughout the ganglion. The result of this sequence would be that those sheaths which were incomplete at the time of cessation would remain in a state of arrested development corresponding to what we actually find in the adult. A study of the developing eighth nerve ganglion in the goldfish, beginning before the appearance of any myelin, would test this proposed explanation.

The Location of Loose Myelin: What factors account for the appearance of loose myelin in the sheath of the single internode containing the cell body, whereas the other internodes of the same nerve fiber are ensheathed in compact myelin? It does not seem useful to distinguish between the satellite cells forming the perikaryal sheath and the Schwann cells forming the axonal sheaths as Cervós-Navarro (3) has done for the dorsal root ganglion of the rat, for both cells are capable of forming compact myelin in the eighth nerve ganglion. Similarly, it cannot be simply assumed that the perikaryal Schwann cells that form compact myelin are basically different from those that form loose myelin, for both types of myelin can occur in continuity in the sheath of a single neuron. The increased diameter of the internode at the site of the perikaryon cannot be responsible, for typical compact myelin occurs precisely on the larger cells, whereas loose myelin is found on the smaller cells. Furthermore, although the internode is enlarged only at the site of the perikaryon, the entire internode is ensheathed in the same manner as the perikaryon. We cannot implicate the frequency at which

FIGURE 21

Part of the cytoplasm of a ganglion cell which has a thick sheath of compact myelin. The myelin is $\sim 0.6\ \mu$ thick and is covered by a single layer of Schwann cell cytoplasm, which has a basement membrane (*bm*). The neuronal cytoplasm contains numerous small mitochondria (*m*), Nissl substance (*ns*) composed of elongated cisternae of the endoplasmic reticulum with associated ribonucleoprotein particles, Golgi membranes (*g*), neurofilaments (*f*), long tubules (*t*) with an outside diameter of 200 A, and both granular (*d*) and vesicular (*v*) inclusion bodies. The packing of organelles is as close here as in Fig. 3, where the cellular sheath consists of only a single thin layer of Schwann cell cytoplasm. Epon embedding. × 26,000.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impulses reach the loosely myelinated internode, since the rate is the same along all segments of a nerve fiber, and the other internodes are all myelinated in the typical way. It also seems unlikely that metabolic peculiarities of the whole ganglion can be responsible, because the ganglion contains typically myelinated axons and large perikarya alongside loosely myelinated cells. Thus internal evidence opposes these numerous possibilities.

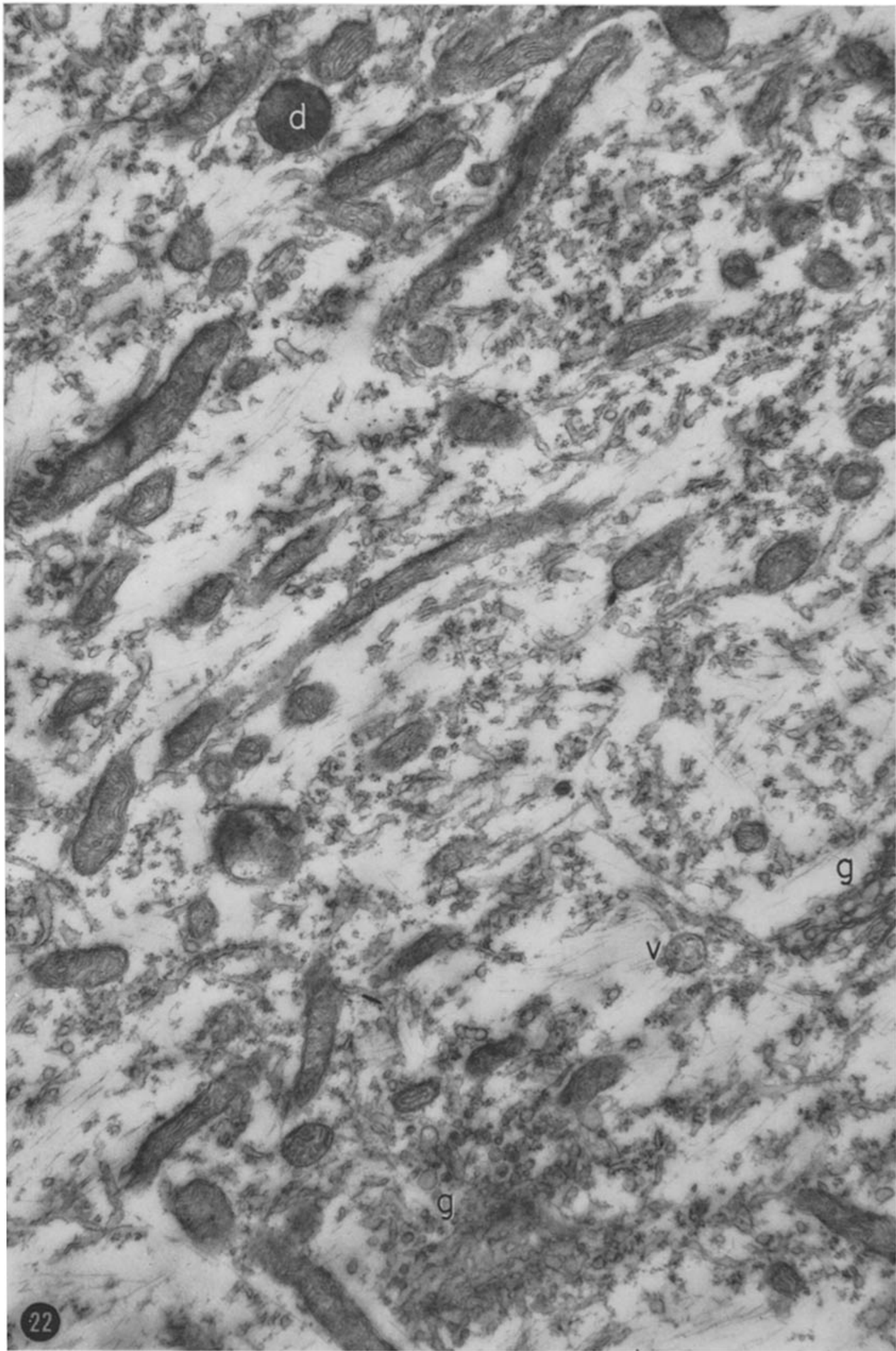
The fact that a given Schwann cell can produce both loose and compact myelin implies that the occurrence of one or the other type depends on factors outside the Schwann cell. It might be inferred that myelinogenesis requires the interaction of Schwann cells with neurons and that variations in the form of myelin ultimately depend on differences among neurons. We might speculate that myelination can occur only when the milieu created by the neuron is appropriate and that specific biochemical differences between neurons may thus determine the nature of the myelin sheaths developed around them. In the present study a search was made for fine structural differences between neurons ensheathed by loose as opposed to compact myelin, on the assumption that such differences might reflect underlying biochemical differences. The only consistent difference found was that the concentration of neurofilaments and tubules was much higher in compactly myelinated cells than in either loosely myelinated or unmyelinated ones. The striking paucity of ribosomes and reduction in the number of mitochondrial profiles in some of the large compactly myelinated cells did not appear in others. Whether these differences are merely the consequences of variations in the myelin sheaths, or are reflections of their cause, or are not related to the myelin sheath cannot be determined from the information at hand.

Contact Areas between Layers in Loose Myelin: When two adjacent and similar cells are apposed in a tissue, their unit membranes appear in electron micrographs to be 150 to 200 A apart. This is the usual interval between independent cells (27), such as those in the intestinal epithelium or in the central nervous system. It is also the usual interval between overlapping parts of the same cell, for example, in the epithelial cells of the renal tubule or of the ciliary body. Although cells that are farther apart are usually separated by a basement membrane or by connective tissue elements, some associated cells can be separated by intervals of 300 or even 1000 A without any visible interposed structure, for instance, the neurons and Schwann cells in dorsal root ganglia (35).

Cells can, however, come much closer together, as in the cardiac muscle of the large veins in the mouse (17) and in human cervical epithelium (16) as recently described by Karrer, where neighboring cells can apparently be separated by a light zone of only 90 to 100 A. In these latter cases, an intermediate line appears between the two limiting membranes and the intercellular space seems to be obliterated. This intermediate line can best be interpreted on the basis of the currently accepted theory of the structure of the plasmalemma. According to Robertson (34), the plasmalemma, or the free surface membrane of a cell, consists of a three-layered structure, the unit membrane, which appears in electron micrographs of sections as a pair of dense lines separated by a light interspace, each approximately 25 A thick. Although this three-layered unit membrane is most clearly and consistently visible in tissues fixed in potassium permanganate, it can also be seen in tissues fixed in osmium tetroxide, especially in certain selected sites, for example, on the surface of microvilli, or at terminal bars. When two adjacent cells are so close together that the intercellular space between them is obliterated, the neighboring plasmalemmas fuse, with the resultant formation of an intermediate line, which represents the fused outer dense layers of the apposed unit membranes. This type of contact has been de-

FIGURE 22

Cytoplasm of a ganglion cell ensheathed in compact myelin. The cytoplasm of this cell is quite distinct from that in Fig. 21. Here the mitochondrial profiles are longer and fewer in number, ribonucleoprotein particles are sparser, and the cisternae of endoplasmic reticulum tend to be smaller and less elongated. Most strikingly, there is a high concentration of neurofilaments. Granular (*d*) and vesicular (*v*) inclusion bodies and Golgi membranes (*g*) are also present. Epon embedding. × 23,000.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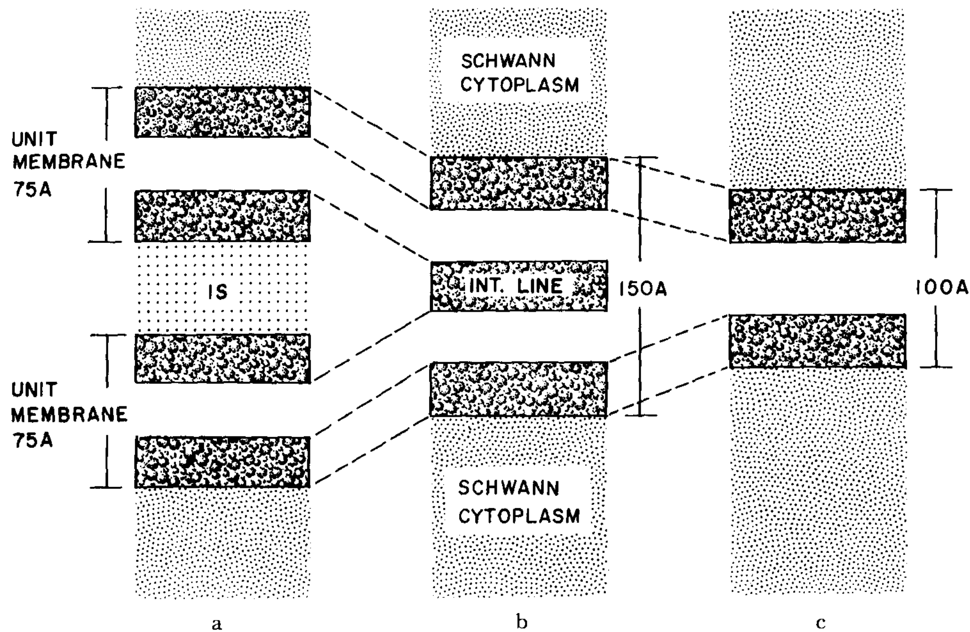

FIGURE 23

Schematic representation of three observed arrangements of apposed Schwann cell membranes. *a*, the two unit membranes are entirely separate, with intercellular space intervening; the width of each unit membrane is approximately 75 A (modified after Robertson, 34). *b*, the two unit membranes have fused, with the formation of an intermediate line; total width approximately 150 A (cf. Figs. 5, 7, and 19). *c*, the two membranes have condensed further; the intermediate line is absent; total width approximately 100 A (*cf.* Figs. 5 and 7).

scribed by Robertson (32, 33) between the axolemma and the Schwann cell plasmalemma at the nodes of Ranvier in peripheral nerves. The same kind of fusion occurs in compact myelin (32, 34) where the neighboring lamellae of the Schwann cell condense to form the intermediate line. In the loose myelin ensheathing the ganglion cells in the eighth nerve ganglion of the goldfish, this type of condensed contact occurs frequently between the limiting membranes of successive layers of Schwann cell cytoplasm (Figs. 7, 17, and 19).

There are, however, areas of contact where the intermediate line is absent, and the two apposed Schwann cell membranes appear even closer together (Figs. 5, 7, and 17). The width of the entire unit, consisting of two dense lines and a narrow zone between, is only about 100 A, whereas the comparable unit when the intermediate line is present is about 150 A wide (Figs. 7 and 19). In Fig. 7 an area of contact between apposed Schwann cell layers is shown (between arrows) in which the two dense lines are separated by about 40 A. Note that at either end of this segment these dense lines are continuous with the *inner* or cytoplasmic sides of the Schwann cell membranes beyond the segment. The *outer* components of the plasmalemmas at either end of this segment have fused to form an intermediate line which, however, terminates short of the narrowing, marked by the arrows. These relationships are schematically represented in Fig. 23. The resultant contact structure can be interpreted in terms of the current theory of the plasmalemma if we assume that in these exceedingly narrow zones two adjacent Schwann cell unit membranes have combined in such a way that their apposed external surface layers have disappeared altogether instead of fusing to form the intermediate line. The width of the combined membranes is accordingly at least 50 A less than would be predicted from the dimensions of the original two unit membranes. The result is a structure resembling a single unit membrane, but slightly thicker. It should be noted that such condensation could result from preparative artifacts, although this seems an unlikely possibility to us. Finean (12) has recently analyzed spacing defects in compact

myelin subjected to controlled dehydration before fixation and has interpreted the collapsed layering in these preparations as due to molecular rearrangement of the myelin layers. In the present instance, dehydration was carried out after fixation, as part of the embedding procedure. Consequently, condensations of neighboring unit membranes in our material need not be related to the collapsed lamellae in Finean's study. Examination of loose myelin sheaths after fixation in potassium permanganate should prove rewarding.

The Myelin Sheaths and Nutrition and Metabolism of the Perikaryon: In the dorsal root ganglion of the rat (2, 3, 35) the neuronal perikarya are completely ensheathed by a single layer of Schwann cell cytoplasm, which is in turn surrounded by a basement membrane. The neuronal surface is nowhere directly exposed to the pericapillary space. Nevertheless a communication of sorts between the surface of the neuron and the pericapillary space is possible through the narrow channels that course deviously in between the satellite cells. Perhaps the entire traffic of nutrients and metabolites to and from the neuron passes through these channels (44). It is also possible that exchange is effected by free diffusion through the satellite cell layer or by pinocytosis across its cytoplasm in vesicles, as suggested by De Robertis and Bennett (4). In the eighth nerve ganglion of the goldfish there are a few cells which are unmyelinated and are thus comparable to the dorsal root ganglion cells. Even in the loose myelin sheaths, the Schwann cell lamellae contain many membrane-limited vesicles which may serve as carriers of materials to and from the encapsulated perikaryon. There are, however, many neurons that are completely surrounded by a thick compact myelin sheath exhibiting no interruptions, no nodes of Ranvier, no devious channels, and no vesicles. Here the route of transfer is obscure. It must be assumed that nutrients and metabolites pass through the myelin sheath or that the exchange occurs at the nearest nodes of Ranvier, a distance of 30 to 100 μ from the center of the perikaryon.

In view of these considerations it might be expected that the fine structure of the heavily myelinated perikarya would appear different from that of unmyelinated or loosely myelinated cells. The present study, however, does not reveal any such differentiation. It is true that some of the largest and most heavily myelinated ganglion cells exhibit a distinctive cytoplasm characterized by fewer mitochondria, a paucity of ribonucleoprotein granules, more prominent neurofilaments, and a dispersed endoplasmic reticulum. But there are also many perikarya ensheathed in the same way in which the cytoplasm is not different from that of completely unmyelinated cells, except for the increased concentration of neurofilaments. If the distinctive cytoplasmic features of these large myelinated cells are related to the presence of the compact myelin sheath, they are not consistent. Other factors, such as the mean distance to the nearest capillary, may be involved.

The Myelin Sheath and Conduction: The role which either loose or compact myelin plays in the function of these ganglion cells has not been directly studied by neurophysiologists. Enough is known, however, about the physiological significance of myelin elsewhere to justify extrapolation to the eighth nerve ganglion. It is well known that myelinated nerve fibers have a much higher conduction velocity than unmyelinated ones. The generally accepted explanation for this difference (43) is that myelin acts as an insulator against the flow of ionic currents. Consequently current circuits set up by action potentials flow between adjacent nodes of Ranvier with little flow across the internodal axonal membrane. The result of this "saltatory conduction" is that long stretches of the axonal membrane are bypassed very rapidly.

In the eighth nerve ganglion the neurons are bipolar, and thus lie directly in the path of impulses coming from the peripheral receptors to the medulla. If the cell body were unmyelinated, conduction velocity would decline in this region. In contrast, in the dorsal root ganglion of mammals, where the neurons are unipolar, the problem is evaded by short-circuiting the impulses past the cell bodies.

Whether loose myelin sheaths function in the same way as compact myelin with respect to impulse propagation has not been determined. It is possible that loose myelin sheaths also enhance conduction velocity but not to the same degree as compact myelin. As a result there may be a spectrum of conduction velocities in the eighth nerve related to the type of sheath surrounding each perikaryal internode. Conduction time from end organ to medulla would be very short in those fibers ensheathed exclusively in compact myelin, somewhat longer in those fibers having one

loosely myelinated segment, and longest in those having an unmyelinated segment. (A comparable spectrum of conduction times is found in spinal nerves, and here different sensory modalities are carried by fibers conducting at different rates. Whether or not there is a corresponding segregation of sensory modalities in the eighth cranial nerve of the goldfish is unknown.) It must be admitted that since the length of the eighth nerve is only 2 to 3 mm., the differences in conduction time might be insignificant as compared with synaptic delays imposed elsewhere in the statico-acoustic system. Nevertheless temporal fractionation or spreading, even though small, might be of considerable importance in the integration of stimuli.

During a part of this study Dr. Rosenbluth was a postdoctoral fellow of the National Foundation.
Received for publication, February 2, 1961.

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