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Ultrastructure of the Nerve Plexus in Flatworms

I. Peripheral Organization

Harold Koopowitz and Paul Chien

Developmental and Cell Biology, University of California, Irvine, California

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Summary. The peripheral nervous system of the polyclad flatworm *Notoplana acticola* is described from electron microscopic observations. There are two components, a subepithelial system and a submuscular plexus. The subepithelial system lies among muscle cells beneath the basement membrane of the epithelium. Axons and processes containing clear or dense-cored vesicles were found. The subepithelial system is in the form of a feltwork of fibers rather than a nerve-net. The submuscular plexus has both specialized and primitive aspects to its organization. In the former category are sheathed axons and complex synaptic configurations while the latter can be seen in the numerous naked axons, somata lying deep in the nerve tissue and islands of neuropil scattered along the nerve tracts.

Key words: Primitive nervous systems— Nerve plexus — Flatworm — Platyhelminthes — Ultrastructure.

Introduction

This paper describes some aspects of the organization of the peripheral nervous system in *Notoplana acticola*, a marine polyclad flatworm. Recent studies on flatworm neuroanatomy have focussed on the brain (Best, 1967; Morita and Best, 1966; Oosaki and Ishii, 1965) which is quite complex. In fact the cerebral ganglia in certain polyclad flatworms reach stages of complexity found in the higher annelids (Bullock and Horridge, 1965). Some researchers consider the flatworms as representing the base of metazoan evolution (Corning and Kelly, 1973; Hansen, 1958 and 1963) and hence of vital importance for understanding the evolution of nervous systems in all higher animals. This is one of the main reasons for the popularity that planarians have enjoyed for comparative studies.

The brains of those flatworms studied are so complex that it seems unlikely that one could get clues about the initial events in the evolution of central nervous systems from this structure. Physiological and behavioral studies, however, indicate that much of the neuronal circuitry underlying behavior is in the peripheral nervous system (Gruber and Ewer, 1963; Koopowitz, 1970, 1973, 1974). As there are no other ganglia besides the brain one might expect the peripheral nervous system to be the most likely site for clues to the evolution of central nervous systems. Superficially the peripheral nervous system, in the flatworms, comprises two parts. There is a submuscular plexus of anastomosing strands and a subepithelial system which in some animals resembles a nerve net (Lentz, 1968). In some species the submuscular plexuses are ladderlike configurations with a net-like plexus between the rungs (Reisinger, 1925); in the polyclad flatworms the

Send offprint requests to: Prof. Harold Koopowitz, Developmental and Cell Biology, University of California, Irvine, Calif. 92664, USA.

plexus is very irregular and the entire system resembles a net. In the most primitive flatworms the entire nervous system is said to be subepithelial and occupying a position reminiscent of that found in the coelenterates (Hyman, 1951).

The coelenterate nervous system, composed of diffusely conducting, naked multi- and bipolar neurones, is generally regarded as the most primitive in the animal kingdom (Bullock and Horridge, 1965). However, even in this seemingly simple group a variety of specialized conducting systems nerve rings and ganglia are to be found (see Josephson, in press, for a recent review). The relationships between the coelenterates and flatworms are controversial (Hadzi, 1963; Hyman, 1951). A comparison of the neural organization of these two phyla might be expected to shed some light on their relationships.

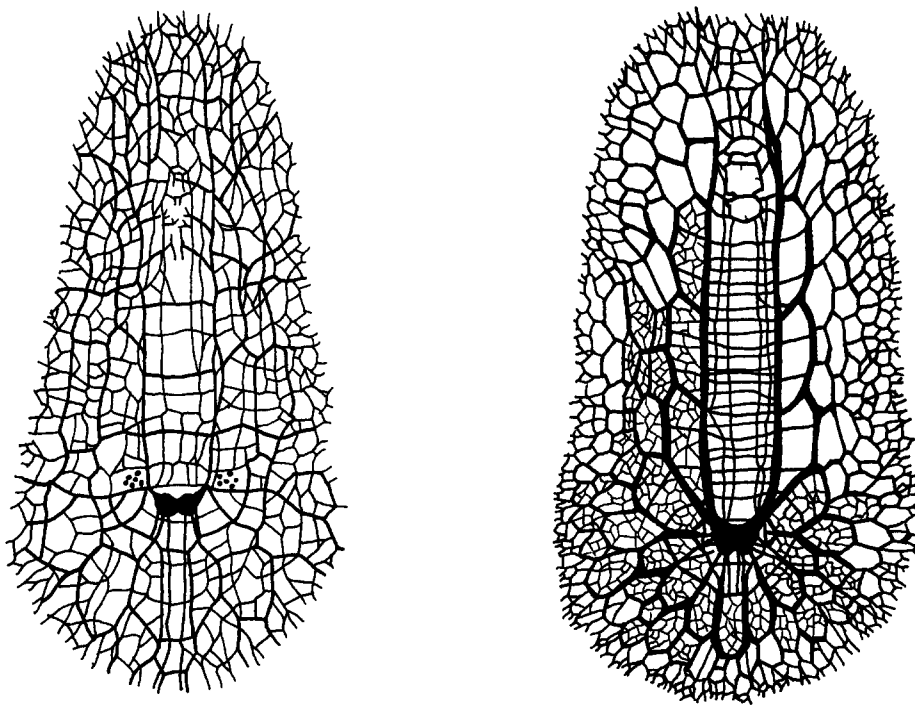
In most of the animal kingdom the nervous system has become centralized following either of two successful plans. The schizocoelous coelomates have evolved a series of discrete ganglia containing an outer rind of somata with an inner neuropile; the ganglia are connected by cords containing axons. The central nervous system in the enterocoelous deuterostomes, on the other hand, is made up of longitudinal cords, which contain somata and neuropile, often in intimate association, as well as axons of passage. At the base of these two major lines of evolution but above the coelenterates are the flatworms, phylum Platyhelminthes. Although they are the first bilaterally symmetrical animals to possess a brain, the flatworms are probably much more closely allied to the schizocoelous protostomes than to the deuterostomes.

At this point there are two questions of interest. (1) Does the flatworm nervous system really share features with those found in the coelenterates? (2) Will this nervous system provide any insight into how nervous tissue originally became centralized? The following report is the first in a series describing the nervous system of the polyclad flatworm *Notoplana acticola* which we believe addresses these problems.

Methods

The methods used were similar to those outlined earlier (Chien and Koopowitz, 1972). Specimens of *Notoplana acticola* were collected beneath intertidal rocks from Corona del Mar, Southern California and maintained in laboratory sea water tanks until needed. These flatworms feed readily on frozen brine shrimp and will live for a number of months. *N. acticola* may reach 4 cm in length and 1.5 cm in width. The material to be fixed was cut from along the sides of the animal between midbody and eye level. Furthermore the tissue was taken from the outer third of the body. This tissue does not contain the major longitudinal nerve cords of the submuscular plexuses. The strip of tissue was placed in 5% triple distilled glutaraldehyde and chopped into little pieces, about 1 mm^2, with a razor blade. These pieces were fixed for two hours in the glutaraldehyde and then transferred to 2% osmium tetraoxide. The fixatives were prepared in 0.1 M phosphate buffer at pH 7.2 or 7.4. Most buffer solutions also contained 0.45 M sucrose; where sucrose was omitted mitochondria and some vesicles became swollen. Following fixation the tissue was washed with buffer, dehydrated in acetone series and embedded in Epon 812.

Glass or diamond knives were used to cut sections on a Reichert OMU2 ultramicrotome. Thick sections, 1–2 μm, were cut and stained with toluidine blue and observed with a phase microscope. If nervous tissue could be seen and if it appeared interesting then gray to silver thin sections were cut. Where possible a number of serial sections were mounted on a grid. Contrast in the thin sections was increased with lead stains (Karnovsky, 1961; Reynolds, 1963). Sometimes aqueous uranyl acetate (2%) preceded the lead citrate stain. A Zeiss EM9A microscope was used to examine the sections.



A B

Fig. 1 A. The dorsal submuscular plexus of *N. atomata*. B. The ventral submuscular plexus of *N. atomata*. Nerve plexuses were drawn after Hadenfeldt (1929)

We had considerable difficulty in obtaining consistent fixation with this tissue. Very often the sides of the animal disintegrated as it was dissected, this behavior is often shown by *N. acticola* when placed in stress situations, e.g., being restrained or gripped. We suggest that workers unfamiliar with these worms cool them down to about 4°C before cutting them for fixation. This seems to stop the disintegration reaction. Orientation with regard to dorsal and ventral surface was not obtained. This was unfortunate because there are slight differences in the gross morphology of the submuscular plexuses of the ventral and dorsal sides of the animal.

Observations

Gross Morphology

There are actually two submuscular plexuses which are visible with light microscopy and suitable staining. The gross form of the nervous system was described for the closely related polyclad *Notoplana atomata* (Figs. 1 A and B) (Hadenfeldt, 1929) and it is essentially the same for the species used in this study. The dorsal plexus is a reticulation of fine nerve fibers while the ventral plexus is comprised of much stouter nerve branches. In the ventral plexus there is also a much finer network of nerves lying between the meshes of thick nerves. Towards the periphery of the animal the nerve trunks become smaller and the meshes reduced. The plexuses at the edge of the animal appear qualitatively quite different under the light microscope but they have not been examined in any

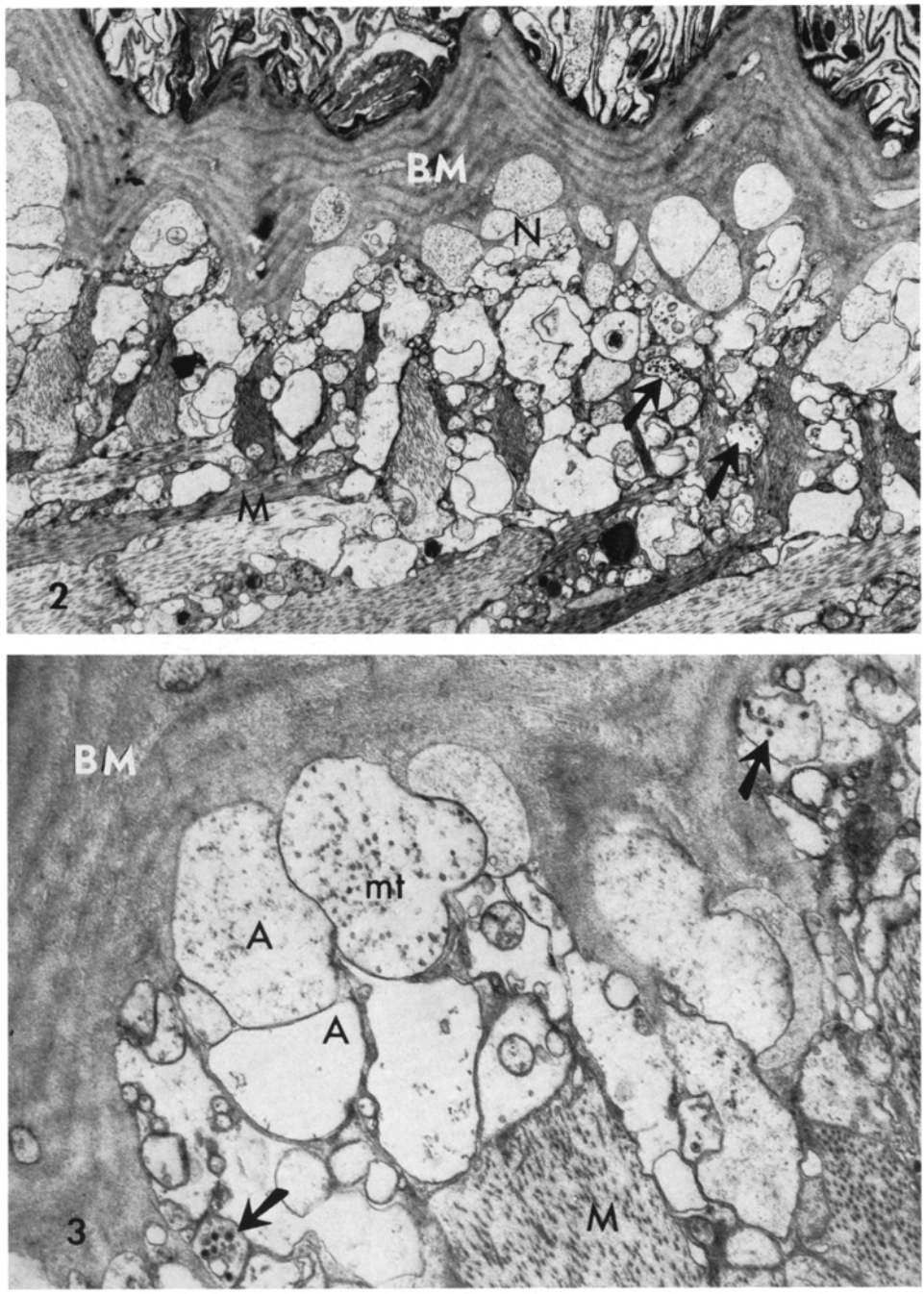


Fig. 2. Transverse section through the epithelium of *Notoplana acticola*. The cell processes at the top of the picture are the bases of the epithelial cells resting on the basement membrane. Beneath the basement membrane is a stratum containing nerve processes. The nerve stratum lies above a layer of muscle cells. Arrows point to processes which contain dense cored vesicles. *BM* basement membrane, *M* muscle layer, *N* nerve cells. ×5400

detail. We do not know if the two plexuses join so that the entire system is a flattened sphere or if there are two separate sheets of nerve. Both plexuses, however, feed into the cerebral ganglia. The submuscular plexus sends branches deep into the muscle fields. We have already described (Chien and Koopowitz, 1972) the peculiar system of neuromuscular innervation that occurs in this flatworm. It is not clear if the nerves end blindly in the muscle fields or if they join up with the subepithelial nervous system on the outer edge of the musculature.

No attempt has been made to examine the structure of sensory cells in the epithelium and we have been unable to distinguish between motor and sensory neurites in the plexuses.

Fine Structure

A. The Subepithelial Nervous System

The ectoderm of flatworms is comprised of tall columnar cells sitting on a laminated basement membrane. Sandwiched between the basement membrane and the underlying muscle fields is a stratum composed of cell processes (Fig. 2). We have interpreted these as nervous in origin. The cytoplasm of the processes (Fig. 3) is very much like that found in nervous tissue (cf. Fig. 7). Microtubules are present in some of the cells (Fig. 3) and structures resembling synaptic vesicles are common (Figs. 2–4) in some of the processes. Both dense cored (Fig. 3) and clear vesicles have been observed (Fig. 4), but definite synapses were not found. We had some concern that much of this tissue might be tails of muscle cells attaching to the basement membrane. Muscle attachments to the basement membrane, however tend to have a very dense staining cytoplasm with filaments.

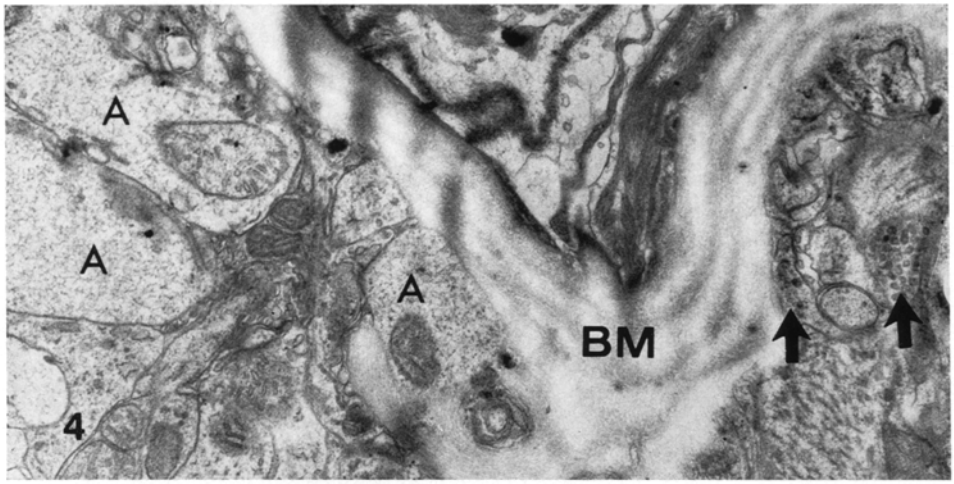
The neuronal tissue is too densely packed to produce a subepithelial nerve-net as has been reported in planarians (Lentz, 1968). It is almost a feltwork of fibers. Likewise one suspects that there are too many to consider them as fibers of passage terminating in sensory dendrites in the epithelium. Although some fibers may be passing through the basement membrane (Figs. 2 and 3) it would be unlikely to find synaptic vesicles in this position if one was dealing only with *en passant* sensory fibers. Only once did we find a cell body that could be designated, with some assurance, as neuronal.

The most important feature of the system is that the cell processes are beneath the basement membrane and situated between muscle cells rather than among the bases of the ectodermal cells as in coelenterates.

B. The Submuscular Plexus

Cell Components. There are at least three different kinds of cells to be found in the nerves making up the submuscular plexus. These are muscle cells, glia and neurites. It is occasionally difficult to identify which of these three types is present as they possess a number of similarities. The criteria that we have used will be given as each kind is discussed. In part, difficulties in interpretation are due to the disorganization of the material which, perhaps, relfects the primitive nature of the organism.

Fig. 3. Cell processes in the sub-epithelial nervous system. Note that a number of the axons contain microtubules. Arrows indicate both clear and dense cored vesicles. *A* axon, *BM* basement membrane, *M* muscle cell, *mt* microtubules. ×20800



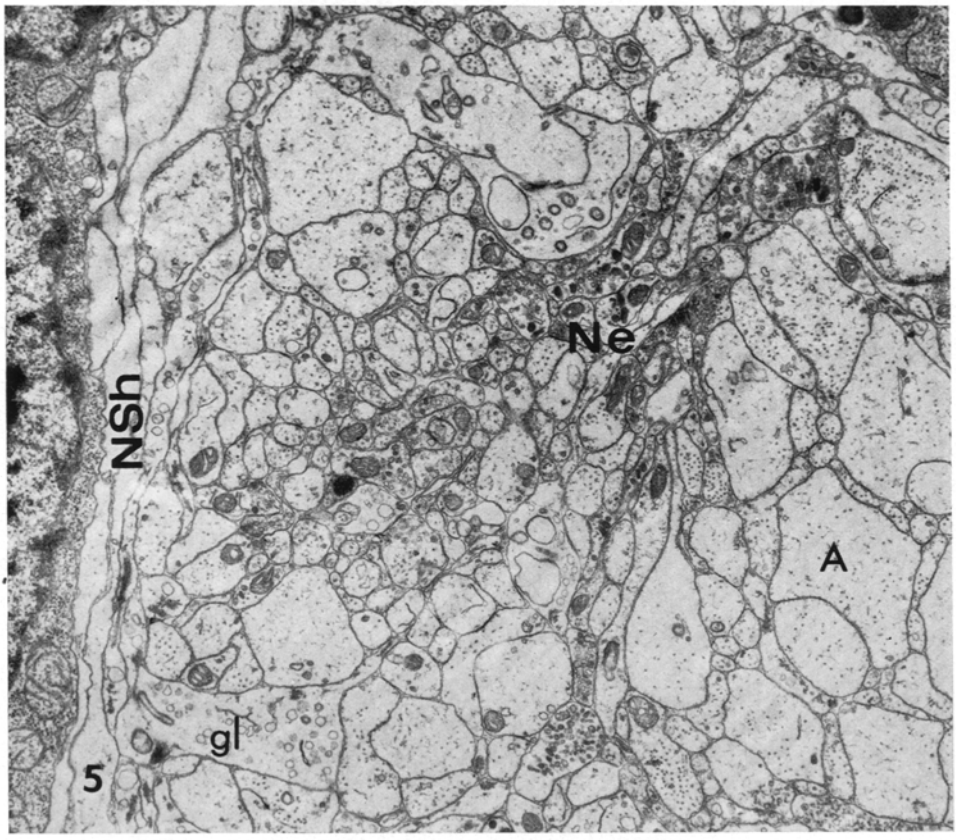


Fig. 4. The subepithelial nervous system. This preparation was from an individual with a very thin basement membrane. Here the axons appear to have more ground substance. The arrows indicate processes containing clear vesicles. *A* axons, *BM* basement membrane. ×18600

Fig. 5. Transverse section through part of a small nerve trunk. Note the abundance of microtubules and cisternae in the axons. In this section individual axons do not appear to be sheathed in glial cells. *A* axon, *gl* glial cell, *Ne* neuropil, *NSh* nerve sheath. ×14700

1. Sheaths and Glia.

Most of the nerves making up the plexus have a sheath of cells around them; however, unsheathed tracts of nerve were also found within the muscle fields. Fig. 5 shows part of a sheath surrounding a small nerve. Typically the sheath is made up of a few layers of cell processes and these contain cytoplasm which is electron transparent. There are few inclusions or organelles other than some irregularly shaped vacuoles. There are no special features which the outer sheath cells possess that distinguish them from the internal glia (Fig. 5). The most obvious glial cell processes are the sheaths that surround some of the axons. Sometimes individual sheathed axons may be difficult to find (Fig. 5) or only thin sheaths may occur around some of the axons (Fig. 6). Occasionally extensive sheathing many layers thick were found around the individual axons (Figs. 7). A single sheet of glia may enclose a number of axons in one bundle or it may contribute to the coverings of separate axons (Figs. 6 and 9). Quite often where there are multiple sheaths as in Fig. 7, the glial processes may be stacked like lamellae. Glial intrusions into large axons are not uncommon (Fig. 8). The most obvious glial elements are those which occur as sheets often containing patches of densely staining cytoplasm (Figs. 7, 8 and 9). Problems in interpretation arise when one considers sections as shown in Fig. 9. Here cells containing small vacuoles, like those in the glia of Fig. 5, are found. While some of the cells contain cytoplasm similar to that of the axon sheaths (Fig. 7) others also contain microtubules.

The configurations shown in Fig. 7 are also of interest. Nerve cell processes appear to run within what appears to be the glia. In one of the cells of Fig. 7 there are two layers of glia around the neurite. One does not know if these represent calyx formations or if the axons merely run for some distance surrounded by the supporting cell. Without the presence of synaptic vesicles one cannot determine whether one is dealing with glia within glia or nerve, or possibly nerve within nerve or glia. Occasionally we have found somata containing dense granular cytoplasm with irregularly shaped nuclei. These are quite different from the nuclei of muscles or neurones and therefore may belong to the glial component.

2. Muscle Cells

Within the muscle fields there is often no nerve sheath and the axons appear to be tucked between the muscle cells. Within these nerves, which are often substantial, as well as within the deeper trunks of the submuscular plexus, one often finds discrete muscle fibers (Fig. 6) deeply embedded in the nerves. This is not really surprising as muscle cells have even been described from within the brain of fresh-water planarians (Morita, 1965). Although the contractile apparatus of muscle cells can easily be discerned, they also have other processes and extensions that cannot be clearly differentiated from neuronal cells (Chien and Koopowitz, 1972). Muscle cells can be divided into three morphologically distinct parts. (1) There is the contractile portion containing thick and thin filaments but towards the tips of the cells there are no contractile elements and the cell is filled with clear cytoplasm rather like some glia or axons. (2) Nuclei belonging to muscle cells are contained in a separate cytoplasmic sac (Chien and Koopowitz, 1972) while, (3) other regions of the cell give off long processes that may travel some distance to the nerve where the neuromuscular junction occurs. These sarcoplasmic ex-

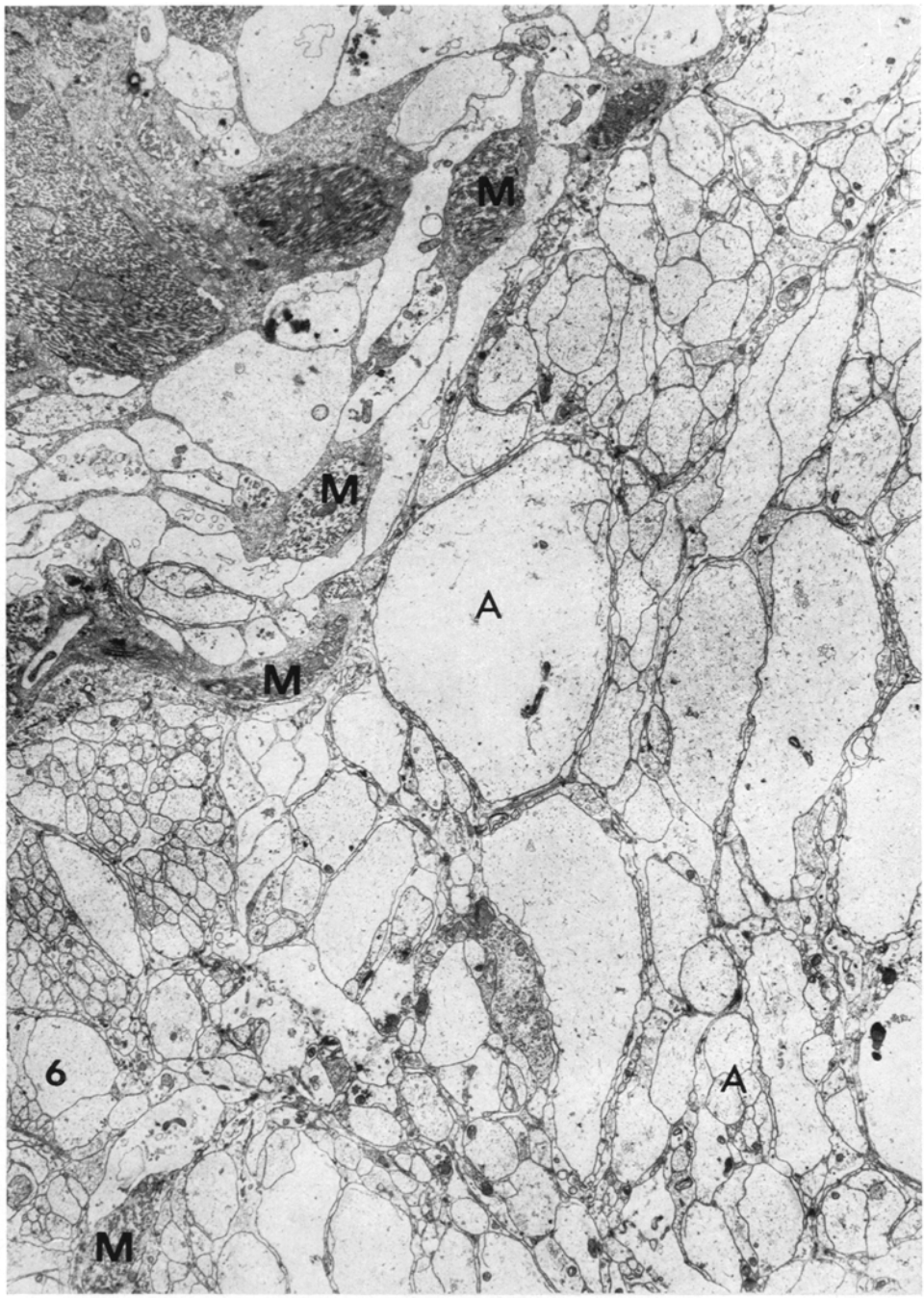


Fig. 6. Transverse section through a portion of a large nerve. Note the considerable variation in diameter of the axons in the nerve. The largest axons tend to be sheathed. Although the distribution of axon size appears to be mixed in the lower right hand portion of the figure, there are areas which contain axons of similar size. There does not appear to be any neuropile in this section. *A* axons, *M* muscle cells, *gs* glial sheath, $\times 6300$

tensions contain clear cytoplasm and microtubules and could be mistaken for axons if the attachment to the rest of the muscle cell is not apparent.

Nerves running deep in the muscle fields often appear to be associated with large numbers of cell bodies containing nuclei. Some of these are clearly neuronal. This point will be discussed further.

3. Neurones

a) Somata. Except for the brain, which has a well defined rind of cell bodies containing nuclei, the somata in the rest of the system appear to be scattered without any apparent order. While a few cell bodies are found at the periphery of the nerve tracts, it is quite common to find them deeply submerged in other neural tissue (Fig. 10). Where a soma does not have a definite axon, we have taken the presence of a glial sheath as a positive indicator for nerve cells. Also these cells tend to have comparatively clear cytoplasm similar to that found in axons. Only very occasionally can structures reminiscent of synaptic vesicles be found within the soma.

Although the brain has a large number of cell bodies there are surprisingly few in the longitudinal cords or the nerve plexus. Neuronal somata can be found in the plexus but one must make a special search for them. Either the vast majority of the axons in the plexus have their cell bodies in the brain or the cell bodies are sequestered in some as yet unidentified place. We cannot answer this question categorically yet, but we suspect that the majority of the cell bodies associated with nerves deep in the muscle fields belong to neurones rather than muscle cells. This point bears further investigation.

From a phyletic point of view the shape of the cell body and the attachment of the axons is of some importance. The nerve cells of coelenterates tend to be mainly multi- or bipolar isopolar cells while the higher protostomes tend to have mainly unipolar cells. All three types of cells have been described in flatworms by light microscopy; unipolar cells usually occur in the rind of the brain in *Leptoplana* (Turner, 1946) while multi- and bipolar cells have been recorded from the brain of *Thysanozoon* (Lang, 1884). We have looked with the electron microscope at the plexus in *Notoplana* and have not been able to find neurones that could unequivocally be called bi- or multipolar. Here the soma of the cell (Fig. 10) has a slight neck, constricting and separating it from the axons. It is a moot point whether or not one could consider this cell as being intermediate between a bipolar cell with its soma suspended between the axons and a unipolar cell with its soma separated from the axons by a stalk. We have, however, found cells that are at least bipolar if not multipolar in another flatworm *Enchridium* (Koopowitz, unpublished data).

As in most invertebrates obvious synapses do not occur on the soma; however, *en passant* axons in close juxtaposition with the soma are not uncommon (Fig. 10), and even occur when the soma is partly sheathed with glia. It is possible that electrical junctions occur on the soma between these cells but good evidence is lacking.

b) Axons. At present we have no means of distinguishing between axons and dendrites in this system and have tended to regard any nerve process that is not obviously a soma or involved with a synapse as being axonal.

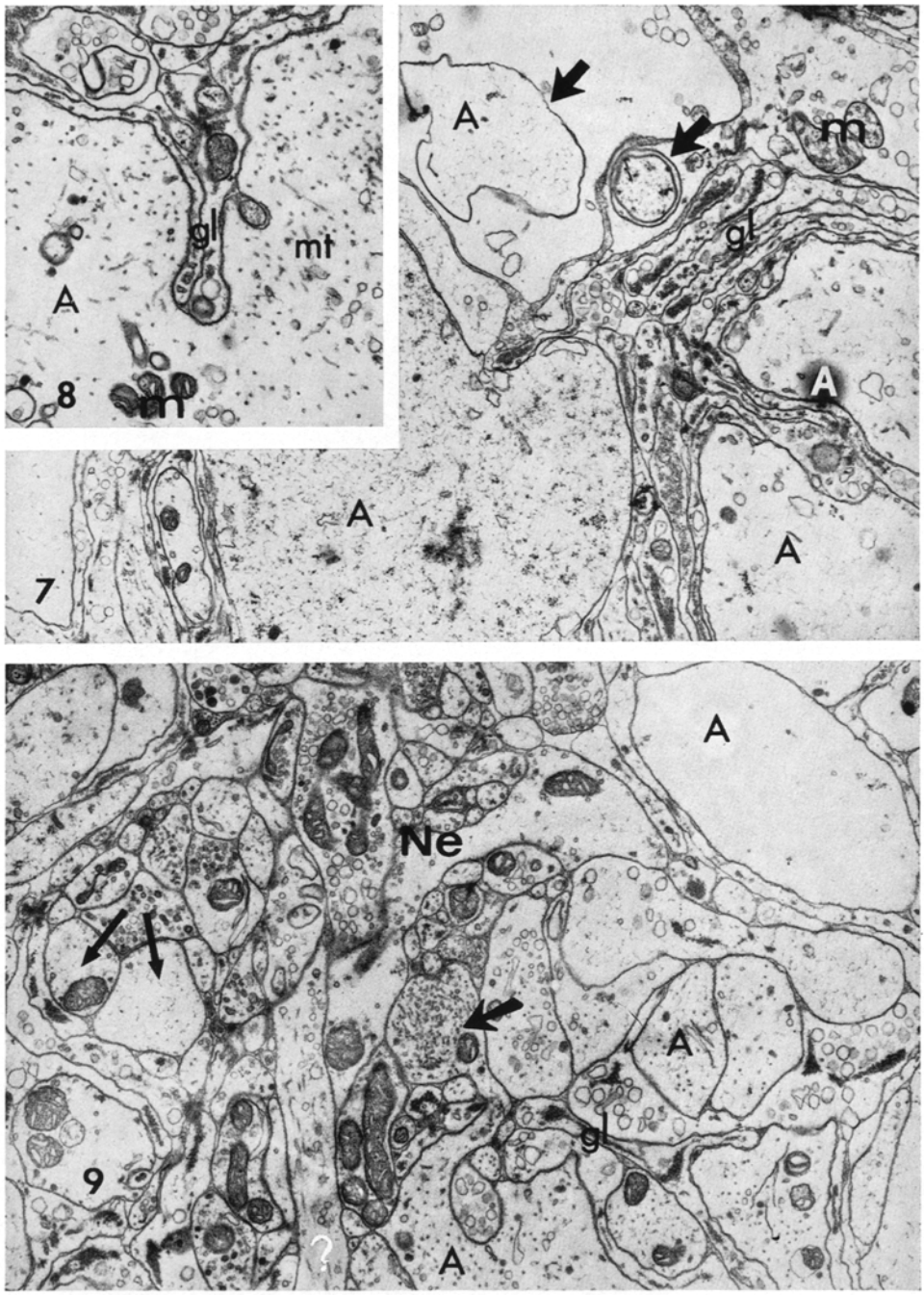


Fig. 7. Sheathing around axons. This figure shows the variety of sheathing configurations which can be found. These range from multiple lamellae of glia to axons submerged in glial cells (arrows). *A* axons, *gl* glia, *m* mitochondria. ×20000

Fig. 8. Glial intrusion into an axon. Note that the intrusion can contain more than one glial extension. *A* axon, *gl* glia, *m* mitochondria, *mt* microtubules. ×16000

The number of axons within a nerve is quite variable and even small branches may have hundreds. Within a nerve the majority of the fibers run parallel to each other. Fig. 5 shows a portion of a cross-section through a fairly large nerve. The range of axon diameter is considerable. The largest axons may be 8 μm in diameter with the smallest less than 0.1 mμ. Usually the very large axons are sheathed and the smaller ones are unsheathed although in the latter case several may be enclosed by a glial extension (Fig. 6). The mean diameters for sheathed axons is 1.6 mμ whereas unsheathed processes average approximately 0.5 mμ. Few axons of smaller nerves (Fig. 5) have individual sheaths. Within a nerve the arrangement of the axons can be variable. Fig. 6 has both homogenous areas where the axons tend to be similar and mixed portions where a variety of axons occur. Whether or not the homogenous areas represent clusters of neurones leaving or entering the nerve is not known.

There is considerable variation in the density of material found in the axons. Basically the electron-opaque material is a webbing of very fine granular material (Fig. 7). Sections through axons have been found which are almost clear of this ground substance (Figs. 3, 6, and 9) while others are quite densely filled (Figs. 2, 4, 7, and 10). It is unlikely that the differences in distribution of ground substance between axons is a fixation artifact because different densities were found in adjacent cells (Fig. 3). With the exception of mitochondria there were comparatively few organelles within the axons. The occurrence of microtubules were variable. In some axons (Figs. 5 and 8) they are quite prominent whereas in other sections they appear to be lacking (Figs. 7, and 10). The microtubules appear to be hollow and about 200 Å in diameter and were rather like the microtubules reported from the neurones of other animals. No neurofilaments have been found. Electron-light irregular vacuoles ranging in size from 400 to 1600 Å were found in axons as well as glia. These can be seen in Figs. 8 and 9 where the vacuoles occur in the same process together with microtubules or synaptic vesicles. Flattened double membrane sheets up to 2 mμ long are often found (Fig. 10). In some respects they resemble the subsynaptic cisternae recorded from other preparations but are not confined to a subsynaptic position. Microtubules were often found in close conjunction to the cisternae but this may have been fortuitous. Synaptic vesicles can be found scattered through the axons.

Discussion

The peripheral nervous system of *Notoplana* is a curious mixture of both primitive and advanced characteristics. Perhaps the primitive nature can be most readily appreciated from the difficulties that arise during interpretation of the material. Muscle, glia and nerve processes share many structural features in

Fig. 9. Transverse section through a part of a small nerve trunk. Note that some axons appear to have little in the way of organelles whereas others have numerous microtubules. Small paired arrows indicate a "shared" synapse. The large arrow points to an ending filled with irregular vesicles. Note that certain processes possess both microtubules and small vacuoles making identification difficult. *A* axons, *gl* glia, *Ne* neuropile, ? processes which could belong to either glia or neurone. ×13800

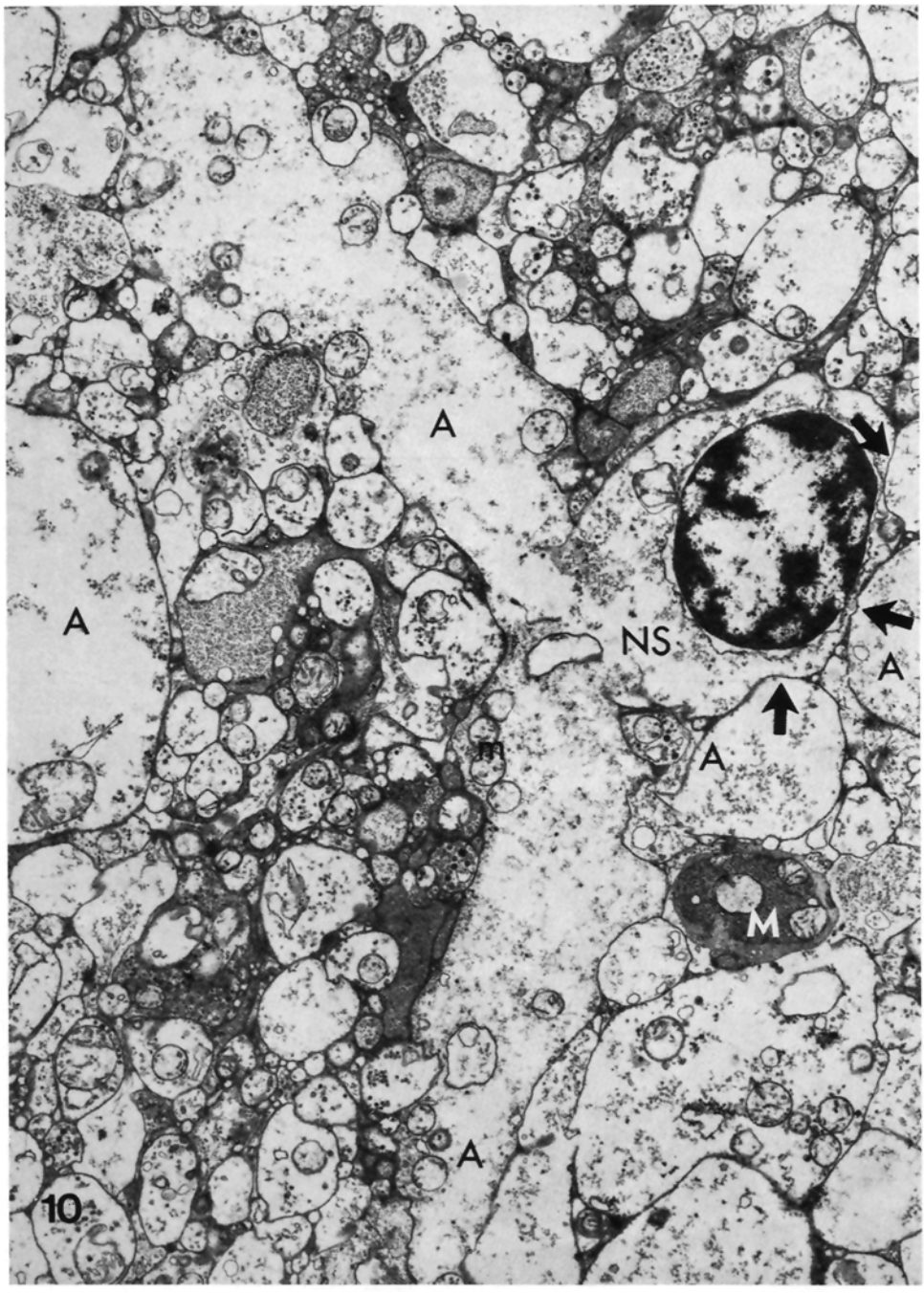


Fig. 10. Section through a nerve cell. This fortuitous section shows the soma connected to two small axons by a short neck. Arrows indicate three axons in close contact with the soma. Note the muscle cell near one branch of the nerve cell. *A* axons, *NS* neural soma, *M* muscle cell. ×11900

common which makes component analysis difficult. Not all of the cells, however, are unspecialized. The tightly sheathed nature of some axons involve highly specialized glial cells and the synaptic configurations indicate a degree of complexity equal to that in the higher protostomes. On an organizational level the primitive nature of the system can be much more readily appreciated. In *Notoplana* nerve plexus there is no compartmentalization of structure that is so clearly apparent in higher protostomes. Somata lie deep in the neural tissue and the cells seem to be scattered through the plexus. Except for the brain we cannot find evidence for a trend towards ganglionation with a rind of clustered cells. Similarly the neuropil is also scattered in small clusters throughout the system. In terms of phylogeny the organization of elements is probably more important than cytological specializations. Even in the coelenterates there is a great variety of neuronal elements (Josephson, in press; Westfall, 1973) and one gets the impression that Lentz (1968) was essentially correct in asserting that advances in the evolution of nervous systems involved rearrangements of basic cellular units. Best (1967) has commented on the great organizational complexity of the brain in fresh water triclads and considers that the differences between these animals and higher organisms in terms of behavioral abilities merely relate to the numbers of cells involved. Polyclad flatworms also show cerebral complexity (Bullock and Horridge, 1965) but it is clear from our observations that the difference in nervous systems between these and higher metazoans is not merely quantitative. The level of organization in the plexus is considerably less than that in the central nervous systems of other protostomes.

At this point it would be worthwhile considering possible relationships between the coelenterates and platyhelminthes. Hyman (1951) considered the nervous system in the most primitive Turbellaria as being "*...in a condition resembling that of the coelenterates*" and occupying "*...the same situation that it possesses in the Cnidaria.*" This description conjures up a picture of a fine network of naked neurones lying between the bases of the epithelial cells. A system like this containing bipolar and multipolar cells has been described for the white planarian *Procotyla* (Lentz, 1968). In *Notoplana* the ectoderm is separated from the mesoderm by an extensive basement membrane. The subepithelial nervous system is actually separate from the ectoderm and lies among mesodermal cells. It appears to be neither analogous nor homologous to the condition described in the coelenterates. This does not imply that the flatworm nervous system is not ectodermal in origin, in fact the brain and submuscular plexus are clearly derived from the ectoderm (Kato, 1940). In the triclads, rhabdocoels and alleocoels the nervous system develops from an ectodermal pair of cerebral ganglia which sink into the mesoderm and the nervous system proliferates and grows out from that organ (Kato, 1968). The development of the subepithelial system is not known. More observations are needed on the development of the subepithelial system before an assessment of its relationship to coelenterate systems can be made. What is of interest is that the submuscular plexus (which has the appearance of concentrated nerve-net) develops out from a *de novo* structure of the brain. Suggesting that although the plexus might represent a structural intermediate between the diffuse cnidarian nets and more centralized systems of higher kinds, it should not be taken as phyletically intermediate.

The platyhelminthes are more closely aligned to the protostome line than to the deuterstomes (Hyman, 1951) and this is clearly evident in the embryology of the phylum. When we compare the attributes of protosome central nervous systems with the organization within the plexus in *Notoplana*, a number of points are evident. Typical protostome nervous systems, i.e., those of annelids and arthropods, are composed of a series of ganglia joined with connectives. Molluscs, the other major protostome group, have the same basic plan but also have a plexus associated with the foot (Bullock and Horridge, 1965). The molluscan plexus bears some scrutiny but, unfortunately has received comparatively little attention at the ultrastructural level. Amphineura, the most primitive molluscs, have a nervous system without discrete ganglia but even here the cell bodies appear to be confined to a rind on the outside of the nerves. In gastropod molluscs which have a variety of plexiform systems in the foot one may find cell somata scattered through the strands but in some species the somata are confined to nodes in the plexus (Bullock and Horridge, 1965). Sub-epithelial plexuses, containing somata, are also present in molluscs but these are thought to be primarily sensory in function. Of the more advanced protostomes, the molluscs appear to have the closest similarities to the flatworms with regard to organization of the nervous system.

Despite the great differences between the protostomes and deuterostomes attempts are often made to derive both groups from the Turbellaria (Hansen, 1958 and 1961). While it may be true that they represent the most primitive group of animals to possess both a brain and bilateral symmetry, the commonly held assertion that the planarians occupy a position of strategic phylogenetic importance (Corning and Kelly, 1973) is not always supported by studies of the lower metazoa (Ax, 1961, 1963; Beklemishev, 1961, 1969). It should be pointed out, however, that there is a flatworm-like animal, *Xenoturbella* (Westblad, 1949), which has similarities in architecture of the ectodermal nervous system with that of the enteropneust hemichordates (Reisinger, 1960). Ax (1963) agrees with Reisinger (1960) that this animal does not belong to the Turbellaria but in some as yet undefined phylum in the deuterostome group.

In summary, the flatworm nerve plexus has both primitive and advanced features. Primitive characteristics can be seen in the organization of neuronal units into a plexus containing dispersed neuropile and submerged somata while advanced features such as complex synapses and sheathed axons also exist. Phylogenetically flatworms do not seem to show affinities, in organization, with the cnidaria and are probably not closely related to the branch that gave rise to the chordates.

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