THE FINE STRUCTURE OF THE ELECTRIC ORGAN OF *TORPEDO MARMORATA*

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ABSTRACT

The fine structure of the electric organ of the fish *Torpedo marmorata* has been examined after osmium tetroxide or potassium permanganate fixation, acetone dehydration, and Araldite embedment. This organ consists of stacks of electroplaques which possess a dorsal non-innervated and a ventral richly innervated surface. Both surfaces are covered with a thin basement membrane. A tubular membranous network whose lumen is continuous with the extracellular space occupies the dorsal third of the electroplaque. Nerve endings, separated from the ventral surface of the electroplaque by a thin basement membrane, contain synaptic vesicles (diameter 300 to 1200 A), mitochondria, and electron-opaque granules (diameter 300 A). Projections from the nerve endings occupy the lumina of the finger-like invaginations of the ventral surface. The cytoplasm of the electroplaques contains the usual organelles. A "cellular cuff" surrounds most of the nerve fibers in the intercellular space, and is separated from the nerve fibre and its Schwann cell by a space containing connective tissue fibrils. The connective tissue fibrils and fibroblasts in the intercellular space are primarily associated with the dorsal surface of the electroplaque.

INTRODUCTION

The electric organ of the fish *Torpedo* consists of stacks of electroplaques, each possessing a dorsal non-innervated and a ventral richly-innervated surface. The plaques are believed to be derived from embryonic muscle tissue in which the contractile elements have vanished, leaving structures homologous with the motor end-plate of voluntary muscle (3, 11, 12, 18). The potential generated by the organ on nervous stimulation is believed to arise from the summation of the postjunctional potentials, generated by each plaque, which are similar in origin to the end-plate potential of voluntary muscle (11). The analogy with respect to the neuromuscular junction extends also to the mode of synaptic transmission: the high content of acetylcholine and cholinesterase (21) in this organ suggests that here, as in voluntary muscle, transmission from the presynaptic terminals to the innervated surface of the plaque is cholinergic. Because of the richness of its innervation, this organ thus provides a unique opportunity for studying cholinergic transmission.

Previous studies of electric organ with the electron microscope (16–18, 39) have been primarily comparative in nature. In such comparative studies details of a given species may become overshadowed by the generally recurring features common to all the species investigated. For this reason, and also as a basis for other work on the mechanism of storage and release of acetylcholine, it was decided to submit the electric organ of

Torpedo to a more detailed morphological study with the electron microscope.

MATERIALS AND METHODS

Electric fish, *Torpedo marmorata*, obtained by air from Naples, were maintained in Cambridge in an aquarium supplied by circulating filtered sea water at 10°C. The fish survived well under these conditions for many weeks.

For study, fish were pinned to a dissection board and immediately pithed with a blunted dissecting needle inserted into the cranial cavity. A steel rod approximately 2 mm in diameter was then inserted into the spinal canal, abolishing all reflex activity. For electron microscopy, entire columns of electroplaques were removed as quickly as possible and fixed for 3 hours according to the following procedures: (*a*) in 2 per cent osmium tetroxide containing 0.25 M sodium chloride and 0.33 M urea; (*b*) in 2 per cent osmium tetroxide in Veronal-acetate buffer (23); (*c*) in 10 per cent formalin in phosphate buffer (28) for 30 minutes followed by postfixation in 2 per cent osmium tetroxide in phosphate buffer (19); and (*d*) in 1 per cent potassium permanganate (15). After fixation was complete, the tissue was rinsed in cold 25 per cent acetone and then placed in 50 per cent acetone. Here, the columns of electroplaques were cut transversely into smaller stacks. The dehydration was completed through 70 per cent acetone in the cold, and the remaining dehydration was done at room temperature. The tissue was embedded in Araldite according to the procedure described by Robertson (32). Sections were cut on an LKB microtome and stained, after mounting on Formvar–carbon-coated grids, with a lead hydroxide–tartrate complex (20) or in 0.5 per cent alcoholic phosphotungstic acid (PTA) (27) solution. The sections were xtamined with the Siemens Elmiskop I (type UM-II ea 80 kv) at original magnifications of 2,000 to 30,000. The micrographs were suitably enlarged.

Sections of formalin-fixed, paraffin-embedded tissue were stained with hematoxylin and eosin, Masson's trichrome, and the periodic acid–Schiff (PAS) technique for examination with the light microscope.

RESULTS

Fine Structure of the Electroplaque

Osmium tetroxide or potassium permanganate preservation reveals a membrane of usual thickness bounding the electroplaques (Figs. 4 and 5). A thin basement membrane, 300 to 500 A thick (Fig. 2), is invariably present on the dorsal surface. A tubular membranous network, primarily localized to the dorsal third of the electroplaque (Fig. 1), branches extensively and its lumen communicates freely with the extracellular space where the network becomes continuous with the plasma membrane on the dorsal surface (Figs. 2 and 5). The diameter of the tubules is 800 to 1000 A, and frequently the basement membrane covering the dorsal aspect of the cell extends into the lumina of this system (Fig. 2). This feature is best seen in material preserved in potassium permanganate.

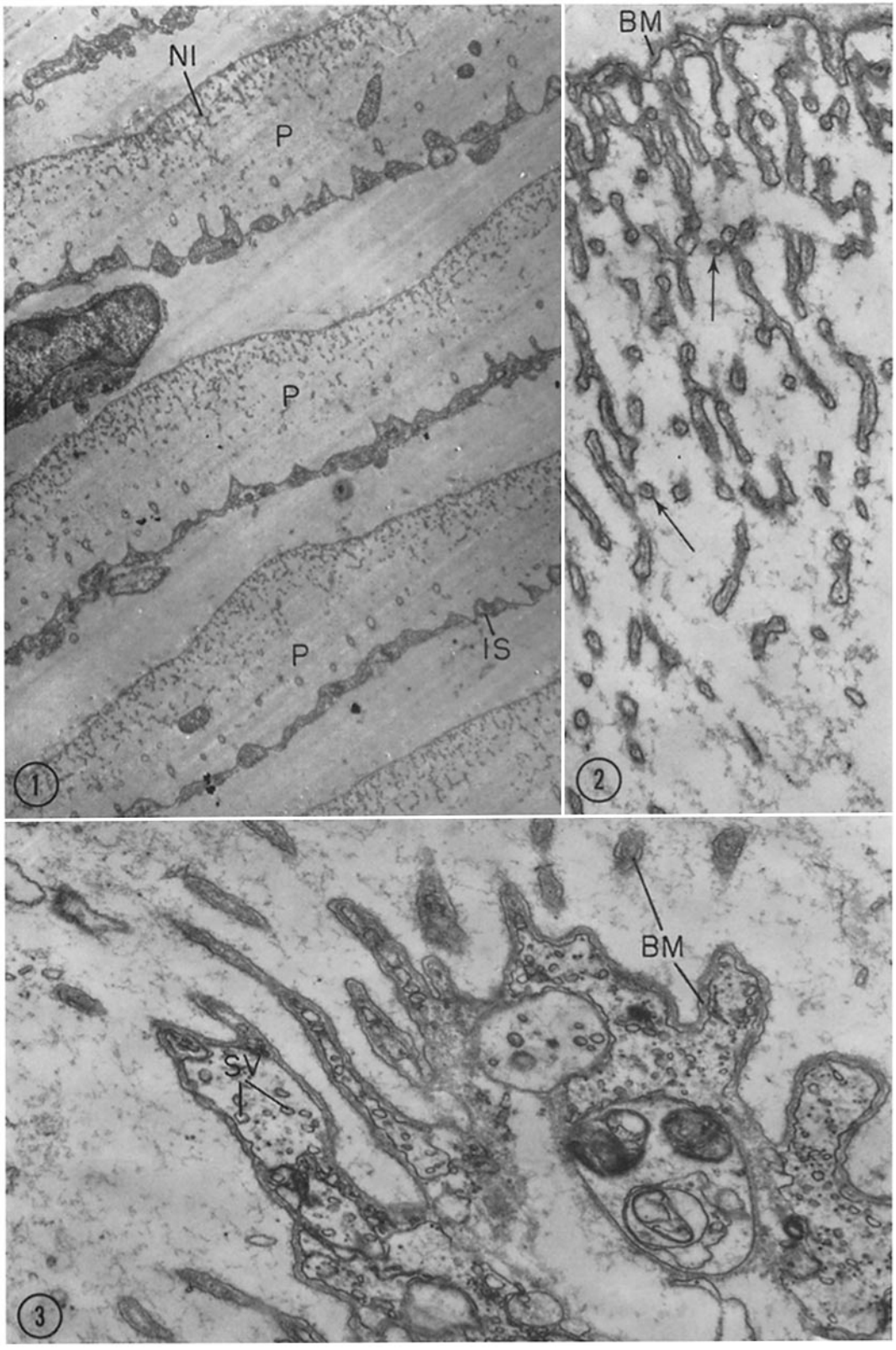
Nuclei are infrequently seen in sections examined by either light or electron microscopy. When seen in thin section, they conform to usual nuclear structure, being 5 to 8 μ in diameter, bounded by a double membrane occasionally exhibiting nuclear pores, and possessing one or more nucleoli (Fig. 4). The tubular system associated with the dorsal surface frequently surrounds the nucleus, and micrographs suggest occasionally that this system is continuous with the outer membrane of the nuclear envelope, as is characteristic of the granular endoplasmic reticulum of other cells (24, 40).

The cytoplasm immediately surrounding the

FIGURE 1 A survey electron micrograph showing parts of five electroplaques (*P*). Non-innervated surface (*NI*); innervated surface (*IS*). OsO_4-fixed, lead-stained. × 4,000.

FIGURE 2 Dorsal non-innervated surface of electroplaque. A basement membrane (*BM*), closely applied to the plasma membrane, extends into the lumina of the tubular network associated with the dorsal surface and is present in cross-sectioned circular profiles of this system (arrows). Potassium permanganate-fixed, lead-stained. × 24,500.

FIGURE 3 Ventral innervated surface of electroplaque. Finger-like invaginations are evident on this surface. Nerve endings are closely applied to the plasma membrane, with an interposed basement membrane (*BM*). Synaptic vesicles (*SV*) are evident. Potassium permanganate-fixed, lead-stained. × 22,000.


NI
P
P
P
IS
1
BM
2
BM
SV
3

nucleus contains more organized structure than the remainder of the cell: occasionally, there are a few strands of the granular endoplasmic reticulum and membranes similar to those of the Golgi complex (Fig. 4). The remaining cytoplasm of these cells is relatively electron transparent, containing only a few scattered mitochondria. The mitochondria of these cells are usually elongated, their length being several microns and their average diameter approximating 1 μ. They are bounded by a double membrane, the inner member of which is reflected inward in the form of blebs, cristae, or tubules (Figs. 1 and 4).

The most complex portion of the electroplaque is the ventral innervated surface. This surface, like the dorsal, is covered with a thin basement membrane immediately external to the plasma membrane (Fig. 3). At irregular intervals, the plasma membrane of this surface infolds as tubular or finger-like invaginations which extend "into" the cell about 2 μ (Figs. 1, 3, and 6). The basement membrane projects into and lines these invaginations (Figs. 3 and 7).

The ventral surface is densely covered with presynaptic nerve endings, occupying shallow trough-like depressions in the ventral plasma membrane (Figs. 1, 3, 4, and 7). Projections from the presynaptic endings occupy the lumina of the inward projections of the plasma membrane (Figs. 3 and 7). This relationship is also evident when the plane of section is parallel to the ventral surface (Fig. 6).

Synaptic vesicles present in the nerve endings vary in diameter from 300 A to 1200 A, with a continuous range between these extremes (Figs. 3 and 7). Occasional micrographs suggest that synaptic vesicles fuse with the plasma membrane of the nerve ending (Fig. 7). Additionally, in some endings a granular component is more prominent than the vesicles. This component is 200 to 300 A in diameter and varies in electron opacity (Figs. 7 and 8). Similar granular structures are seen in non-myelinated axons presumably near nerve terminals (Fig. 9). Small, elongate mitochondria are a constant feature of the nerve ending (Fig. 7).

The Space Between the Electroplaques

The space between two adjacent electroplaques measures approximately 3 μ and contains a number of structures related to the bounding plaques (Fig. 1).

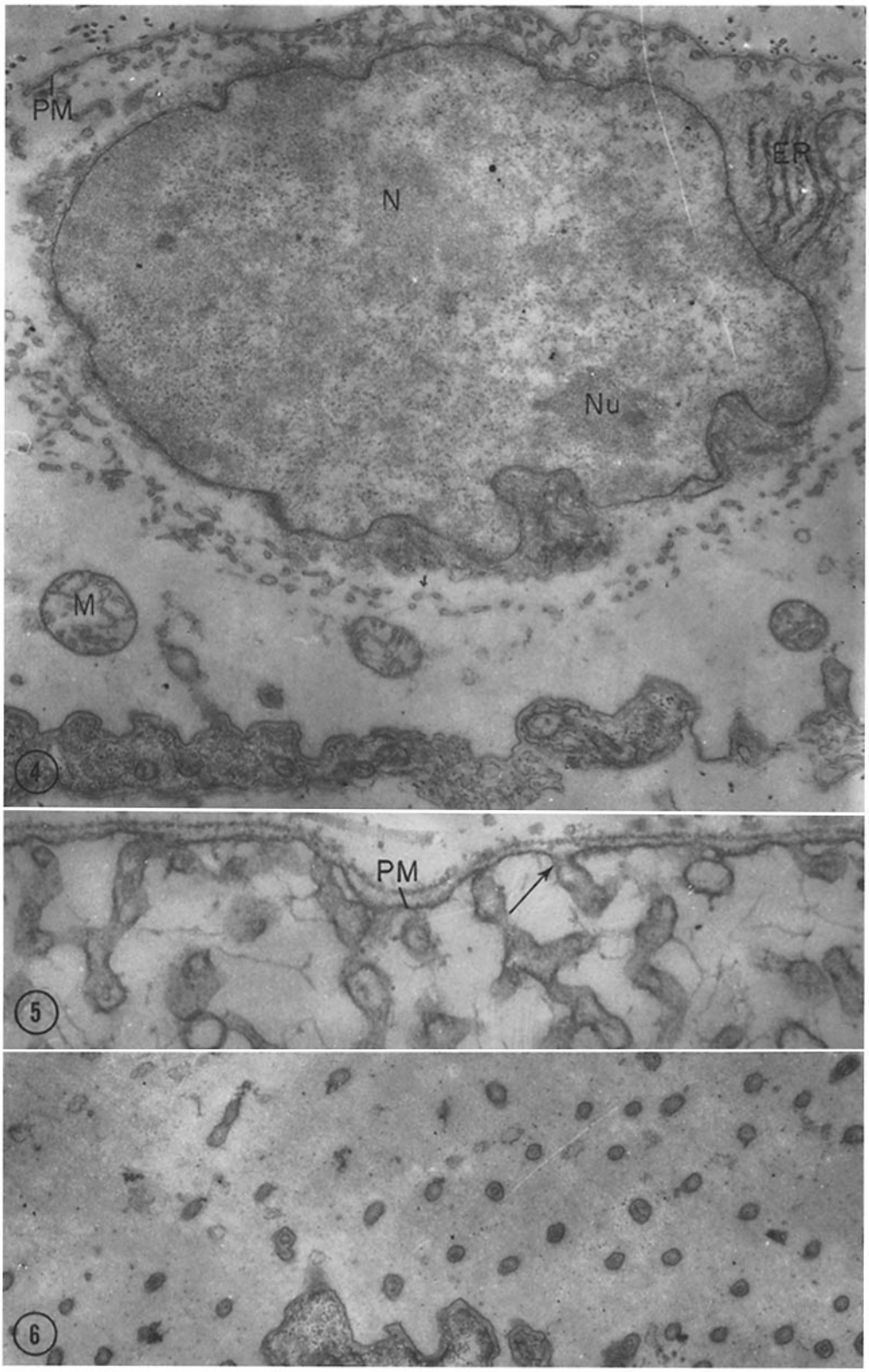
Fibrils are regularly seen associated with the dorsal surface of the electroplaques (Fig. 15) as well as with small nerve fibres (Fig. 8) and with blood vessels in this space. These fibrils are collagen or reticulum, as they demonstrate a 640 A periodicity after PTA "staining," are PAS-positive, and stain light blue with Masson's connective tissue stain. Flattened, elongate fibroblasts also are occasionally applied to the dorsal surface of the electroplaques.

The space between the plaques contains abundant nerve fibres, of both myelinated and non-myelinated types, completely surrounded by a characteristic "cellular cuff" (Figs. 10, 11, and 13) which is separated from the axon by a space approximately 0.5 μ wide containing collagen or reticulum fibrils. The cuff usually has one or two layers (Figs. 9 and 13) but it sometimes has as many as five (Figs. 11 and 12). The cell body of the cuff cell envelope has a nucleus of usual structure surrounded by moderately dense cytoplasm. The thin attenuated cytoplasm of the remainder of the cell has been seen at least once to envelop two nerve fibres and perhaps a third

FIGURE 4 Part of an electroplaque showing a nucleus (*N*) with its nucleolus (*Nu*). A thin basement membrane is evident just external to the plasma membrane (*PM*). Infrequently, strands of the granular endoplasmic reticulum (*ER*) are encountered. Mitochondria (*M*) are evident. Note the PTA-stained fibrils associated primarily with the dorsal surface above. OsO_4-fixed, PTA-stained. × 14,500.

FIGURE 5 Dorsal surface of electroplaque. The tubular network here is continuous (arrow) with the plasma membrane (*PM*). OsO_4-fixed, lead-stained. × 48,000.

FIGURE 6 This micrograph is of a section almost parallel to the ventral innervated surface of electroplaque. The finger-like invaginations are cut in cross-section. Cytoplasmic matrix occupies the space between the cross-sectioned "fingers." OsO_4-fixed, lead-stained. × 11,000.


PM
N
ER
Nu
M
4
PM
5
6

(Fig. 10). These cells contain vesicular structures which appear similar to the synaptic vesicles of the nerve endings (Fig. 9).

The space between the plaques also contains a third cell type (Figs. 16 and 17) different from the fibroblast and the "cuff" cell associated with the axon. This cell has a well developed system of cytoplasmic membranes, including what is believed to be a Golgi apparatus (Fig. 17). The membranous system consists of a profusion of vesicular structures of varying size. Lipid droplets and mitochondria are present (Fig. 16) and myelin figures are occasionally seen in these cells (Fig. 16).

DISCUSSION

Among the teleost and elasmobranch fishes endowed with electric organs, the elasmobranch *Torpedo* has received little attention with the electron microscope. Of the four reports (16–18, 39) concerned with the electron microscopy of this organ, three include electron micrographs of *Torpedo* (16–18). All of these studies have been primarily concerned with comparative cytology and have stressed the similarities of the dorsal and ventral surfaces of the electroplaques among the species investigated.

The fish electroplaques thus far examined with the electron microscope show a tubular or canalicular network associated with the non-innervated surface. Perhaps Luft (17) has devoted the most attention to this system. He described in electroplaques of *Torpedo* and *Electrophorus* a "highly developed system of caveolae, or cave-like blind tubules and vesicles." According to him, the caveolae are blind, elongate, sac-like indentations of the plasma membrane of the cell. He also described as associated with this system, completely intracellular vesicles of approximately the same diameter as the caveolae.

Electron micrographs included in this report of the *Torpedo* electroplaques show a similarly highly developed tubular network associated with the dorsal plasma membrane. While circular membranous profiles are observed in the preparations, they are interpreted as being cross sections of the tubular network instead of vesicles, as interpreted by Luft (17). This interpretation is supported by the observation that the thin basement membrane on the dorsal surface of the electroplaque extends into and lines the tubular membranous system. The finding that within almost all of these structures there is an electron-opaque material comparable in density with the basement membrane justifies the assumption that the vesicles described by Luft are merely cross-sections of this tubular system. Luft (17) indicates that this specialization of the non-innervated plasma membrane is the only feature common to species thus far examined. Indeed, it is evident from these studies that this system enormously increases the surface area and must certainly serve an important physiological function, perhaps associated with ion movement (12).

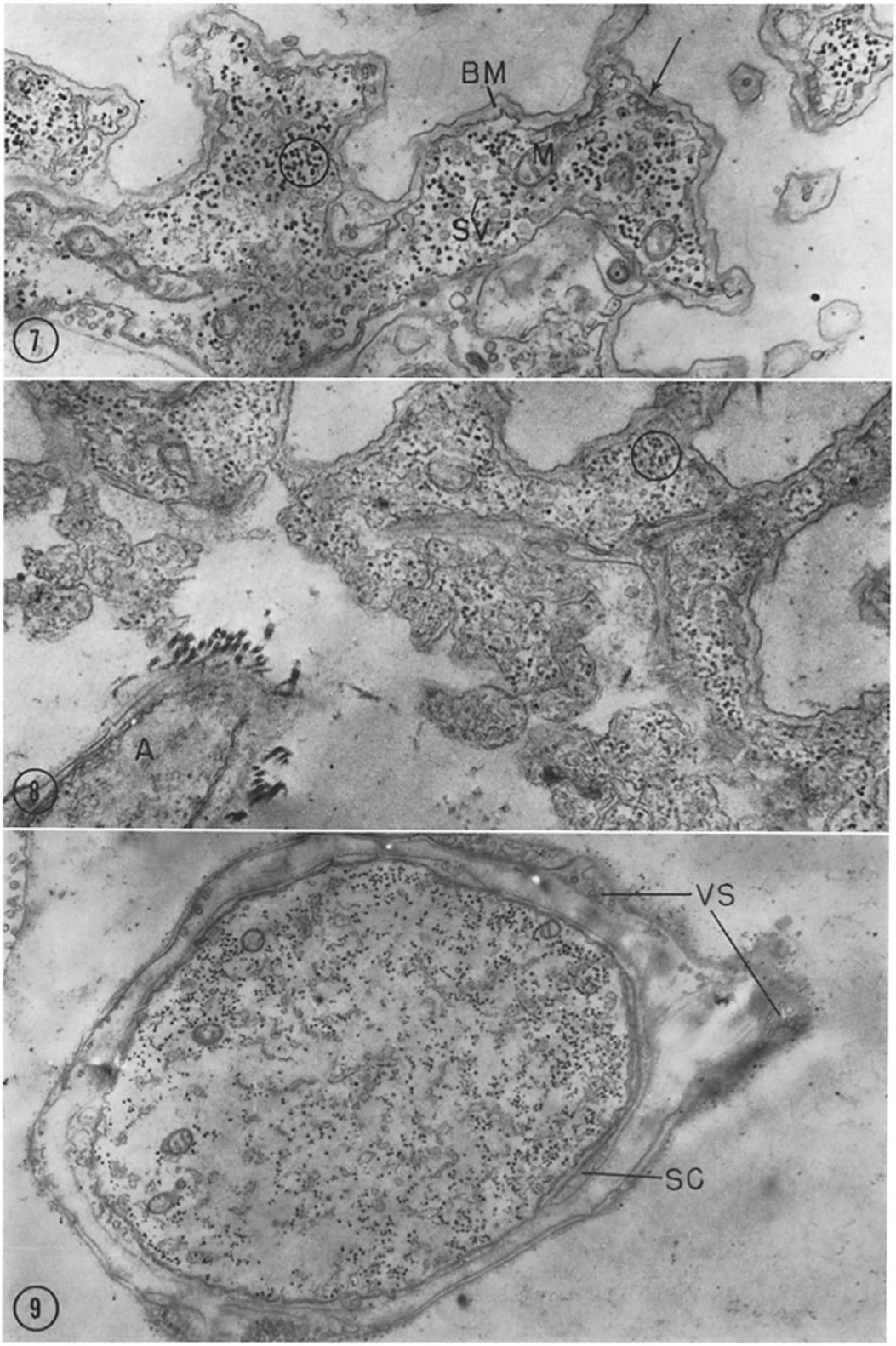
The synaptic relationship at the ventral surface of the electroplaques resembles that of a myoneural junction (2, 4, 6, 30, 31) with some simplification.

The relationship of the synaptic ending to the

FIGURE 7 A nerve ending associated with the ventral surface of the electroplaque. A basement membrane (*BM*) is present between the nerve ending and the plasma membrane. Synaptic vesicles (*SV*) and mitochondria (*M*) are evident. Note the high concentration of electron-opaque granules, some of which are shown in the circle. At the heavy arrow are shown vesicular profiles in close apposition to the bounding membrane, and at places there are suggestions that vesicles have fused with the membrane and give it an undulated appearance. OsO_4-fixed, lead-stained. × 23,000.

FIGURE 8 Area similar to that in Fig. 7 except that the preparation is PTA-stained. Again, granules (circle) are evident. A non-myelinated axon (*A*), visible in the lower part of the micrograph, is devoid of the enveloping cuff. OsO_4-fixed, PTA-stained. × 23,000.

FIGURE 9 Cross-section of a small non-myelinated axon containing an abundance of electron-opaque granules. Surrounding the axon and its thin adhering Schwann cell (*SC*) is a space containing fibrils. Immediately external to this space is a cellular cuff which at places possesses vesicular structures (*VS*) resembling similar structures in the nerve ending. OsO_4-fixed, lead-stained. × 10,500.


BM
M
SV
7
A
8
VS
SC
9

electroplaque in fishes has been described by Mathewson *et al.* (18) and Wachtel *et al.* (39). They showed that the presynaptic terminals lie in "troughs or furrows" (with unbranched inpocketings) of the postsynaptic membrane. According to them, the presynaptic ending extends only to the base of the inpocketings and is not present in the finger-like inward projections of the ventral plasma membrane which have been described here. Wachtel *et al.* (39) described "finger-like" invaginations of the ventral membrane of electroplaques in *Narcine*. These reports have indicated that the synaptic nerve ending does not extend into these invaginations to their fullest extent. The findings reported here indicate that the synaptic ending is in intimate contact with the ventral membrane of the electroplaque, being present in the finger-like invaginations of this surface. In sections both parallel and perpendicular to the ventral surface this relationship is shown, as well as the presence of a basement membrane which extends into this system and is evident between the synaptic ending and the postsynaptic membrane. Mathewson *et al.* (18) reported the presence of a "dense formation of unknown nature" in the synaptic space in *Raja* and *Astrocopus*. These authors consider this dense formation homologous to the dense line described by Gray (9) in axo-dendritic synapses. Examination of their micrograph (Fig. 10 in reference 18) reveals that this dense band is present in places devoid of nerve ending. It is suspected that this structure is similar to the structure here interpreted as a basement membrane and bears little if any relation to the electron-opaque line in axo-dendritic synapses previously described (9).

The usual structures encountered in nerve endings associated with neuromuscular junctions or interneuronal cholinergic synapses are an abundance of synaptic vesicles approximately 500 A in diameter, a few small filamentous mitochondria, and occasionally fine neurofilaments (2, 4, 6, 7, 25, 26, 30, 31). The vesicles of the nerve endings described in this report vary more in size than do similar structures in nerve endings of other species. Here there is a wide and continuous variation in diameter (300 A to 1200 A). This variation cannot be dismissed as artifactual, because the same variation is present in tissue fixed in four different ways. Similar structures have also been observed in negatively stained preparations derived from homogenized and fractionated material (41).

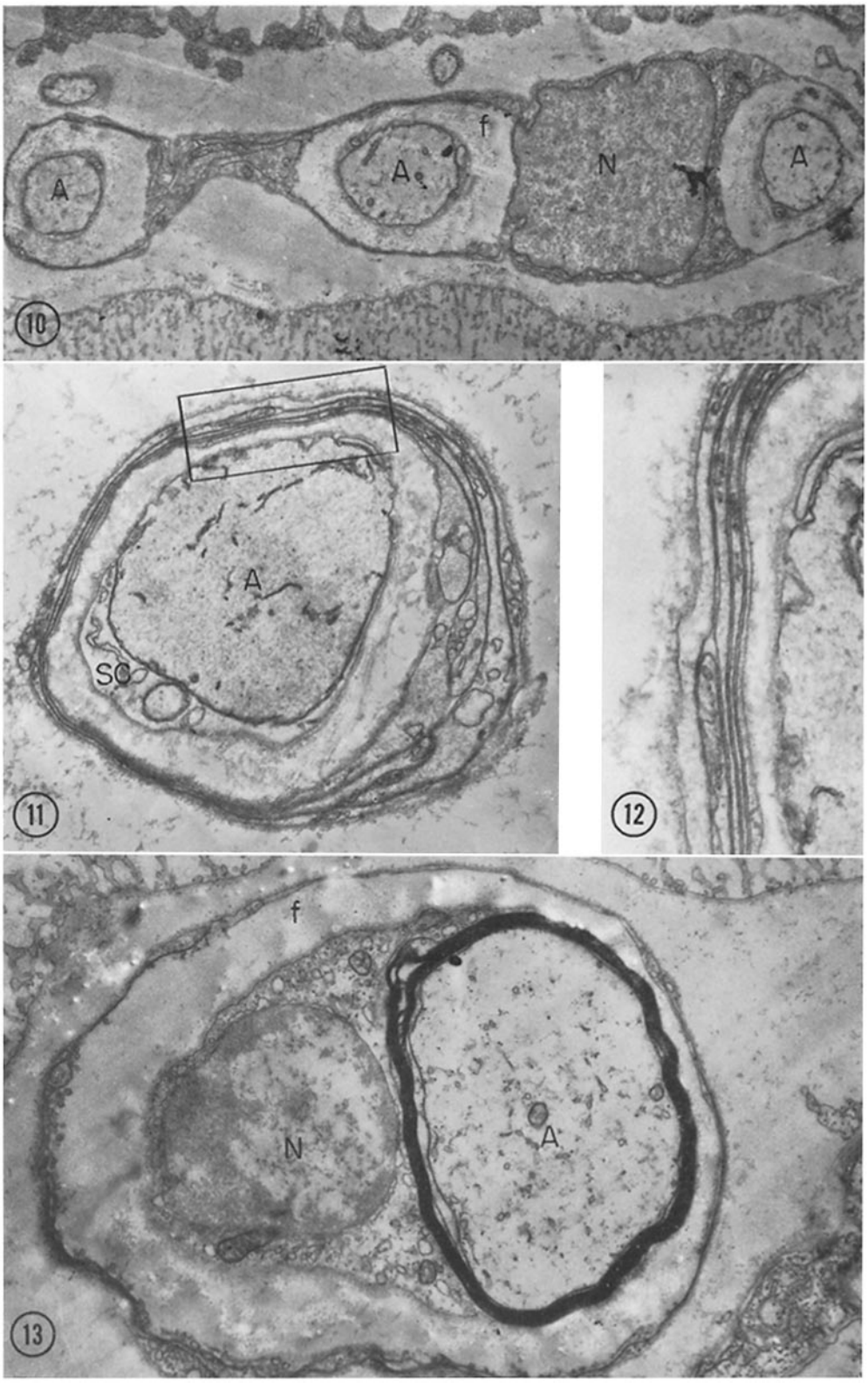
Perhaps the most unusual feature of the nerve ending in the electric organ of *Torpedo* is the presence of a granular component. This component has also been seen in structures identified as small nonmyelinated axons, and occasionally in the ventral portion of the electroplaque. The granules are approximately 300 A in diameter and demonstrate a variation in electron opacity. While the granule is evident after primary fixation in formalin, after fixation in osmium tetroxide, and after fixation in permanganate, its presence is greatly enhanced after "staining" of the thin sections with either lead or phosphotungstic acid. Luft (reference 16, in the legend to his Fig. 6) noted the presence of granules in the synaptic ending in the electric organ of *Torpedo occidentalis*. Luft (17, in Fig. 10) also showed granules in the electroplaque of *Malapterurus* which he identified as Palade's particles. Examination of his micrograph reveals that most of these particles actually measure well over 300 A, more than double the

FIGURE 10 This survey electron micrograph shows two non-myelinated axons (*A*) and perhaps a third (to the extreme left) enveloped by a single cuff cell. The nucleus (*N*) of the cuff cell is evident. Fibrils (*f*) occupy the space between the Schwann cell and the cellular cuff. OsO_4-fixed, lead-stained. × 5,000.

FIGURE 11 Non-myelinated axon (*A*) with its adhering thin Schwann cell (*SC*). The enveloping cellular cuff is evident. Potassium permanganate-fixed, lead-stained. × 15,000.

FIGURE 12 Photographic enlargement of the area indicated in Fig. 11. The cuff in places consists of as many as five layers. × 34,000.

FIGURE 13 Myelinated axon (*A*) with an associated Schwann cell and its nucleus (*N*). The space between the Schwann cell and the cuff contains fibrils (*f*). OsO_4-fixed, lead-stained. × 12,000.


f
A
A
N
A
10
A
SC
11
12
f
N
A
13

usually quoted 150 A diameter for the Palade particle. These particles as well as the larger vesicles of the nerve ending have also been identified with negative staining techniques in this laboratory (41).

Small granules (100 to 150 A) have previously been noticed in the spinal cord of the rat and cat (10) and in sympathetic ganglia of the frog (36, 42). Robertson *et al.* (32) described granules (150 to 250 A) in the axon caps of the goldfish Mauthner cell; according to these authors, the smaller granules are ribosomes and the larger ones possibly represent glycogen. Gray (10) speculates that the granules he described are glycogen on the basis of their similarity to those seen in the mouse liver fixed in permanganate (15) and identified as glycogen.

In light of the work of Revel *et al.* (29), it would appear that the granules previously observed as well as those reported here are glycogen and correspond to the β-particles described by Drochmans (8). However, as regards a structure so little understood physiologically as the nerve ending, caution must be used in specifically identifying structures such as this. Revel *et al.* (29) indicated that a differentiation between glycogen granules and RNP particles could be made by a comparison of permanganate- and osmium tetroxide–fixed materials. The observation that glycogen is well preserved with permanganate fixation while RNP granules are lost (29) supports the concept that the granules in the nerve ending in the electric organ of *Torpedo* might be something other than glycogen, in that they are seen to best advantage after osmium tetroxide fixation. Likewise, in sympathetic ganglia synapses of frogs Yamamoto (42) has observed granules which are best preserved with osmium tetroxide. He identifies these granules as glycogen and states that the difference in appearance between the structures he sees and those described in previous reports by other workers may be due to differences in the procedures used.

A cellular cuff usually envelops small bundles of axons and individual myelinated and non-myelinated axons in the extracellular space. This relationship either has been overlooked in other studies of similar tissue, or is not present in species having structures with similar electrophysiological properties. Robertson (30) showed in amphibian nerve a similar structure associated with the myoneural junction and he described it as endoneurium. Shanthaveerappa and Bourne (34, 35) dismiss Robertson's interpretation and give convincing microscopic evidence of a perineural epithelial sheet, a lepto-meningeal extension, which surrounds nerve fascicles in several specific incidences. They prefer to interpret the endoneurium of Robertson's micrographs as extensions of the perineural epithelium.

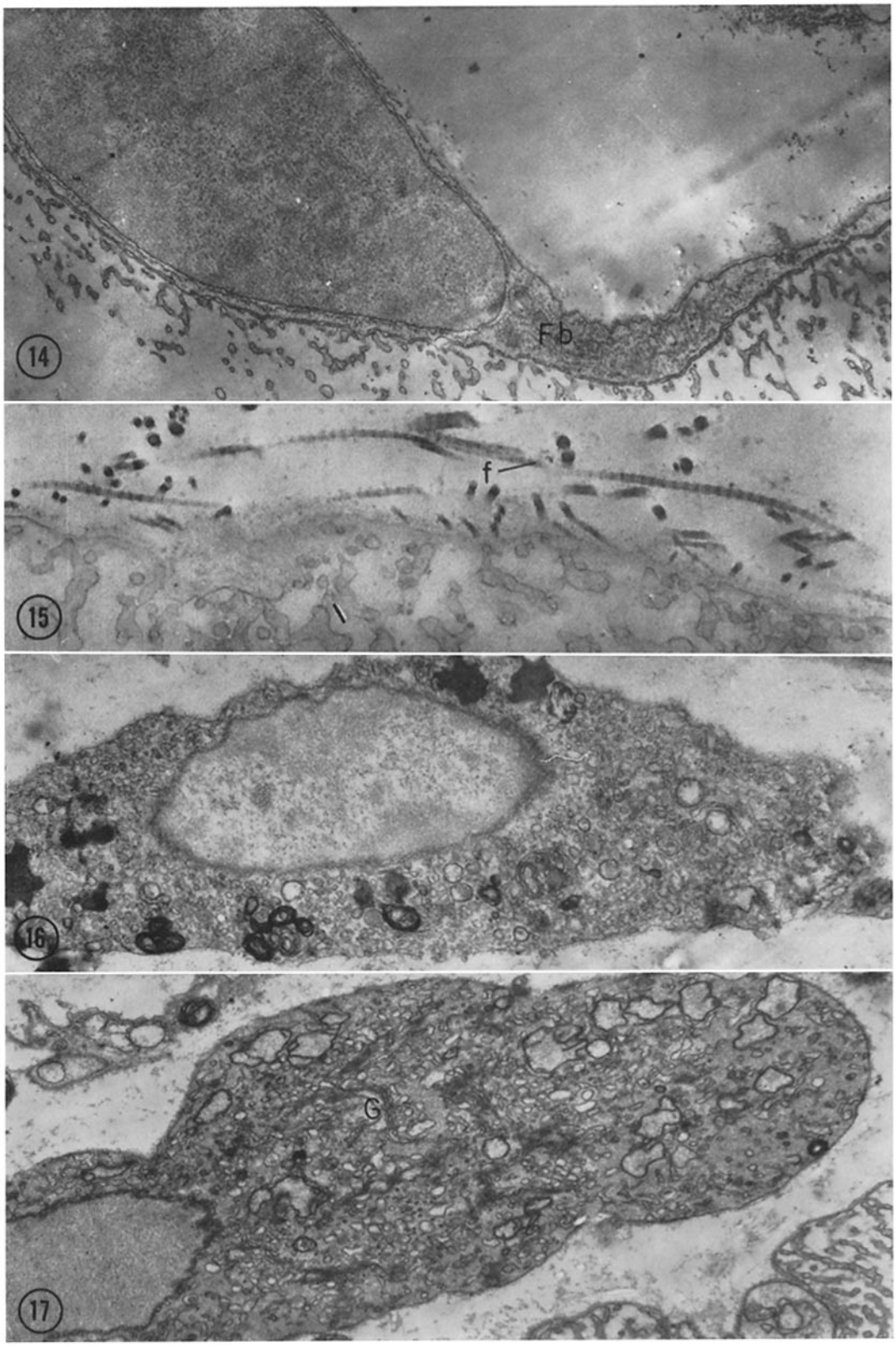
Textbooks of histology usually define the endoneurium as penetrations of connective tissue from the perineurium into the nerve fascicle surrounding individual nerve fibres. If this definition be accepted, it would appear that Robertson's interpretation over-simplifies the relationship, since no evidence is given that the structure he calls endoneurium is a fibroblastic tube. Terry and Harkin (37, 38), studying Wallerian degeneration, described a perineural or laminar sheath which they believe to be of fibroblastic origin. The fact that connective tissue fibrils are observed in the space between the nerve fibre and the cellular cuff lends some support to the concept of the fibroblastic origin of the cuff. However, the literature suggests that under extreme conditions of stress the

FIGURE 14 A flattened cell, presumably a fibroblast (*Fb*), is closely applied to the dorsal surface of the electroplaque. OsO_4-fixed, lead-stained. × 17,000.

FIGURE 15 The dorsal surface of an electroplaque, demonstrating the presence of fibrils (*f*). OsO_4-fixed, PTA-stained. × 29,000.

FIGURE 16 A cell of the type frequently encountered in the intercellular space, demonstrating an abundance of membranous vesicular structures. Two lipid droplets and several myelin figures are present. OsO_4-fixed, lead-stained. × 12,000.

FIGURE 17 A cell similar to that in Fig. 16. A well organized Golgi apparatus (G) is present. Potassium permanganate-fixed, lead-stained. × 11,000.


Fb
14
f
15
16
G
17

Schwann cell itself is capable of producing collagen fibrils (22).

Assuming that this sheath is something other than a modified connective tissue tube, it is interesting to speculate what its function might be. Shanthaveerappa and Bourne (34, 35) point out that confusion exists in the literature, concerning the presence of a diffusion barrier in the peripheral nerve. Some studies indicate that the epineurium is the barrier (5), while others indicate that ions diffuse readily through this structure (14). Krnjević (13) demonstrated in frog sciatic nerve a barrier to perfusion of a number of compounds, and he attributed this barrier to the perineurium. It is possible that the enveloping cuff observed here is in fact a further projection of the perineural epithelium and represents a barrier to the diffusion of at least some ions at the individual axonal level.

Torpedo marmorata is a species of electric fish which is incapable of being stimulated directly electrically (12). For a thorough discussion of this physiological property the reader is referred to the work of Altamirano *et al.* (1) and the review of Grundfest and Bennett (12).

Histochemical studies of electric organs at the electron microscopical level have been reported by Wachtel *et al.* (39) and Mathewson *et al.* (18) who demonstrate that esterase is limited to the synaptic cleft. In view of the advances made in the techniques of electron microscopic histochemistry (33) further study of the electric organ from this aspect should be rewarding.

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BIBLIOGRAPHY

1. Altamirano, M., Coates, C. W., and Grundfest, H., Mechanisms of direct and neural excitability in electroplaques of electric eel, *J. Gen. Physiol.*, 1955, **38**, 319.
2. Andersson-Cedergren, E., Ultrastructure of motor end plate and sarcoplasmic components of mouse skeletal muscle fibres, *J. Ultrastruct. Research*, 1959, Suppl. **1**, 1.
3. Biedermann, W., Electrophysiology, (F. A. Welby, translator), London, The Macmillan Co., Ltd., 1898, **2**.
4. Birks, R., Huxley, H. E., and Katz, B. The fine structure of the neuromuscular junction of the frog, *J. Physiol.*, 1960, **150**, 134.
5. Causey, C., and Palmer, E., The epineural sheath of a nerve as a barrier to the diffusion of phosphate ions, *J. Anat., London*, 1953, **87**, 30.
6. Couteaux, R., Morphological and cytochemical observations on the postsynaptic membrane at motor end plate and ganglionic synapses, *Exp. Cell Research, Suppl.*, 1958, **5**, 294.
7. de Robertis, E., Submicroscopic morphology of the synapse, *Internat. Rev. Cytol.*, 1959, **9**, 61.
8. Drochmans, P., Technique for the isolation of particulate glycogen and its examination in the electron microscope, *in* Methods of Separation of Subcellular Structural Components, *Biochem. Soc. Symp. No. 23*, 1963, 127.
9. Gray, E. G., Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscopic study, *J. Anat., London*, 1959, **93**, 420.
10. Gray, E. G., Electron microscopy of presynaptic organelles of the spinal cord, *J. Anat., London*, 1963, **97**, 101.
11. Grundfest, H., The mechanisms of discharge of the electric organ in relation to general and comparative electrophysiology, *Prog. Biophysics and Biophysic. Chem.* 1957, **7**, 1.
12. Grundfest, H., and Bennett, M. V. L. Electrophysiology of marine electric fishes. Studies on the morphology and electrophysiology of electric organ, *in* Bioelectrogenesis, (C. Chagas and P. Paesde Carvalho, editors), Amsterdam, Elsevier Publishing Company, 1961, 57.
13. Krnjević, K., Some observations on perfused frog sciatic nerve, *J. Physiol.*, 1954, **123**, 338.
14. Lorente De Nó, R., The ineffectiveness of the connective tissue sheath of nerve as a diffusion barrier, *J. Cell. and Comp. Physiol.*, 1950, **35**, 195.
15. Luft, J. H., Permanganate: a new fixative for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 799.
16. Luft, J. H., The fine structure of the electric organ of the electric eel and torpedo ray, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4 suppl., 229.

17. LUFT, J. H., The fine structure of electric tissue, *Exp. Cell Research, Suppl.*, 1958, **5**, 158.
18. MATHEWSON, R., WACHTEL, A., AND GRUNDFEST, H., Fine structure of electroplaques, *in* Bioelectrogenesis, (C. Chagas and P. Paesde Carvalho, editors), Amsterdam, Elsevier Publishing Company 1961, 57.
19. MILLONIG, G., Advantages of a phosphate buffer for OsO_4 solutions in fixation, *J. Appl. Physics.*, 1961, **32**, 1637.
20. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 736.
21. NACHMANSOHN, D., Chemical and molecular aspects of bioelectrogenesis, *in* Bioelectrogenesis, (C. Chagas and P. Paesde Carvalho, editors), Amsterdam, Elsevier Publishing Company, 1961, 237.
22. NATHANIEL, E. J. H., and PEASE, D. C., Collagen and basement membrane formation by Schwann cells during nerve regeneration, *J. Ultrastruct. Research*, 1963, **9**, 550.
23. PALADE, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95**, 285.
24. PALADE, G. E., The endoplasmic reticulum. *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4 suppl. 85.
25. PALAY, S. L., Synapses in the central nervous system, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4 suppl., 193.
26. PALAY, S. L., The morphology of the synapse in the central nervous system, *Exp. Cell Research, Suppl.*, 1958, **5**, 275.
27. PEASE, D. C., Histological Techniques for Electron Microscopy, New York, Academic Press, Inc., 1960.
28. PEASE, D. C., Buffered formaldehyde as a killing agent and primary fixative for electron microscopy, *Anat. Rec.*, 1962, **142**, 342.
29. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron micrographs of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 575.
30. ROBERTSON, J. D., The ultrastructure of a reptilian myoneural junction, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 381.
31. ROBERTSON, J. D., Electron microscopy of the motor endplate and the neuromuscular spindle, *Am. J. Physic. Med.* 1960, **39**, 1.
32. ROBERTSON, J. D., BODENHEIMER, T. W., and STAGE, D. E., The ultrastructure of Mauthner cell synapses and nodes in Goldfish brains, *J. Cell Biol.*, 1963, **19**, 159.
33. SABATINI, D. D., BENSCH, K. G., and BARRNETT, R. J., Cytochemistry and electron microscopy, *J. Cell. Biol.*, 1963, **17**, 19.
34. SHANTHAVEERAPPA, T. R., and BOURNE, G. H., A perineural epithelium, *J. Cell. Biol.*, 1962, **14**, 343.
35. SHANTHAVEERAPPA, T. R., and BOURNE, G. H., The perineural epithelium. A metabolically active, continuous, protoplasmic cell barrier surrounding peripheral nerve fasciculi, *J. Anat., London*, 1962, **96**, 527.
36. TAXI, J., Etude de l'ultrastructure des zones synaptiques dans les ganglions sympathiques de la Grenouille, *Compt. rend. Acad. sc.*, 1961, **252**, 174.
37. TERRY, R. D., and HARKIN, J. C., Regeneration of peripheral nerve sheaths following Wallerian degeneration, *Exp. Cell Research*, 1957, **13**, 193.
38. TERRY, R. D., and HARKIN, J. C., Wallerian degeneration and regeneration of peripheral nerves, *in* Biology of Myelin, (S. R. Korey, editor), Hoeber-Harper Inc., New York, 1959, 303.
39. WACHTEL, A., MATHEWSON, R., and GRUNDFEST, H., Electron microscopic and histochemical comparison of the two types of electroplaques of *Narcine Brasiliensis*, *J. Biophysic. and Biochem. Cytol.* 1961, **11**, 663.
40. WATSON, M. L., The nuclear envelope—its structure and relation to cytoplasmic membranes, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
41. WHITTAKER, V. P., and SHERIDAN, M. N., unpublished observations, 1964.
42. YAMAMOTO, T., Some observations on the fine structure of the sympathetic ganglion of bullfrog, *J. Cell Biol.*, 1963, **16**, 159.