ULTRASTRUCTURE OF SOMATIC MUSCLE CELLS IN *ASCARIS LUMBRICOIDES*

II. Intermuscular Junctions, Neuromuscular Junctions, and Glycogen Stores

JACK ROSENBLUTH

From the Department of Anatomy, Harvard Medical School, Boston. Dr. Rosenbluth's present address is Department of Anatomy, Albert Einstein College of Medicine, New York

ABSTRACT

Somatic muscle cells of *Ascaris lumbricoides* consist of three differently specialized components referred to as the *fiber*, which contains the contractile apparatus (described previously), the *belly*, and the *arm*. The belly is shown to be a sac of glycogen, which is depleted during starvation of the animal. The arm extends to a nerve cord where it establishes a myoneural junction characterized by giant mitochondria and clusters of vesicles in the nerve fibers and by a 500 A neuromuscular gap. The arms, which have been shown to be "electrically interconnected" in the vicinity of the nerve cord, form "tight junctions" with one another in just this region. At high magnification, these junctions can be resolved into several types. In some there is fusion of the outer leaflets of the membranes with formation of an intermediate line. Others resemble septate desmosomes in that a residual extracellular space ~20 A in width remains between the membranes, but the outer leaflets are interconnected across the gap. It is suggested that the term "tight junction" encompasses a variety of structures distinguishable only at high magnification and that the different variations are not necessarily equivalent functionally.

INTRODUCTION

Multiple specialization in metazoan cells is particularly well demonstrated by the body muscle cells of the parasitic nematode, *Ascaris*. Each of these enormous cells is divided into three morphologically distinct and spatially separate parts subserving different functions. The spindly *fiber* contains the obliquely striated contractile apparatus (28, 29); the sac-like *belly* houses the nucleus, and, as will be shown, serves in addition as a glycogen storage depot; the elongated *arm*, or innervation process, conducts signals from a distant myoneural junction to the contractile part of the cell (5). So extreme are the cytological differences among the several parts of the cell that one would scarcely suspect that the fiber, belly, and arm are interconnected at all. The structural intricacies of the contractile apparatus in the fiber have already been presented in a separate report, and the present paper will therefore be concerned only with the remaining constituents of the cell, the belly and the arm, and with the neuromuscular junction.[1]

The innervation processes are of special interest because of physiological data indicating that they

[1] These findings were presented as a demonstration at the third annual meeting of the American Society for Cell Biology held in November 1963 (28).

are "electrically interconnected" (5); *i.e.* ionic currents apparently flow readily from one to the next in a manner corresponding to "ephaptic transmission" between invertebrate nerve cells, as described by Arvanitaki more than 20 years ago (1), and to "electrical transmission" or "electrical coupling" as described more recently (3, 12, 14). In the case of the *Ascaris* muscle arms, the physiological data suggest that the interconnections occur at the distal extremities of the arms in the region where they converge on the nerve cord (5). This particular region was therefore studied with special interest in an effort to ascertain the structural basis for the phenomenon in this animal in comparison with others.

MATERIALS AND METHODS

Specimens of *Ascaris lumbricoides*, var. suum, obtained from a local slaughterhouse, were fixed with osmium tetroxide either immediately or after 6 days at 37°C. in Kronecker's solution (5). Details of fixation, dissection, and further preparation for electron microscopy are described elsewhere (29). Electron micro-

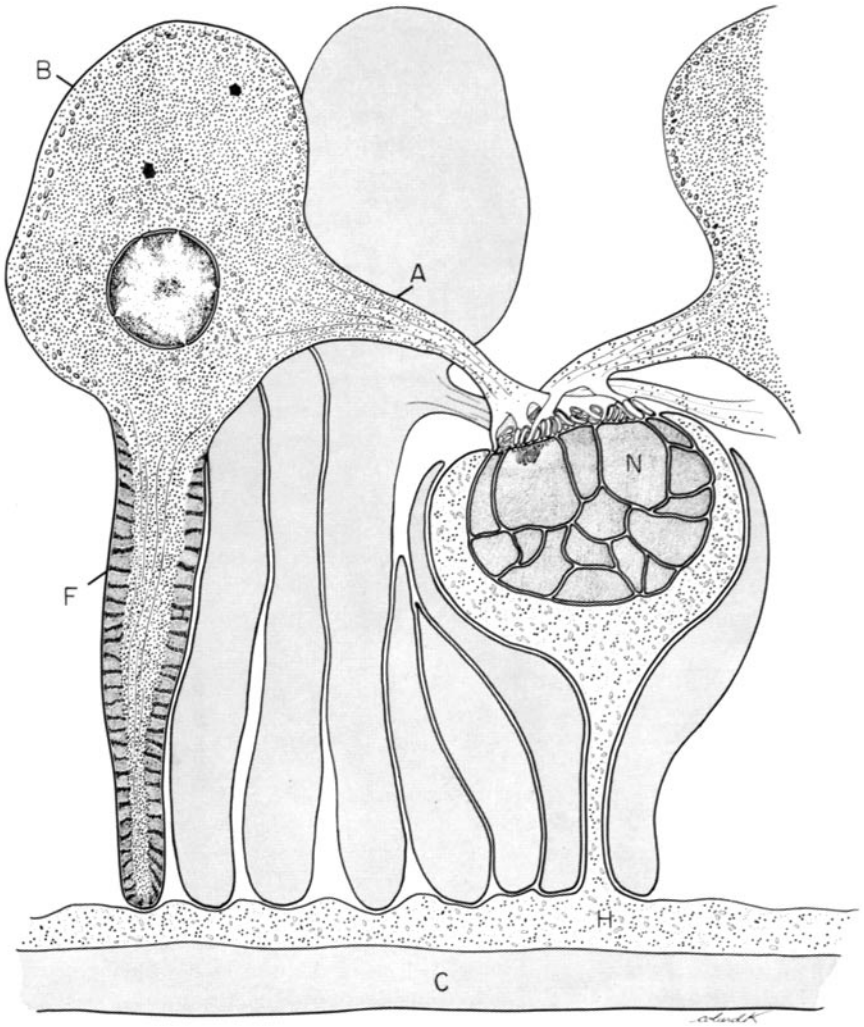


FIGURE 1 Diagram of muscle cells and myoneural junctions in transverse section. The belly (*B*) containing the nucleus of the muscle cell is continuous with the core of the striated fiber (*F*) and with the elongated arm (*A*). The arm subdivides as it approaches the nerve cord (*N*). The individual axons comprising the nerve cord are embedded in a trough-like extension of the hypodermis (*H*) which underlies the animal's cuticle (*C*).

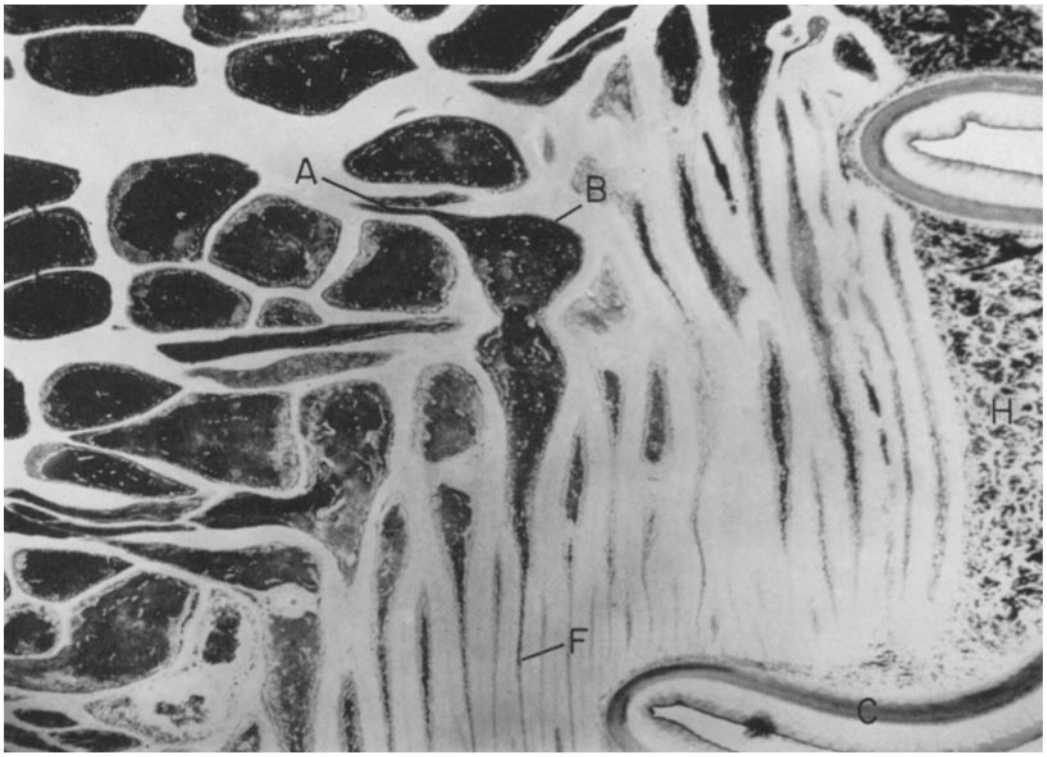


FIGURE 2 Photomicrograph of PAS-stained somatic musculature (oblique section). Muscle bellies (*B*) are intensely stained. PAS-positive material extends also into the muscle arms (*A*) and into the sarcoplasmic core of the fibers (*F*). The contractile cortex of the fibers appears unstained at this magnification. Patches of PAS-positive material occur in the hypodermis (*H*), and one lamina of the cuticle (*C*) is moderately stained. × 160.

graphs were taken with an RCA EMU 3G at initial magnifications of 4500 to 12,000. For light microscopy, 1 μ Epon embedded sections were stained with 0.5 per cent toluidine blue in a 1 per cent solution of sodium borate according to the method of Richardson, *et al.* (21), and examined with either phase-contrast or bright field optics. PAS staining was carried out according to Lillie (13) with prolongation of the total time in each reagent to 30 minutes. Iodine staining was carried out by covering 2 μ sections for 30 seconds with a drop of stain (4 per cent iodine in an 8 per cent solution of potassium iodide), then blotting dry and mounting with Permount. The sections studied were taken from the anterior third of the body.

OBSERVATIONS

The structural organization of the somatic musculature at the histological level has been reviewed recently by DeBell *et al.* (5). Fig. 1 shows the several components of a somatic muscle cell diagrammatically. The contractile apparatus resides in the striated cortex of the muscle fiber (*F*), shown here in transverse section. The contractile cortex surrounds a core of sarcoplasm which continues into the muscle belly (*B*). The latter contains the nucleus and gives rise to a tubular arm (*A*) which extends to a nerve cord (*N*). There the arm breaks up into several finger-like processes which interdigitate with those from other arms and which make synaptic contact with nerve fibers. This mode of innervation is known to occur only in nematodes and in *Amphioxus* (11).

MUSCLE BELLY: In unstarved animals, muscle bellies appear, in toluidine blue–stained sections, as large, homogeneous, metachromatic sacs with smooth contours (29). Occasionally the section passes through the nucleus, which is located in the belly, but aside from this, the belly exhibits no discrete internal structure. In PAS-stained sec-

tions (Fig. 2) the belly, the core of the fiber, and the part of the arm adjacent to the belly all appear intensely red. PAS-positive material also extends from the fiber core into the "dense bands" of the contractile cortex but otherwise the contractile apparatus remains unstained. Iodine-stained sections develop a warm brown coloration with the same distribution as the PAS-positive material. In sections taken from starved animals, no PAS or iodine staining can be demonstrated in any part of the muscle cells. In addition the muscle bellies in the latter animals are shrunken and shriveled.

In electron micrographs, the portions of the muscle cell that exhibit such a strong affinity for PAS and iodine stains are found to contain a great abundance of particles several hundred A in diameter, which are not associated with membranes (Fig. 3). A striking feature of this material is that the particles are extremely dense in lead stained sections, but appear only as negative images after uranyl staining (*cf.* reference 29, Fig. 10). On the basis of size, configuration, and staining properties (19) this particulate material appears to be glycogen, large quantities of which have been demonstrated previously in *Ascaris* by biochemical methods (see reference 18 for review). The glycogen particles fill the belly almost completely, extending nearly up to the plasma membrane, and leaving only a thin cortical region which contains fibrillar bundles of the cytoskeleton (17, 29) and a row of small mitochondria. Occasional lipid droplets and mitochondria also occur deeper in the belly. In the starved animals no such particles are visible in the muscle cells. Only the mitochondria remain in the shrunken muscle belly and in the sarcoplasmic core of the fiber where they appear in high concentration. In both starved and unstarved animals, muscle bellies contain very few membrane-limited vesicles and cisternae.

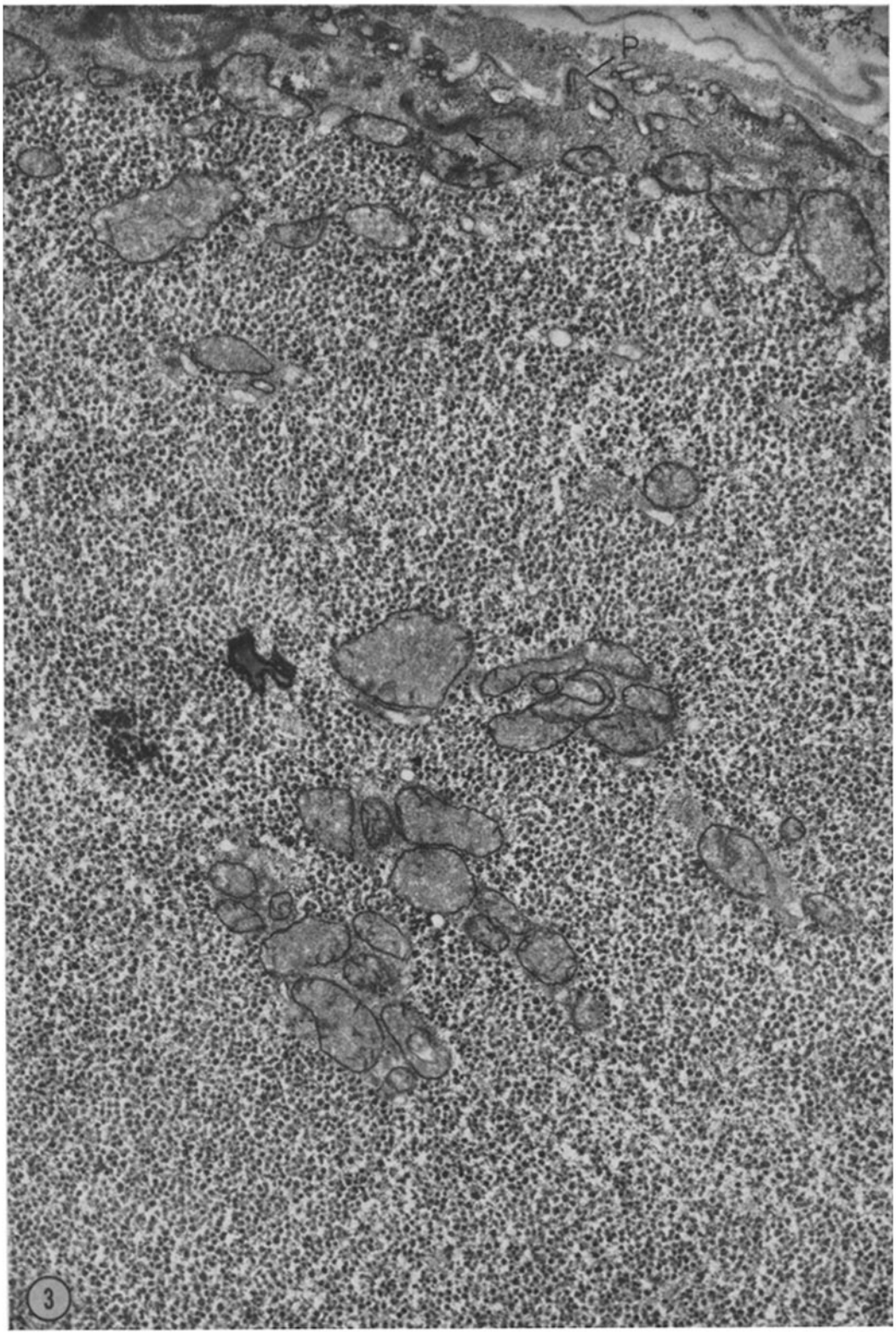
INNERVATION PROCESS: The muscle arm, or innervation process (Fig. 4) may originate from almost any portion of the belly including the junction between the belly and fiber. At least some bellies give rise to more than one arm. The arms contain patches of glycogen and fibrillar bundles except in their distal extremities. Like the muscle bellies they are limited by a plasma membrane and are separated from one another by wide extracellular spaces which contain broad bands of moderately dense ground substance (Fig. 5).

As the arms approach the nerve cord, they become thinner in caliber and the space between them diminishes somewhat. In the immediate vicinity of the nerve cord, the muscle arms branch several times over, and the subdivisions become intertwined forming a cap of interlocking processes over the nerve cord. Occasional desmosomes occur between the muscle processes. Some of the arms appear to be attached to the lips of the hypodermis adjacent to the "bare area" of the nerve cord. (See below.)

The space separating the preterminal processes from one another is usually ~100–200 A. Examples can be found, however, in which neighboring processes become extremely closely apposed along stretches of several microns; *i.e.*, the extracellular space between them is either completely or virtually obliterated (Fig. 5, *cf.* reference 2). These "tight junctions" (10), or "nexuses" (8), sometimes have the configuration of mortise and tenon joints (Fig. 8). Unlike the *zonulae occludentes* (10) that occur near the apices of certain epithelial cells the tight junctions here appear to be circumscribed patches rather than encircling bands. Because of this configuration it does not seem likely that they serve to isolate the surface of the nerve cord from the body cavity. Indeed, solutions of acetylcholine (6) and piperazine (7) are apparently able to diffuse from the body cavity to the vicinity of the nerve cord, albeit slowly. Moreover, in fortunate sections, it is possible to trace patent channels from the surface of the nerve cord through the cap of muscle processes into the body cavity.

At high magnification (Figs. 6, 7) the plasma membranes of the muscle arm processes can be

FIGURE 3 Portion of a muscle belly. Just beneath the plasma membrane (*P*) there is a thin finely granular zone containing fibrillar bundles (arrow), which probably belong to the cytoskeleton, and a row of mitochondria. The mitochondria here and deeper in the belly have few cristae and an abundant matrix. Aside from these organelles, an occasional lipid droplet, and a few vesicles and cisternae, the belly is entirely filled with particulate glycogen. In lead-stained sections such as this, the particles appear dense. × 13,000.


P
3

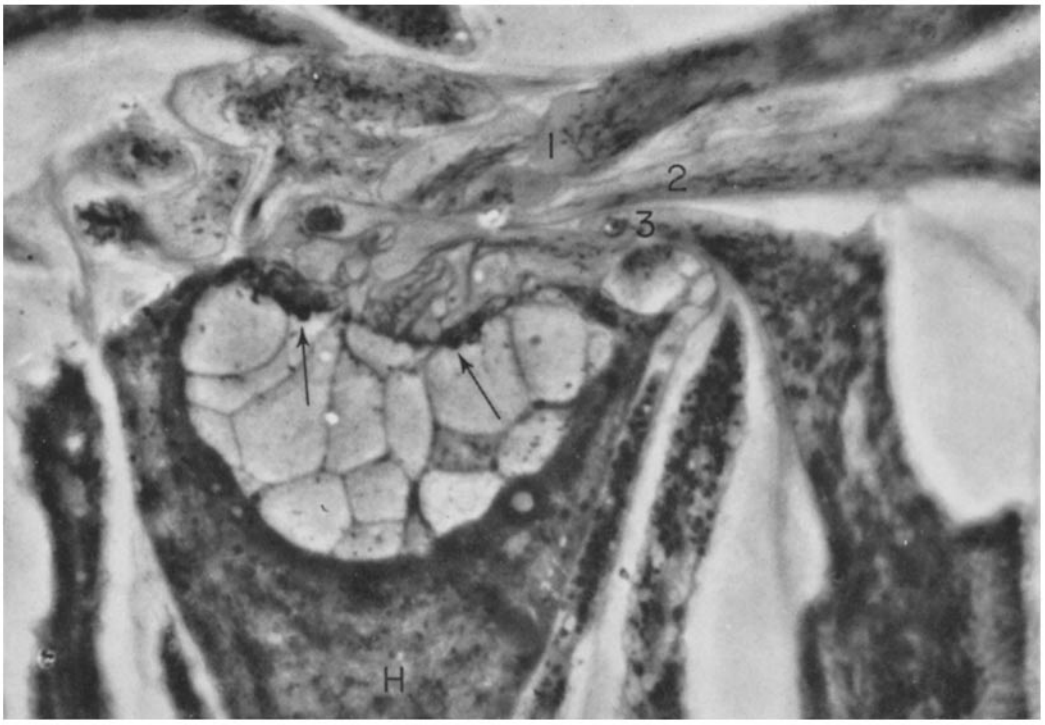
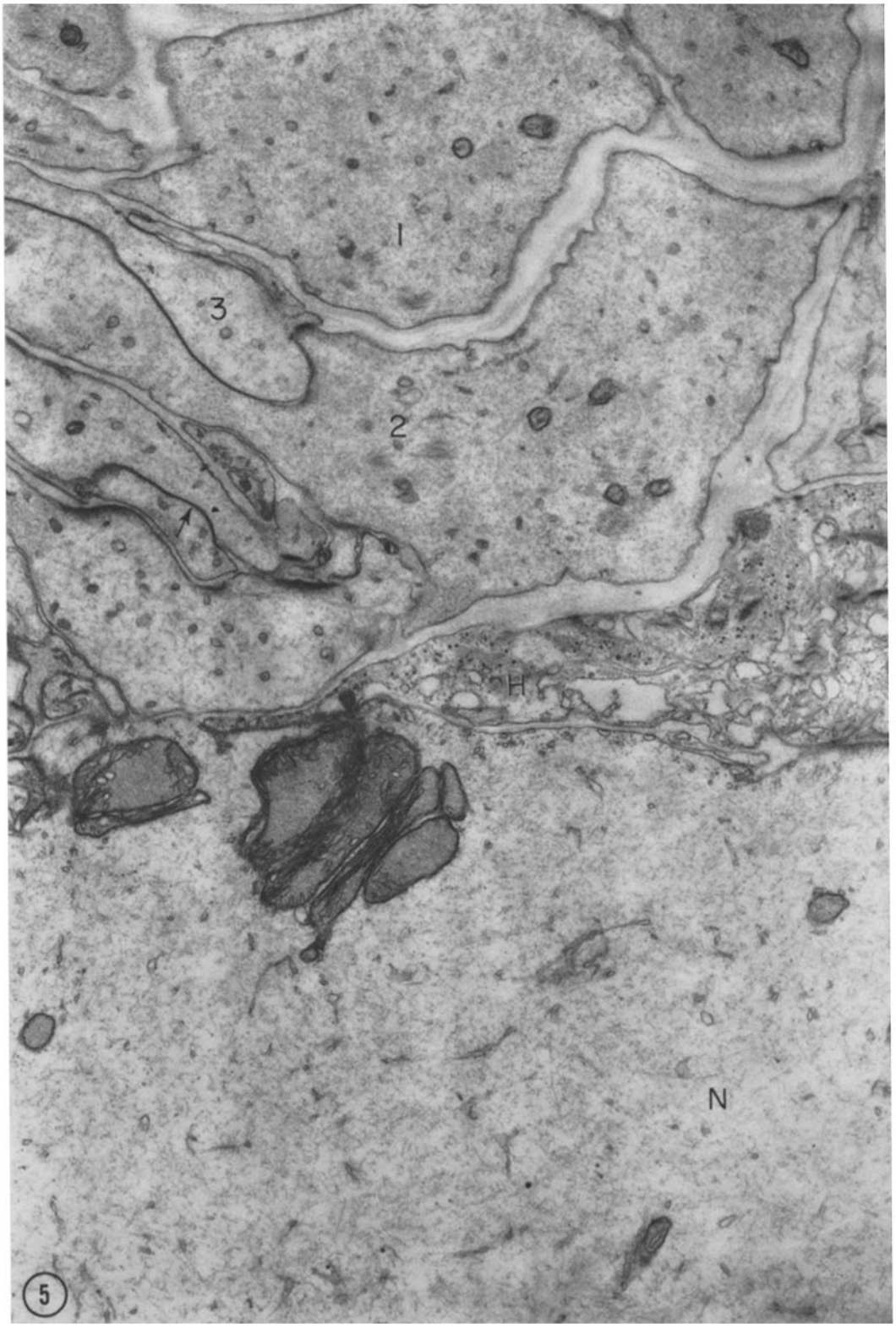


FIGURE 4 Photomicrograph showing muscle arms converging on a nerve cord. At least three separate arms (*1*, *2*, *3*) approach from the right and probably two or three others from the left. The subdivisions of the arms interlace tightly with one another forming a cap over the nerve cord. Several large dense granules (mitochondria) occur in the nerve fibers just at their junction with the muscle arms (arrows). *H*, hypodermis. × 1800.

resolved into trilaminate unit membranes (22) even after osmium tetroxide fixation. The membranes appear to be composed of subunits (Fig. 7, upper bracket; *cf.* Smith, reference 31), and sometimes a radial pattern with a period of ~70 A is visible in them especially when they are cut obliquely (Fig. 6, circle; *cf.* Robertson, reference 24). At tight junctions the intermediate line, which at low magnification may appear to be a single lamina or may not be visible at all, can in some places be resolved into two layers separated by a residual extracellular gap of ~20 A (Figs. 6, 7; *cf.* Elfvin, reference 9). This space is, however, bridged by radial elements (Fig. 7, lower bracket) and, in addition, the intermediate line, whether single or split, exhibits interruptions (Fig. 7, arrows). As a result the middle light components of the respective plasma membranes appear to be connected as they are at septate desmosomes (34).

A vague, radially oriented material is applied

FIGURE 5 Muscle arms near the myoneural junction. The nerve fiber (*N*) in the bottom half of the field is separated from the muscle arms in the top half by a tongue of the hypodermis (*H*) which contains ergastoplasm and fibrillar bundles. Three muscle arms are labeled (*1*, *2*, *3*). *1* is separated from *2* by an extracellular gap of 2500 A. There appears to be no gap at all between *2* and *3*, however. Arrow indicates another such tight junction between two arms. Notice the marked difference in size between the small mitochondria that occur in the muscle arms and deep in the nerve fiber and the large mitochondrial complex at the surface of the nerve fiber. × 14,000.


1
3
2
H
N
5

to the cytoplasmic surfaces of the membranes at tight junctions (Figs. 6, 7) but aside from this poorly delineated coating, no cytoplasmic specializations occur in association with these junctions.

MYONEURAL JUNCTIONS: At the surface of the nerve cord, the final subdivisions of the muscle arms terminate against nerve fibers, which, unlike the C fibers of vertebrates, are not separated from one another by Schwann cell processes. The fibers in the nerve cord travel longitudinally in the animal, contacting muscle arms at the surface *en passage*. In contrast to vertebrate myoneural junctions, each nerve fiber here is very much larger than the individual muscle arm processes with which it is in synaptic contact. The entire cap of muscle arm processes appears to function as a syncytium, however (5).

The nerve cord is surrounded on three sides by a trough-like extension of the hypodermis (reference 5 and Fig. 4). The latter extends partially over onto the fourth side, but leaves a "bare area" of variable size at which nerve fibers are covered by the intertwined muscle arm processes. In this bare area (Fig. 9) only an extracellular space ~500 A in width separates muscle processes from nerve fibers.[2]

In the region of the myoneural junction, the nerve fibers exhibit a prominent cytoplasmic specialization consisting of two elements: crowds of small vesicles ~500 A in diameter, some of which contain dense material, and giant mitochondria with longitudinally oriented cristae (Fig. 9). The two may occur separately, but are usually seen together. The mitochondria are 2 to 3 μ in size and are readily visible in light micrographs (Fig. 4). They may be very closely apposed to the inner surface of the axolemma (Fig. 9, inset). Like all the mitochondria in this animal, these exhibit an abundant, rather dense matrix surrounding the cristae. The vesicles cluster against the axolemma, and, on occasion, one can be found which has the form of a pocket continuous with the axolemma (Fig. 9, arrow). Multivesicular bodies containing vesicles quite like those free in the axoplasm also occur in this region of the axon. The muscle arm processes contain scattered vesicles, somewhat larger in size than those in the axon, but they are not specifically associated with the junctional region. Thus the cytoplasmic specializations characteristic of this junction occur entirely on the neuronal, or presynaptic side. Elsewhere in the nerve fibers one finds filaments, canaliculi, and small mitochondria. The specialized organelles of the junction are not separated from these latter structures by any discrete boundary.

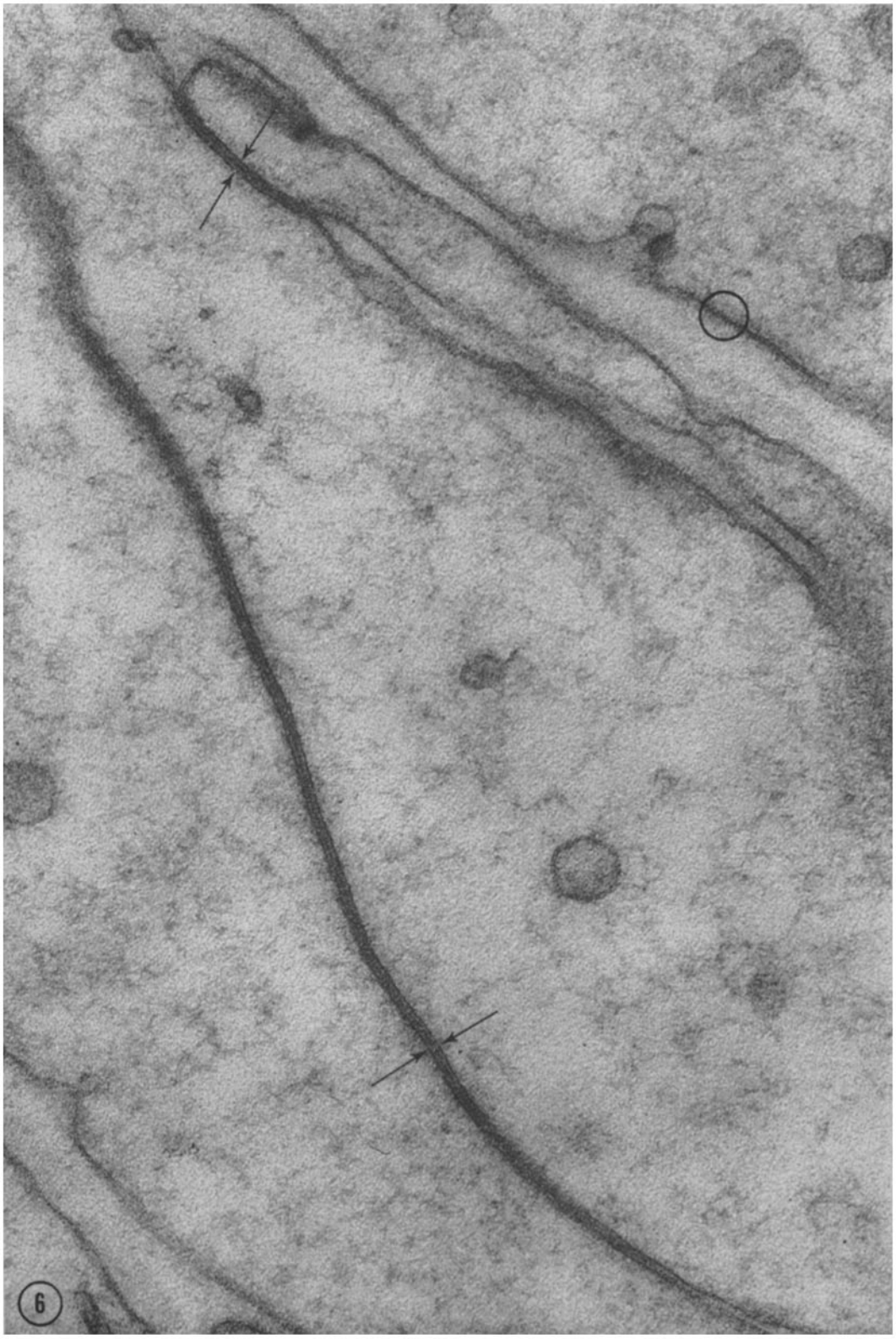
[2] In one instance a slender muscle process was found which invaginated a nerve fiber forming a tight junction with it.

DISCUSSION

FUNCTION OF THE MUSCLE BELLY: Of the findings reported here, one appears to have straightforward significance: From the presence of glycogen in large quantity in the muscle belly and its depletion under conditions of starvation, one can hardly escape the conclusion that the muscle belly serves as a glycogen storage depot which is utilized either by the muscle cell itself or by the animal as a whole when an external food supply is not available. In the natural situation, the nutritional status of the worm depends on the eating habits of its host. Presumably the glycogen supply in the muscle belly of the worm provides for those exigencies in which the host animal eats either irregularly or infrequently.

ULTRASTRUCTURAL CORRELATES OF SYNAPSES AND EPHAPSES: These muscle cells exhibit two types of intercellular junction, which have been correlated with two different physiological mechanisms for cellular interaction. The first type of junction is characterized by crowds of vesicles and mitochondria in a presynaptic element, and by the presence of a gap several hundred Angstrom units wide between the pre- and postsynaptic membranes. Such is the junction

FIGURE 6 Detail of Fig. 5 showing tight junctions between muscle arms. Between the upper pair of arrows the outermost leaflets of the apposed membranes have fused to form a single intermediate line. Between the lower pair of arrows, the outermost leaflets remain separated by a residual extracellular space of ~20 A. Unit membrane structure is visible in both plasma membranes and intracellular membranes. One part of a plasma membrane (circle) has a radial pattern. × 79,000.


6

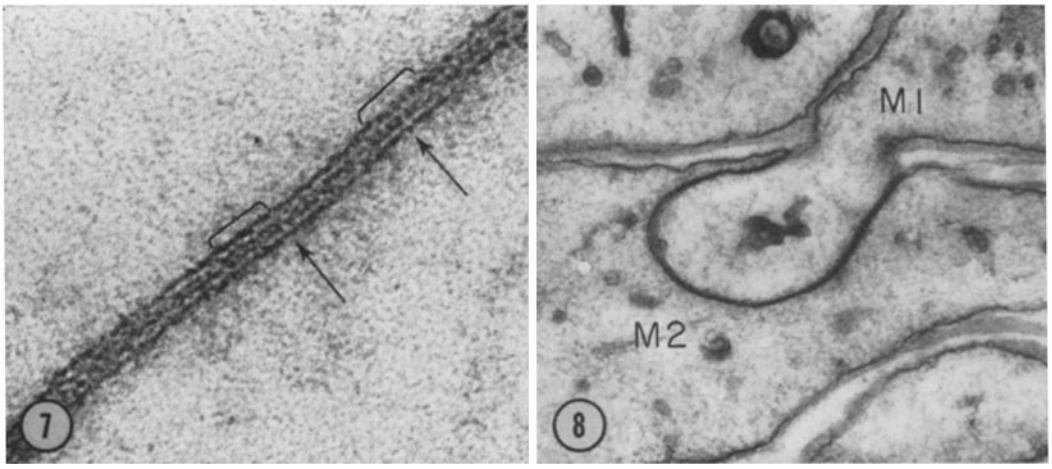


FIGURE 7 Detail of Fig. 6 showing subunit structure in a plasma membrane (upper bracket), cross-bridges connecting the outer leaflets of the apposed membranes (lower bracket), and interruptions in the intermediate line (arrows). × 190,000.

FIGURE 8 Tight junction between interlocking muscle cell processes (*M1* and *M2*). Although not visible at this magnification, a "split" intermediate line occurs at this junction. × 24,000.

between the terminal divisions of the muscle arms and the axons of the nerve cord. Here, as in the myoneural junctions of vertebrates (4, 20), there is evidence that neurohumors liberated by the nerve fibers alter the electrical potential across the muscle cell membrane[3] (5–7). The basic ultrastructural similarity between neuromuscular junctions in *Ascaris* and in vertebrates serves to emphasize the generality of the structural features associated with chemical neuromuscular transmission even in situations where the architecture is atypical and the size relationships of the cellular elements involved are reversed.

The second type of junction occurs between the muscle arms themselves. Physiological studies indicate that ionic currents flow readily between neighboring muscle cells, the shunt pathway apparently occurring near the distal ends of the muscle arms (5). It is precisely in this region that one finds examples of tight junctions—structures which in other locations have been considered the morphological basis for electrical, or ephaptic, interconnections in both nerve and muscle (3, 8, 23, 25). The results reported here are consistent with this correlation.

[3] The *Ascaris* muscle resembles vertebrate cardiac muscle more than skeletal muscle in that it has its own pacemaker and has both excitatory and inhibitory innervation (5–7).

However, the present study also indicates that tight junctions, which at low magnification all appear identical, exhibit a considerable variety at high magnification. In particular, they may show splitting and interruptions of the intermediate line. Some resemble septate desmosomes. In other locations (30) the outer leaflets of the apposed membranes may be missing altogether. One wonders whether all of these different kinds of tight junction are equivalent or whether only certain ones should be regarded as ephapses.

Clearly, not all tight junctions constitute low-resistance pathways for ions. Compact myelin, for example, consists of a stack of plasma membranes sealed tightly together, but the specific resistance across a myelin sheath (presumably also including the Schmidt-Lantermann clefts) is 10^5 ohm·cm^2 in the case of frog A fibers (32), and this figure is probably a simple multiple of the specific resistance of a single Schwann cell membrane and the number of such membranes comprising the sheath (26). In this instance, mere contact between the apposed cell membranes is not by itself a *sufficient* condition for ionic shunting across them. One is therefore not justified in automatically ascribing an ephaptic function to every tight junction wherever it occurs.

In addition, there is now also some question about the *necessity* for tight junctions at ephapses.

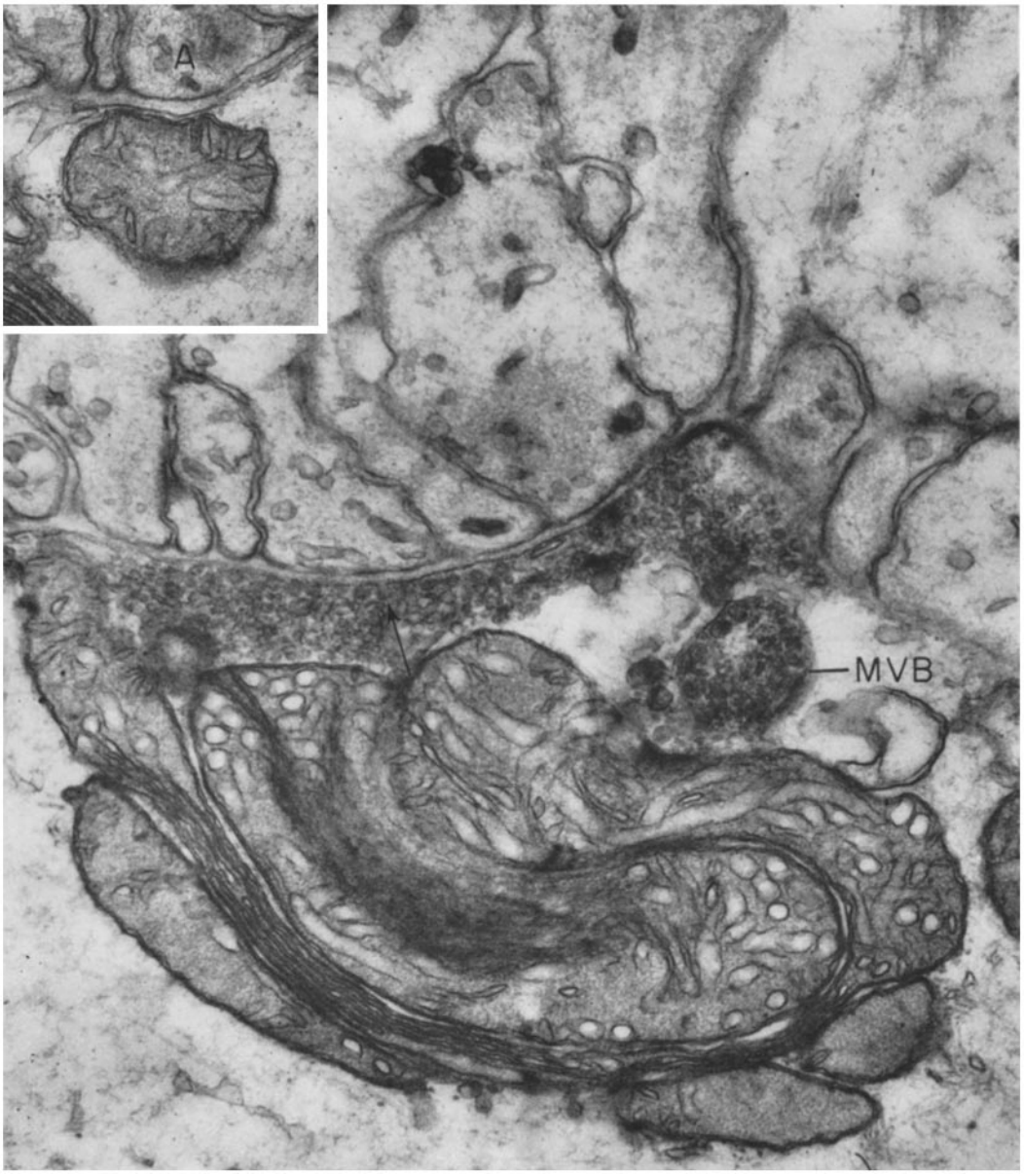


FIGURE 9 Myoneural junction. The terminal subdivisions of the muscle arms fill the upper half of the figure and a nerve fiber occupies the lower half. The two are separated by a gap of ~500 A. The muscle arms contain fine filaments and scattered vesicles. The nerve fiber contains fine filaments, canaliculi and smooth surfaced cisternae, some of which are flattened against the plasmalemma. The most striking organelle in the nerve fiber is a huge mitochondrial complex containing longitudinally oriented tubular cristae. Between this complex and the plasmalemma is a horde of tightly packed vesicles ~500 A in diameter, one of which (arrow) appears to open into the synaptic cleft; *MVB*, multivesicular body. × 27,000.

Inset, myoneural junction showing close apposition of a mitochondrion in a nerve fiber to the plasmalemma. *A*, muscle arms. × 31,000.

Studies of electrical coupling in epithelial cells of *Drosophila* show no tight junctions between the cells. In this case septate desmosomes are thought to underlie the phenomenon (33). To complicate matters further, Maunsbach has recently shown that the presence or absence of tight junctions in fixed tissues may depend heavily on the fixative used (16). Without an independent method for checking conflicting results, there can be no assurance that tight junctions are not either created or obliterated purely by preparative procedures (*cf.* reference 27).

These considerations suggest that one should not consider tight junctions *per se* to be the morphological basis for ephaptic transmission, but rather some other, presumably finer structure, which may be associated with certain tight junctions, but not all, and which may also occur in the absence of tight junctions. Robertson has described radially oriented substructures within the plasma membranes of tissues fixed or stained with permanganate (24), and in the present study similar substructures are seen after osmium tetroxide fixation as well. Luzatti and Husson (15) have described aqueous channels in "liquid crystals" formed from both natural and artificial lipids. Pores of this kind might occur in plasma membranes but remain unseen either because of their small size or because of their lability.

Although such plasma membrane substructures are attractive candidates for an ephaptic function, it may be too optimistic to think that they can be visualized and described accurately at this time. Considering the technical limitations imposed by the necessity for processing biological specimens before they can be examined by electron microscopy, it is possible that, far from being preserved, some substructures are created by the technical procedures now in use. For the present it may prove necessary to rely on physiological methods not only for the demonstration of ephaptic connections between cells, but also for quantitative descriptions of the dimensions and other properties of these pathways.

The author wishes to thank Mr. Richard Hebert for his valuable technical assistance. This work was completed at the author's present address. Supported by Grants NB-03659, NB-05219, and 5T1-MH-6418 from the United States Public Health Service.

Received for publication, December 28, 1964.

Addendum: Further studies of the "nexus" in various locations have been published by Dewey, M. M., and Barr, L. (*J. Cell Biol.*, 1964, **23,** 553, and *Anat. Rec.*, 1965, **151,** 343).

REFERENCES

1. ARVANITAKI, A., Effects evoked in an axon by the electric activity of a contiguous one, *J. Neurophysiol.*, 1942, **5,** 89.
2. AUBER-THOMAY, M., Structure et innervation des cellules musculaires de Nematodes, *J Micr.*, 1964, **3,** 105.
3. BENNETT, M. V. L., ALJURE, E., NAKAJIMA, Y., and PAPPAS, G. D., Electrotonic junctions between teleost spinal neurons: electrophysiology and ultrastructure, *Science*, 1963, **141,** 262.
4. BIRKS, R., HUXLEY, H. E., and KATZ, B., The fine structure of the neuromuscular junction in the frog, *J. Physiol.*, 1960, **150,** 134.
5. DEBELL, J. T., DEL CASTILLO, J., and SANCHEZ, V., Electrophysiology of the somatic muscle cells of *Ascaris lumbricoides*, *J. Cell. and Comp. Physiol.*, 1963, **2,** 159.
6. DEL CASTILLO, J., DE MELLO, W. C., and MORALES, T., The physiological role of acetylcholine in the neuromuscular system of *Ascaris lumbricoides*, *Arch. Internat. Physiol. et Biochim.*, 1963, **71,** 741.
7. DEL CASTILLO, J., DE MELLO, W. C., and MORALES, T., Mechanism of the paralysing action of piperazine on *Ascaris* muscle, *Brit. J. Pharmacol.*, 1964, **22,** 463.
8. DEWEY, M. M., and BARR, L., Intercellular connections between smooth muscle cells: the nexus, *Science*, 1962, **137,** 670.
9. ELFVIN, L.-G., The ultrastructure of the nodes of Ranvier in cat sympathetic nerve fibers, *J. Ultrastruct. Research*, 1961, **5,** 374.
10. FARQUHAR, M. G., and PALADE, G. E., Junctional complexes in various epithelia, *J. Cell Biol.*, 1963, **17,** 375.
11. FLOOD, P. R., A peculiar mode of muscular innervation in amphioxus. Light and electron microscopic studies of the so-called ventral roots, *J. Comp. Neurol.*, 1965 (in press).
12. FURSHPAN, E. J., and POTTER, D. D., Transmission at the giant motor synapses of the crayfish, *J. Physiol.*, 1959, **145,** 289.
13. LILLIE, R. D., Histopathologic Technic, New York, Blakiston Division, McGraw-Hill Book Co., Inc., 1954.
14. LOWENSTEIN, W. R., and KANNO, Y., Studies on

an epithelial (gland) cell junction. I. Modifications of surface membrane permeability, *J. Cell Biol.*, 1964, **22**, 565.

15. LUZATTI, V., and HUSSON, F., The structure of the liquid-crystalline phases of lipid-water systems, *J. Cell Biol.*, 1962, **12**, 207.
16. MAUNSBACH, A. B., Comparison of renal tubule ultrastructure after perfusion fixation with different fixatives, *J. Cell Biol.*, 1964, **23**, 108A.
17. REGER, J., The fine structure of the fibrillar network and sarcoplasmic reticulum in smooth muscle cells of *Ascaris lumbricoides (var. suum)*, *J. Ultrastruct. Research*, 1964, **10**, 48.
18. REID, W. M., Comparison between *in vitro* and *in vivo* glycogen utilization in the fowl nematode *Ascaridia galli*, *J. Parasitol.* 1945, **31**, 406.
19. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron micrographs of thin tissue sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 575.
20. RICHARDSON, K. C., The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscles, *Am. J. Anat.*, 1964, **114**, 173.
21. RICHARDSON, K. C., JARETT, L., and FINKE, E. H., Embedding in epoxy resins for ultrathin sectioning in electron microscopy, *Stain Technol.*, 1960, **35**, 313.
22. ROBERTSON, J. D., The ultrastructure of cell membranes and their derivatives, *Biochem. Soc. Symp.*, 1959, **16**, 3.
23. ROBERTSON, J. D., Ultrastructure of excitable membranes and the crayfish median-giant synapse, *Ann. New York Acad. Sc.*, 1961, **94**, 339.
24. ROBERTSON, J. D., The occurrence of a subunit pattern in the unit membranes of club endings in Mauthner cell synapses in goldfish brains, *J. Cell Biol.*, 1964, **19**, 201.
25. ROBERTSON, J. D., BODENHEIMER, T. S., and STAGE, D. E., The ultrastructure of Mauthner cell synapses and nodes in goldfish brains, *J. Cell Biol.*, 1964, **19**, 159.
26. ROSENBLUTH, J., The fine structure of acoustic ganglia in the rat, *J. Cell Biol.*, 1962, **12**, 329.
27. ROSENBLUTH, J., Contrast between osmium-fixed and permanganate-fixed toad spinal ganglia, *J. Cell Biol.*, 1963, **16**, 143.
28. ROSENBLUTH, J., Fine structure of body muscle cells and neuromuscular junctions in *Ascaris lumbricoides*, *J. Cell Biol.*, 1963, **19**, 82A.
29. ROSENBLUTH, J., Ultrastructural organization of obliquely striated muscle fibers in *Ascaris lumbricoides*, *J. Cell Biol.*, 1965, **25**, 495.
30. ROSENBLUTH, J., and PALAY, S. L., Fine structure of nerve cell bodies and their myelin sheaths in the eighth nerve ganglion of the goldfish, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 853.
31. SMITH, D. S., The structure of flight muscle sarcosomes in the blowfly *Calliphora erythrocephala* (Diptera), *J. Cell Biol.*, 1964, **19**, 115.
32. TASAKI, I., New measurements of the capacity and the resistance of the myelin sheath and the nodal membrane of the isolated frog nerve fiber, *Am. J. Physiol.*, 1955, **181**, 639.
33. WIENER, J., SPIRO, D., and LOWENSTEIN, W. R., Studies on an epithelial cell (gland) junction. II. Surface structure, *J. Cell Biol.*, 1964, **22**, 587.
34. WOOD, R. L., Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 343.