THE FINE STRUCTURAL ORGANIZATION OF NERVE FIBERS, SHEATHS, AND GLIAL CELLS IN THE PRAWN, *PALAEMONETES VULGARIS*

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ABSTRACT

In view of reports that the nerve fibers of the sea prawn conduct impulses more rapidly than other invertebrate nerves and look like myelinated vertebrate nerves in the light microscope, prawn nerve fibers were studied with the electron microscope. Their sheaths are found to have a consistent and unique structure that is unlike vertebrate myelin in four respects: (*1*) The sheath is composed of 10 to 50 thin (200- to 1000-A) layers or laminae; each lamina is a cellular process that contains cytoplasm and wraps concentrically around the axon. The laminae do not connect to form a spiral; in fact, no cytoplasmic continuity has been demonstrated among them. (*2*) Nuclei of sheath cells occur only in the innermost lamina of the sheath; thus, they lie between the sheath and the axon rather than outside the sheath as in vertebrate myelinated fibers. (*3*) In regions in which the structural integrity of the sheath is most prominent, radially oriented stacks of desmosomes are formed between adjacent laminae. (*4*) An ~200-A extracellular gap occurs around the axon and between the innermost sheath laminae, but it is separated from surrounding extracellular spaces by gap closure between the outer sheath laminae, as the membranes of adjacent laminae adhere to form external compound membranes (ECM's). Sheaths are interrupted periodically to form nodes, analogous to vertebrate nodes of Ranvier, where a new type of glial cell called the "nodal cell" loosely enmeshes the axon and intermittently forms tight junctions (ECM's) with it. This nodal cell, in turn, forms tight junctions with other glial cells which ramify widely within the cord, suggesting the possibility of functional axon-glia interaction.

INTRODUCTION

The nerve fibers of invertebrates display two kinds of structural modifications that may be related to their speed of conduction. First, in many invertebrate species there occur nerve fibers of such large diameter that they are called "giant fibers" (1). These giant fibers are generally considered to possess improved cable properties and increased speeds of conduction (1, 9). Second, in a few invertebrate species there occur heavily ensheathed nerve fibers. These fibers often appear similar in the light microscope to vertebrate myelinated axons with thick semicrystalline sheaths interrupted periodically by nodes. According to a theory widely favored, myelin sheaths act as "insulators" and alter the cable properties of axons to such an extent that rapid saltatory conduction can occur (12). The application of electron microscope techniques to the study of invertebrate nerve sheaths has substantiated some of the conclusions reached by light microscopy. McAlear

(14) studied nerve sheaths in the brain of a crab with the electron microscope and observed that they appear strikingly similar to vertebrate myelin sheaths. Nevertheless, Hama (8) studied the nerve sheaths of the earthworm with the electron microscope and found that they are structurally unlike vertebrate myelin, since they are composed of many loosely arranged cytoplasmic sheets and are not interrupted by nodes. He proposed, however, that these cytoplasmic sheets spiral around the axon, and thus are somewhat analogous to vertebrate myelin.

Holmes (10) presented a classical light microscope study of the nerve cord of the common sea prawn (*Palaemonetes vulgaris*), based on a study by Johnson (13) of the giant nerve fibers of this animal. Holmes concluded that many of the prawn nerve fibers were myelinated and differed from vertebrate nerves only "in having a nucleated inner sheath between the axon and the myelin layer and in having no Schwann nuclei between the myelin layer and the connective tissue." Previous to this study, Holmes, Pumphrey, and Young (11) found the speed of conduction in prawn nerve fibers with an average diameter of 35 μ to be about 20 m per sec, a speed greater than any recorded in fibers of a corresponding size in other crustaceans, but markedly less than that of the largest fibers of even the cold-blooded vertebrates.

We studied the nerve cord of the prawn with the electron microscope and report here that the sheaths of its nerve fibers are significantly different from vertebrate myelin. In spite of drastic differences in the appearance of prawn sheaths and vertebrate myelin, however, we present evidence for the existence of sufficient homologues in the structure to reconcile earlier investigators' reports of similarities in their function.

MATERIALS AND METHODS

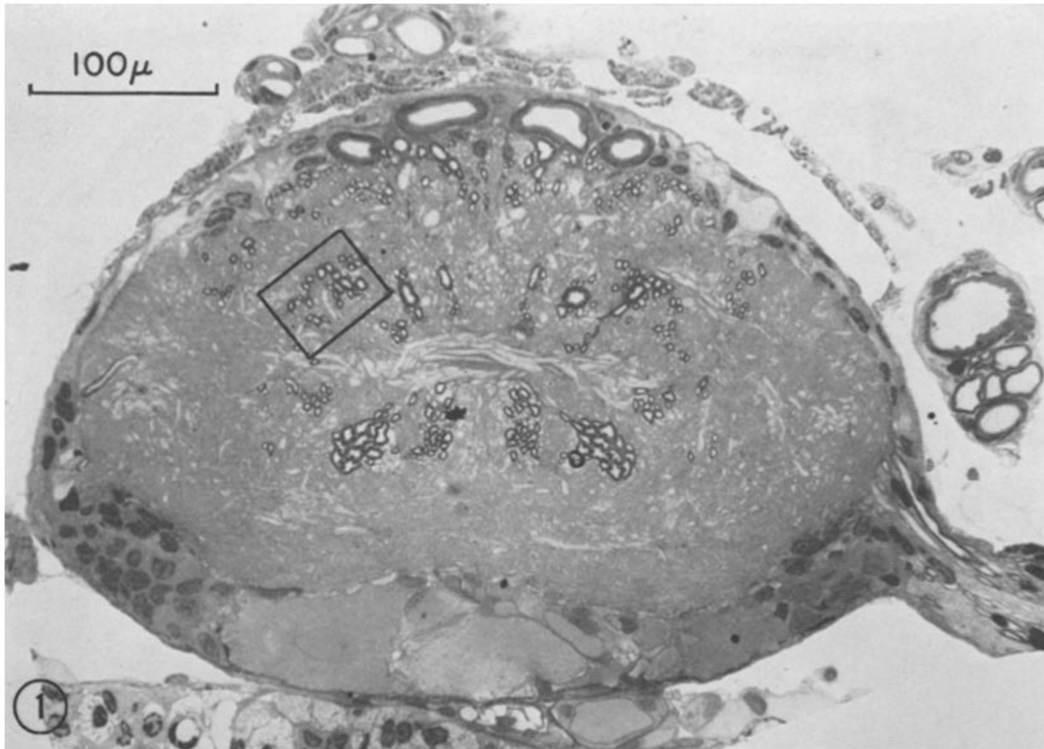
Small sea prawns (*Palaemonetes vulgaris*) were obtained from the Woods Hole Marine Biological Laboratory. They were kept alive in a small sea water aquarium and fed fish food. Each animal was anesthetized by cooling, and the abdominal portion of its ventral nerve cord was rapidly dissected out, still attached to the hard ventral exoskeleton which prevented the cord from shortening during fixation. This small block of tissue was immersed immediately in cold potassium permanganate fixative and fixed for 3 hr. The fixative consisted of *2 parts* of 2% potassium permanganate dissolved in filtered natural sea water and *1 part* of 0.1 M *s*-collidine buffer. Since this fixative decomposes within a few hr, the two parts were mixed just before use. The final concentration was 1.33% $KMnO_4$ and 0.06 M collidine buffer in sea water diluted to 800 milliosmols, pH 7.4. Other fixatives were applied to the prawn, and the results of their use are reported elsewhere (6). The tissues were dehydrated rapidly in acetone and embedded directly in Araldite according to a modified method of Glauert et al. as reported by Robertson (24). Silver sections were cut on a Sorvall MT-2 microtome with glass knives made from strips of Solex glass (Pittsburgh Plate Glass Company). Sections were collected on 100-mesh grids previously coated with a carbon film, stained with Venable and Coggeshall's lead citrate stain (28), and studied in a Siemens Elmiskop I equipped with a pointed filament and employing a 200-μ condensor aperture and a 50-μ objective aperture.

OBSERVATIONS

General

LIGHT MICROSCOPE: Fig. 1 is a light micrograph of the prawn ventral nerve cord sectioned in the region of an abdominal ganglion. It demonstrates that the cord contains a few hundred axons, the majority of which are small (1 to 5 μ in diameter) and have no sheaths visible in the light microscope. Some of the larger axons are surrounded by thick, dense sheaths that are interrupted intermittently by nodes. Four giant axons measuring up to 50 μ in diameter are present in the dorsal region of the cord. Their organization is similar to giant fiber systems in other invertebrates that innervate rapid escape muscles (1). These four giant axons are also surrounded by thick (10 to 20 μ) dense sheaths, but the sheaths are not observed to be interrupted by nodes. This is undoubtedly an important variation, but we did not study these four giant fibers in detail and will not describe them further here. The nerve cord is expanded in each abdominal segment by a ganglion. Here, many of the smaller nerve fibers terminate in a dense neuropil whereas larger ensheathed fibers pass through without apparent interruption. The neurons and their associated glial cells in these ganglia have been studied with the electron microscope (15).

ELECTRON MICROSCOPE FIXATIVES: Fig. 2 is a low power electron micrograph of a portion of the nerve cord. It demonstrates that the permanganate fixative used for this study reduces the cytoplasm, of both neural and glial elements, to a



The magnification markers indicate 1 μ unless otherwise stated.

FIGURE 1 Light micrograph of the prawn nerve cord at a segmental ganglion. Dorsally are four giant nerve fibers and numerous smaller fibers, some of which have densely staining sheaths. Ventrally are nerve cell bodies which supply segmental nerve fibers. Between these regions is a thick neuropil. On the right, one nerve root is sectioned longitudinally as it leaves the ganglion, and another root is found in cross-section to contain a giant fiber. The rectangle encloses an area equivalent to Fig. 2. × 250.

similar amorphous condition in which it contains only remnants of mitochondria and sparse elements of the endoplasmic reticulum. Better preservation of cytological detail can be achieved with the commonly used aldehyde and osmium tetroxide fixatives; but we have demonstrated elsewhere (6) that these fixatives consistently disrupt the membranes contained within the prawn nerve sheaths, producing artifactual vesicles and broken membranes. Also, these fixatives often produce extensive swelling or shrinking of different cell types. In contrast, the permanganate fixative employed in this study was chosen because it, alone, can achieve delicate preservation of membranes as well as excellent maintenance of the structural organization of the tissue.

EXTRACELLULAR SPACE: Extensive extracellular space surrounds all of the nerve fibers in the cord and ganglia. Holmes (10) demonstrated that this space is similar in its staining characteristics to the collagenous endoneural connective tissue sheath around vertebrate nerves. Its constituent fibers, however, are finer than those in vertebrate collagen; they stain only faintly with trichrome stains, but are impregnated sharply by a silver method specific for reticular fibers of vertebrate connective tissue. In support of Holmes' report of its similarity to connective tissue, we observe thin collagenlike fibers throughout the extracellular space. These are ~50 to 200A thick and lack readily demonstrable periodic striations. With permanganate fixation, however, these fibers are not well preserved (see only Fig. 16) and the extracellular space appears very dense and amorphous. This dense extracellular space (labeled * in the figures) completely surrounds all

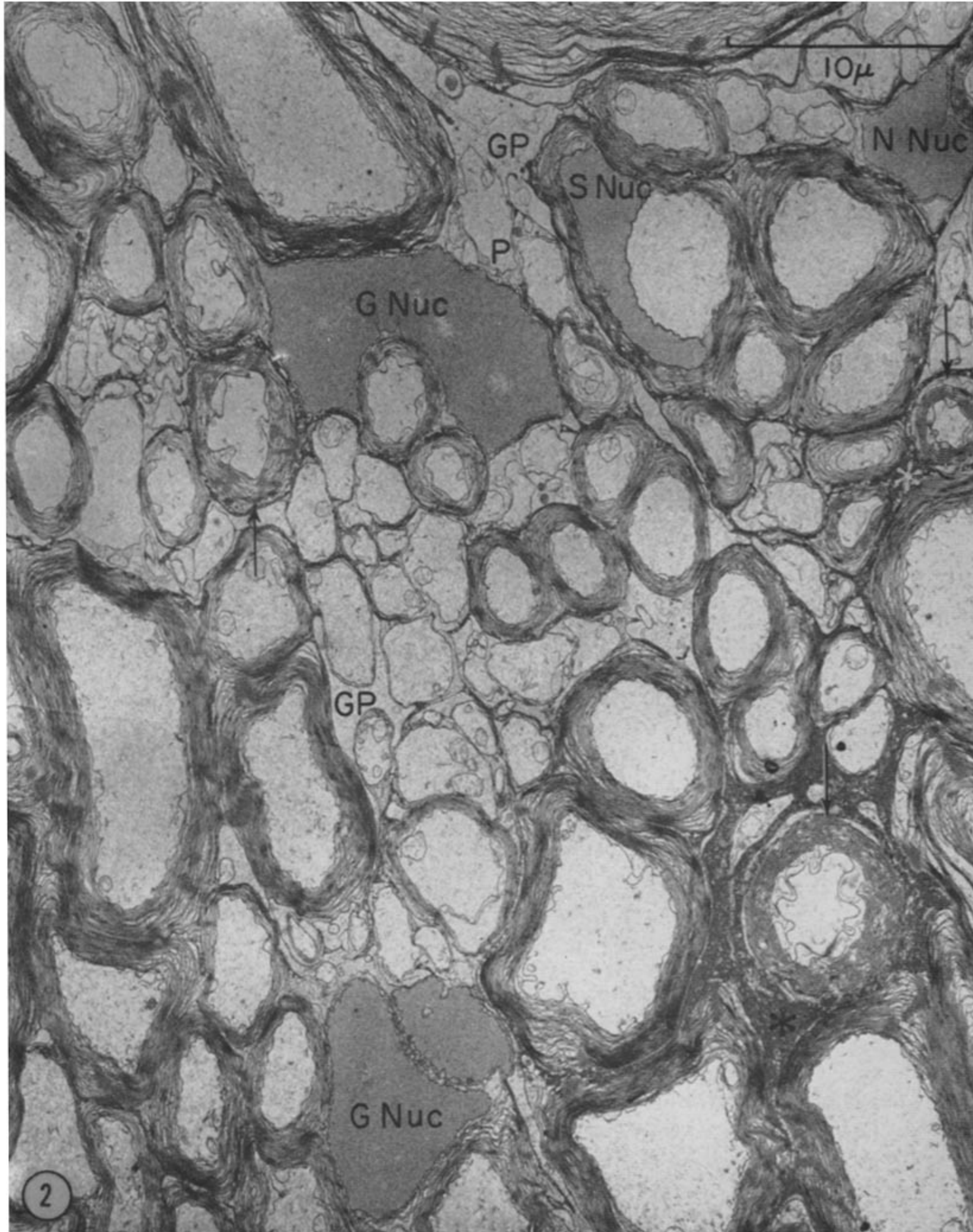


FIGURE 2 Low power view of a portion of the nerve cord equivalent to the area in the rectangle in Fig. 1, showing nerve fibers with simple and complex sheaths (see Figs. 4 and 5). Extracellular spaces (*) and nonspecific glial processes (*GP*) ramify among the nerve fibers. Three types of glial cell nuclei are present: nuclei of the nonspecific glial cells (*G Nuc*), a nucleus of a glial cell which forms the complex sheaths (*S Nuc*), and a nucleus of the glial cell which surrounds axons at nodes (*N Nuc*). At the arrows are three axons surrounded by complex sheaths undergoing structural changes characteristic of their nodal regions (see Fig. 13). × 3300.

nerve fibers and separates them from each other. Vascular spaces ramify throughout the cord and ganglia, but they appear always to be separated from the extracellular spaces by an intact layer of endothelial cells. The vessels have an internal basement membrane and contain an interesting variety of amoeboid-type cells, a pattern similar to that seen in other arthropod nerve cords (1).

GLIAL CELLS: Three different forms of glial cells are associated with the axons in the prawn nerve cord. (Several other forms of glial cells are found in neuropil and ganglia, but are not considered here.) Figs. 2 and 4 demonstrate that certain glial cells form cytoplasmic processes which compose the complex sheaths that surround many axons. Figs. 14 and 15 demonstrate that a distinct form of glial cell replaces the complex sheath at nodes and enmeshes the axon in a unique fashion. Figs. 2, 4, and 5 demonstrate that other nonspecific glial cells form processes that ramify throughout the cord, partially filling the extracellular interstices among nerve fibers and forming the simple sheaths around small axons. Each of these is described in more detail below.

Simple Nerve Sheaths

The majority of smaller axons have simple sheaths. These axons are enveloped by one or two glial sheets which vary in thickness, but are often as thin as 0.1 μ (Fig. 3). Each glial sheet wraps entirely around the axon to form a short mesaxon like a "seam." There is an ~200-A gap between the axon and the glial membranes and between the two component membranes of the mesaxon or seam. The glial sheet thus separates the axon from the surrounding extracellular spaces except at its open seam in which the extracellular space is continuous with the ~200 A axon-sheath gap. We cannot distinguish any cytoplasmic differences between the glial processes contributing to these simple sheaths and the nonspecific glial cell processes which ramify throughout the cord. Moreover, in many instances a single glial process can be shown to ramify among larger axons as well as envelop a small axon. Thus, it is possible that both these forms of glial processes originate from the same type of glial cell perikarya.

Complex Nerve Sheaths

Many of the axons, including most of the larger fibers, are surrounded by complex sheaths which ave a consistent and unique structure. Figs. 4 and 5 are cross-sections of the sheaths of typical small axons, Fig. 6 is a portion of the sheath of a larger axon, and Fig. 7 is a diagram summarizing the sheath structure. The prawn sheath is unlike the vertebrate Schwann cell myelin sheath of peripheral (19) and central (2, 18) nervous systems, in four basic respects.

1. CYTOPLASM IN SHEATH: The sheath is a laminated structure composed of many thin cellular sheets which extend along the axon and wrap concentrically around it. Each of these sheets or laminae is composed of a thin layer of cytoplasm with two limiting membranes. Their cytoplasm sometimes contains recognizable organelles like the mitochondrion in Fig. 6, and in tissue preserved with aldehyde fixatives displays many longitudinally oriented microtubules (6). The laminae vary in thickness between 2000 and 100 A, those in the inner regions of the sheath being generally thicker. In larger nerve fibers, the cytoplasm is frequently completely squeezed out of portions of the outer laminae, and the cytoplasmic dense strata of each lamina's membranes merge to form a major dense line analogous to that of vertebrate myelin (21). Thus, in such fibers the laminae in outer regions of the sheath are composed simply of two membranes adhering along their cytoplasmic surfaces. This is clearly seen only in tissue fixed with osmium tetroxide, since permanganate seems to alter the natural contact relationships of these membranes, as reported elsewhere (6).

Holmes (10) anticipated our observation of cytoplasm in this sheath by noting that when lipids are not preserved during fixation the prawn nerve fibers differ from vertebrate fibers by displaying a thick layer of "neurokeratin" protein between the axon and sheath that does not occur in vertebrate myelin. We can now interpret his observation to be a result of the increased proportions of cytoplasm in the laminae near the axon. The inclusion of cytoplasm in the sheath also explains why the prawn sheath displays weaker birefringence than the more compact vertebrate myelin (6, 11, 26).

This arrangement, within sheaths, of distinct layers of cytoplasm between membranes is observed occasionally in other invertebrate sheaths (1), notably the earthworm giant fiber sheaths (3, 8). It is very rarely observed in vertebrate nerves, although an analogous arrangement is the "loose myelin" surrounding certain bipolar nerve peri-

karya described by Rosenbluth (24) and by Rosenbluth and Palay (25).

2. CONCENTRIC STRUCTURE OF THE SHEATH: The most unique feature of the prawn sheath is that the laminae are clearly not arranged in a spiral of the kind commonly seen in vertebrate myelin (2, 16, 18, 19, 27). Each lamina appears instead to be a distinct cellular process isolated from laminae adjacent to it in the sheath. Figs. 4, 5, and 7 show that each lamina of the complex sheath is like the single glial sheet of a simple sheath, as it wraps entirely around the axon and meets itself to form a short mesaxon or seam. This produces a highly organized concentric arrangement of laminae. Moreover, in most sheaths the seams of alternate laminae are situated on the side of the axon opposite to the seams of adjacent laminae, thus creating a strikingly regular arrangement of the seams in each sheath (Figs. 5 and 8).

3. EXTRACELLULAR SPACES IN SHEATH: Adjacent sheath laminae are attached to each other in a characteristic manner. This is most clearly seen in Fig. 6. In the outer regions of the sheath, the laminae are so closely apposed that all extracellular space between them is squeezed out and the external dense strata of their adjacent unit membranes merge, forming a single intraperiod line characteristic of external compound membranes (ECM's) (20) and myelin sheaths (2, 18, 21). This is structurally similar to the zonula occludens of epithelial cells described by Farquhar and Palade (7). Robertson (19) showed that during myelination in vertebrate fibers the Schwann cell mesaxon forms a similar ECM as the gap between its component membranes becomes obliterated before it has fully elongated and the Schwann cytoplasm has been displaced. Hama (8) reported finding similar ECM's between adjacent cytoplasmic sheets in the sheaths surrounding earthworm giant fibers.

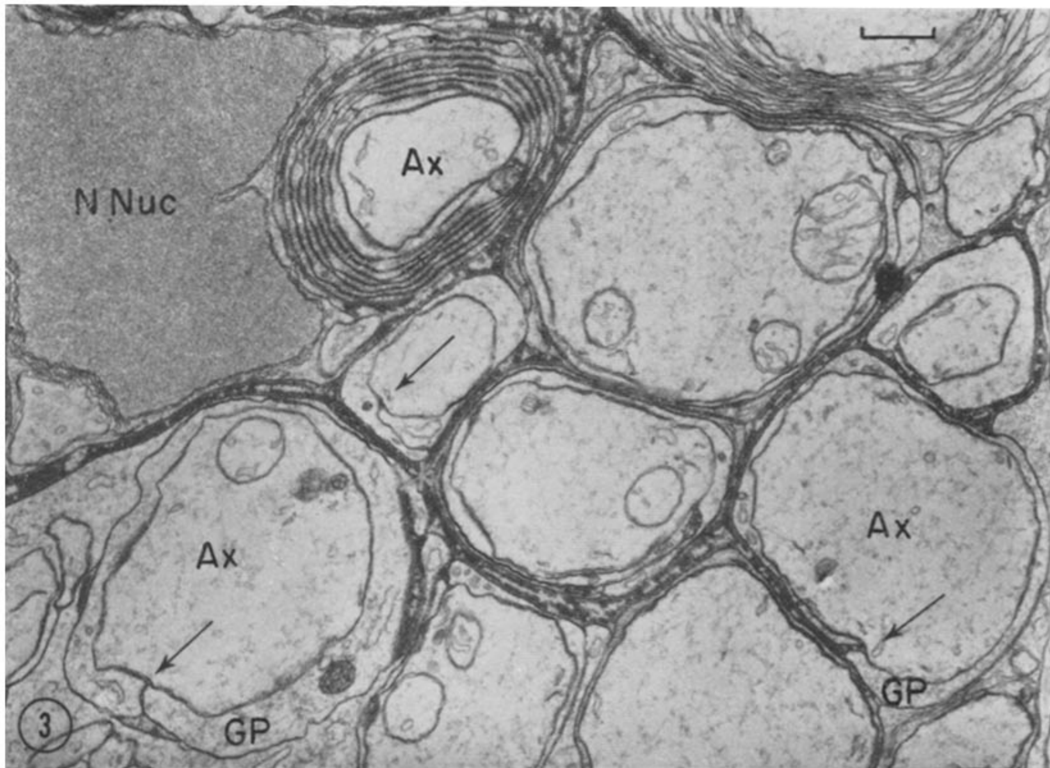
In the inner portions of the sheath, near the axon, the laminae are usually separated by a 100 to 200 A extracellular gap (Fig. 6). The innermost lamina is separated from the axon by the usual 100 to 200 A extracellular gap seen in axon-Schwann complexes in other invertebrate as well as vertebrate nerve fibers.

It is worthy of mention that in a few sheaths we observe bodies which distort their regular structure (Fig. 9). When one of these bodies occurs, it is found in the extracellular space between adjacent sheath laminae, pushing them apart and causing a bulge in the sheath. It is composed of several continuous circles of membrane, concentrically arranged to form a large 1- to 5-μ body. It is not clear whether or not there is cytoplasm contained within this body, but its appearance suggests that it represents the cross-section of several concentrically arranged tubes of cytoplasm. This body is considered further in the Discussion.

4. NUCLEI OF SHEATH: A fourth difference between prawn and vertebrate nerve sheaths was described by Holmes (10). He found that in prawn nerve fibers nuclei frequently occur between the axon and sheath. He called them "inner sheath nuclei" in order to distinguish them from the Schwann cell nuclei of vertebrates which occur only outside the sheath. We find that these inner sheath nuclei lie in the innermost lamina of the

FIGURE 3 A group of nerve fibers with simple sheaths. The axons (*Ax*) are enveloped by one or two glial sheets (*GP*) which separate them from the surrounding extracellular spaces (*) except at the mesaxons or seams of the glial sheets (arrows) where the extracellular space is continuous with the ~200-A axon-sheath gap. At the upper left is an axon surrounded by a complex sheath undergoing changes characteristic of its nodal region (see Fig. 13). The adjacent nucleus (*N Nuc*) belongs to a unique glial cell found at nodes (see Fig. 14). × 9500.

FIGURE 4 A group of nerve fibers with complex sheaths. The sheaths are composed of many concentrically arranged glial sheets or laminae, each of which is similar to the single glial sheet surrounding nerves with simple sheaths (Fig. 3). Note that the seams of alternate laminae are regularly aligned (arrows). Surrounding these fibers are extracellular spaces (*) within which glial processes (*GP*) ramify and become closely apposed to each other (at circles). Higher magnification of these regions demonstrates that external compound membranes (ECM's) or tight junctions are formed between the processes. × 12,500.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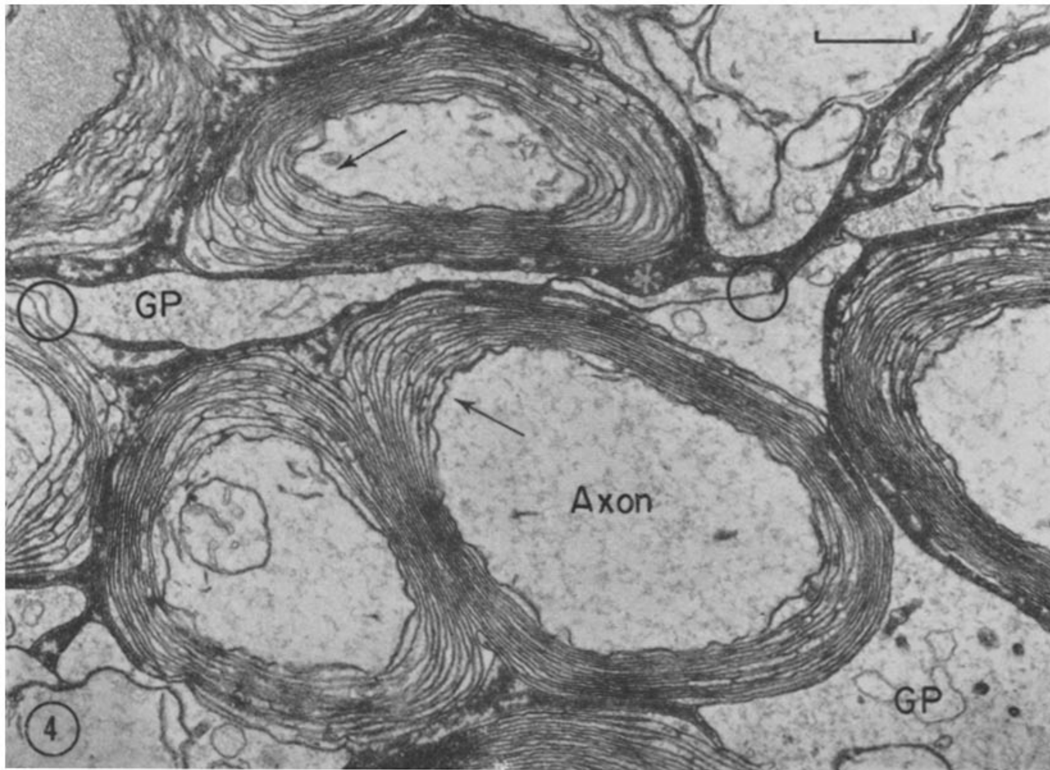
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sheath (Figs. 7 and 10). Examination of serial sections through the region of the sheath nucleus, including Fig. 10, indicates that the nucleus greatly distorts the normal organization of the axon and sheath. In this region, the axon decreases in diameter drastically (to 0.1 μ in Fig. 10) and the sheath expands to two or three times its normal diameter, as some of its laminae open up along their seams and fail to completely encircle the axon and nucleus.

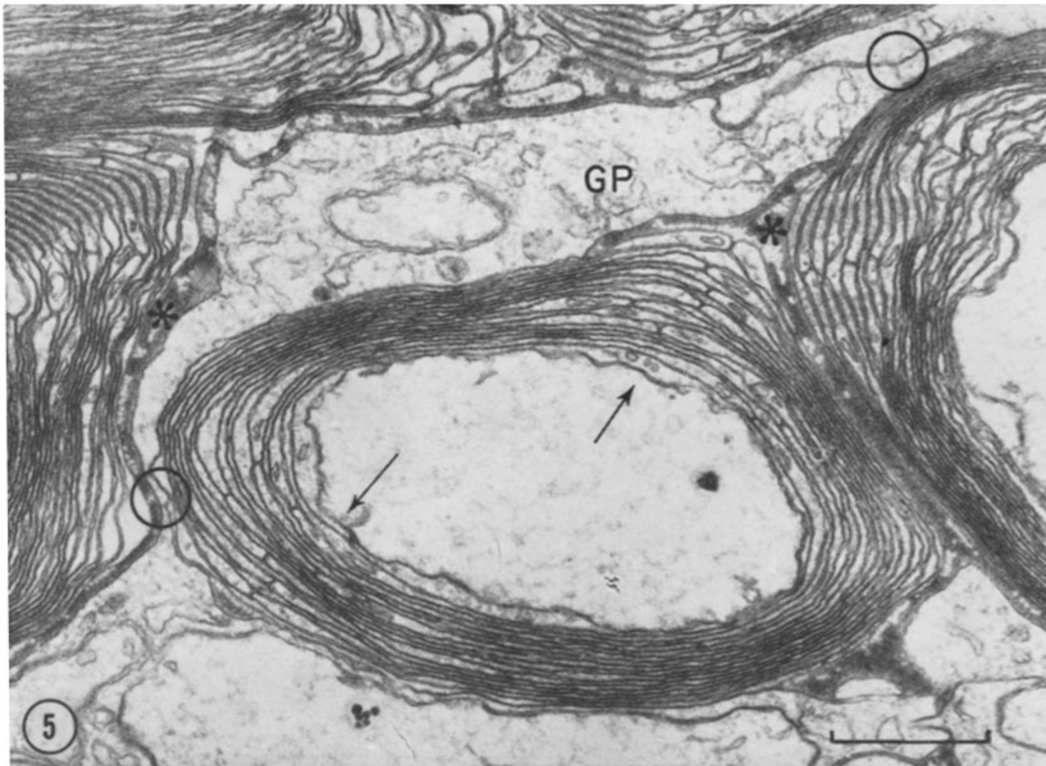
Many other nuclei are found outside nerve sheaths (Figs. 2 and 10); but the processes which lead away from their perikarya either ramify loosely among the nerve fibers or form simple sheaths around small axons. They do not contribute to the complex nerve sheaths. Thus two distinct glial cell forms are distinguishable in these figures: the nonspecific glial cell with its nucleus occurring between nerve fibers (i.e., outside of nerve sheaths), and the sheath cell with its nucleus occurring in the innermost lamina of the complex sheath. We call the nucleus of the sheath cell simply the "sheath nucleus" rather than "inner sheath nucleus" which incorrectly suggests that there is an outer sheath nucleus supplying the complex sheath.

ATTACHMENT ZONES IN SHEATH: We reported earlier (5) that occurring in these sheaths are intermittent areas of differentiation which pass radially across many laminae (Figs. 6, 8, and 11). These areas resemble stacks of 0.5 μ in diameter desmosomes or maculae adherentes (7) and thus are termed "radial attachment zones" (RAZ's). In these RAZ's, adjacent laminae are separated by a very constant ~150-A gap containing a small amount of densely staining material, their membranes are very distinct and regular, and their cytoplasm is more dense. Two observations suggest that the radial attachment zones function to provide mechanical strength and rigidity to the sheath, as desmosomes are thought to maintain cell-to-cell structural relationships. First, in spite of extensive damage to the sheaths during various dissection and fixation procedures, the radial attachment zones and the membranes within them remain intact long after all other sheath structures have been destroyed (5, 6). Second, the proportion of sheath laminae involved in this differentiation increases dramatically in areas in which the maintenance of structure would seem crucial, as near nodes (Fig. 13). The nodes are described below.

Similar areas of differentiation are observed in the nerve sheaths of several unrelated phyla and in all cases are thought to provide mechanical strength to the sheaths. Hama (8) observed similar attachment zones among the loose sheath laminae of earthworm giant fibers. Rosenbluth and Palay (25) described attachment zones between the loose laminae of Schwann cell processes that surround bipolar neurons in the eighth cranial ganglion of the goldfish. Rosenbluth (24) found radially oriented attachment zones in the loose myelin surrounding bipolar nerve cell bodies in the spiral and vestibular ganglia of the adult rat. At nodes in this myelin, he also observed desmosomes between the paranodal terminal loops of Schwann cell cytoplasm. Thus it appears generally true that if a nerve sheath is composed of a loose array of cytoplasmic processes, it may contain specialized

FIGURE 5 A typical small nerve fiber with a complex sheath composed of 16 laminae of glial sheets, showing the concentric and discontinuous nature of the laminae and the regular alignment of the seams of alternate laminae (arrows). A large glial process (*GP*) adjacent to this fiber partially fills the extracellular spaces (*) and forms external compound membranes (ECM's) or tight junctions with the sheath and with an adjacent glial process (at circles). × 21,000.

FIGURE 6 A portion of the sheath of a larger nerve fiber, demonstrating the inclusion of a large mitochondrion within one lamina, and cytoplasmic remnants within others. The axon and sheath are separated by an ~200-A gap. Between the inner laminae there is often a 100- to 200-A extracellular gap (*GAP*), but between outer laminae this gap is largely obliterated and external compound membranes (ECM) are formed. Laminae near the axon tend to contain more cytoplasm than outer laminae. In one portion of the sheath (between arrows) a small radial attachment zone is formed among several laminae (see Fig. 11). × 50,000.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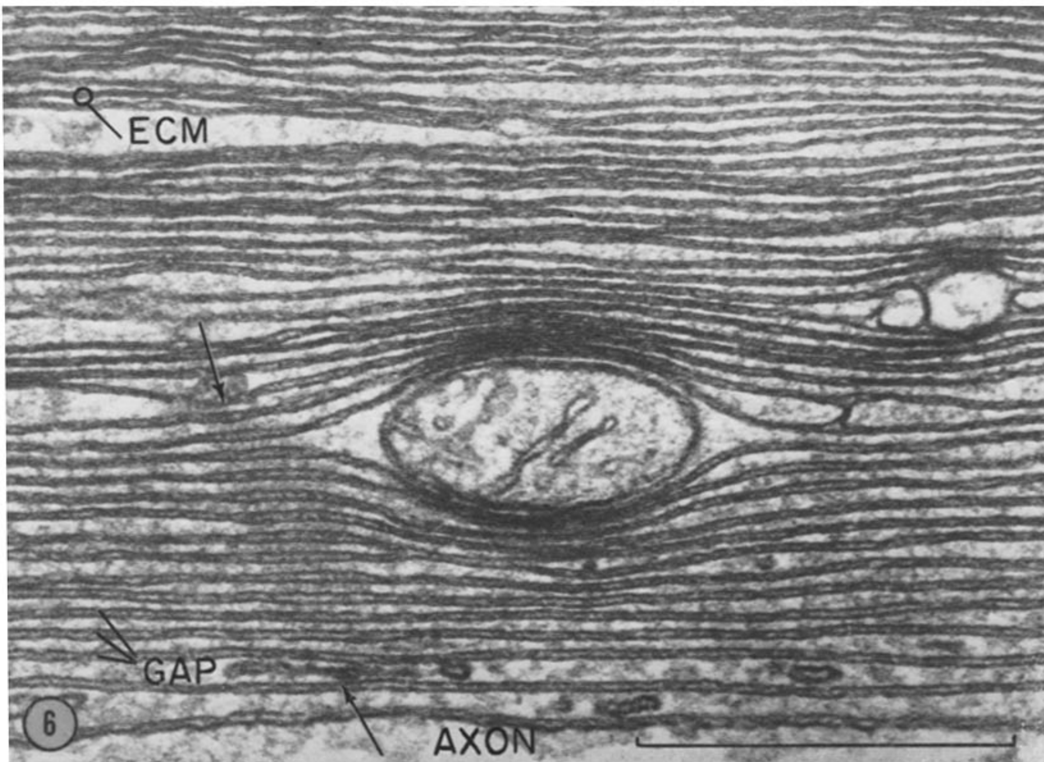
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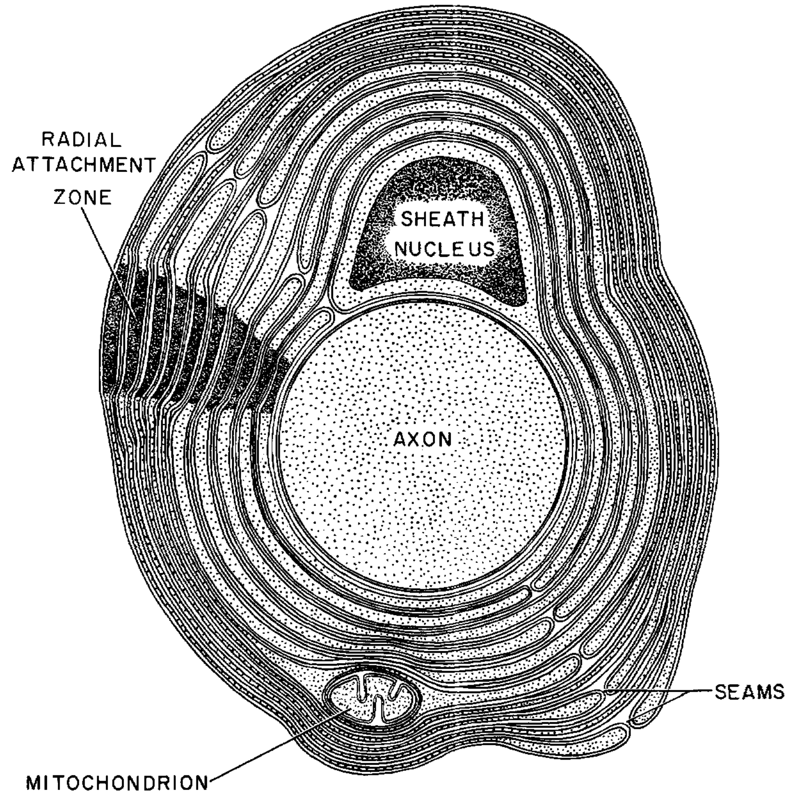


FIGURE 7 A diagram of a cross-section of a prawn nerve fiber. Its sheath is composed of many laminae, each an isolated cellular process that contains cytoplasm and wraps concentrically around the axon to form a simple mesaxon or seam. The seams are arranged in a highly organized manner, as the seams of alternate laminae are in close register and are on the side of the axon opposite the seams of adjacent laminae. A radial attachment zone, analogous to a stack of desmosomes, is formed between adjacent laminae in one region. The nucleus of the cell that forms the sheath (shown here schematically and much smaller than actual size) is located in the innermost lamina. Extracellular spaces occur around the axon and among the inner sheath laminae only.

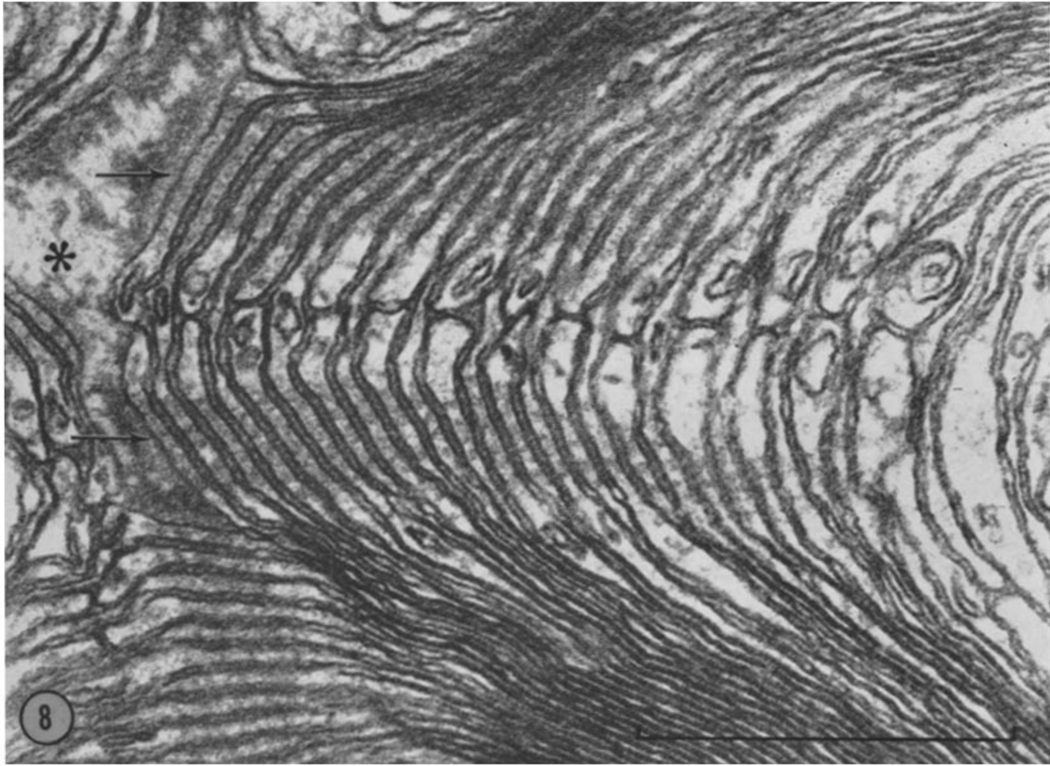
regions that look and function like a stack of desmosomes.

NODES

The complex sheaths of prawn nerve fibers are interrupted at intervals by nodes. Holmes (10) observed these nodes and reported that they appear similar in the light microscope to vertebrate nodes of Ranvier. By studying serial sections, he could conclude only that the internodal distances vary considerably, even along a single fiber (11). We studied these nodes with the electron micro-

FIGURE 8 A portion of a nerve sheath, showing the characteristic alignment of the mesaxons or seams of alternate laminae. On both sides of the seams are found radial attachment zones (arrows) (see Fig. 11). Extracellular space (*) is present around the sheath. × 50,000.

FIGURE 9 A nerve fiber (*Ax*) with its sheath expanded by the sheath cell nucleus (*S Nuc*) (see Fig. 10). The sheath is distorted by a body (arrow) composed of many concentrically arranged rings of membranes, which is discussed in the text. × 20,000.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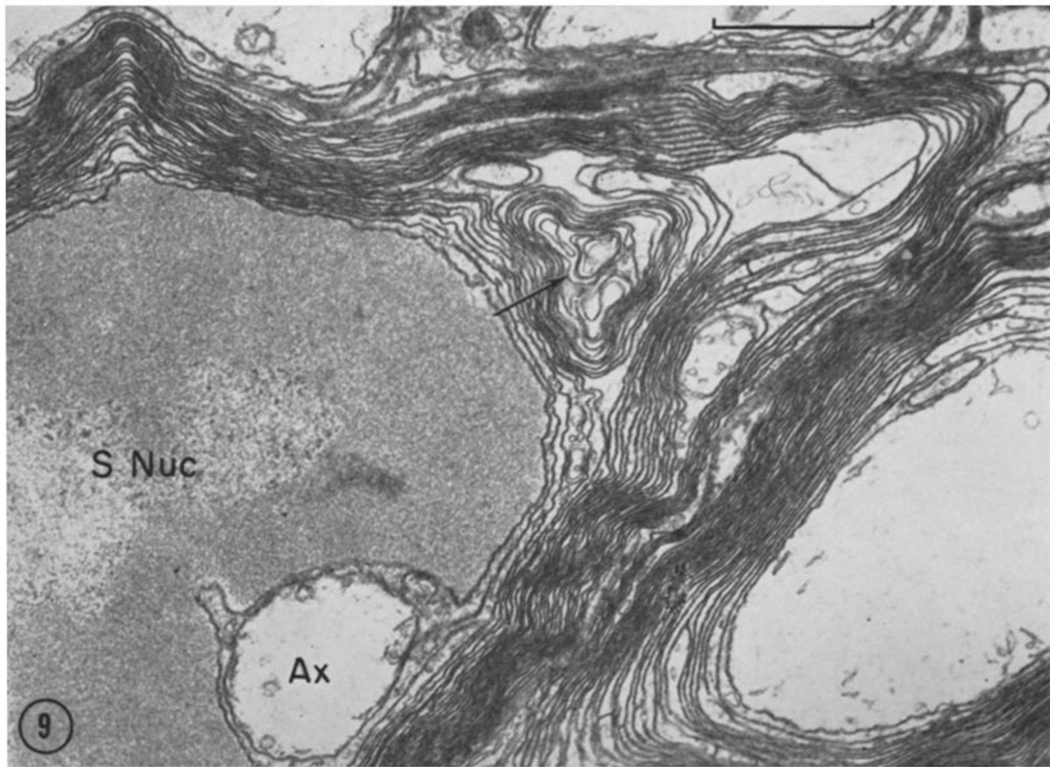
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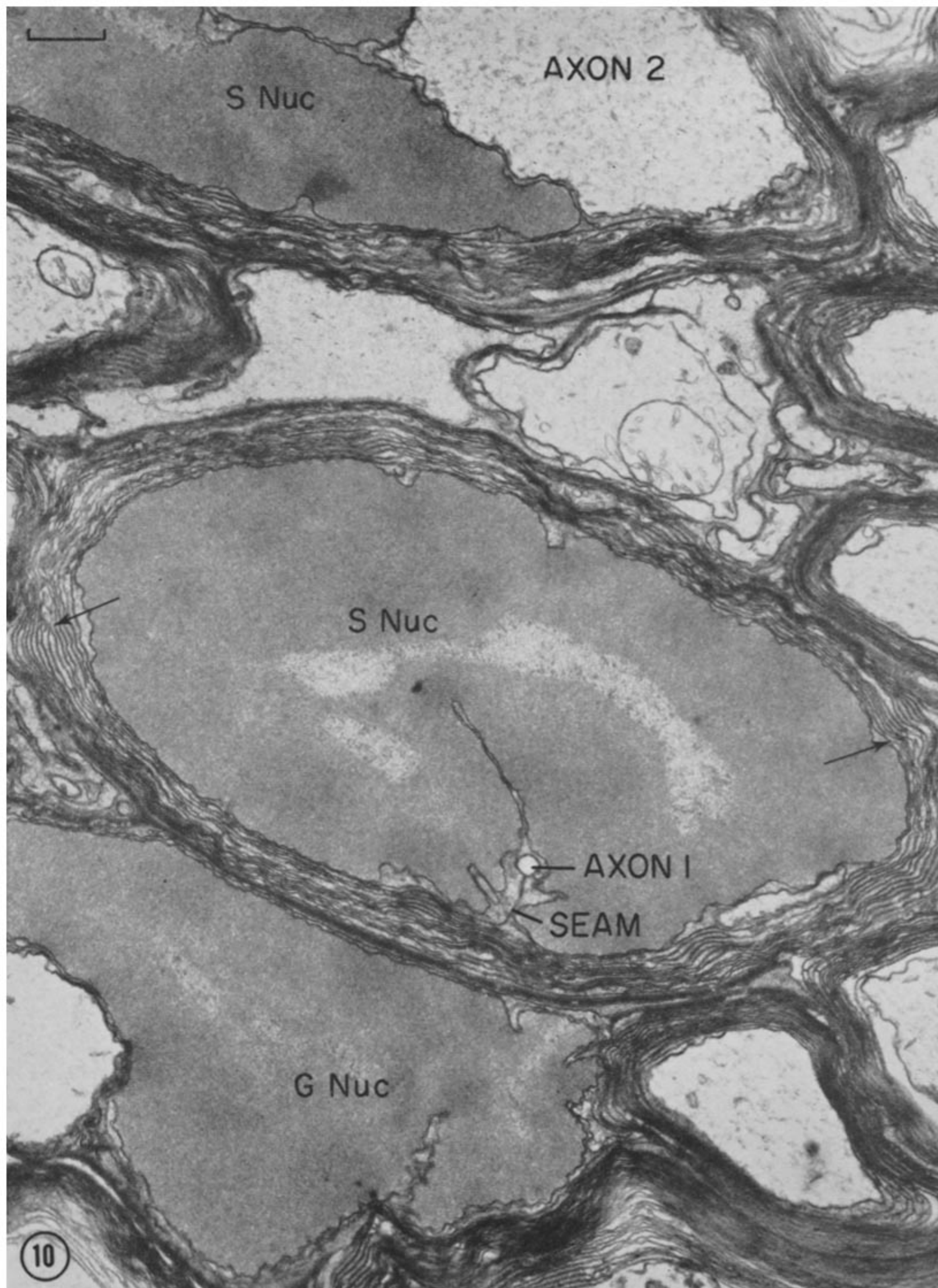


FIGURE 10 A group of nerve fibers and three associated glial cell nuclei. Two of the nuclei (*S Nuc*) are nuclei of the specialized glial cells forming the sheaths around axons *1* and *2*. They are located in the innermost lamina of their respective sheaths (the mesaxon or seam of the innermost sheath lamina around axon *1* is indicated). The third nucleus (*G Nuc*) is the nucleus of a nonspecific glial cell, distinguishable from the others because it is located outside of any sheaths. The sheath nucleus of axon *1* has greatly swollen its sheath, causing laminae to open along their seams and fail to encompass it (at arrows), and has compressed the axon to a surprisingly small diameter (0.1 μ). $\times$ 11,500.

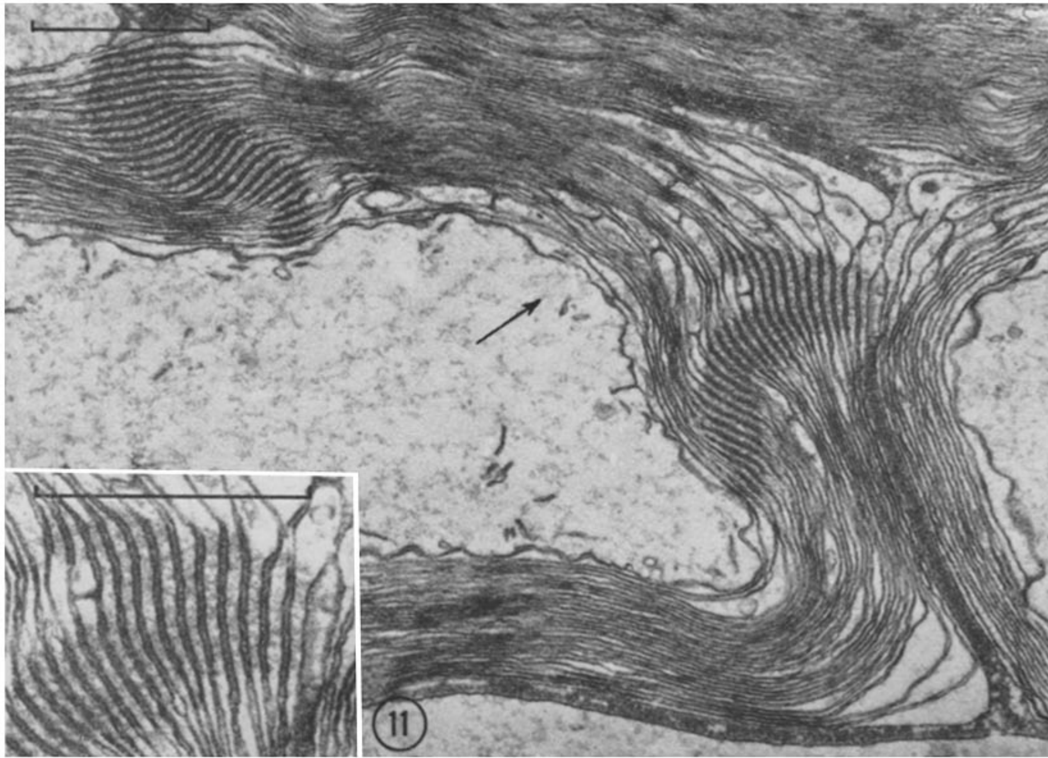


FIGURE 11 A portion of a nerve fiber whose sheath contains two attachment zones passing radially through all laminae of the sheath. Most prominent in these regions is a dense precipitate within the cytoplasm and a stabilization of the laminar membranes (see inset and Figs. 6 and 8). Seams of alternate sheath laminae are found in approximate orientation with the arrow. × 23,000; inset, × 36,000.

scope and report that they differ from vertebrate nodes in several respects. Figs. 13 to 15 are serial cross-sections and Figs. 16 and 17 are longitudinal-sections through the nodal regions of individual nerve fibers. Our observations are summarized diagrammatically in Fig. 12.

SHEATH TERMINATION: By following a single fiber throughout multiple serial sections it can be shown that as the nodal region of a nerve sheath is approached, several radial attachment zones (RAZ's) appear in the sheath and progressively expand to include greater portions of its laminae until practically the entire circumference of the sheath is held rigidly within an attachment zone (Fig. 13). At the node, each lamina terminates independently against the axon, beginning with the innermost lamina and progressing outward (Figs. 16 and 17). In this manner, the laminae overlap and the outer ones come nearest to the node (Fig. 12). Figs. 13 and 14 are cross-sections near the node which demonstrate that the laminae do not terminate evenly along the axon, but reach nearer the node on the sides opposite their seams. Thus in serial cross-sections, the laminae first appear to open up along their seams and then to slowly shrink, covering less and less of the axon circumference until they finally terminate.

As each lamina terminates against the axon, the ~200-A extracellular space which normally separates the axon from its sheath is altered. In longitudinal-sections this space contains several small ~20-A wide dense bars which pass perpendicularly between the axon and sheath membranes and appear to connect them (Fig. 17, inset). In cross-sections this space appears to contain an amorphous dense material that manifests itself (in Fig. 14, insets) as an indistinct dense line running between the unit membranes. A combination of these views suggests that this extracellular space is occupied by

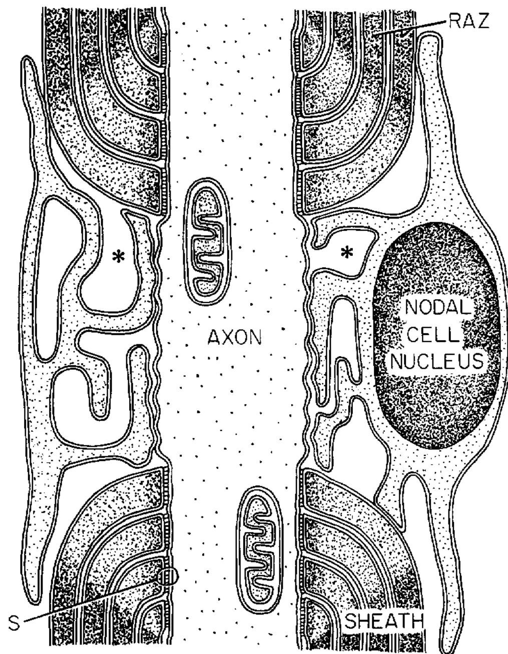


FIGURE 12 Diagram of a longitudinal-section of a node in the prawn nerve sheath. Near the nodal region radial attachment zones (*RAZ*) occur among laminae of the sheath. Laminae form septate structures (*S*) as they terminate near the axon. At the node the axon surface becomes highly rugose, and the axon is loosely enmeshed by a characteristic type of glial cell called the "nodal cell." The nodal cell processes separate the axon from the surrounding extracellular spaces (*) over much of its surface and intermittently adhere to the axon and sheath by forming external compound membranes (ECM's) or tight junctions.

several dense ridges which run circumferentially around the axon and connect axon and sheath cell membranes. Furthermore, in these regions the axon and sheath membranes are very distinct and the sheath cytoplasm stains somewhat more densely. Thus at a node each sheath lamina terminates against the axon in a structure which looks similar to the radial attachment zones (RAZ's) seen among sheath laminae, and similar also to the septate desmosomes seen in Hydra by Wood (29) (see Fig. 12). This is unlike nodes of Ranvier in the vertebrate peripheral or central nervous system, since Robertson (22) and Peters (18) showed that where a myelin sheath terminates at a node, the extracellular space between the axon and Schwann cell is completely obliterated as their membranes form a tight junction.

NODAL AXON: At the node, the axon's structure is markedly altered (Figs. 15 to 17). Its surface becomes highly rugose with numerous small infoldings or deeper invaginations which increase its surface area considerably. Its membrane becomes distinctly more dense and thus appears to be somewhat thickened ($\sim$120 A). The axon contains increased amounts of unrecognizable vesicular and membranous material and often a large mitochondrion. We do not observe a specific clustering of vesicles at these nodes like that observed in some vertebrate nodes of Ranvier by Robertson (22).

GLIAL CELLS AT NODE: Even though the axon's complex sheath terminates as described to form nodes, the axon is not left entirely naked and exposed directly to extracellular space. Replacing the complex sheath at every node is a unique type of glial cell which surrounds the axon (Figs. 14 and 15). Fig. 14 shows that the perikaryon of this "nodal cell" lies a few microns from the axon and is practically filled by its nucleus. Figs. 15 and 16 show that the sparse cytoplasm of the nodal cell forms many thin pseudopodia or processes that coat much of the axon's surface and fill in many of its surface irregularities with a single layer of cytoplasm. This thin cytoplasmic coating thus separates the axon, over most of its surface area, from the extensive extracellular spaces which occur at the node. Interestingly, the pseudopodia of the nodal cell often come into intimate association with the axon, as their membranes adhere at many points to form external compound membranes (ECM's) or tight junctions (Fig. 15, right inset). The structure and relations of this nodal cell with the axon are shown diagrammatically in Fig. 12.

The nodal cell appears to send processes only toward the one axon which it engulfs. However, processes of the nonspecific glial cells which ramify throughout the cord and surround small axons often abut on the nodal cell body. Here also an intimate attachment appears to be formed between the two glial cell types, as their membranes adhere to each other to form ECM's or tight junctions. The functions of these nodal cells is unknown, but may be related to their intimate contacts with both the axon at the node and the glial cells that ramify widely throughout the cord and surround other axons. These contacts or tight junctions are thought to offer low resistance to intercellular ion flow and thus to allow direct electrotonic spread of membrane depolarizations between cells. They have been described in a

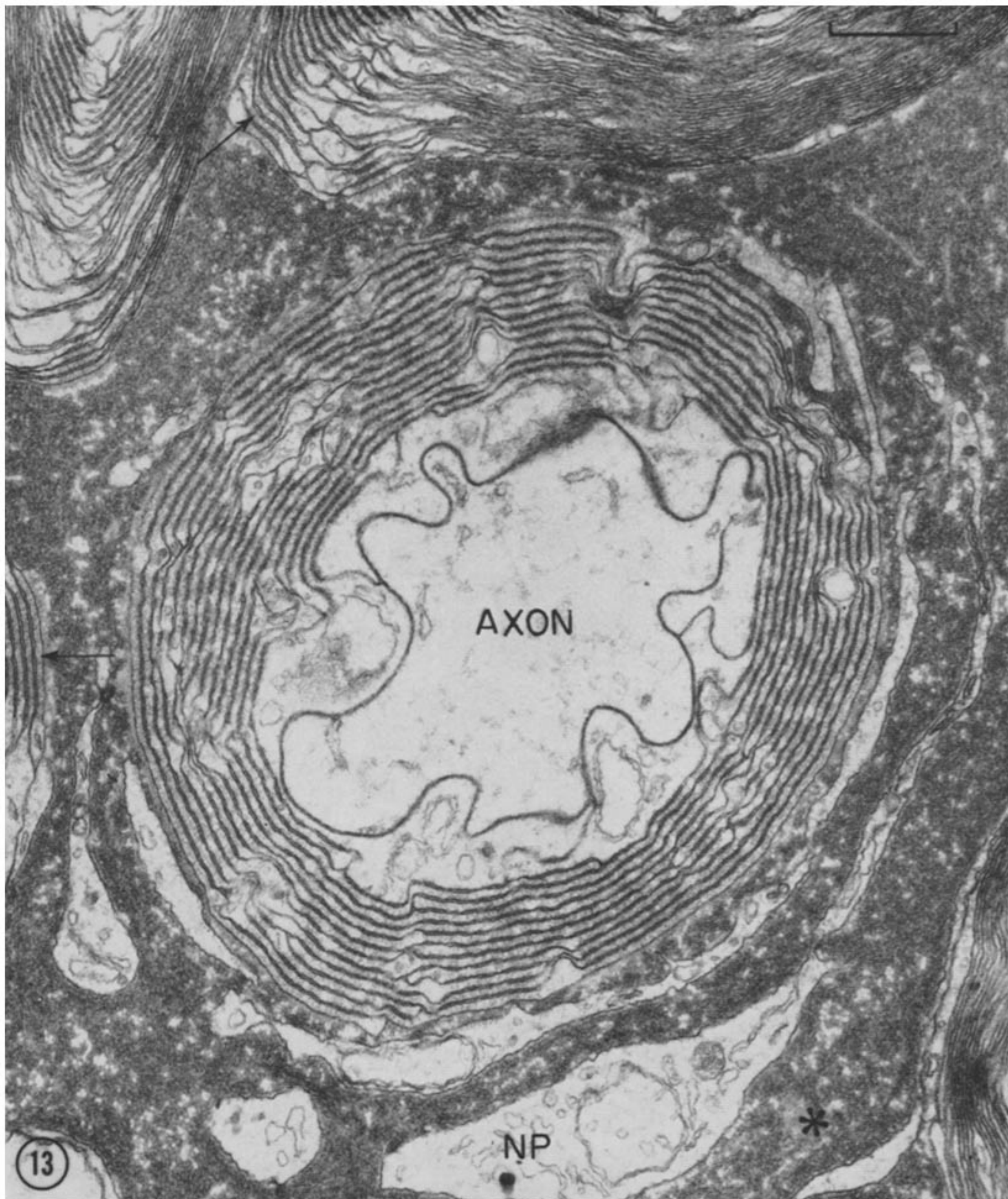


FIGURE 13 A nerve fiber sectioned near the termination of its sheath at a node. Around the entire circumference of the sheath the laminae are held rigidly in a large radial attachment zone that is structurally similar to the small attachment zones in the sheaths of adjacent fibers (arrows). Some laminae do not extend entirely around the axon, but appear to be shrinking or "unwrapping" in the process of termination. The sheath is surrounded by extensive extracellular space (*) in which are found glial processes of the nodal cell (*NP*). A similar fiber is seen in Fig. 3. × 17,000.

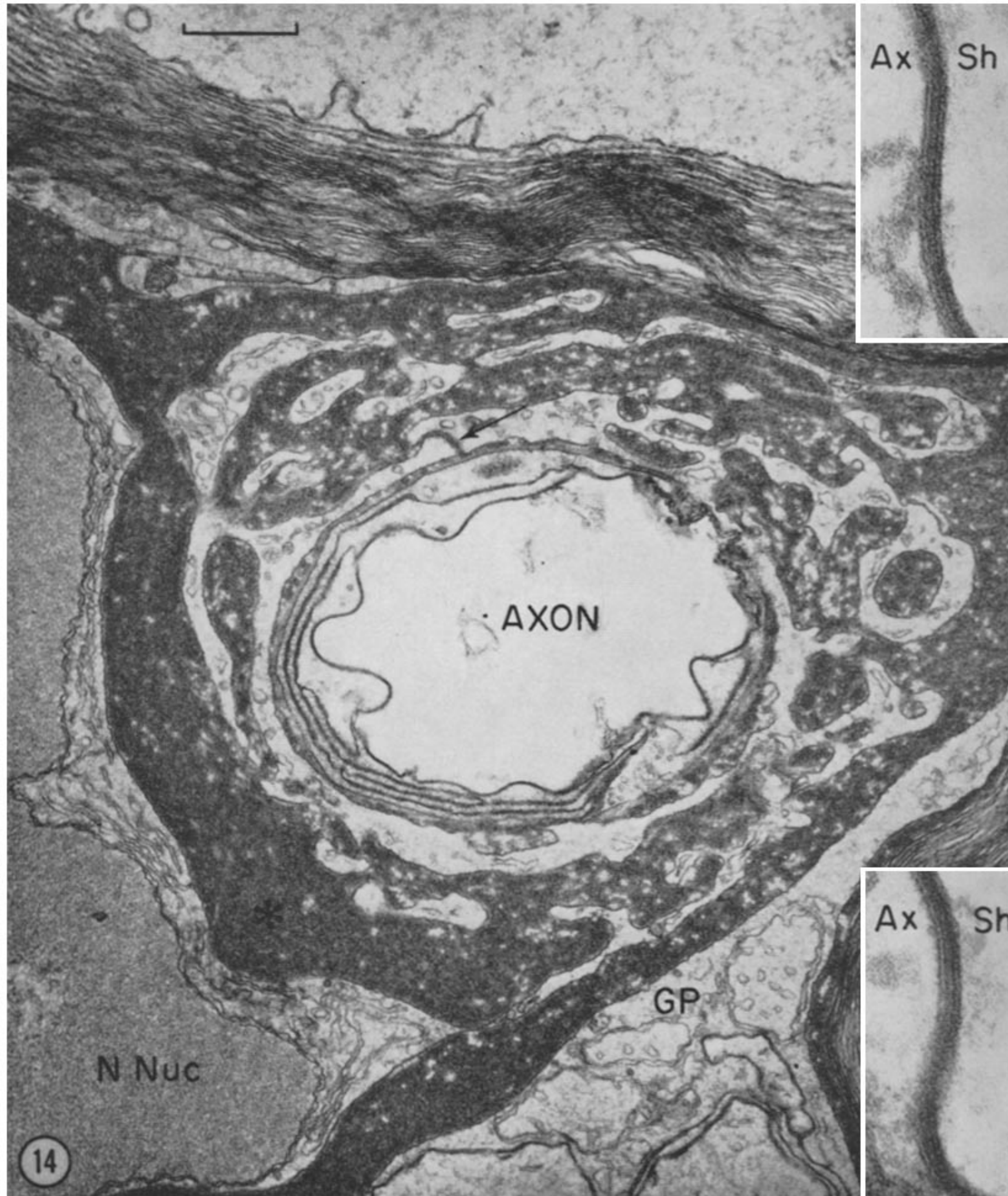


FIGURE 14 Nerve fiber sectioned near the termination of the last sheath laminae at a node. On the left side of the fiber, four sheath laminae partially surround the axon. Insets show that at the termination of a sheath lamina (*Sh*) against the axon (*Ax*) their trilaminar unit membranes are separated by an ~75-A space containing an amorphous dense material appearing in places as an indistinct dense line. On the right side of the fiber, no sheath laminae occur and the axon surface is differentiated into the rugose pattern characteristic of nodes. Replacing the sheath in this region is the nodal cell. Portions of its perikaryon containing sparse elements of endoplasmic reticulum and a large nucleus (*N Nuc*) are present. Its cytoplasm loosely enmeshes the axon and ramifies within the extensive extracellular spaces (*) around the axon. A desmosome appears formed at one point (arrow). Fig. 15 is a later serial section of this fiber. *GP*, nonspecific glial processes. × 15,500; insets, × 100,000.

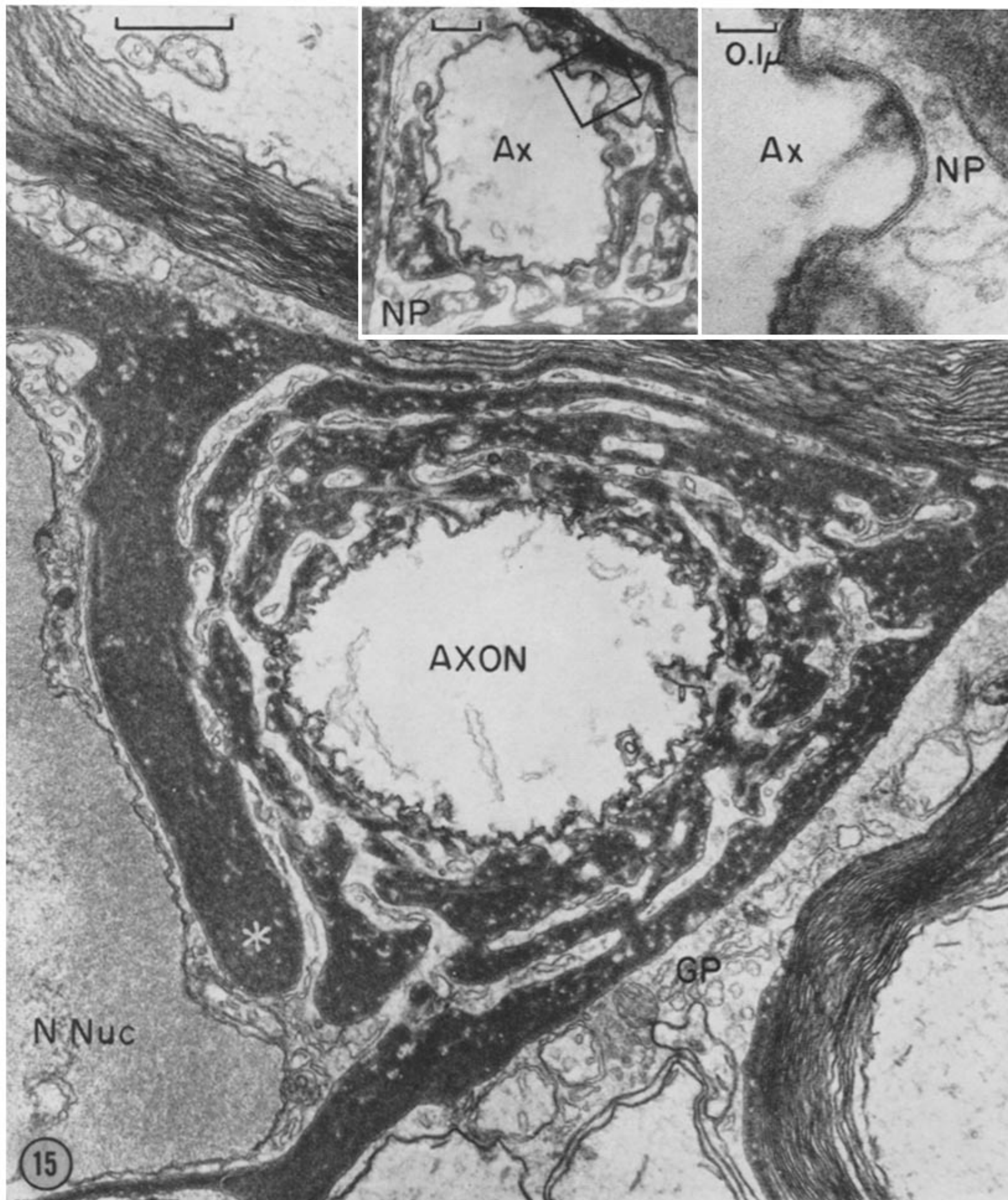


FIGURE 15 The same nerve fiber as in Fig. 14, sectioned within the node. The axon surface is extremely rugose. Its many surface irregularities are filled or coated by slender cytoplasmic processes that closely approach the axon and separate it from extracellular spaces (*). These processes emanate from the nodal cell, whose perikaryon and nucleus are present. The left inset is another fiber (*Ax*) at a nodal region where it is coated by nodal cell processes (*NP*). The area in the rectangle, magnified in the right inset, includes a portion of the axon (*Ax*) and a nodal cell process (*NP*) and shows that their unit membranes have adhered to form an external compound membrane (ECM) or tight junction. *GP*, nonspecific glial processes. × 15,500; left inset, × 6000; right inset, × 75,000.

variety of nerves and muscles, from invertebrates as well as vertebrates, in which "electrical" transmission of nerve impulses is thought to occur (23, 4). Thus, the nodal cell in the prawn may provide an excellent system for the direct observation of possible glial-mediated, axon-to-axon electrical interactions.

The relation of the nodal cell to the nodal axon in the prawn is similar to the relation of glial cells to nodes of Ranvier in the central nervous system of amphibians (16–18) and mammals (27, 2). In these vertebrates it is reported that the central nervous system nodes are unlike peripheral system nodes in which Robertson (22) showed that the axons are surrounded by a collar of minute, interdigitating, fingerlike processes of the two Schwann cells. Instead, at central nervous system nodes the axons are denuded of sheath cell processes and basement laminae, and are closely apposed to adjacent myelinated or unmyelinated axons and glial cells. Recently Metuzals (17) reported the close apposition of nodal axons with what appeared to be dendrites in the frog spinal cord and proposed that a tight junction was formed between the axon and dendrite. The observation of distinct tight junctions at prawn nodes in which a similar axon-glial apposition occurs may contribute to understanding the occurrence and function of such appositions in vertebrate central nervous system nodes.

DISCUSSION

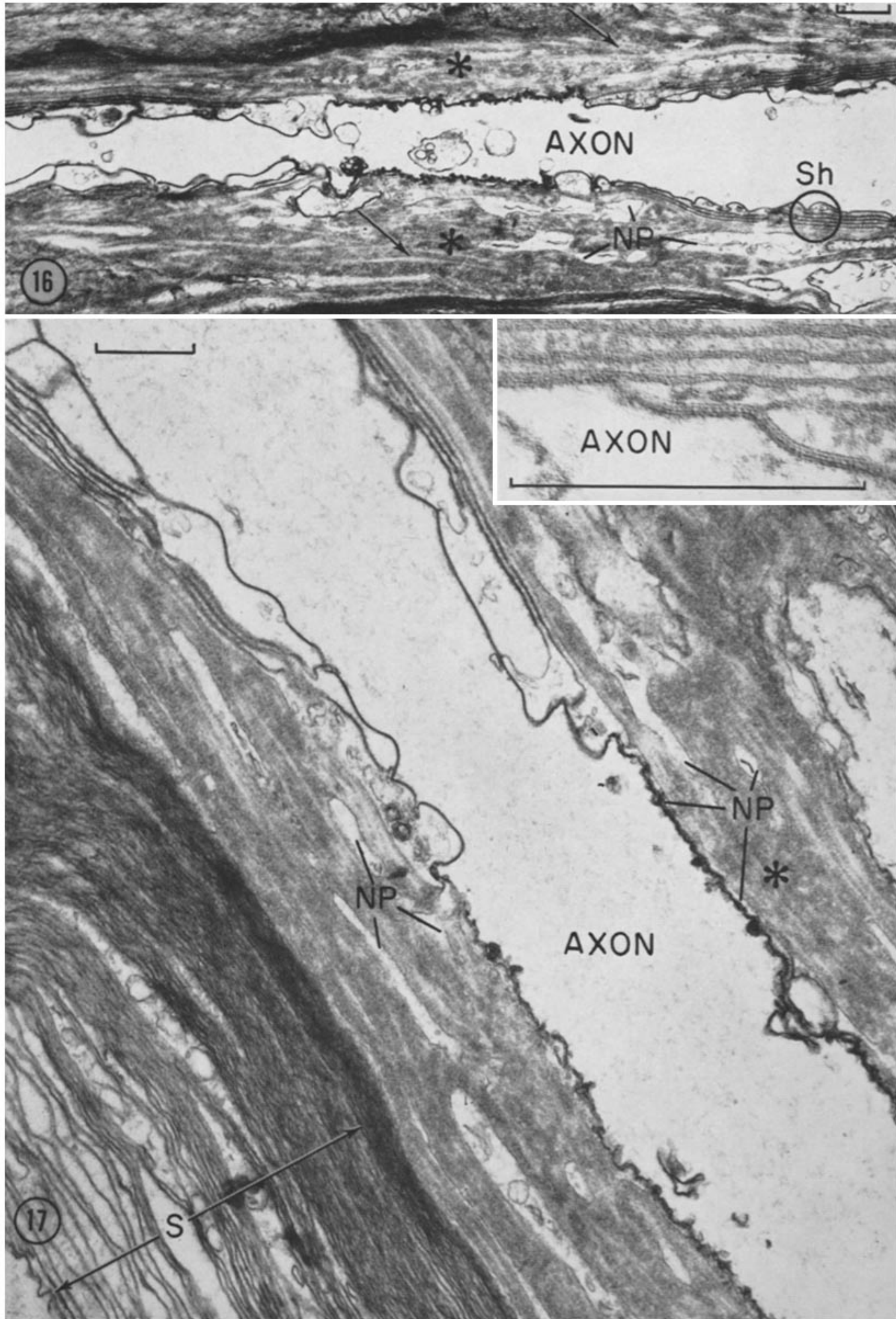
Sheath Structure

Our observations leave an important aspect of the sheath structure unexplained: how can this sheath be composed of cytoplasmic laminae that always appear to be isolated from each other? We have tentatively concluded, on two bases, that this observed structure represents the natural state of the sheath. First, we have not been able to conceive of an artifactual process that could break down a native spiral configuration of cytoplasm into the observed discontinuous concentric configuration. Second, the regularity in occurrence and location of distinct seams in all the lamina of each sheath strongly suggests that they are not artifacts.

One explanation for the observed structure is that the laminae in each sheath are, in fact, isolated from each other, that several cells are involved in forming the sheath in a single internodal region, and that each cell supplies one or a few of the laminae. We observe, however, only one nucleus in each internodal portion of the sheath, although the existence of more than one nucleus has not been ruled out by complete serial sections through an entire internode. This one nucleus is always located in the innermost lamina of the sheath. Thus, we must tentatively conclude that the laminae are not isolated from each other and that each lamina in an internode is somewhere

FIGURE 16 A longitudinal-section through a node, showing the termination of sheath laminae (*Sh*) (circle) and the differentiation of the axon surface at the node. The raspberrylike bodies in the axon are thought to be tangential sections of the axon surface. Surrounding the axon at the node are extensive extracellular spaces (*) containing fibrils (arrows) which appear negatively stained as a result of intense reaction of permanganate with the surrounding ground substance. In this space are found glial processes (*NP*) presumably originating from the nodal cell perikaryon not in the plane of this section. × 8000.

FIGURE 17 A longitudinal section of a nerve fiber, showing the termination of sheath laminae and their replacement by extracellular spaces (*) at a node. The axon surface is clearly differentiated into a rugose form and covered over much of its surface by thin cytoplasmic processes from the nodal cell (*NP*). A portion of the sheath of an adjacent fiber is shown (*S*). The inset is a higher magnification of a longitudinal section through a node, showing the termination of sheath laminae against the axon. In these regions, the ~200-A axon-sheath gap is not obliterated, but is somewhat narrowed and differentiated into a septate structure similar to a septate desmosome. × 14,000; inset, × 55,000.


AXON
Sh
NP
16
AXON
AXON
NP
NP
AXON
S
17

connected with the innermost, nucleated lamina of that internode.

This connection might be manifested in several possible forms. The simplest possible form of connection would be direct cytoplasmic continuity between adjacent laminae, as diagrammed in Figure 18 *a* (arrow). This, however, has never been observed. A second possible form of cytoplasmic connection is suggested by the membranous body that distorts the nerve sheath in Fig. 9. This sheath is diagrammed in Figure 18 *b1*, next to the two alternative forms which it might

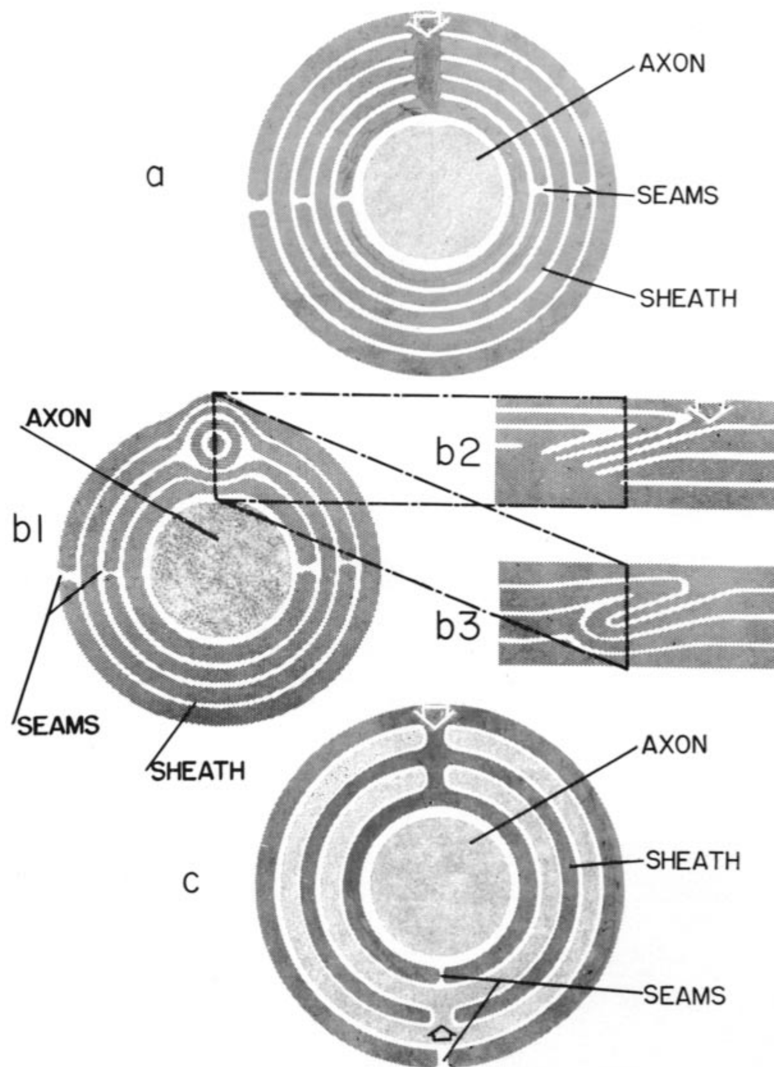


FIGURE 18 Diagram of three possible types of cytoplasmic connections among laminae of a sheath seen in cross-section. For simplicity, cytoplasm is shown as stippled areas, and membranes are omitted. In *a*, laminae are connected directly (at arrow). In *b1*, the circular body located between the sheath laminae is a possible representation of the membranous body in Fig. 9. On *longitudinal* section, this body may appear either as *b2*, tubular connections of outer laminae with the inner nucleated lamina (at arrow), or *b3*, a simple variation or warping of the sheath laminae. (The vertical dotted lines indicate the location of the section in the opposite plane). In *c*, alternate laminae are connected through an opening in the seam of the intervening lamina (at arrows), and two different cells (shown by different shading) make up one sheath.

display if it were sectioned longitudinally. The body within this sheath could be a cross-section through several concentrically arranged cytoplasmic tubes that connect outer laminae to the inner nucleated lamina, as diagrammed in Fig. 18 *b2*. It is equally possible, however, that this body is a cross-section through a simple distortion of the normal sheath, as diagrammed in Fig. 18 *b3*. In order to determine which of these alternatives is correct, it will be necessary to obtain longitudinal sections through one of these membranous bodies. For technical reasons this has not been accomplished.

A third possible form of cytoplasmic connection would be cytoplasmic continuity between alternate laminae through a split in the seam of the intervening lamina, as diagrammed in Fig. 18 *c*. This form would suggest that the sheath was formed by a very regular, alternate overlapping of cytoplasmic sheets from two separate glial cells (shown with different shading in Fig. 18 *c*). The connection would be quite unlike those connections in Figs. 18 *a* and *b*, since they suggest that the laminae of one internode are interconnected and hence part of the same glial cell. Although this third form of cytoplasmic continuity has not been observed, it is an attractive possibility and, if observed, would warrant careful serial sectioning of an entire internode to positively determine the number of cells contributing to one internode and the location of their nuclei. This type of connection would offer an attractive explanation for the occurrence of seams in sheath laminae and their regular alignment (Fig. 8). Initially, these connections between alternate laminae could occupy a large fraction of the length of the sheath and limit the expansion around the axon of intervening laminae. As development proceeds, these connections might not grow as the sheath laminae elongate with the axon, but they could produce a pattern that would continue along the length of the sheath, a pattern of regularly aligned seams.

Since the area involved in any of these possible cytoplasmic connections would undoubtedly be only a small proportion of the total sheath area, it is unlikely that we would have observed them in routine sections. So, in order to explain adequately how this sheath is formed, it will be necessary to study its embryogenesis and development. If ultimately no cytoplasmic connections of any sort can be found among laminae, it would be necessary to conclude that the laminae are isolated cellular fragments with no nucleus, and the nature of their formation would be intriguing.

Sheath Function

The prawn nerve fibers' highly organized sheaths and nodes probably permit them to propagate nerve impulses by saltatory conduction. But conduction in prawn fibers is slower than in vertebrate myelinated fibers that are known to employ saltatory conduction (11, 12, 26). In attempting to understand this discrepancy, we considered the various structural factors, which are thought to influence the rate of conduction in all nerve fibers, and their apparent differences in prawn and vertebrate nerves. As discussed in the Introduction, it is commonly accepted that the rate of conduction of impulses in a nerve fiber is governed by its "cable properties." These cable properties are thought to be related to two structural factors. One cable property is the axon's internal resistance to ion flow, which is found to be inversely proportional to the axon's cross-sectional area when other factors are held constant (9). Since prawn fibers are larger than vertebrate myelinated nerves, it is unlikely that this cable property is significant in explaining the slower rate of conduction in prawn fibers.

A second cable property is the nerve sheath's capacitance and resistance (or its reciprocal, conductance) which is thought to control the proportion of ions "lost" from the axon as ions flow along it. It is commonly held that a reduction in the capacitance and conductance of the nerve sheath will increase the internal current flow in the axon and increase the rate at which ionic activity at one node can spread electrotonically along the axon to exert effects on adjacent nodes, thereby increasing the rate of conduction. The myelin sheath is thought to achieve this reduction in capacitance and conductance by aligning several tightly packed unit membranes in series with the axon membrane. This thesis offers an attractive explanation for the slower rate of conduction in prawn fibers: that their sheaths are less effective than vertebrate myelin in reducing the fiber's capacitance and conductance. Our observations suggest that this may be true. We have shown that the prawn sheath is structurally different from the tightly packed lipoprotein lamellae of vertebrate myelin in several respects, three of which may significantly affect the sheath's capacitance and conductance.

First, the prawn sheath is, for the most part, composed of a loose arrangement of laminae with distinct layers of cytoplasm between membranes. Second, the prawn sheath contains areas of extracellular space, especially between the inner laminae, which are continuous with the axon-sheath gap. Since cytoplasm and extracellular space can act both as a reservoir for ions and as low resistance pathways for ion flow, their presence in the sheath could vitiate the improved cable properties brought about by the membranes of the sheath. Moreover, their presence in prawn sheaths means that, in a given sheath thickness, fewer membranes can occur in a prawn sheath than in a vertebrate sheath. So, classical studies (11, 26) which showed that prawn sheaths are nearly as thick as vertebrate sheaths can no longer be interpreted to mean that the prawn sheath offers as great a resistance as the vertebrate sheath.

Third, the prawn sheath is separated from the axon at the node by an ~200 A gap. This could allow a passage of ions from the node to the axon-sheath gap; this ion flow is presumably prevented in vertebrate myelinated nerves by complete closure of the axon-Schwann gap at the node (22, 18). Nevertheless, the septate desmosomelike structure observed between sheath and axon at a prawn node may function like a gap closure to prevent such a flow of ions.

Finally, it is important to compare differences in the spacing of nodes in the prawn and vertebrate sheaths. We have not measured the internodal distance in prawns. Earlier workers (11) reported that the internodal distances in prawns are not related to fiber size as they are in vertebrates and vary considerably, but suggested that they are shorter than those usually found in vertebrates. If future work verifies this, then the slower rate of conduction in prawn nerve fibers could partially be explained by postulating a less efficient saltatory conduction (12).

In summary, we can suggest only that these structural features may make the prawn sheath less effective than vertebrate myelin in reducing the axon's capacitance and conductance and thus help to explain its slower rate of conduction. It seems that the prawn sheath offers a good opportunity to study by neurophysiological and ultrastructural techniques the mode of operation of a high resistance sheath in rapid nerve conduction.

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Mr. Heuser is a member of the Class of 1968, Harvard Medical School. During the course of these studies, Dr. Doggenweiler was on leave from Universidad Catolica de Chile.

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