A Fine Structural Analysis of the Ventral Nerve Cord and Associated Sheath of *Lumbricus terrestris* L. [1]

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ABSTRACT This study is a fine structural analysis of the earthworm nervous system and its sheath. The bulk of the findings consists of the addition of fine structural detail to the extant descriptions of the general organization of this system. These findings were of particular interest, (1) that the nervous system possesses one basic type of supportive glial cell, (2) that the zonula occludens was not found anywhere in the structures studied here, and (3) that the sheath of the ventral giant axon is not myelin but consists of a thick covering of many tiny axons and synaptic terminals.

The relatively simple metameric organization of the nervous system of the earthworm was well described before the end of the ninteenth century (e.g., Claparéde, 1869; Friedländer, 1888). Certain cytological features of this ventral nerve cord could not be described in these classical studies, however, because many of the glial cells, capillaries and smaller axons are beyond the reach of the light microscope. This important aspects of the organization of the nerve cord can only be studied with profit at the level of the electron microscope. This study, which is an electron microscopic analysis of the cytologic organization of the earthworm ventral nerve cord and its sheath, will not only add fine structural detail to already extant descriptions but can also contribute to comparative anatomical knowledge of the annelid nervous system. For example, the leech nervous system, which has the same general size and organization as that of the earthworm, is avascular and possesses a specific number of large glial cells associated with various parts of the neuron (e.g., Apathy, 1897). The nervous system of the earthworm, on the other hand, is vascular and the glial cells are small and numerous. Attention will thus be paid to the cytological structure and distribution of the blood vessels to see if meaningful insights into glial-vascular and glial-neuronal relationships in these animals can be gained. Finally, this nervous system is relatively small and simple with a repetition of certain aspects of the neuronal organization in each segment. These features, plus the presence of large easily identifiable axons, the dorsal and ventral giants, make this nervous system a favorable site to begin the analysis of a neuropil at the level of the electron microscope.

MATERIALS AND METHODS

Worms labeled *Lumbricus terrestris*, L. were obtained from a biological supply house. The identification was kindly confirmed by Dr. Gordon E. Gates of the Department of Zoology at Harvard University. Lengths of the nervous system were carefully exposed and either fixative was placed on the nerve cord *in situ* or long pieces were dissected free and immersed directly in the fixing fluids. The fixatives used were osmium tetroxide buffered with collidine (Bennett and Luft, '59), cacodylate buffered glutaraldehyde (Gordon, Miller and Bensch, '63), or a 1% potassium permanganate solution in distilled water. The lengths of fixed nervous system were dehydrated in ethanol and embedded in epon or araldite. Sections, 0.5–1.0 μ thick, were cut for light microscopy and stained with 1.0% toluidine blue in a 1.0% sodium borate mixture (Richardson, Jarrett and Finke, '60). For electron microscopy, gold to gray sections were cut, then picked up on bare grids and examined in RCA-EMU 3 E, F or G microscopes. The stains employed for increasing the con-

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trast of these thin sections were: (1) a saturated solution of uranyl acetate in distilled water or a half-saturated uranyl acetate solution in 50% ethanol, (2) a potassium permanganate solution (Lawn, '60) or (3) various lead stains (Karnovsky, '61; Millonig, '61; Reynolds, '63). The most frequently used lead stain, however, was a modification of the lead citrate mixture (Reynolds, '63) for it is the easiest lead stain to make up fresh.[2] To make this stain, 0.02 gm of lead citrate (K & K Laboratories, Plainview, New York) and three drops of five normal sodium hydroxide are added to 10 cm^3 of singly distilled water. This mixture is shaken for two minutes and gives a stain equal in contrast and delicacy to any of the published lead stains. Uranyl acetate followed by one of the above lead stains was also frequently used.

OBSERVATIONS

The ventral nerve cord of the earthworm is a cylinder of relatively uniform diameter, and its segmental nature is revealed by a repeating pattern of both the peripheral roots and its cytologic structure (e.g., Stough, '26). It is encased in a complex trilaminar sheath (fig. 1). The peripheral region of the ventral nerve cord contains the three dorsal giant axons and the ganglion cells but consists principally of the interlacing processes of small glial cells (figs. 1, 2). The *supportive glial zone* ("Bindegewebstoff" of earlier authors) surrounds the neuropil which consists mainly of axons and synaptic processes (fig. 1). The most obvious features of the neuropil are two giant axons located ventrally on either side of the midline.

The trilaminar sheath

The outer layer of the sheath that encloses the earthworm nervous system is part of the coelomic lining. Beneath the coelomic lining is a prominent layer of muscle cells and beneath this, directly investing the nervous system, is a fibrous capsule. Small nerves, blood vessels and cells with distinctive cytoplasmic inclusions are interspersed among the muscle cells.

The coelomic lining. The free surface of the squamous epithelium that lies between the nervous system and the coelomic cavity is relatively flat and without surface specializations (figs. 3, 4). The nuclei of these cells are filled with sharply outlined clumps of chromatin set against a finely granular background. Single round nucleoli, which consist of dense particles approximately 150 A in diameter and a more finely granular material, are sometimes seen. Except for variations in size, this description can serve for the nuclei and nucleoli of all the cell types that make up this nervous system and its capsule. The cytoplasm of the coelomic lining cell contains an inconspicuous Golgi apparatus, some cisternae of the granular endoplasmic reticulum and an occasional small mitochondrion. The most prominent cytoplasmic feature is a basal feltwork of fine filaments which are 50 A in diameter and indefinitely long (fig. 3). These filaments, which in their size and organization resemble tonofilaments in terminal webs, for example, are presumably cytoskeletal in function; but they do not attach to the lateral junctional complexes of these cells.

The lateral junctions between coelomic lining cells differ in certain respects from those found between the vertebrate epithelial cells. At the coelomic surface of this squamous epithelium, the two apposed cell membranes are separated by a gap of approximately 200 A (figs. 3, 4). No material can be seen between these membranes but there is an osmiophilic condensation of the cytoplasm adjacent to them. No filaments attach to this part of the complex. This structure is always found at the coelomic surface and favorable sections allow it to be followed for some distance around the periphery of the cell. It is thus a zonula adhaerens (Farquhar and Palade, '63). Previously, the zonula adhaerens has been reported as the distal of an epithelial junctional complex only in the ependymal lining of the rat brain (Brightman and Palay, '64). Usually a zonula occludens (Farquhar and Palade, '63) forms the outer part of an epithelial junction.

In the depths of the zonula adhaerens described above, the plasma membranes suddenly approach each other so that they

[2] This modification was developed with Dr. John Venable of the Department of Veterinary Anatomy, Oklahoma State University, Stillwater, Oklahoma.

are separated by a gap of 50 A (fig. 5). The dark and light layers of the apposed unit membranes are 25 A in thickness and the inner and outer dark layers are of equal thickness and density no matter what fixative or stain is used. Thus a junctional complex of 200 A overall width is formed which appears in electron micrographs as four dark lines separated by three light lines, the middle light line being the intercellular gap. This junctional relationship has a marked similarity to the seven layered junction between axonal and Schwann cell membranes at the node of Ranvier in the cat (Elfvin, '61). However, at the node, the outer dark layers of the apposed unit membranes are thinner and less dense than the cytoplasmic dark layers; whereas, in the coelomic lining, both outer layers of the unit membranes are equally thick and dense. Because of these differences, the earthworm junction will not be referred to as a seven layered junction but rather as a *close membrane apposition* (figs. 4, 5). This close apposition extends the depth of the epithelium from the base of the zonula adhaerens to the basal lamina or "basement membrane" that underlies these cells, except for occasional regions where the two epithelial cells are pulled apart for short distances (fig. 3). The zonula occludens and macula adhaerens have never been seen in this epithelium. Studies on the earthworm gut and integument are being undertaken to see whether other epithelial junctions in this animal are the same.

Specialized junctions at the basal surface of the coelomic lining cells are sparse. Half-desmosomes are occasionally found where these cells lie directly on extensions of the fibrous capsule that invests the nervous system. Where the cells lie on muscle or connective tissue cells, however, junctional complexes between the different cell types are not apparent. Staubesand, Kuhlo and Kersting ('63) report, on the other hand, that the different types of contiguous cells attach to each other by means of "Haftplatten" or desmosomes, but their pictures do not adequately support this statement. Desmosomes were never found between these cells in the material described here.

Finally, a thin *basal lamina* (Coggeshall and Fawcett, '64, footnote 2) underlies these cells where they lie on the fibrous capsule (fig. 8), but no extracellular coating is seen between the coelomic lining cells and muscle or other cells in the interior of the capsule.

The muscle of the sheath. A layer of muscle cells lies between the coelomic lining and the fibrous capsule investing the nervous system. The nucleus and nucleolus of the muscle cell resemble those described above. The perinuclear cytoplasm contains a prominent Golgi apparatus, some cisternae of the granular endoplasmic reticulum and many dense particles approximately 150 A in diameter. A few small mitochondria are found here or at the periphery of the cell (fig. 7), but they are never seen among the myofilaments.

The bulk of the muscle cell cytoplasm is occupied by myofilaments, elements of the sarcoplasmic reticulum and structures made of an ill-defined dense material (figs. 6, 7). The myofilaments are of two kinds, the more numerous of which are 50 A in diameter and the less numerous 200–500 A in diameter (figs. 6, 7). As in striated muscle, the two filament types interdigitate, but a constant spacing or number of thin filaments around a thick filament is not seen (figs. 6, 7). Another similarity of this myofilament arrangement with that of striated muscle is that all filaments are aligned parallel to the long axis of the muscle cell. This myofilament pattern differs from that of striated muscle, however, in that each thick filament is set forward a short distance from the preceding one, and thus the cross banding is not perpendicular to the long axis of the myofilaments, as in striated muscle, but is oblique to this axis. With this arrangement of myofilaments the muscle is appropriately termed "obliquely striated" (Rosenbluth, '65), although in the classification proposed by Hanson and Lowy ('60) it would be termed a helical smooth muscle.

Only the thin filaments enter that region of the cell occupied by the sarcoplasmic reticulum and denser material, and it is in this region that a structure equivalent to the Z line of striated muscle would be found. The thin filaments do not appear to insert into the sarcoplasmic reticulum

or the denser structures, however, and nothing else that could be equated with the Z line in striated muscle is seen.

The sarcoplasmic reticulum consists of a series of tortuous tubules that penetrate between the myofibrils from the region of the sarcolemma (fig. 7). Near the sarcolemma, the tubules often open into a larger vesicle whose outer surface lies within 100 A of the sarcolemma (*S. R. and Ves.*, fig. 7). No cytoplasmic specializations, such as tiny vesicles (Hanson and Lowy, '61), are seen between the vesicle membrane and the plasma membrane but, with osmic acid or glutaraldehyde fixation, the vesicle membrane is darker than the adjacent plasma membrane (fig. 7). Permanganate fixation shows separate trilaminar unit membranes of the sarcolemma and vesicle, thus ruling out a membrane fusion, but this fixation does not reveal the density difference seen after other fixatives.

The ill-defined dense structures that are interspersed with the sarcoplasmic reticulum in the myofilamentous region of the cell usually merge into the surrounding cytoplasm so that boundaries cannot be delineated with great certainty (fig. 6). Occasionally tonofilaments (described below) enter the myofilamentous region and appear to blend with the dense structures. It was not possible to determine, however, whether the dense material is made of matted tonofilaments or whether the tonofilaments and dense material are different. Various descriptions of these structures have been given in other helical smooth muscles. They are referred to as "dense bodies" in a tonic oyster muscle (Hanson and Lowy, '61) and *Ascaris* body wall muscle (Rosenbluth, '65), as "dark sarcotubules" in leech (Pucci and Afzelius, '62) and earthworm (Staubesand, Kuhlo and Kersting, '63) body wall muscle, as "cross fibers" in leech body wall muscle (Röhlich, '62), and as "fibrillar networks" in *Ascaris* body wall muscle (Reger, '64). It is not yet clear whether these descriptions indicate different structures in the same relative position in different helical smooth muscles or whether there is a common structure that is being interpreted in different ways.

The muscle cells attach to the surrounding fibrous framework by means of half-desmosomes (Staubesand and Kersting, '64) (fig. 6). The latter consist of darkened plaques of cytoplasm next to the sarcoplasm next to the sarcolemma with opposing darknesses in the external lamina or "basement membrane" that coats these cells. Cytoplasmic tonofilaments, gathered into curving bundles, insert into the dark cytoplasmic plaques. On occasion, the tonofilaments also enter the periphery of the myofilamentous region of the cell and extend into the cytoplasm between the myofilaments.

The fibrous capsule. Staubesand, Kuhlo and Kersting ('63) clearly show that the material most closely investing the nervous system is not a cuticle, as early investigators reported (e.g., Friedländer, 1888; Stough, '26), but is instead a fibrous capsule (fig. 2). The fibers have a diameter of approximately 150 A, are indefinitely long, and have a dark periphery surrounding a lighter core. They are usually spaced some distance apart suggesting that a substance in which they were embedded in life has not been preserved by the fixatives used in this study. At certain points, however, small bundles of tightly packed fibers are found and they presumably increase the strength of the fibrous capsule without greatly reducing its flexibility. All the fibers stain intensely with uranyl acetate and longitudinal views of these intensely stained fibers reveal a 300 A cross banding. The fibrous material that forms the cuticle around the entire earthworm is collagenous in nature (Laverack, '63). Although the arrangement of the outer fibrous material differs in several respects from that of the fibrous material around the nervous system, the latter material is also collagen (Rudall, '55).

In addition to closely investing the nervous system, the fibrous tissue also ramifies throughout the capsule and forms broad sheets under the coelomic lining cells. The sheets are connected by thick septa with the investing capsule. Thus the commonest support of the coelomic lining is a collageneous layer, but there are points where the coelomic lining cells lie directly on muscle or other cells. The fibrous tissue

also penetrates the nervous system and forms an important part of the *supportive glial zone* (fig. 17).

The nerves within the capsule. Groups of small cell processes are commonly found interspersed among the muscle cells in the interior of the sheath (fig. 8). The majority appear clear but small mitochondria, a few filaments and occasional vesicles are seen in some. These processes are presumed to be axons that originate in the nervous system and innervate the capsular muscle. Extensive search has failed to disclose the neuromuscular junctions.

Capsular cells with distinctive cytoplasmic inclusions. In the interior of the capsule two cell types that are noteworthy because of the presence of distinctive cytoplasmic inclusions are sometimes found. The first contains inclusions filled with a crystalline material (fig. 10). The crystals are sometimes closely invested by a triliminar unit membrane (fig. 10), while in other instances polygonal masses of this material float free within large cytoplasmic vacuoles. The number of cells containing this crystalline material, its amount and its internal pattern vary from animal to animal and in different regions in the same animal. The reasons for these variations are not known. Presumably the inclusions represent different states of aggregation of a crystalline protein of unknown composition and function.

The second cell type contains large numbers of vacuoles, 0.2–1.0 μ in diameter, filled with a dense flocculent precipitate (fig. 9), and the presence of so many in a single cell results in such a striking picture that attention is called to them here. The numbers of these cells also vary from animal to animal and the reasons for the variations are not known.

The blood vessels. Blood vessels, whose diameters range up to 50 μ, course lengthwise down the sheath. Capillaries that vascularize the supportive glial zone originate from and terminate in the capsular vessels. Early investigators described the vascular pattern of various parts of the body and determined the direction of flow in the larger vessels (e.g., Johnston, '03), but they were unable to agree on the nature of the endothelium and cuticle that line the vessels. Hama ('60), reexamining the vessels with the electron microscope, noted that the endothelial cells, which he defined as those cells that line the blood vessels, contain cytoplasmic filaments that were approximately 250 A wide. In the endothelial cells around the large vessels, the filaments were organized into myofibrils, and so he concluded that these cells are myoendothelial in nature and all are capable of contraction. He further noted that these cells have a prominent "basement membrane" on their luminal surface in contrast to the vertebrate endothelium where it is on the tissue side of the vessel. This "basement membrane" or external lamina corresponds to the cuticle of earlier authors. He also described the particulate structure of the erythrocruorin particles which make up the blood pigment of the earthworm. All these findings are here confirmed.

The endothelial cells of the capsular vessels have an irregularly rounded nucleus with a nucleolus and chromatin pattern that are not markedly different from other cells in the capsule (fig. 11). The diameter and density of the endothelial myofilaments is the same as the thick myofilaments in the capsular muscle but, as yet, thin myofilaments have not been seen (fig. 12). There are densities in the endothelial cytoplasm next to the plasmalemma that faces the external lamina (fig. 12). Although no filaments attach to these densities, they presumably represent points of firmer attachment of the endothelial cell to the external lamina. The external lamina is approximately 2,000 A wide and long fibers, 150 A thick, are embedded in its luminal surface (Luminal fibers, fig. 11). Since the diameter and density of these fibers is approximately the same as that of the erythrocruorin particles, it is only on longitudinal section that they can easily be recognized. A basal lamina on the tissue side of the myoendothelium is not present.

Low power pictures of the endothelial junctions show that the two plasmalemmas are closely approximated (fig. 11). Higher power pictures show that, with osmic acid or glutaraldehyde fixation, the apposed unit membranes are approximately 75 A wide and are separated by a

gap of 75 A instead of the 120 A or more that separates most contiguous cells in the earthworm. The material between the membranes has an appreciable density, but no intermediate dense line can be visualized. The inner and outer dark lines of the unit membranes are equally thick and dense no matter which fixative or stain is used. In this respect they resemble the membranes involved in the close apposition presumably described between coelomic lining cells.

After permanganate fixation, the unit membranes of the posed endothelial cells are 75 A wide and 75 A apart, but more structure is visible in the intercellular "gap." At some points in the "gap," an intermediate line 25 A wide appears, thus making a nine layered junction with five dark lines alternating with four light lines, each 25 A in width (figs. 14, 15). At other points, only a dense granular or amorphous material is seen. In summary, the endothelial junctions consist of the apposition of two symmetrical unit membranes, each about 75 A wide, separated by a 75 A "gap" which is filled with a material of appreciable density and is occasionally bisected by an intermediate line.

Amoeboid cells are common in the lumen of the vessels (fig. 12). These were described by Hama ('60), and to his description it should only be added that cytoplasmic processes indicative of amoeboid movement and possibly of pinocytosis and phagocytosis are usually present (fig. 12).

The nervous system

The nervous system, which is enclosed by the capsule described above, can be divided into an outer supportive glial zone and the neuropil in the interior (figs. 1, 2). Early anatomists stated that the outer glial zone consisted of an amorphous material in which were embedded glial cells, blood vessels, ganglion cells and the dorsal giant axons. They referred to this outer zone as a "Bindegewebstoff" or connective tissue (e.g., Friedländer, 1888). Now, however, the increased resolution provided by improved light microscopic techniques and especially by the electron microscope reveals that the bulk of the amorphous material consists of the intertwined processes of small glial cells. With these newer techniques, the six components that form the supportive glial zone can be described in some detail. They are (1) the supportive glial cells, (2) the migratory glial cells, (3) the capillaries, (4) the inward extensions of the fibrous capsule, (5) the ganglion cell bodies, and (6) the dorsal giant axons.

The supportive glial zone

The supportive glial cell. The commonest cell in the supportive glial zone is small and has a nucleus 3–4 μ in diameter. The cytoplasm is relatively clear but contains a small Golgi appartus, cisternae of the granular endoplasmic reticulum, particulate material presumed to be glycogen, an occasional tubule or vesicle and infrequent small mitochondria (figs. 16, 17). The prominent cytoplasmic components are large masses of fine filaments that are approximately 50 A in diameter and insert into the junctional specializations that attach these cells either to extracellular fibrous tissue or to one another.

These small cells, with one exception to be described below, cover and surround the fibrous capsule and its extensions into the nervous system (figs. 16, 17). Where the glial processes are exposed to the extracellular fibrous tissue, they are covered by a thin external lamina (Coggeshall and Fawcett, '64, footnote 2) 200–500 A thick. Fibers of the capsule are embedded in the outer surface of the external lamina. Half-desmosomes are very prominent along the interface of the glial cell and its external lamina (figs. 16, 17), and many of the fine glial filaments mentioned above are the inserting tonofilaments of these structures.

Another type of junction occurs where one supportive glial cell joins with another. Here the plasma membranes tend to be straight and are separated by a constant gap of approximately 100 A. A faint line, approximately 25 A thick, is sometimes seen bisecting the intercellular gap while, in other instances, the intercellular gap appears clear (figs. 20, 24). The cytoplasm immediately adjacent to the membranes of this junction is dark and the cell membranes themselves also appear dark although it is difficult to be certain about the latter statement because of the dark

cytoplasm (figs. 16, 20, 24). Large numbers of glial filaments insert into the darkened cytoplasm often after running some distance parallel to the plasma membrane. Many of these attachment regions are large and some can be followed for a micron or more (fig. 20). These junctions resemble desmosomes (macullae adhaerentes) but are atypical in that the intercellular gap is narrower in the earthworm than in other animals described heretofore.

Although intercellular junctions of this kind are seen in all parts of the supportive glial zone and are particularly common in regions where ganglion cell bodies are found (fig. 20), they are most prominent in the sheath of the dorsal giant axon (figs. 23, 24). Hama described the fine structure of the sheath and the junctions therein in 1959 and this study confirms many of his basic findings. The sheath consists of flattened cytoplasmic processes of small cells wrapped around the axon (figs. 17, 21, 23, 24). Though the cytoplasm of any given process can be very tenuous, it is never completely excluded. The membranes therefore do not come together to form the dark and light lines of regular dense myelin. Instead, this is a loose myelin sheath like that around the neurons in the eighth nerve ganglia in the goldfish (Rosenbluth and Palay, '61). Each individual glial wrapping forms desmosome like junctions with the gyres above and below it (fig. 24). In central and posterior regions of the nerve cord, where the axon is large and its sheath is thick and prominent (fig. 21), the junctions are aligned more or less in register and form columns extending part way or completely across the sheath (fig. 24). In cranial parts of the nerve cord, where the giant axons are small and appear unsheathed (Stough, '26), the supportive glial junctions are numerous around the axon but the cytoplasmic processes of the glial cells are not regularly and concentrically arranged so as to form loose myelin (fig. 23).

The nuclei of the cells that make up the sheath of the dorsal giant axon are usually located in the adjacent supportive glial zone though they are occasionally found in one of the outer layers of the sheath. The nucleus and cytoplasm of the sheath cell are indistinguishable from those of the supportive glial cell. These findings; namely (1) that the cell body of the sheath cell is usually located in the supportive glial zone, (2) that the nucleus and cytoplasm of the two cell types are identical in appearance, (3) that the intercellular junctions between the sheath cells and between the supportive glial cells appear identical, and (4) that when the dorsal giant axons are small, they are not wrapped in loose myelin but are surrounded by supportive glial cells that are not disposed circumferentially; lead to the conclusions that the two cell types are identical and that this nervous system possesses one basic type of supportive glial cell.

As regards the relationship of the innermost flattened glial process to the dorsal giant axon, there are frequent dark cytoplasmic patches in the glial cytoplasm next to the plasmalemma that faces the giant axon (fig. 24). Tonofilaments insert into these dark patches. There is also a slight darkening in the adjacent axoplasm into which a few axonal filaments insert but these filaments are so sparse that it is impossible to say whether they are neurofilaments or a separate population of axonal tonofilaments. It seems reasonable to assume that this glial-axonal junction is a point of attachment of the sheath to the axon.

The migratory glial cell. Interspersed among the other components of the connective glial zone and the neuropil is a small cell whose nucleus cannot be distinguished from the supportive glial cell but whose cytoplasm is distinctive (fig. 16). The cytoplasmic matrix is dark by comparison and formed cytoplasmic elements include a prominent Golgi apparatus, many cisternae of the granular endoplasmic reticulum, numerous mitochondria and many 150 A in diameter particles. Large numbers of vacuoles that range from 0.2–1.0 μ in diameter are found in most of these cells. The contents of the vacuoles are heterogeneous and may be breakdown products of various materials ingested by the cells. Only scattered cytoplasmic filaments are present and there are no plasmalemmal specializations that might attach the cells to other structures. Thus, these

cells are probably migratory scavengers but this speculation should be tested by examining the behavior of these cells after injury to the nervous system.

The capillaries. The capillaries within the supportive glial zone originate from and terminate in the larger vessels that travel in the capsule. These capillaries are smaller and have thinner walls than their parent vessels but their cytologic structure and junctional complexes are the same (fig. 17). Erythrocruorin particles fill the lumen of the capillary, and contrary to the report of Hama ('59), amoeboid cells are frequently seen in the lumen of the capillary. Since the amoeboid cells seem to be motile and may very well be scavenging cells, their presence in the capillaries of the nervous system raises the possibility that the migratory glial cells are amoeboid cells that have left the lumen of the vessel and entered the nervous system. However, no cells have ever been observed entering or leaving vessels and the cytoplasm of these two cell types appears somewhat different. Thus this speculation is not supported by evidence and needs to be verified by study of the responses of both these cells to injury of the nervous system. Finally, the capillaries in the nervous system are usually surrounded by supportive glial cells but neither a basal lamina nor any attachment device has been observed between the glial cells and the endothelial cells (fig. 17).

Exhaustive search by light and electron microscopy revealed no vessel within the neuropil. Thus the neuropil is avascular and substances needed for the maintenance and functioning of the axons and synaptic processes in the neuropil must diffuse through extracellular channels or be transported intracellularly over long distances by the neurons and glia.

Inward extensions of the fibrous capsule. Columns and sheaths of fibrous tissue arise from the fibrous capsule surrounding the nervous system and penetrate the supportive glial zone (fig. 17). As with the blood vessels, these penetrations of connective tissue are found only in the supportive glial zone and not in the neuropil. Although they are scattered everywhere in the glial zone, they are most prominent just ventral to the dorsal giant axons (fig. 17). There, large sheets of collageneous fibers extend inwards to form a massive but incomplete septum which, at the light microscopic level, gives the impression that the dorsal giant axons are isolated from the rest of the nervous system. Electron micrographs reveal extensive gaps in this septum, however, through which glial processes pass quite freely.

Though processes of the supportive glial cells usually surround the fibrous penetrations, there is one place where a neuron is directly exposed to the fibrous stroma. At the site where a process from the large median cell rises to join the dorsal giant axon, it comes into direct contact with the sheets of fibrous tissue that incompletely enclose the dorsal giant axons (fig. 22). The neuronal process is here covered by a thin external lamina approximately 200 A thick.

The ganglion cells. The ganglion cells are flask-shaped unipolar neurons whose cell bodies are located in the supportive glial zone and whose axons are directed centrally into the neuropil (fig. 2). The nuclei and nucleoli are large but otherwise unremarkable (fig. 18). There is a prominent granular endoplasmic reticulum whose cisternae are often gathered together in an array (fig. 20) reminiscent of the fine structure of a Nissl body in a vertebrate neuron (Palay and Palade, '55). Innumerable mitochondria, small dense irregular particles presumed to be glycogen, vesicles filled with membranous or amorphous debris, multi-vesicular bodies and several Golgi complexes are scattered throughout the cytoplasm. There is little zonation of these cytoplasmic structures except that the granular endoplasmic reticulum tends to be located at the periphery of the cell. Identations of the cytoplasm by supportive glial cell processes are common, particularly in the larger ganglion cells.

This general cytoplasmic organization is common to all ganglion cells, but two cytoplasmic specializations modify the basic pattern in some cells. First, there are large cytoplasmic regions that are essentially free of formed elements. The size of these regions is proportional to the size of the neuron and they reach their greatest development in the median giant cells where

they are responsible for the mottled appearance of the cell as seen under the light microscope (fig. 1). It is not known whether these areas of low density represent artifactitious swellings of certain areas of the cytoplasm or whether they represent a reasonably accurate portrayal of the structure of the living cell. Secondly, large numbers of ganglion cells have much of their cytoplasm occupied by membrane-bounded granules. These granules sometimes displace all other cytoplasmic elements except the Golgi apparatus (figs. 18, 19). The vesicles range from 1,200–2,400 A in diameter and the majority of them fall into one of two categories; vesicles whose contents are only slightly more dense than the cytoplasmic matrix ("pale granules," B. Scharrer, '63) (fig. 19) and those filled with dense black cores ("dark or osmiophilic granules," B. Scharrer, '63) (fig. 18).

The cells with dark granules are similar to those described by Scharrer and Brown ('61) in the subesophageal ganglion of *Lumbricus*. The cells with pale granules are like those illustrated and described by Röhlich, Aros and Vigh ('62), also in the subesophageal ganglion of *Lumbricus*. Three additional findings can now be reported.

First, the membranes of the pale granules are frequently broken. This damage is not random in its distribution; rather, in some cells nearly all granule membranes are ruptured while in others they are almost all intact. Second, with the exception of the few cells laden with dark granules, all ganglion cells so far examined have significant volumes of cytoplasm occupied by pale granules. Those cells with fewest granules have each group of Golgi lamellae surrounded by many vesicles (pale granules). Other cells have larger regions filled with these granules and, not infrequently, cells are found whose cytoplasm is fully occupied by an extensive Golgi apparatus, pale granules and a nucleus (figs. 18, 19). Finally, cells are sometimes found that have both pale and dark granules and intermediate forms in their cytoplasm (fig. 20).

It is tempting to call those cells that are full of granules neurosecretory cells, but it is obvious that, by this criterion, all ganglion cells in this animal are more or less neurosecretory (are more or less heavily laden with granules). The neurosecretory cells will have to be identified by the demonstration of the release of their secretory product into the circulation of the earthworm (see E. Scharrer, '62, for a general discussion of the definition of neurosecretion).

The dorsal giant axons. The three dorsal giant axons originate in the cranial part of the nerve cord near the subesophageal ganglion (Stough, '26). The lateral giant axons are only 4 μ wide in these cranial regions (fig. 23) but, in posterior regions of the nerve cord, the lateral giant fibers can be 50 μ in diameter (fig. 21) and the median giant fiber, 100 μ in diameter. The sheath of the giant axons is loose myelin (see the section on the supportive glial cell and figures 17, 21 and 24). No structures resembling the nodes of Ranvier in a vertebrate myelinated axon have ever been observed with either the light or electron microscopes.

A few mitochondria, scattered tubules of the agranular endoplasmic reticulum and small bundles of neurofilaments are visible in the axoplasm (figs. 21, 23, 24). Compared to the abundance of neurofilaments in axons of smaller size, the numbers of neurofilaments in giant axons are small in relation to the total volume of the processes. Oblique septa compartmentalize the dorsal giants. Certain ganglion cells in each segment of the nerve cord are connected with the corresponding segment of the axon (Stough, '26). The axoplasm on one side of the septum is reported to be darker than on the other (Stough, '26; Adey, '51), but the cytoplasmic components responsible for this staining difference were not revealed by the light microscope. This electron microscopic study confirms this basic observation and can only add that the difference in axoplasmic staining is apparently due to the greater concentration of mitochondria, tubules, neurofilaments, amorphous ground substance, etc., on the darker side. There are no new cytoplasmic elements and the proportions of the usual cytoplasmic elements are not changed. It is perhaps easiest to explain this by assuming a lower water content in the dark axoplasm which results

in a closer packing of the more stable axoplasmic elements. Since this morphologic polarization is not associated with rectification of the nerve impulse, however, the functional implication of this phenomenon is not clear.

The septum across the giant axon, which appears as a single line in light micrographs, consists of two apposed plasma membranes separated by an intercellular gap (figs. 25, 26). This septum is usually regarded as having a low resistance to the flow of ions (an "electric" synapse, see discussion) and so the membrane relationships here are of particular interest. At the periphery of the septum, the two membranes are separated by 150 A or more which is the usual distance between contiguous cells in this nervous system. In central regions of the septum, however, there are large regions where the two plasmalemmas are separated by only 50 A although intercellular gaps ranging anywhere from 50–150 A can be found. The trilaminar unit structure of these membranes is not well revealed with osmic acid or glutaraldehyde fixation and so, with these fixatives, the central part of the septum appears to consist of two plasma membranes, each approximately 75 A wide, separated by a gap of approximately 50 A. Thus, Hama's ('59) description of the septum in *Eisenia foetida* is confirmed. Hama also described small vesicles in the cytoplasm adjacent to both membranes but these have not been found in the present study, even after extensive search. It is not clear whether this discrepancy is due to generic differences between animals (*Eisenia foetida* vs. *Lumbricus terrestris*), to different buffers for the osmic acid (vononal acetate vs. collidine), to different embedding media (methacrylate vs. epon or araldite) or to the fact that different regions of the septum were observed.

In permanaganate fixed material, the trilaminar unit membranes can be seen. Here, also, at the periphery of the septum, the unit membranes are separated by 150 A or more whereas near the center of the axon they are 50 A apart (figs. 25, 26). Though a search was made in material fixed in several different ways, membrane fusion was never observed. These findings are contrary to those reported by Dewey and Barr ('64) after permanganate fixation of this same septum. This discrepancy will be taken up in the discussion.

The neuropil

The neuropil, which is located on either side of the ventral nerve cord (fig. 1), is surrounded by the supportive glial zone but no barrier, such as a fibrous septum is interposed between these two regions (figs. 1, 2). The non-neuronal elements within the neuropil consist of supportive and migratory glial cells identical to those which have already been described. The cell body of either of these cell types is often found in one region while some of its processes extend into the other. In the neuropil, both the supportive and migratory cells are sparse so that there are regions where large numbers of neuronal processes are contiguous with one another (e.g., figs. 27, 29). Finally, it should be restated that neither blood vessels nor extensions of the fibrous capsule are found within the neuropil.

The neuronal processes, which form the bulk of the neuropil, are separated from each other and from the glial cells by the 150 A interspaces that separate most cells in this nervous system (fig. 27). In non-synaptic regions the axons have a clear cytoplasm of very low density with a few mitochondria, some tubular profiles and bundles of tightly packed neurofilaments (figs. 27, 28). Interspersed among the clear axonal profiles are processes filled with mitochondria and synaptic vesicles (small clear vesicles, the majority of which are 200–500 A in diameter) (figs. 27, 29). On occasion a process filled with mitochondria and synaptic vesicles abuts on a clear process and the plasma membranes of the two processes appear thickened (fig. 29). It is unlikely that the membranes themselves are thicker, however, since, if the plasmalemma were this thick, its trilaminar structure should be obvious. Since the trilaminar structure cannot be seen, it is probable that a small rim of darkened cytoplasm is directly apposed to the plasma membranes. These regions are identified as synapses on the basis of their similarity to synapses in the vertebrate nervous system (Palay, '56, '58).

The pale and dark granules described in the section on ganglion cell border are commonly found in nerve fibers (fig. 28) where they may occur alone or be associated with myriads of synaptic vesicles (fig. 27). Also, vesicles 600 A wide and with dense cores are occasionally found.

The most obvious components of the neuropil are the two ventral giant axons, each 10–20 μ in diameter, located singly on either side of the midline (figs. 1, 2). Each axon is surrounded by a sheath that stains densely with osmic acid or iron hematoxylin, but on light microscopic examination, the sheath has a different texture and color than vertebrate myelin or the sheath of the dorsal giant axons (Friedländer, 1888). Electron micrographs reveal that the sheath is not glial as in the dorsal axons but consists of innumerable tiny unmyelinated axons that surround the large axon and run parallel to it (figs. 2, 29). It is not surprising therefore, that its tinctorial properties differ from those of myelin.

The synaptic relationships between the ventral giant and its tiny satellite axons are unusual. The small fibers form many presynaptic terminations on the giant axon (fig. 29). At various points the ventral giant also forms presynaptic terminals back onto the small axons (fig. 29). In random cross sections of many ventral giants, the presynaptic endings on the giant were 10–20 times as numerous as the presynaptic endings that the giant formed on the small axons that surround it. Smallwood ('30) demonstrated that many collaterals from motor ganglion cells (ganglion cells whose major process leaves the nervous system in a peripheral root) end on this axon and also that many processes of a sensory cell arborization terminate here. As yet, however, no data are available on the origin and major terminations of the ventral giant fiber itself.

DISCUSSION

At many places within the earthworm neuropil, there are two apposed neuronal processes, one filled with synaptic vesicles and mitochondria, the other relatively clear. In these processes symmetrical patches of dark cytoplasm adjacent to the apposed plasma membranes face each other across the 150 A intercellular gap. This constellation of structures is generally accepted as defining a synapse (Palay, '56, '58). The major difference between earthworm and vertebrate synaptic structure is the width and symmetry of the dark cytoplasmic patches. In the vertebrate, they are quite prominent and usually asymmetrical while in the earthworm they are only 25–50 A thick and usually symmetrical. The functional correlate of this morphologic difference is not known.

The synapse described above presumably transmits the impulse by the release of a presynaptic transmitter which results in a permeability change in the post-synaptic membrane. The other common way an impulse can be transmitted is to have the local currents in one cell directly affect the membrane polarization of a neighboring cell. Such an event probably occurs at the earthworm septal synapse. Physiologic investigations (e.g., Rushton, '46; Kao and Grundfest, '57) reveal that the impulse can pass in either direction over the septum and that any synaptic delay, if present, is brief. The simplest way to explain these findings is to assume that the septum provides little resistance to the flow of ions. Hama ('59) investigated the fine structure of the septum in order to make a more precise morphologic correlation with the physiologic data. He found that it consists of two plasmalemmas, each approximately 75 A wide, separated by a gap of 45–85 A. These findings, though not disproven, have not been generally accepted for it has been pointed out that the outer leaflet of some trilaminar unit membranes cannot be seen in unstained sections of material fixed in osmic acid and embedded in methacrylate (Robertson, '61; Brightman and Palay, '63). Thus, especially since the intercellular gap is so narrow, it is felt that Hama did not rule out the presence of a membrane contiguity or fusion as occurs in the zonula occludens. Dewey and Barr ('64) examined this septal junction after permanganate fixation and reported that a nexus (tight junction, fascia occludens) joined the two apposed plasma membranes. However, their high power micrograph of this junction does not clearly demonstrate a touching of the outer dark lines of the apposed unit membranes. In

this study, large regions were found where the two unit membranes are clearly separated by 50 A and no membrane fusion was ever seen. It is probable that the discrepancies in the two studies are due to differences in fixation and thickness of the plastic sections used for electron microscopy. This study, then, like that of Hama's ('60), shows that the two unit membranes are closely apposed but not fused and thus this topographical relation of two membranes with a 50 A gap is regarded as the morphologic basis of what is generally accepted as an "electric" synapse.

It was somewhat surprising to find that the cells forming the earthworm septal synapse never touch because, where a low electrical resistance exists between cells, a fusion of the outer leaflets of the membranes is usually found (Furshpan, '64). Recently, however, a low resistance connection between epithelial cells in *Drosophilia* was demonstrated (Lowenstein and Yoshinobu, '64). In this case there was an extensive interdigitation of the two cell membranes at the basal surface of the epithelium and a large septate desmosome on the lateral boundary between these cells (Wiener, Spiro and Lowenstein, '64). While the precise relation of the septa to the unit membranes is not clear, a typical zonula occludens was not reported anywhere between the two cells. Thus, there is precedence for the association of a low resistance between cells joined by a junctional complex that does not contain a membrane fusion.

Although it was surprising not to find a fascia occludens or membrane fusion in the septum of the dorsal giant axon, it was even more surprising not to find one in the epithelium lining the coelomic cavity. The zonula occludens or 5-layered junction was first described by Karrer ('59) in human cervical epithelium. Since then, all vertebrate epithelia whose fine structure has been examined show zonulae occludentes, usually near the outer surface of the epithelium (Farquhar and Palade, '63). Nevertheless, in the earthworm coelomic linging, which is a squamous epithelium, the two unit membranes do not touch but are separated over long distances by a gap of 50 A. The fact that a zonula occludens was not found in an earthworm epithelium is indirect evidence that a fascia occludens or membrane fusion was not missed in the septal synapse, for this finding raises the possibility that cell membranes do not fuse anywhere in the earthworm. Further studies will be necessary to confirm this speculation. This earthworm epithelial junction and the septate desmosome (Wood, '59; Overton, '63; Wiener, Spiro and Lowenstein, '64) are two epithelial junctional complexes that differ significantly from those described and collated by Farquhar and Palade ('63). As the invertebrate kingdom is explored, junctions of this type may turn out to be as or more widespread than the triad of the zonula occludens, zonula adhaerens, and macula adhaerens, which is characteristic of vertebrate epithelia.

Another interesting finding is the nine-layered junction between myoendothelial cells in the earthworm. The endothelial cells possess myofilaments, and rhythmic pulsations pass down the larger vessels in the living animal. Although hundreds of vessels have been examined, no structure that could represent neuronal endings on the myoendothelial cells was found. Also there are no cytoplasmic specializations in these cells that resemble the "chemical synapse" or the neuromuscular junction. It is probable, therefore, that the depolarization accompanying contraction in one cell set up local currents that depolarize the next cell. The earthworm myoendothelial junction, however, resembles neither the vertebrate endothelial junction (Muir and Peters, '62) nor the septal synapse, a presumed low resistance junction in the earthworm. Thus it would be of interest to determine whether the resistance to the flow of current between myoendothelial cells is low (an "electric" synapse) and also whether any differences between this junction and the septal synapse of the giant axon can be found. Although the myoendothelial cells in the vessels of the sheath and the capillaries of the nervous system are too small, the cells of the dorsal blood vessel are larger and should be easy to impale with microelectrodes thus allowing appropriate measurements to be made.

Early investigators noted that the nervous system of the earthworm is vascular (e.g., Claparéde, 1869; Friedländer, 1888).

In 1944, Scharrer demonstrated that these vessels are end-arteries, in that they form non-anastomosing loops, much like the cerebral vessels of marsupials. This study demonstrates that the vascular loops are restricted to the supportive glial zone. In regard to these findings, it would be instructive to compare the earthworm nervous system to that of the leech, which has the same general thickness and organization but is avascular (e.g., Coggeshall and Fawcett, '64). The neuropil and connective tissue of these animals are different in that the leech possesses four large glial cells that are associated with the axons and synaptic terminals whereas, in the earthworm, the glial cells are small and numerous. The cytologic characteristics of these two cell types are similar, however, in that tonofilaments occupy the bulk of the cytoplasm in both and there is little evidence in their fine structure that they act as more than mechanical supports. By contrast, there are great differences in the cytologic structure of the supporting cells in regions where the ganglion cell bodies are located, the packet in the leech and the supportive glial zone in the earthworm. In the earthworm, the glial cells are again numerous, small, and laden with tonofilaments while, in the leech, a single packet glial cell laden with mitochondria, vesicles, and endoplasmic reticulum surrounds many ganglion cell bodies. Another major difference between the two systems is that the endothelium over the leech nervous system is markedly vesicular and possesses deep basal cytoplasmic infoldings while the coelomic lining over the earthworm nervous system is quite simple. The resemblance of the endothelial and packet glial cell in the leech to known active transport cells plus the lack of neuronal capillaries led Coggeshall and Fawcett ('64) to speculate that these two cells facilitated the transfer of nutrients from the blood sinus in which the nervous system is suspended to the neurons. This speculation is indirectly supported by the findings in the earthworm. Here, a similarly organized nervous system has blood vessels that enter the region of the ganglion cell body, and the glial and coelomic lining cells do not resemble active transport cells. It is difficult to escape the conclusion that the blood vessels in the earthworm and the packet and endothelial cells in the leech have many common functions. Further anatomical studies on other invertebrates to determine if there is a relationship between the presence or absence of capillaries in the region of the ganglion cell body and the presence or absence of probable active transport cells would be desirable.

The annelid neuropil forms an interesting contrast with that of the vertebrate for, while there are many basic similaries, the vertebrate neuropil contains both neuronal perikarya and capillaries, structures that are absent from the annelid. The relationship between these two elements in the vertebrate is not clear, but it is obvious that certain invertebrates do not need blood vessels to maintain a normal neuropil but they presumably need some sort of specialized transport apparatus to nourish the ganglion cell bodies.

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PLATE 1

EXPLANATION OF FIGURE

1 A light micrograph of a cross section through the posterior part of the ventral nerve cord of the earthworm. A trilaminar sheath envelops the nervous system. The supportive glial zone, which consists principally of the interlacing processes of small glial cells, surrounds the neuropil. The ganglion cells, including the median giant cell, and the dorsal giant axons are contained within this glial zone. Note the thick myelin sheath around the dorsal giant axons and the thinner sheaths of different texture around the ventral giant axons. Osmic acid fixation, $\times$ 250.

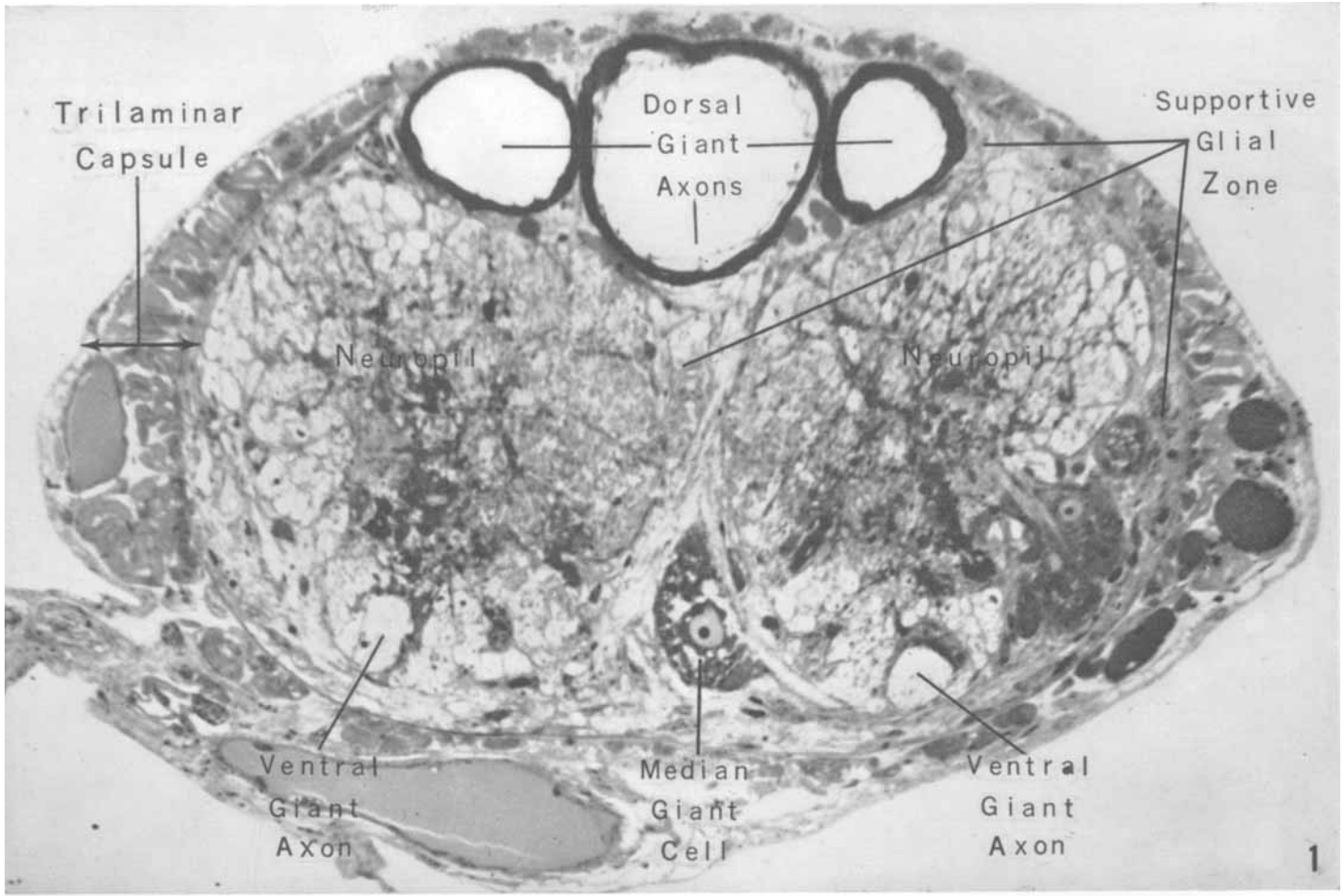

Trilaminar
Capsule
Dorsal
Giant
Axons
Supportive
Glial
Zone
Neuropil
Neuropil
Ventral
Giant
Axon
Median
Giant
Cell
Ventral
Giant
Axon
1

PLATE 2

EXPLANATION OF FIGURE

2 A low power electron micrograph of a ventrolateral corner of the nerve cord. The fibrous capsule that directly invests the nervous system is well seen here. The difference between the cytologic structure of the supportive glial zone and the neuropil should be noted. The flask shaped ganglion cell bodies are located within the supportive glial zone and send their processes into the neuropil. Finally, note that the sheath of the ventral giant axon is made up of many tiny axons. Glutaraldehyde fixation, × 1,500.

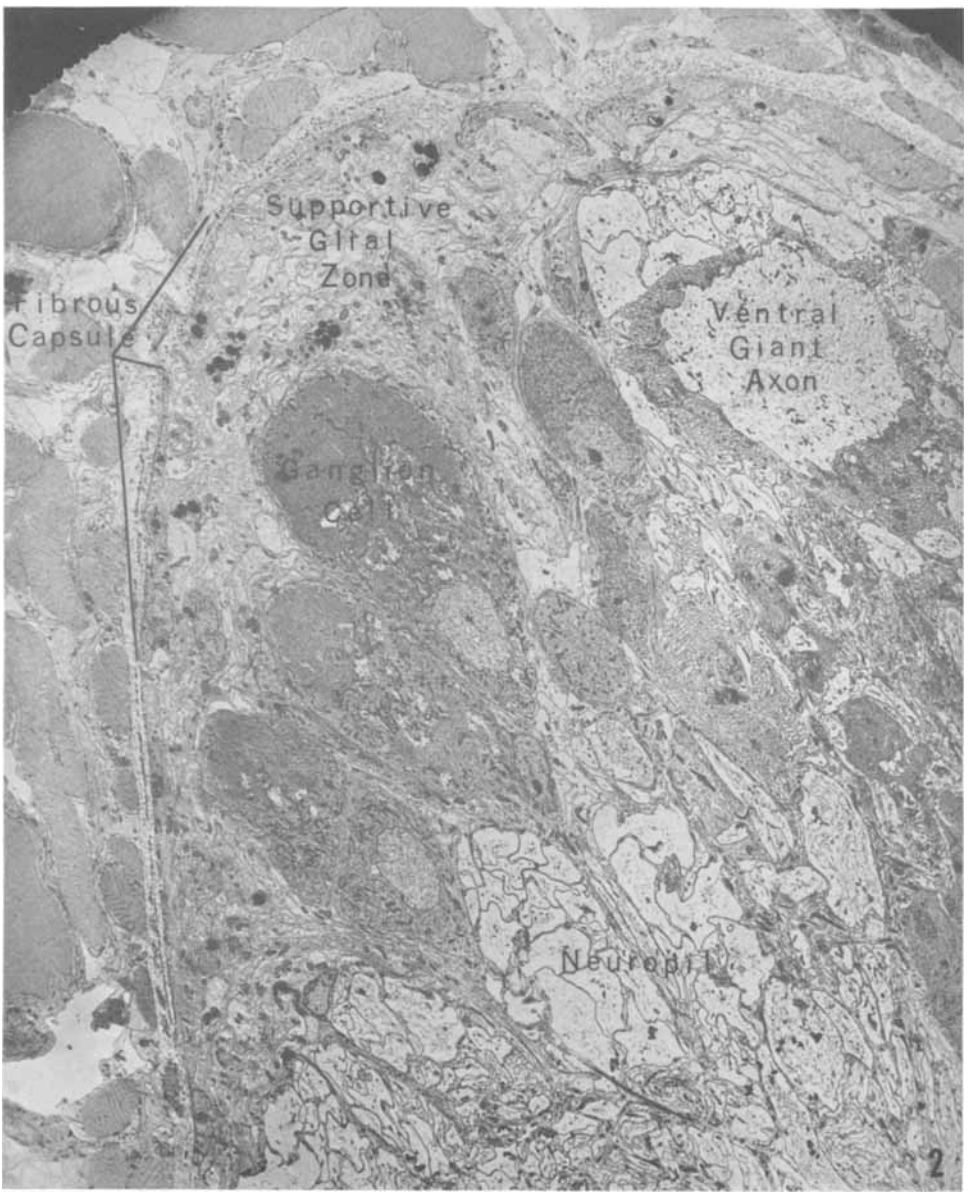

Supportive
Glial
Zone
Fibrous
Capsule
Ventral
Giant
Axon
Ganglion
Cell
Neuropil
2

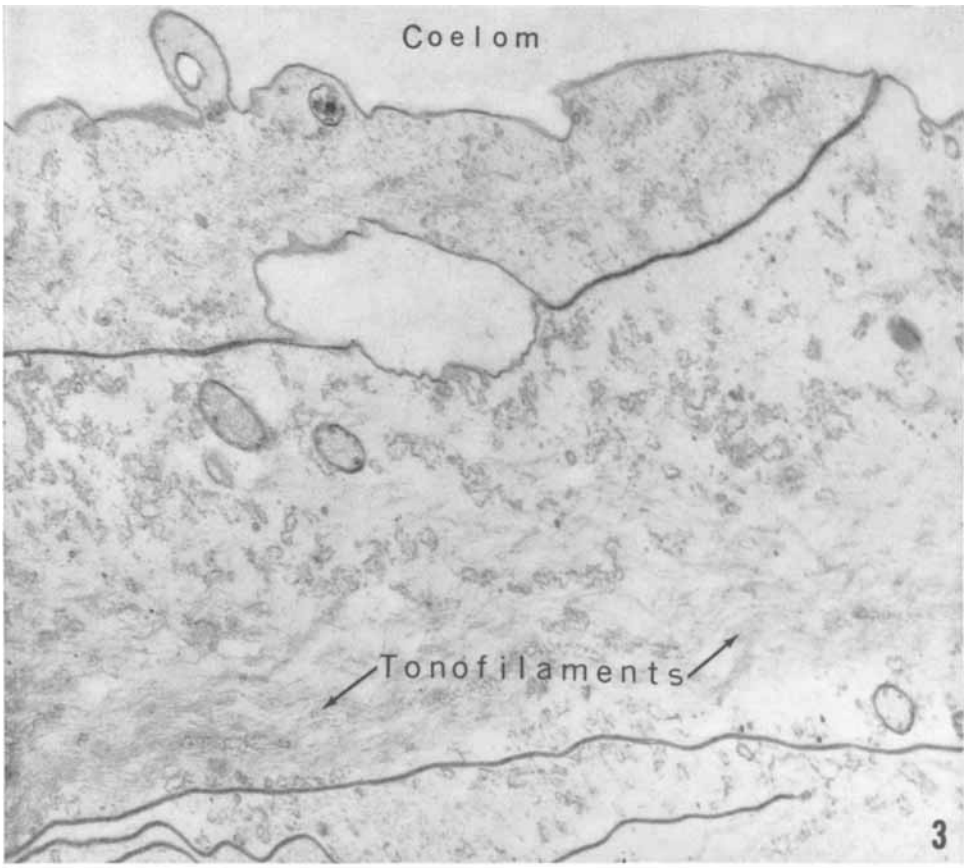
PLATE 3

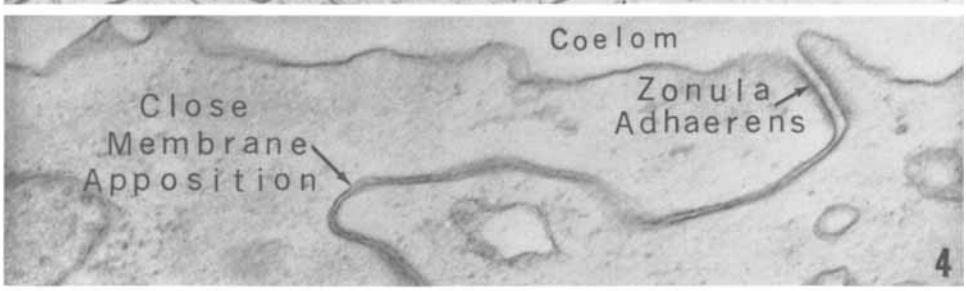
EXPLANATION OF FIGURES

3 The outer layer of the trilaminar sheath that surrounds the nervous system is made of coelomic lining cells. Note the intercellular junctional complex, the relative absence of cytoplasmic organelles and the tonofilaments. Osmic acid fixation, × 25,000.

4 A zonula adhaerens is the part of the junctional complex that faces the coelom in this animal. The intercellular gap in the zonula adhaerens is approximately 200 A. Note that the bulk of the intercellular junctional complex is a close membrane apposition. Osmic acid fixation, × 70,000.

5 A high power picture of the unit membrane structure of the close membrane apposition between apposed coelomic lining cells. Each of the two trilaminar unit membranes of these apposed cells is 75 A wide and the intercellular "gap" is approximately 50 A wide. Osmic acid fixation, × 300,000.


Coelom
Tonofilaments
3


Coelom
Zonula
Adhaerens
Close
Membrane
Apposition
4

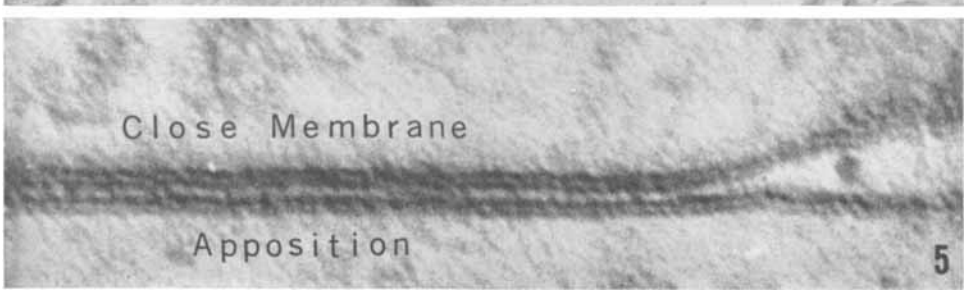
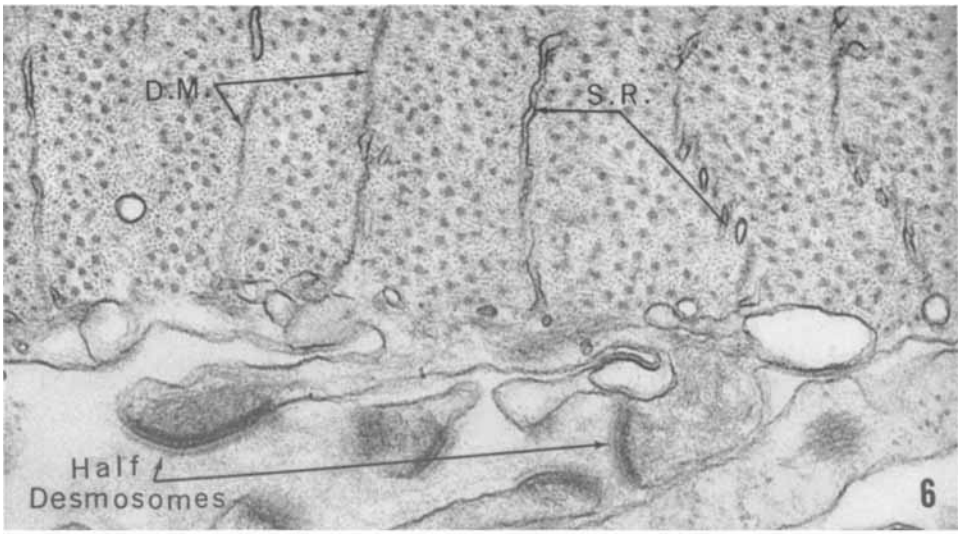

Close Membrane
Apposition
5

PLATE 4

EXPLANATION OF FIGURES

6 The bulk of the cytoplasm of the muscle cell of the sheath is occupied by myofilaments, elements of the sarcoplasmic reticulum (S. R.) and ill-defined dense structures (D. M.). Note the half-desmosomes that attach this muscle to extracellular supporting structures. Osmic acid fixation, × 32,000.

7 A view of the edges of two muscle cells. There are two sizes of myofilaments; the first thick, 200–500 A wide, and the second thin, 50 A wide. Note that the thin filaments do not have a consistent relationship with the thick filaments. A tubule of the sarcoplasmic reticulum is continuous with a large vesicle at the edge of the cell (S. R. and Ves.). The vesicles lie within 100 A of the plasma membrane, and the difference in density between the plasma membrane (P. M.) and the Vesicle (Ves.) membrane should be noted. Osmic acid fixation, × 65,000.


D.M.
S.R.
Half
Desmosomes
6

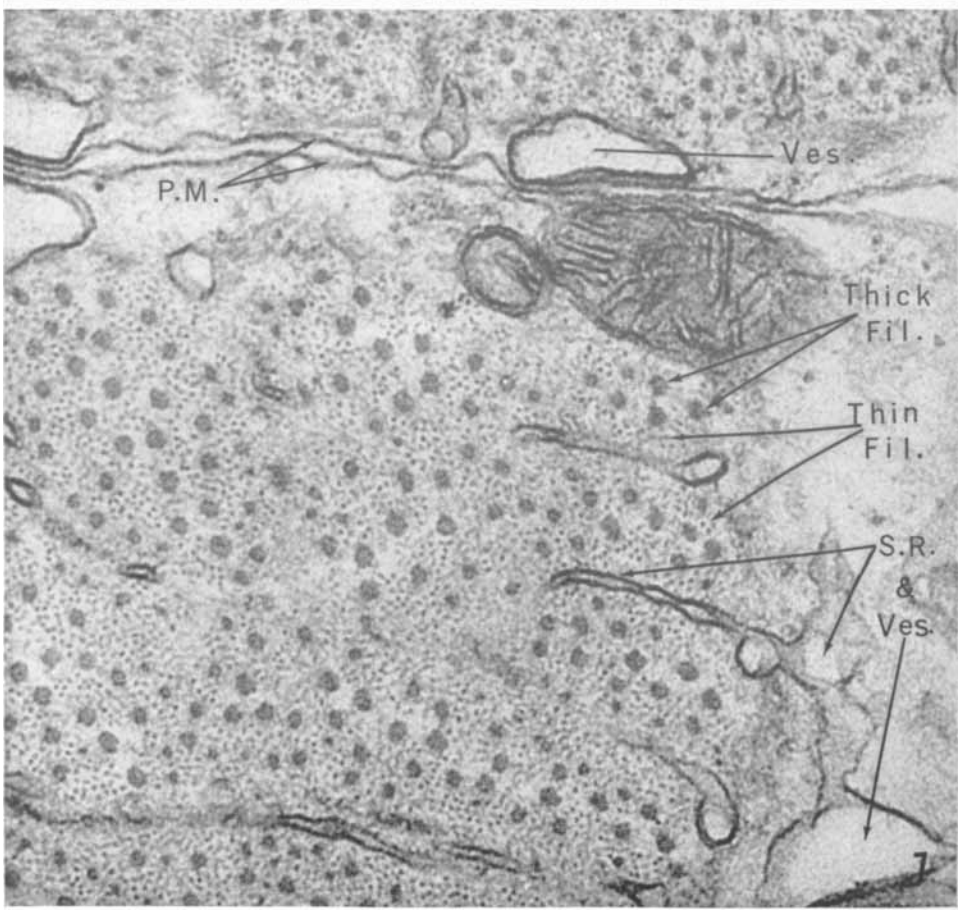

P.M.
Ves.
Thick
Fil.
Thin
Fil.
S.R.
&
Ves.
7

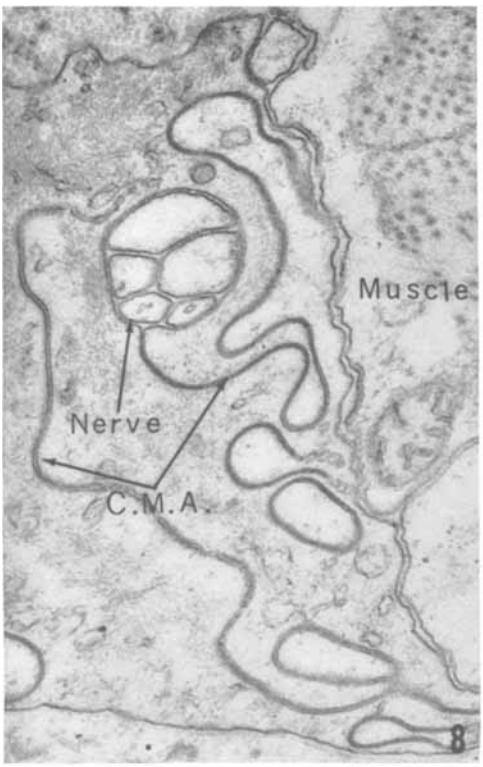
PLATE 5

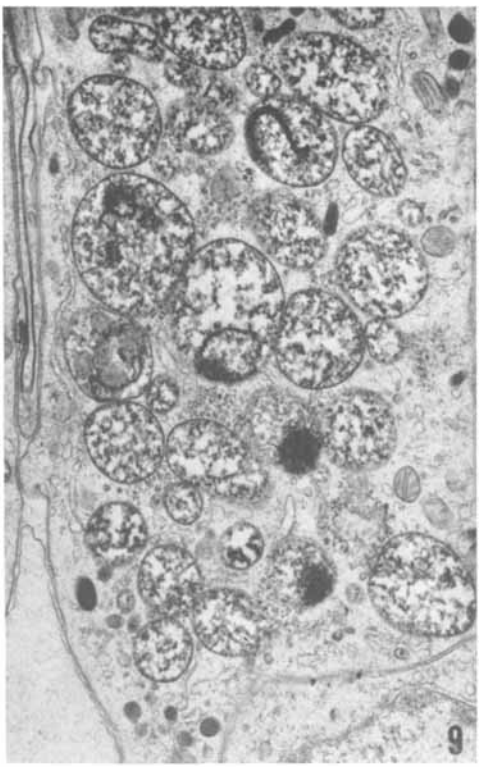
EXPLANATION OF FIGURES

8 A small bundle of axons are here embedded in folds of coelomic lining cells which can be distinguished by their tonofilaments and the close membrane apposition (C.M.A.). At other points, these nerves indent muscle cells or travel free between the other cell types in the interior of the trilaminar sheath. Note the basal lamina that underlies these cells. Glutaraldehyde fixation, × 52,000.

9 This is a cytoplasmic process of a cell that is filled with vacuoles filled with a flocculent precipitate. Osmic acid fixation, × 21,000.

10 Many cells in the interstices of the capsule possess packets of crystalline material that are often lined by a smooth trilaminar membrane (A.E.R.). In other instances this material floats free in large cytoplasmic vacuoles. Osmic acid fixation, × 102,000.


Muscle
Nerve
C.M.A.
8


9

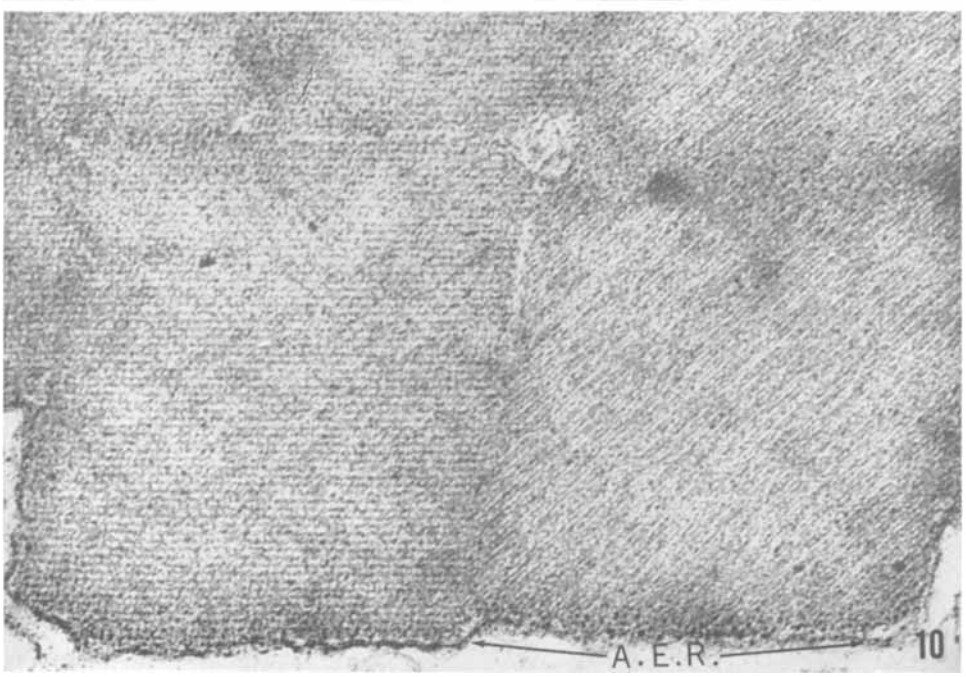
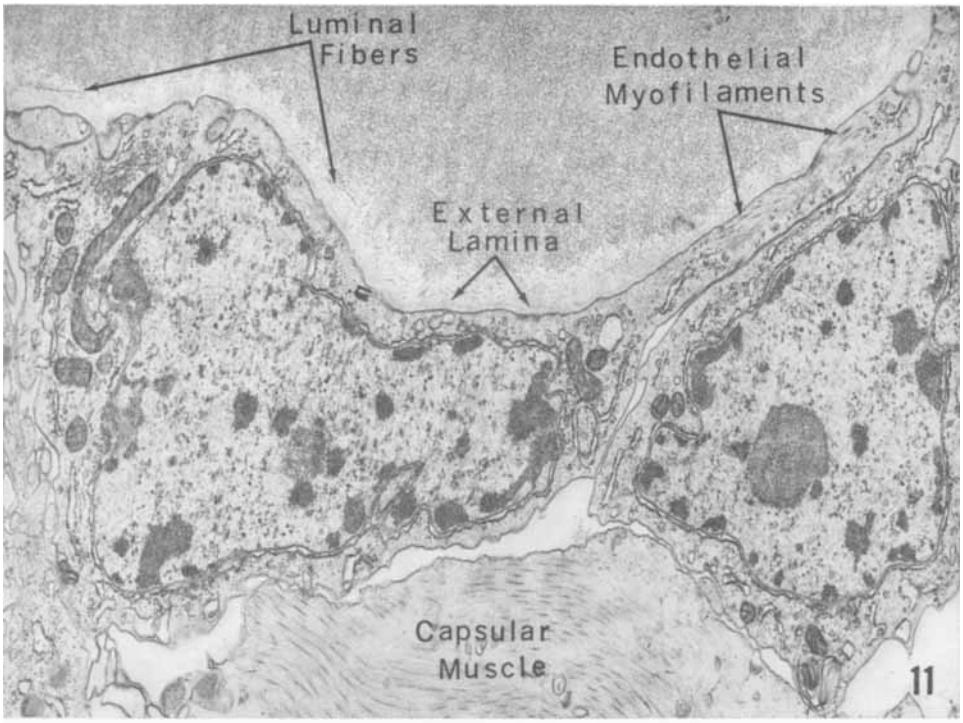

A.E.R.
10

PLATE 6

EXPLANATION OF FIGURES

11 This is a picture of two endothelial cells. Note the narrow intercellular junction and the prominent external lamina with fibers embedded in its luminal surface (luminal fibers). The endothelial myofilaments are approximately the same diameter as the thick myofilaments of the capsular muscle. The vessel is filled with erythrocuorin particles. Glutaraldehyde fixation, × 15,000.

12 The amoeboid cell with its many processes occupies part of the lumen of a blood vessel. The external lamina is here 1,500–2,000 A wide. Note the endothelial myofilaments and thickenings. Glutaraldehyde fixation, × 50,000.


Luminal
Fibers
Endothelial
Myofilaments
External
Lamina
Capsular
Muscle
11

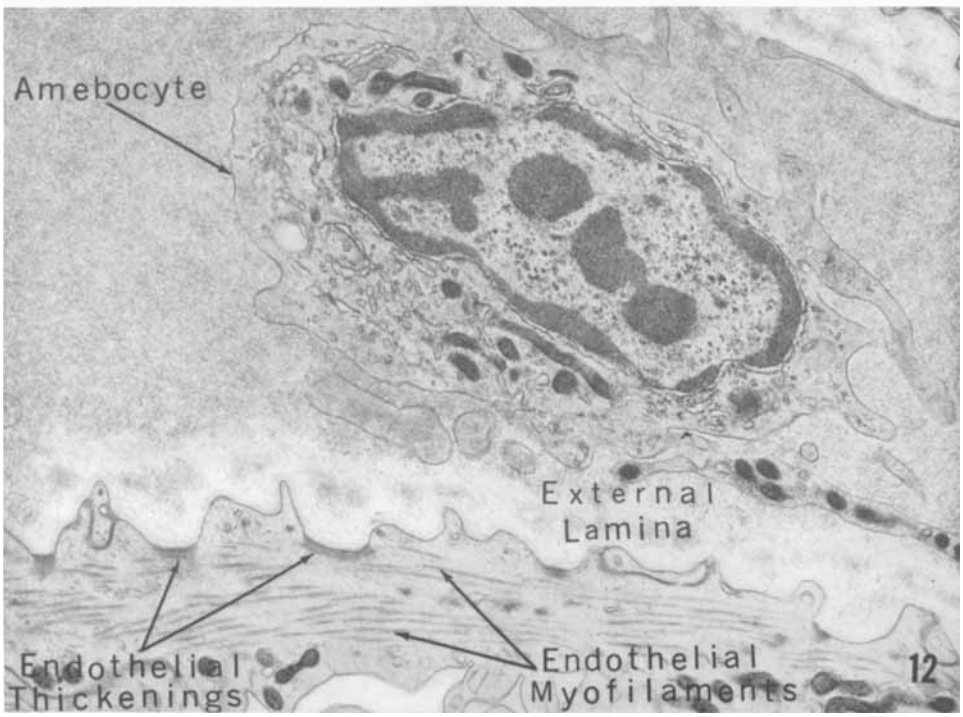
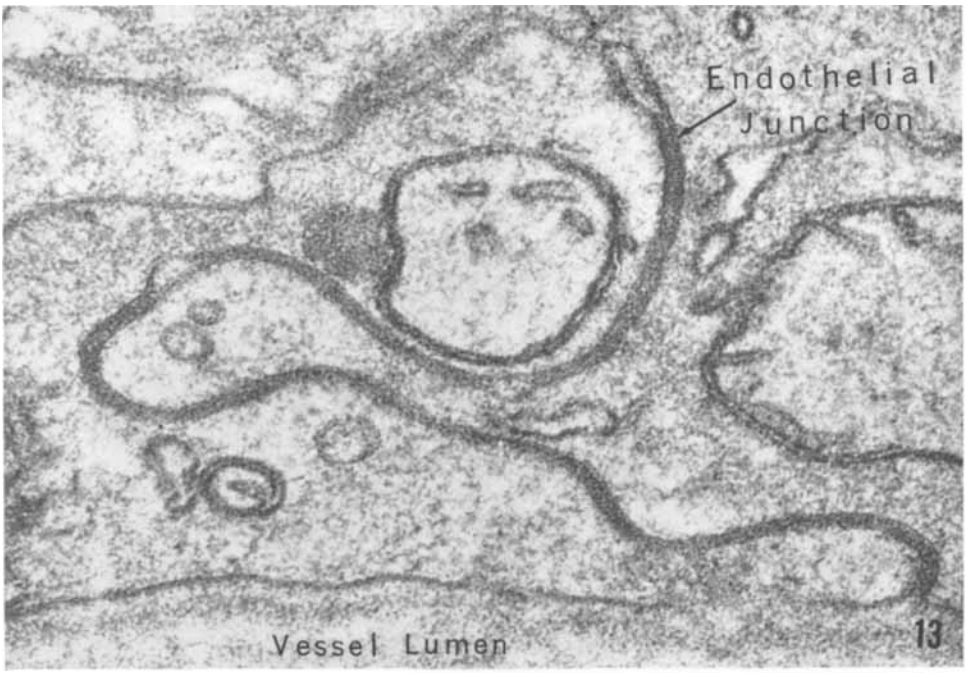

Amebocyte
External
Lamina
Endothelial
Thickenings
Endothelial
Myofilaments
12

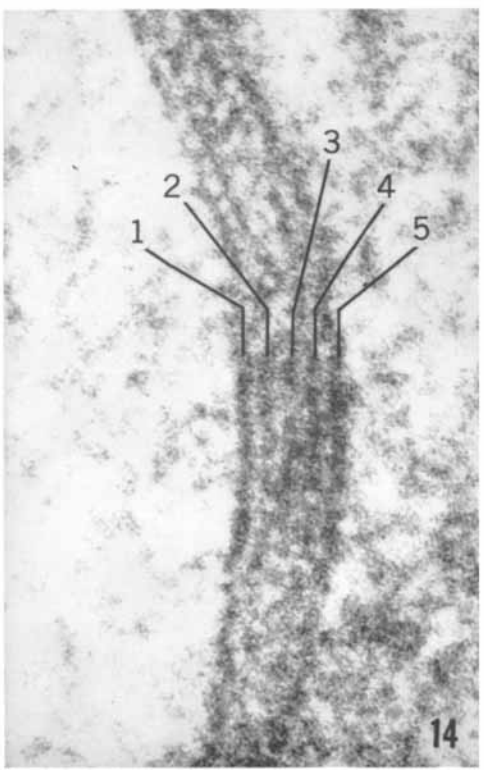
PLATE 7

EXPLANATION OF FIGURES

13 A typical endothelial junction as seen after permanganate fixation. Note that all membranes appear trilaminar and that there seems to be some material between the membranes of the two endothelial cells. Permanganate fixation, × 105,000.

14–15 Two high power views of the endothelial junction. Note that the density between the apposed endothelial cells is here resolved into an intermediate line (3) approximately 25 A wide. Here, therefore, the junction has nine layers, five dark lines (1–5) separated by four light lines, each 25 A in width. Permanganate fixation, × 910,000.


Endothelial
Junction
Vessel Lumen
13


1
2
3
4
5
14

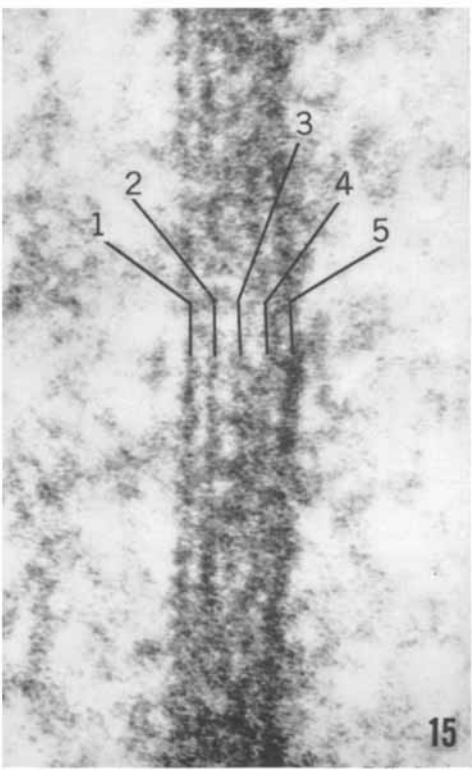

1
2
3
4
5
15

PLATE 8

EXPLANATION OF FIGURE

16 A picture of the supportive glial zone at the edge of the nervous system. The majority of the cellular processes, in which the relative lack of cytoplasmic organelles and predominance of glial filaments should be noted, are parts of supportive glial cells. The denser, filament-free, organelle packed cytoplasm of the migratory glial cell contrasts strongly with the supportive glial cell cytoplasm. Osmic acid fixation, × 18,000.

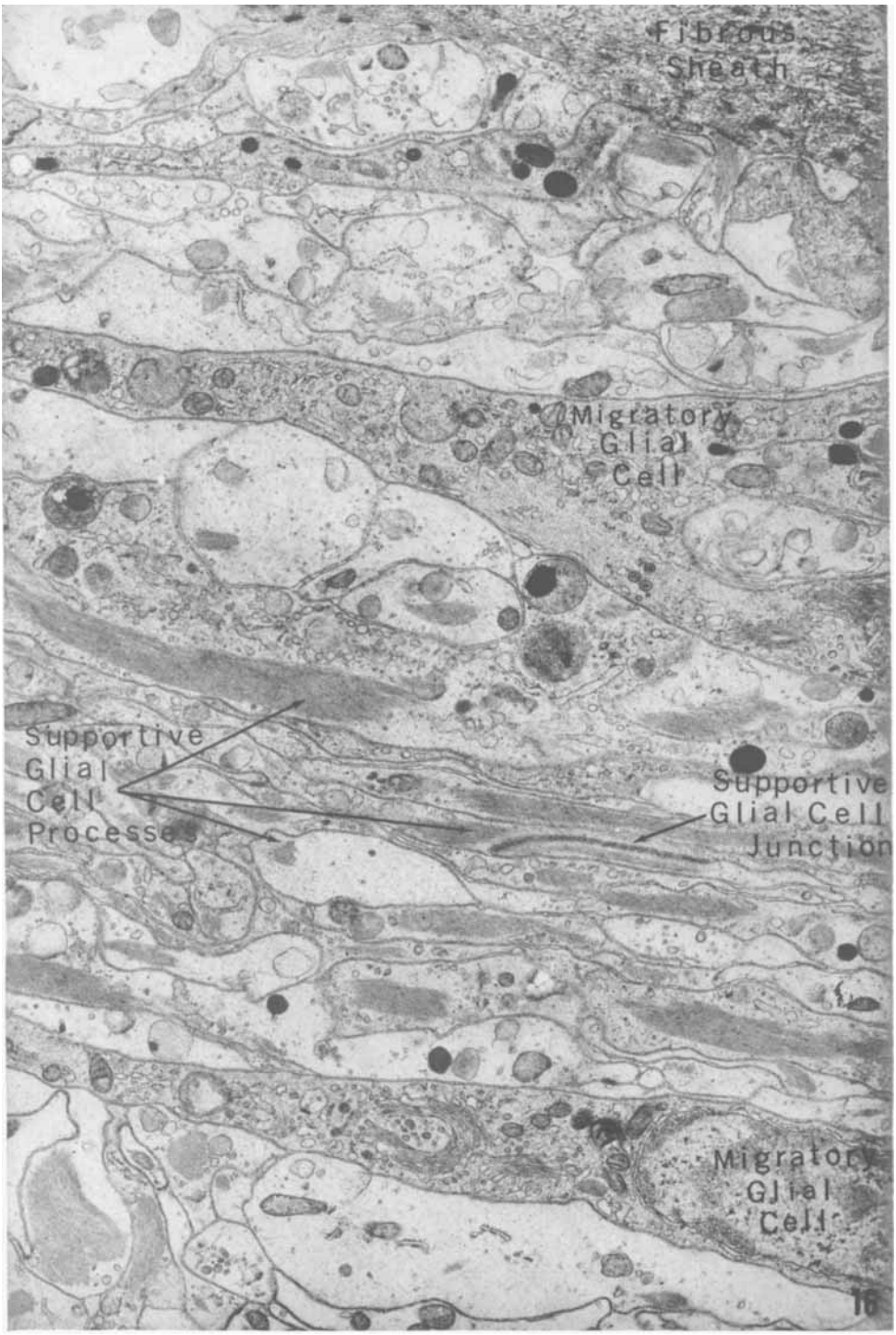

Fibrous Sheath
Migratory Glial Cell
Supportive Glial Cell Processes
Supportive Glial Cell Junction
Migratory Glial Cell
1µ

PLATE 9

EXPLANATION OF FIGURE

17 This is a view of the supportive glial zone between the dorsal giant axon and the neuropil. A small blood vessel occupies the center of the field and this vessel has a structure similar to the larger vessels of the sheath. Fibrous tissue (F.T.) is found in several places in the supportive glial zone and, at many points, supportive glial cells attach with half-desmosomes (H.D.) onto this fibrous tissue. Note that the lamellae of the loose myelin sheath of the dorsal giant axon are intact with this fixation. Finally, in the bottom center of the picture, some processes of the supportive glial zone enter the neuropil. Glutaraldehyde fixation, × 17,000.

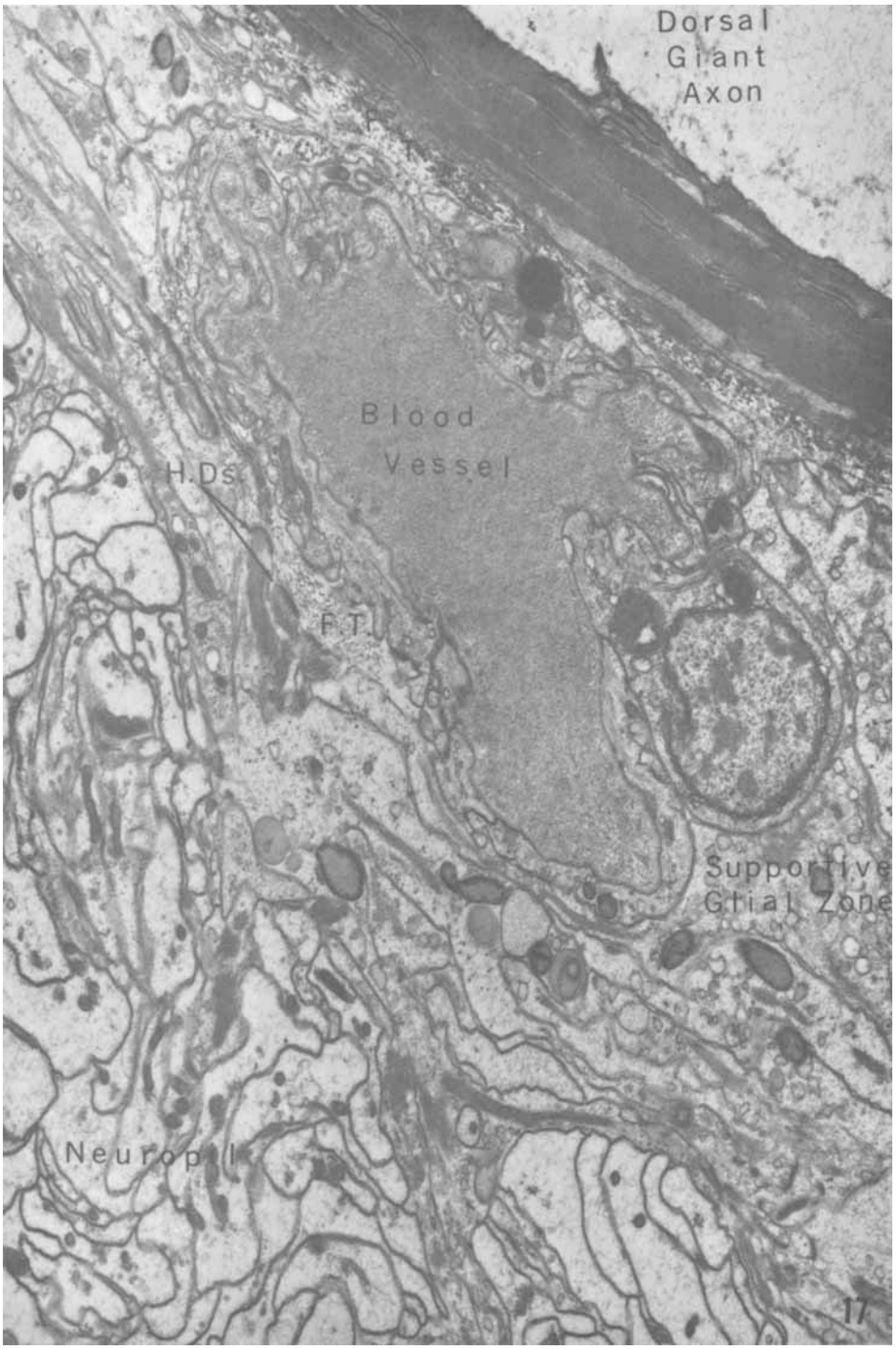

Dorsal
Giant
Axon
Blood
Vessel
H.Ds.
F.T.
Supportive
Glial Zone
Neuropil
17

PLATE 10

EXPLANATION OF FIGURE

18 All ganglion cells in this nervous system have a significant number of granules in their cytoplasm. The most extreme cases are those cells that are filled with almost nothing but granules, a Golgi complex (G.C.) and a nucleus. Such a cell, laden with dark granules, is shown here. The arrows indicate connections of one of the few cisternae of the granular endoplasmic reticulum with perinuclear cisternae. Glutaraldehyde fixation, × 32,000.

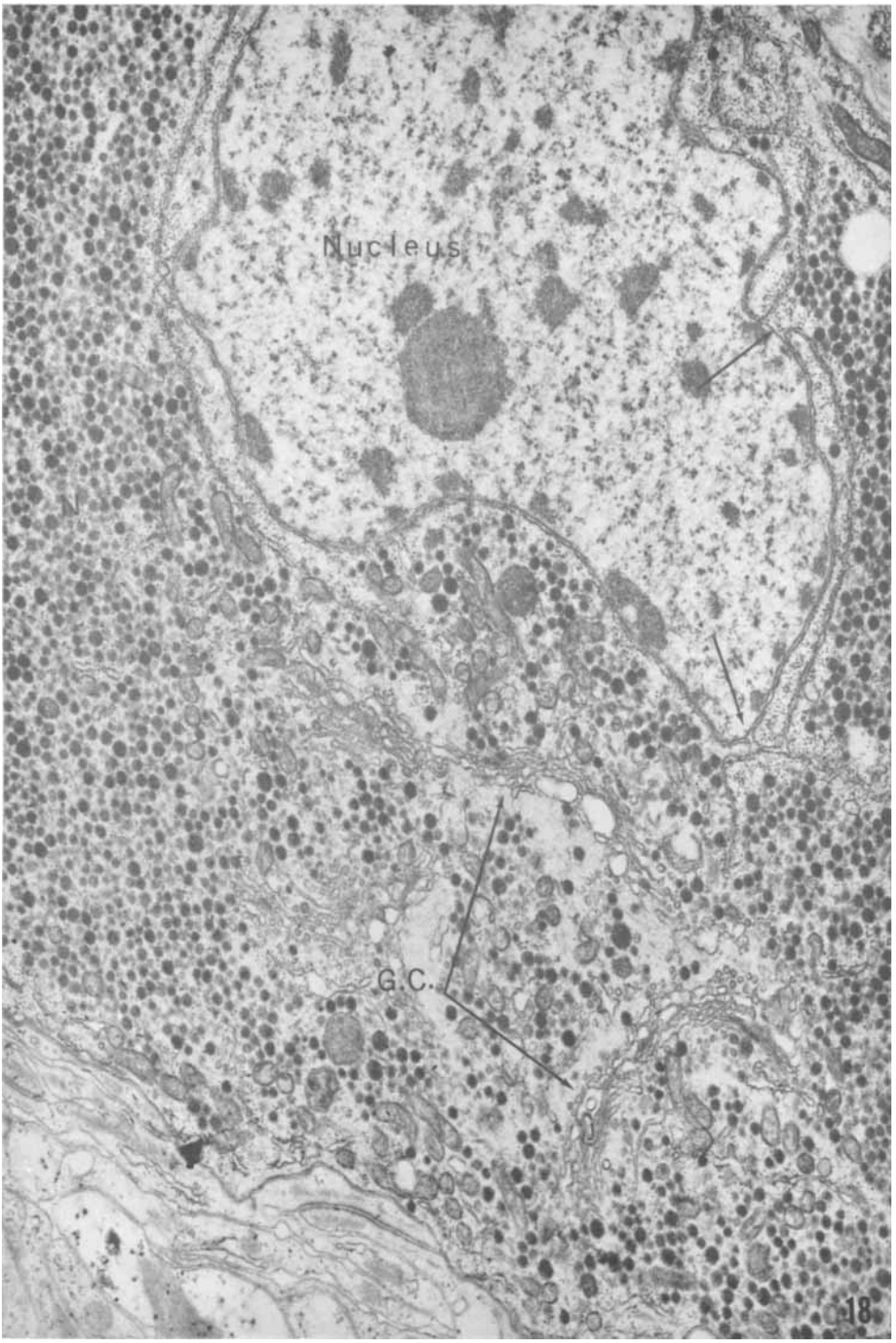

Nucleus
N
G.C.
18

PLATE 11

EXPLANATION OF FIGURES

19 Another ganglion cell is shown. This one, however, is characterized by pale granules. Note the Golgi complex (G.C.) Glutaraldehyde fixation, × 18,000.

20 In those ganglion cells not completely filled with granules, the cisternae of the endoplasmic reticulum (E.R.) often line up in an array similar to that of a Nissl body in a vertebrate neuron. Also this ganglion cell contains both pale and dark granules plus intermediate forms. Note that the extensive supportive glial cell junction in the lower part of the picture is not bisected by an intermediate line. Glutaraldehyde fixation, × 44,000.

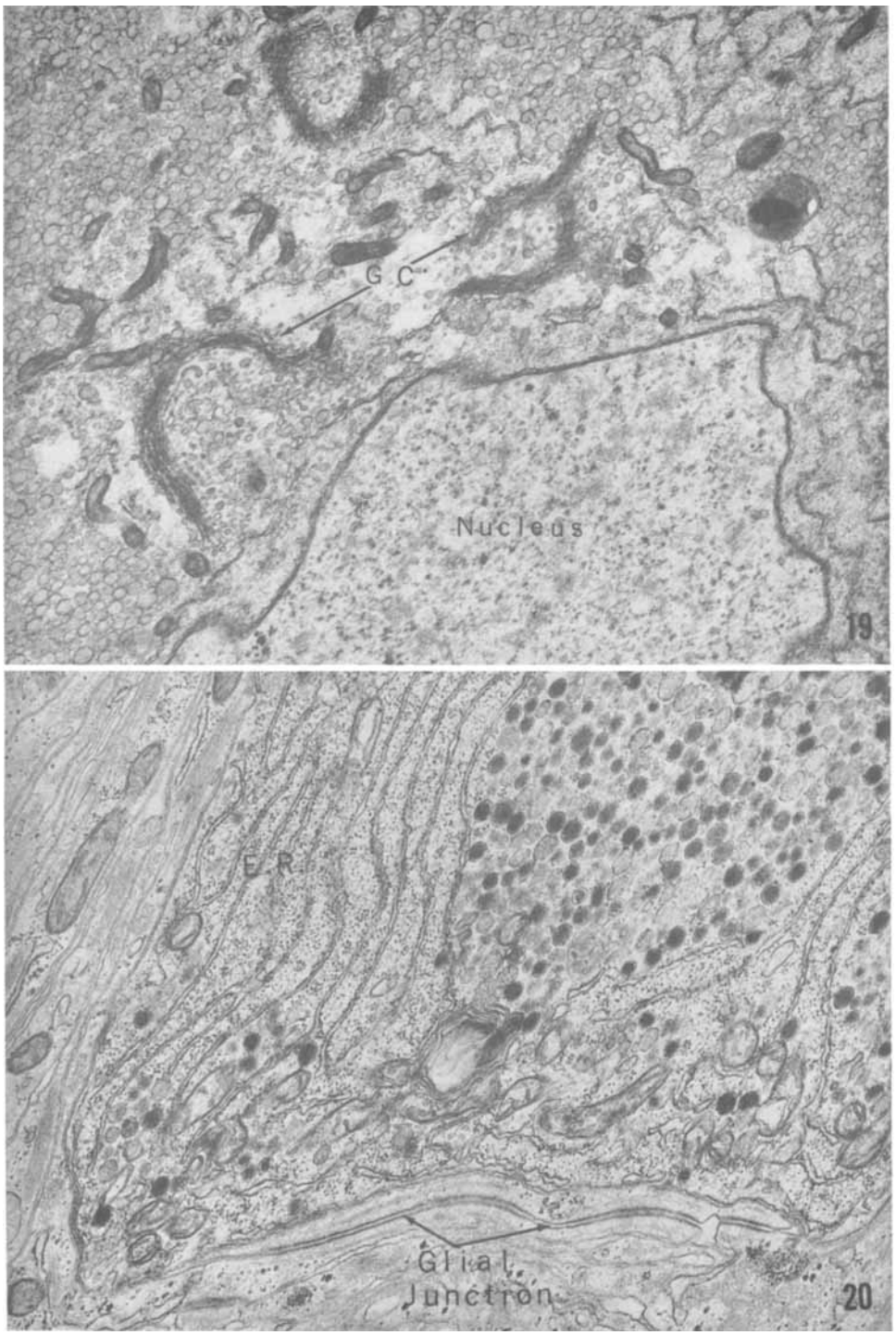
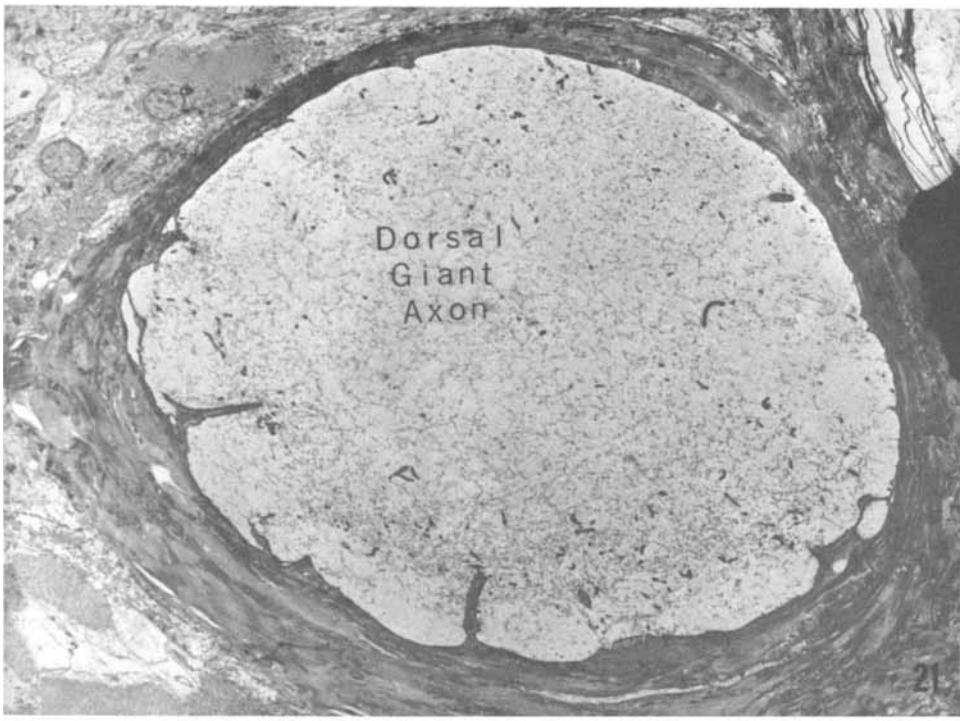

G C
Nucleus
19
E R
Glial
Junction
20

PLATE 12

EXPLANATION OF FIGURE

21 A view of a large dorsal giant axon in the posterior part of the nerve cord. The diameter of the axon is approximately 50 μ. Glutaraldehyde fixation, $\times$ 1,800.

22 At the point where a process of the median giant cell penetrates the fibrous lamina that lies between the dorsal giant axons and the rest of the nervous system, it loses its covering of supportive glial cells and is directly exposed to the fibrous extracellular material. After the median cell process passes through the fibrous tissue, it dilates to form a segment of a dorsal giant axon and here is covered by loose myelin as can be seen in this figure. Glutaraldehyde fixation, $\times$ 12,000.


Dorsal
Giant
Axon
21

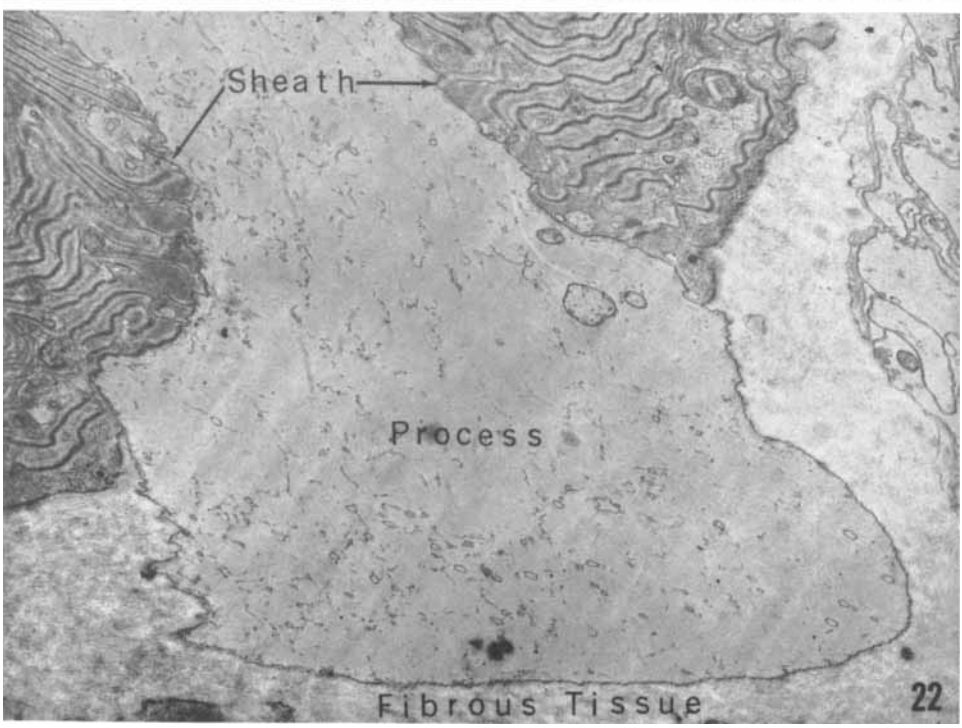
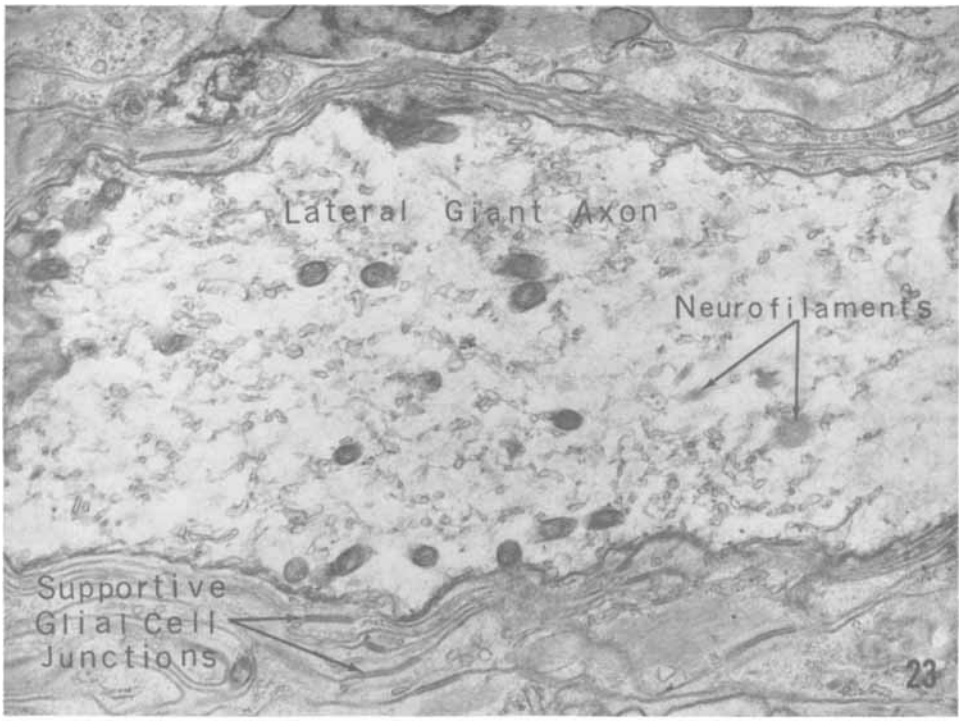

Sheath
Process
Fibrous Tissue
22

PLATE 13

EXPLANATION OF FIGURES

23 This is a lateral dorsal giant axon in the cranial nerve cord. Here the axon is quite small measuring about 5 μ in its greatest diameter. Mitochondria, smooth surfaced tubules and bundles of neurofilaments are the main cytoplasmic components of the axon. Note that, while the supportive glial cells surround the axon, the cytoplasmic processes of these supporting cells are not circumferentially arranged so as to form myelin. Osmic acid fixation, $\times$ 12,000.

24 This is a view of two attachments of a supportive glial cell process to a dorsal giant axon. On the glial side is half a supportive glial junction while on the axonal side there is only a slight thickening of the axoplasm. Note that the glial-glial junctions tend to be in register. The membranes of the supportive glial cells are here vesiculated. This phenomenon is characteristic of osmic acid fixation and not of glutaraldehyde or permanganate fixation. Osmic acid fixation, $\times$ 66,000.


Lateral Giant Axon
Neurofilaments
Supportive
Glial Cell
Junctions
23

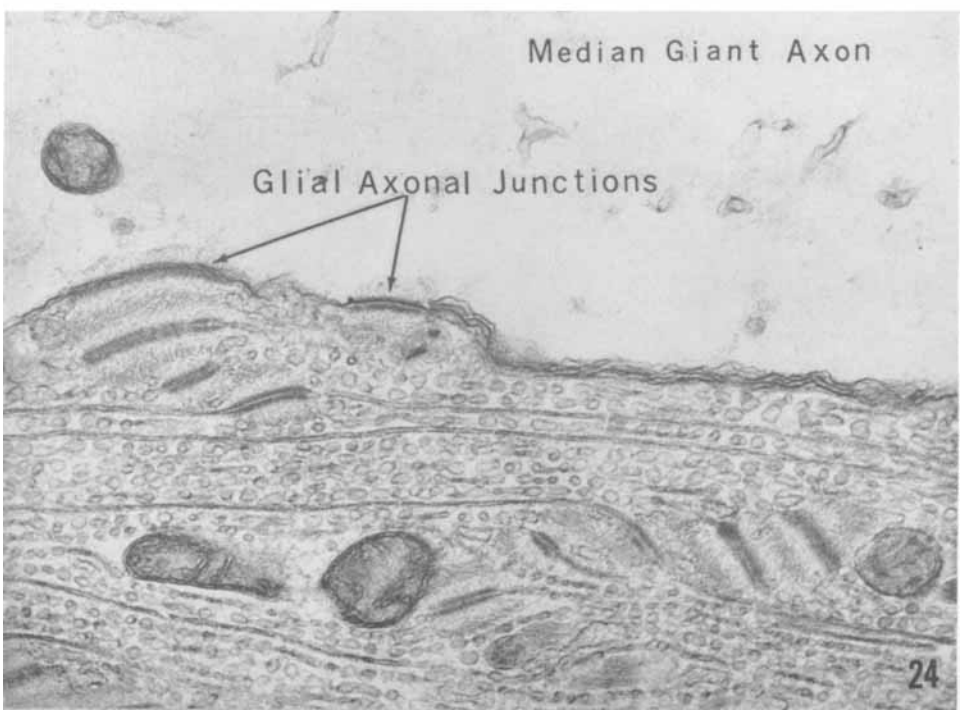

Median Giant Axon
Glial Axonal Junctions
24

PLATE 14

EXPLANATION OF FIGURES

25–26 High power views of a septum across a dorsal giant axon. The "gap" that separates each of these 75 A wide plasma membranes is approximately 50 A. This is the closest that these membranes come to one another. Note that the unit membranes appear distinctly beaded. Also note that a similar beaded pattern can be faintly seen in the cytoplasm. Permanganate fixation, × 480,000.

27 A view of the neuropil showing the different types of cell processes that characterize this region. Dark granules (D.G.), pale granules (P.G.), neurofilaments (N.F.), glial filaments (G.F.) and synaptic vesicles (S.V.) can be seen. Osmic acid fixation, × 9,000.

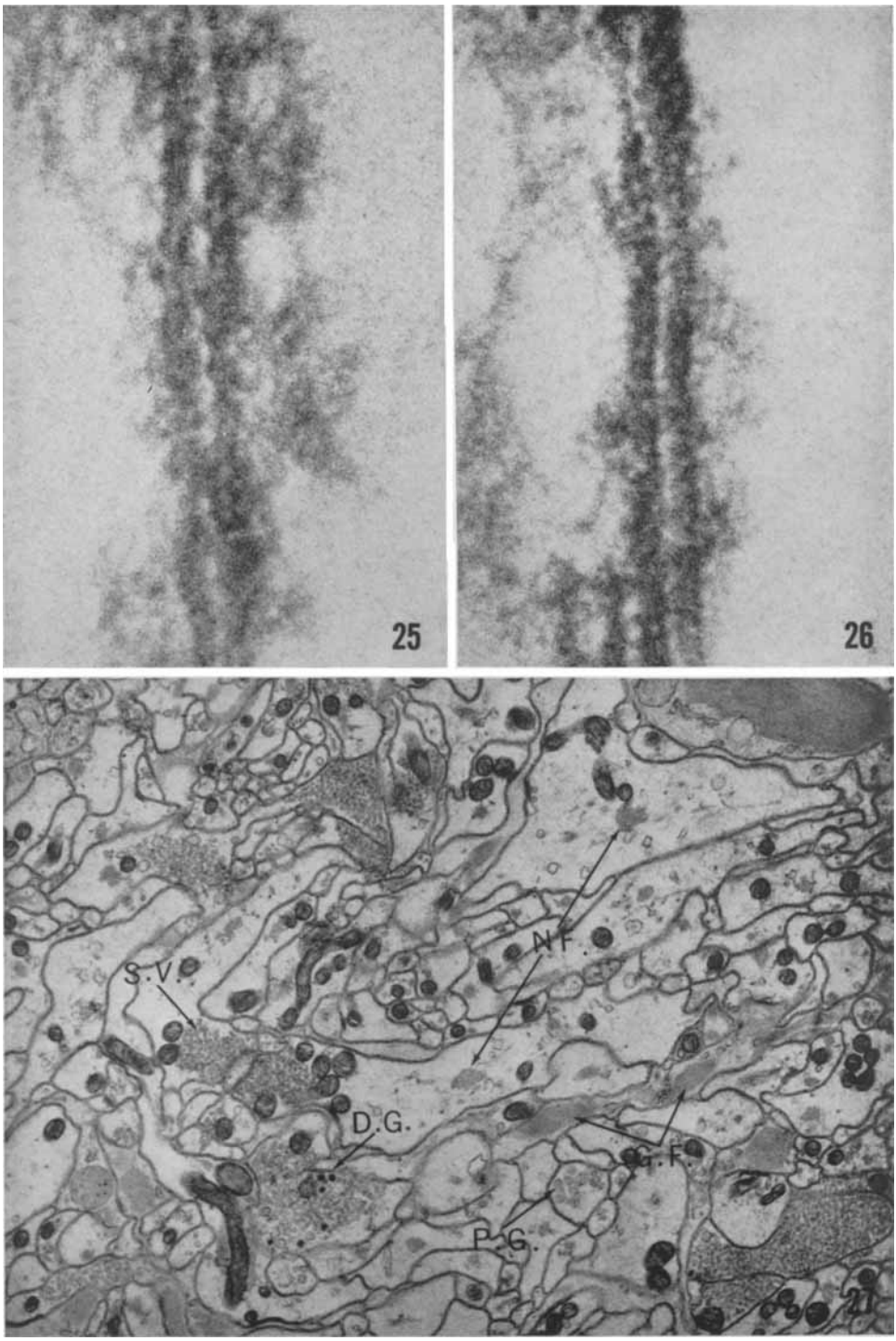
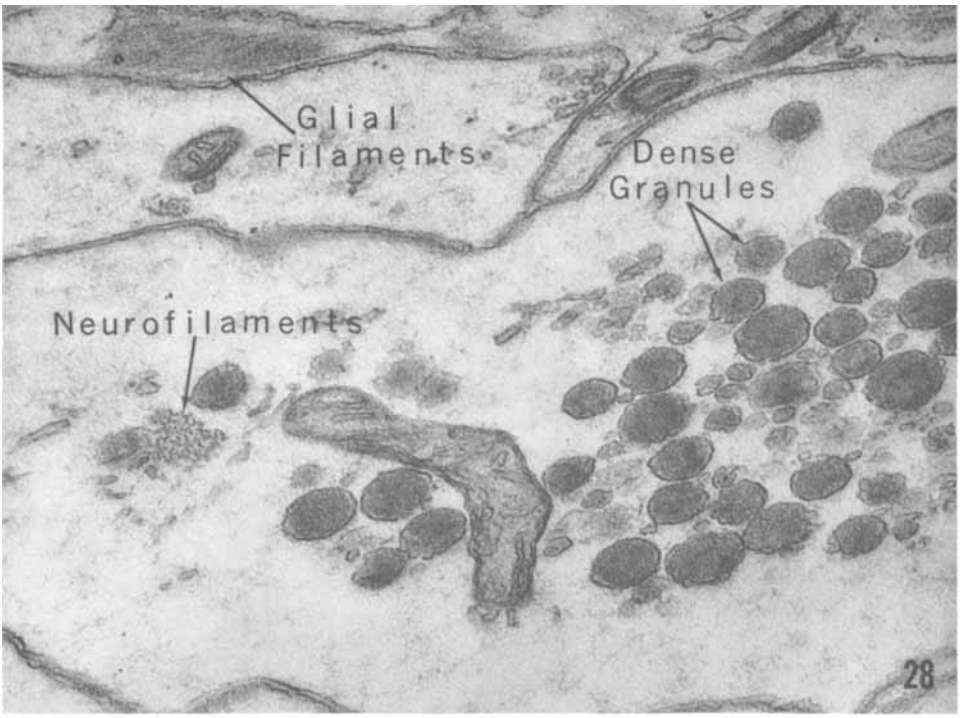

25
26
S.V.
N.F.
D.G.
G.F.
P.G.

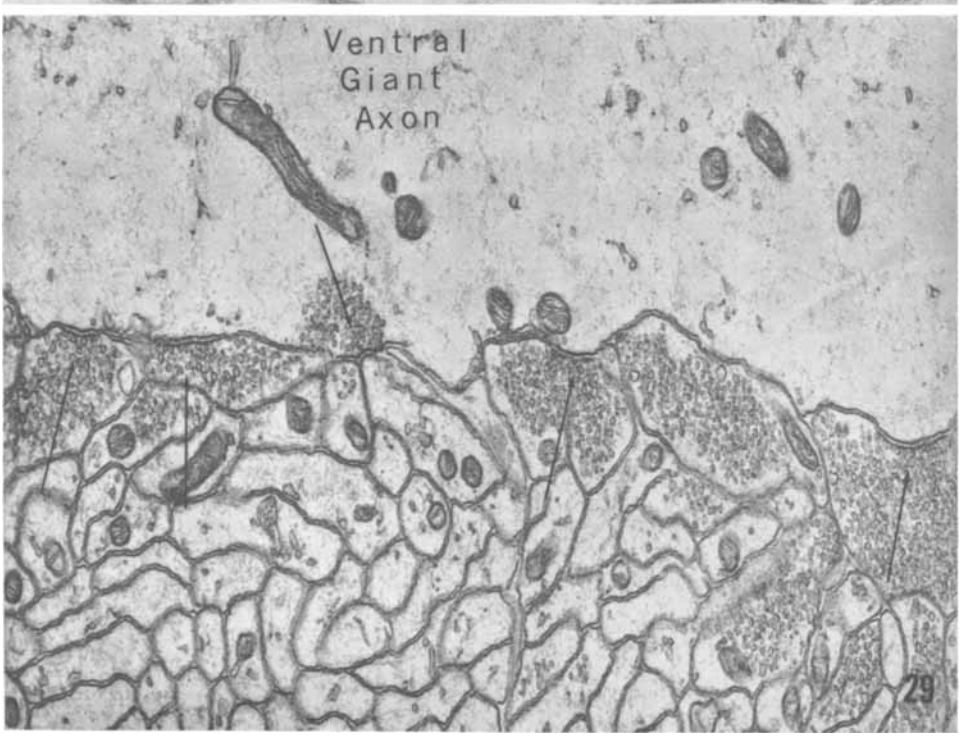
PLATE 15

EXPLANATION OF FIGURES

28 A non-synaptic region in the neuropil with a large axon containing dark granules and neurofilaments. Note that the neurofilaments are closely packed and compare the neurofilaments with the glial filaments. Glutaraldehyde fixation, × 54,000.

29 A ventral giant axon and its many satellite axons. The majority of the small axons are 0.1–0.2 μ in diameter. Arrows indicate synaptic endings and the presumed direction of synaptic transmission. Osmic acid fixation, × 48,000.


Glial
Filaments
Dense
Granules
Neurofilaments
28


Ventral
Giant
Axon
29