ELECTRON MICROSCOPIC AND HISTOCHEMICAL COMPARISON OF THE TWO TYPES OF ELECTROPLAQUES OF *NARCINE BRASILIENSIS*

ALLEN WACHTEL, ROBERT MATHEWSON, and HARRY GRUNDFEST, Ph.D.

From the Cell Research Laboratory, The Mount Sinai Hospital, New York, and the Department of Neurology, College of Physicians and Surgeons, Columbia University, New York. Mr. Mathewson's present address is Lerner Marine Laboratory, American Museum of Natural History, Bimini, Bahamas

ABSTRACT

The torpedine electric fish *Narcine brasiliensis* has two morphologically distinct electric organs (*main* and *accessory*) which also differ with respect to a number of electrophysiological properties. The fine structure of the electroplaques of these organs has been examined by electron microscopy and by a histochemical method for localizing esterase activity with a high degree of resolution. In both kinds of electroplaques the innervated surface (ventral in those of the main organ, dorsal in those of the accessory) is the only site of esterase activity. The latter is further confined to the regions of synaptic contact between vesicle-containing axon terminals and the electroplaque membrane. The synaptic apparatus is similar to, but less elaborate than, that of neuromuscular junctions. The axon terminals and electroplaque membranes are free of connective tissue envelopments. The membrane of the uninnervated surfaces forms a continuum with a dense canalicular network which penetrates deeply into the 7 μ thick electroplaques of the main organ. The canalicular network has about the same thickness in the 20 μ electroplaques of the accessory organ. Except for this difference, the two kinds of cells appear to have the same fine structure. This finding is discussed in relation to the electrophysiological data on functional differences.

Electric organs of all electric fishes have in common the capacity, unique among the electrogenic tissues, for summation of the voltages that are produced during the synchronized discharge of the individual cells, or electroplaques (18). However, recent electrophysiological studies have revealed numerous variations in electrical activity in the different families and even in different genera of the same family of fish (3–8, 10, 21, 23). These differences can be clearly demonstrated and characterized by intracellular recordings from single electroplaques. They are ascribable to specifically different functional properties of the cell membranes in various electroplaques, and even to differences in different regions of the cell membrane in a single electroplaque (3, 7, 19–21). Some of these varieties of functional manifestation appear to be adaptive, and correlated with special requirements associated with evolutionary differences in gross morphology of the electric organs or of their electroplaques among the various electric fishes.

It seemed desirable to seek for possible morphological correlates of these differences by means of electron microscopy and by a histochemical method (14) which localizes esterase activity with

a high degree of resolution at magnifications obtainable with the electron microscope (25). As will be shown, it is likely that many of the functional differences lie at molecular levels which are as yet beyond the range of any available morphological techniques.

The South Atlantic torpedine *Narcine brasiliensis* was chosen for the initial study because material was readily available for working out the details of the various procedures. Furthermore, this form, unlike all other torpedine electric fish examined thus far (8, 15, 27, 28), possesses an accessory organ whose electroplaques differ both in gross structure and in mode of functioning from the cells of the main organ (5). Thus, the material offers greater scope than do other forms for a comparative study of the correlations between functional and morphological properties.

METHODS

The fish[1] were anesthetized with 0.1 per cent Tricaine methane sulfonate[2] in sea water (16). Some fish were sacrificed and entire organs removed; others were kept for repeated biopsies of the main organ. Fish subjected to the latter procedure survived indefinitely in aquaria in which the sea water was vigorously recirculated. The excised tissue was treated in one of three ways:

1. Cubes approximately 1 cm on edge were fixed in formalin, embedded in paraffin, and sectioned for light microscopy.

2. For electron and phase contrast microscope preparations, slices no more than 5 mm square and 1 to 2 mm thick were cut either parallel or perpendicular to the plane of the electroplaques, which lie in ordered array in the organs. This material was kept at 0–5°C while it was fixed by either of two methods: (i) in a 2 per cent solution of OsO_4 in distilled water (adjusted to pH 7.2 with dilute NaOH) for 0.5 to 10 hours, or (ii) in 10 per cent formalin in M/15 Sørenson phosphate buffer (pH 7.2–7.3) for 0.5 to 10 hours, followed by postfixation in OsO_4 as above. After a brief rinse in distilled water the tissue was dehydrated in a graded series of ethanol solutions and embedded in *n*-butyl methacrylate (30) with uranyl nitrate (75 to 100 mg/100 ml) (32).

Sections were cut with glass knives on a Porter-Blum microtome. For electron microscopy they were mounted on carbon-coated grids and examined in a Philips 100 B electron microscope. All the electron micrographs shown were taken at 100 kv accelerating voltage.

3. For esterase localization a modification of the pararosanilin method (14) described by Lehrer and Ornstein (25) was used. The material, blocks of tissue 1 to 3 mm on edge, was kept at 0–5°C throughout. It was fixed for 0.5 to 3 hours in buffered formalin or in 2 per cent OsO_4. The blocks were then incubated for 30 minutes to 1 hour in a saturated solution of α-naphthyl acetate in M/15 phosphate buffer (pH 6.8–6.9) to which was added 1 to 2 per cent of a solution of hexaazotized pararosanilin.[3] After incubation the blocks were washed for 5 to 10 minutes first in dilute buffer and then in distilled water. Tissue which had been fixed in formalin and was to be used for electron microscopy was postfixed in 2 per cent OsO_4 solution for 30 minutes. Further treatment for microscopy was as described above.

OBSERVATIONS

Electroplaques of the Main Organ

In gross structure, as well as function, the electroplaques of the main organ of *Narcine* resemble the electroplaques of *Torpedo nobiliana* (5, 8). All the regions described by Fritsch (15) in *Torpedo* electroplaques (Fig. 1) may also be observed by phase contrast microscopy in an unstained 1 μ thick section of a *Narcine* electroplaque (Fig. 2). However, the higher resolution of electron microscopy furnishes data which lead to a different interpretation of these structures from that given by Fritsch.

The *stratum granulosum*, which he described as a layer of globules on the ventral, innervated surface of the electroplaque, is revealed in low power

[1] *Narcine* were supplied by the Marineland Research Laboratory, St. Augustine, Florida, through the courtesy of Mr. F. G. Wood, Jr., Curator of the Laboratory. We wish to thank Mr. Wood and his staff for their kindness.

[2] MS222, obtained from Sandoz Pharmaceuticals, Inc., Hanover, New Jersey.

[3] Both solutions were prepared immediately before use. Since the solubility of α-naphthyl acetate in water is low, an emulsion was prepared by adding a small volume of water to the solid, heating in a water bath until the melting point of the solid was reached, and shaking vigorously. This emulsion was added to the buffer solution with stirring. After cooling to ice bath temperature, the solution was filtered to remove excess reagent. Hexaazotized pararosanilin was prepared by adding an equal volume of a 4 per cent aqueous solution of $NaNO_2$ to a 4 per cent solution of pararosanilin (CI676) in 2 N HCl. The pH of the incubating solution was then adjusted to 6.8–6.9. The hexaazo compound is relatively stable at room temperatures and is light yellow to straw color at this concentration.

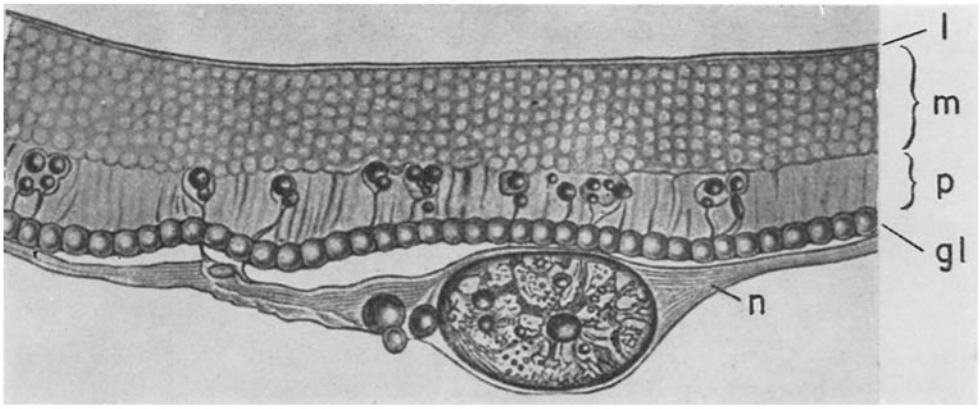


FIGURE 1

Cross-section of *Torpedo* electroplaque reproduced from Fritsch (15, Fig. 56). The structure across the bottom (*n*) represents an axon. Fritsch termed the three layers of the electroplaque above it stratum granulosum (*gl*), palisade layer with nerve endings (*p*), and stratum moleculare (*m*), respectively; *l* is connective tissue.

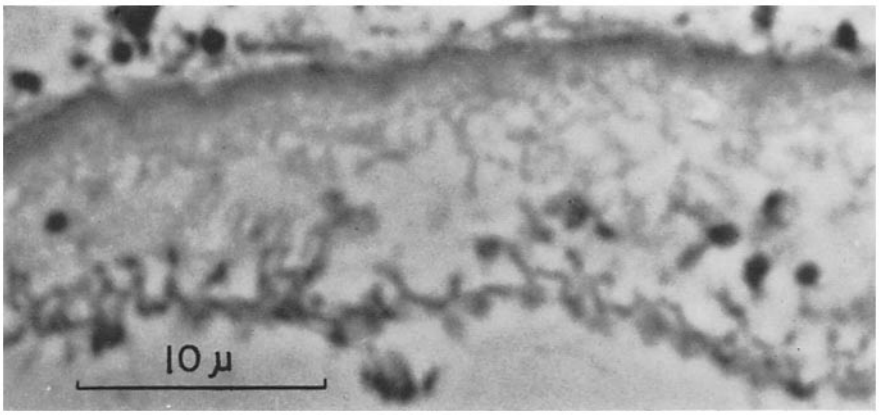


FIGURE 2

A phase contrast micrograph of a cross-section of *Narcine* main organ electroplaque. Dark objects outside the cell are dense collagen deposits. Mitochondria appear as equally dark objects within the cell. The fingers of the ventral innervated membrane, and the canalicular network of the uninnervated dorsal membrane, may also be seen. × 3,350.

electron micrographs of thick (*ca.* 0.3 μ) sections (Fig. 3) as the troughs and indentations of the postsynaptic membrane and the dense presynaptic terminals of its nerve supply. The clear layer of faintly outlined *palisades*, amid which Fritsch saw globules connecting to the ventral surface (Fig. 1) and which he thought were the innervating nerve terminals, is the cytoplasmic region which lies immediately dorsal to the innervated membrane of the electroplaque. Mitochondria (Fig. 2) and the deeper inpocketings of the synaptic troughs (Fig. 3) account for Fritsch's globular "nerve terminals." The reticulated *stratum moleculare*, which Fritsch pictured as occupying about the dorsal two-thirds of the cell, is a dense network of interconnected canaliculi (Fig. 3). The membranes of these structures are continuous with the uninnervated dorsal membrane of the electroplaque. Opening into the extracellular space (*cf.* Fig. 5) and extending deep into the cell body, the canalicular network increases enormously the membrane area of the uninnervated surface.

Electroplaques of the Accessory Organ

At all levels of magnification the electroplaques of the accessory organ show the same arrangement of structures (Fig. 4) as do those of the main organ. However, since the dorsal surface is the innervated one in the electroplaques of the accessory organ (28), their orientation is inverted relatively to the cells of the main organ. The electroplaques of the accessory organ are also thicker (*ca.* 20 μ). The additional thickness is

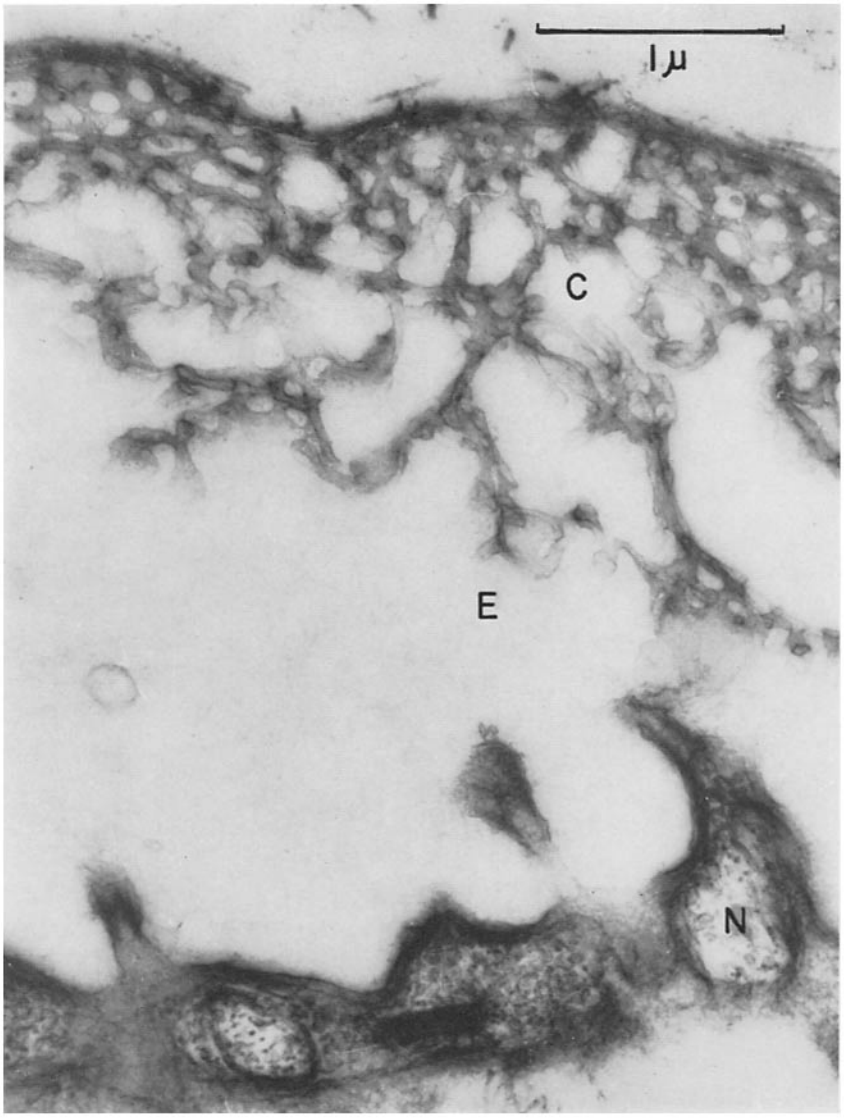


FIGURE 3

An electron micrograph of an electroplaque from the main organ. Fibrillar material is scattered in the spaces outside the electroplaque. The canalicular network (*C*) extends from the membrane of the uninnervated (dorsal) surface far into the body of the electroplaque. The interconnections of the canaliculi may be readily followed in this thick (*ca.* 0.3 μ) section. The cytoplasm of the electroplaque (*E*) is very rich in fibrillar material. Numerous presynaptic nerve terminals (*N*) are closely applied to the innervated (ventral) surface. The terminals are densely packed with vesicles. Three deep indentations of the innervated membrane are seen in this section. They are not invaded by the axon terminals. × 33,000.

mainly contributed by a larger central cytoplasmic (or palisade) region, and the canalicular network has about the same absolute extent in both kinds of cells.

Fine Structure of the Electroplaques

The details of cell structures are similar in the two kinds of electroplaques. The infrequent nuclei are found near the uninnervated surface whether this be ventral, as in the cells of the accessory organ, or dorsal, as in the electroplaques of the main organ. They lie (Fig. 4) in a relatively clear cytoplasmic area which is surrounded, but not invaded, by the canalicular network and which contains some mitochondria. Most of the mitochondria, however, are found in the palisade region in the central part of the electroplaque.

The canalicular network appears discontinuous in thinner electron microscope sections, taking on the aspect of vesicles (Fig. 5). This appearance is enhanced by bulbous enlargements which are sometimes found at the intersections of the tubules. The walls of the canaliculi are clearly seen to be continuous with the membrane of the uninnervated surface. In the thin electroplaques of the main organ the network frequently extends close to the deepest inpocketings of the innervated surface, but connections between the canaliculi and the innervated membrane have not been observed. The palisade layer contains numerous fibrils and granules which have not been further identified as yet.

The Synaptic Apparatus

The innervated surfaces of both kinds of electroplaques are densely covered with presynaptic terminals. However, the structure of the synaptic apparatus is simpler than it appears to be in many vertebrate neuromuscular junctions (*cf.* 11, 31). The presynaptic terminals lie in shallow troughs of the postsynaptic membrane which may be homologous with the "synaptic gutter" of end-plates. Deep indentations and finger-like projections of the membrane which add some complexity to the synaptic structure are probably similar to the "junctional folds" of end-plates. The axons follow the shallower of the inpocketings, so that the synaptic area is considerably increased over that which would be presented by simple appositional contact. The nerve terminals are free of any connective tissue or Schwann sheath.

Although the details of the innervating nerve supply differ in the electroplaques of the main and accessory organ (5), the finer structures of the synaptic contacts between the presynaptic terminals and the postsynaptic electroplaque membrane are similar. The terminals contain numerous synaptic vesicles (Figs. 5 and 6) and are thereby clearly distinguishable from cross-sectioned projections of the electroplaque, and from the larger axonal branches (Fig. 6). The former present themselves as oval or indented clear areas, frequently containing a peripheral zone of fine, radially oriented fibrils. In oblique sections spaces between the digitations are seen in which the presynaptic terminals lie (Figs. 6 and 7). The nerve fibers do not invade the deepest of these inpocketings of the electroplaque (Figs. 5 and 7).

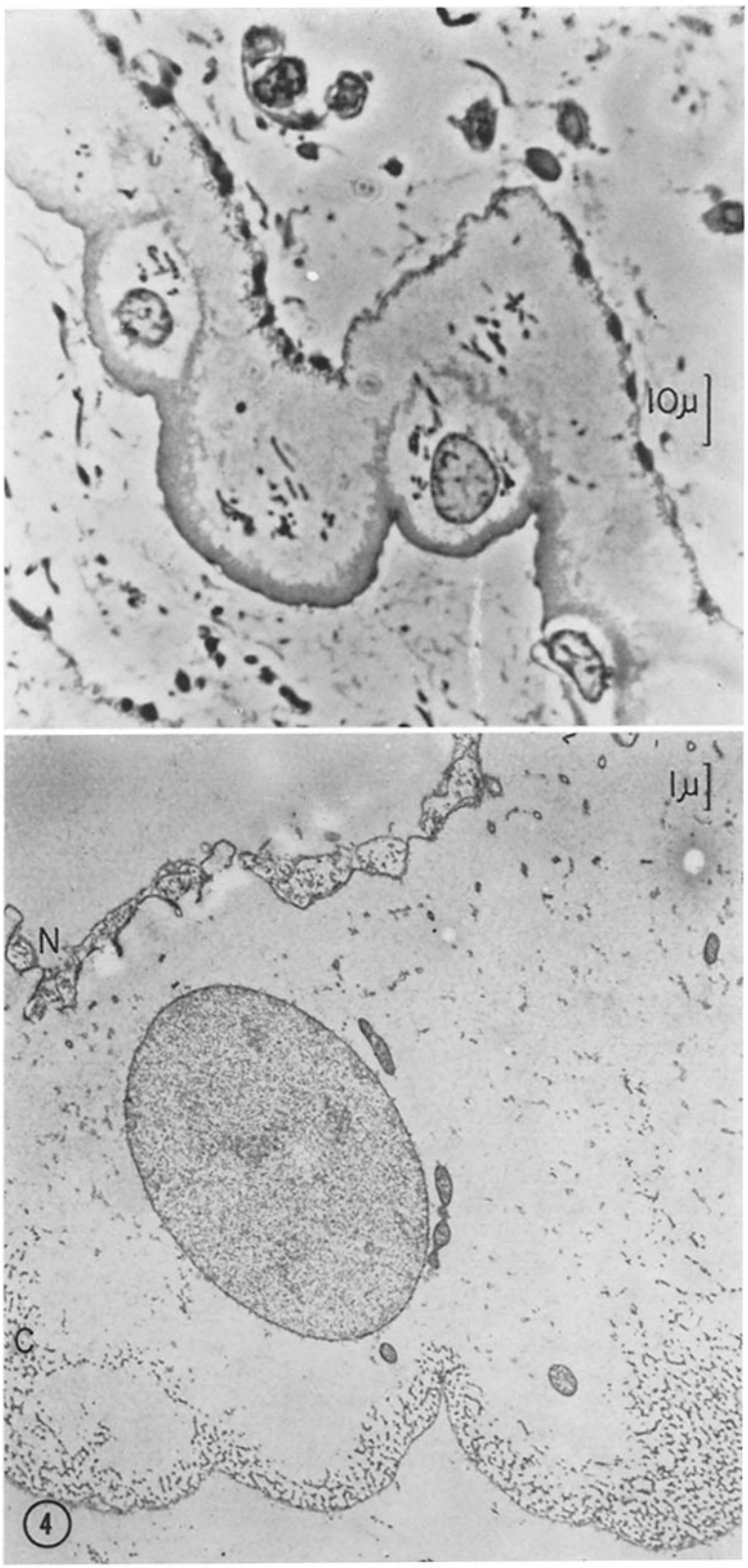
Localization of Esterase

Dye was deposited only on the innervated surface of the electroplaque (Fig. 8), forming a pattern which delineated the regions of synaptic contact. The dye followed the inpocketings of the surface, and was deposited along the narrow channels even of the deepest inpocketings (Fig. 9). In electron as in phase contrast micrographs, no dye could be observed at the opposite, uninnervated surface, nor was it seen in regions of the innervated surface that were free of presynaptic terminals.

DISCUSSION

There seem to be no marked differences in fine structure of the electroplaques of the main and accessory organs, except for the differences relating to the inversion of the dorsal-ventral orientation of the cells, and the greater thickness of the electroplaques of the accessory organ. The similarity in fine structure may be ascribed to the common origin of both kinds of electroplaques from muscle tissue, albeit from different muscles. Embryological studies now in process should help to elucidate the differences between the dorsal innervation of the electroplaques of the accessory organ and the ventral innervation in the main organ.

That the synaptic apparatus appears to have the same fine structure in both kinds of electroplaques is also noteworthy for other reasons. Not only is the innervation on opposite surfaces in the two kinds of cells (28), but the arrangement of the


10μ
1μ
N
C
4

innervation is also different (5). The electroplaques of the main organ are supplied by four to seven axons, and, as in the electroplaques of other torpedine fishes (8, 15), each axon approaches the ventral surface of the electroplaque from a different point at its periphery and each innervates a separate area of the membrane. Overlap of innervation, if at all present, is small. In contrast, the dorsal surfaces of the electroplaques of the accessory organ are innervated by two or three nerve trunks, each containing several axons. The nerve fibers from the different trunks interlace on the surface of the cell. With respect to their contacts with the postsynaptic membrane, however, the differences of innervation appear to be of no significance. Furthermore, no differences were observed in the number and size of the synaptic vesicles. However, quantitative studies on such preparations remain to be done.

The similarity of the synaptic apparatus is also noteworthy because the responses of the two types of electroplaques to neural stimuli are markedly different (5). Excitation of a single axon to an electroplaque of the main organ results in a maximal or nearly maximal discharge of that part of the synaptic membrane which is innervated by the axon. The response is a depolarizing pulse about 50 to 60 mv in amplitude, lasting about 5 msec. There is little or no facilitation in the response to a second stimulation of the axon. In contrast, a single stimulus to an axon of the accessory organ electroplaque results in a negligible response. However, there is marked facilitation, and maximal responses develop after some 10 to 12 stimuli at 60 to 100 per second. The discharges are considerably longer than those of the electroplaques of the main organ, each lasting about 15 msec. instead of about 5 msec. Since these functional differences are not reflected in the structural details discerned in the present work, it must be concluded that they depend upon differences that are not resolved by the morphological techniques employed.

The canalicular network of the uninnervated membrane of *Narcine* electroplaques is a striking structural feature. It also has its counterpart in other electroplaques (29), but in very thin sections the canaliculi appear as vesicles or as the "caveoli" that have been described in various electroplaques (26). Since electroplaques of all electric fishes derive from muscle fibers (29), the canaliculi may represent (*cf.* 21, 28) the disorganized remnants of the tubules of the sarcoplasmic reticulum which, it is postulated (22), mediate the spread of excitation-contraction coupling of muscle fibers. Fibrillar elements which are probably homologous with the I band elements of muscle fibers are present in the electroplaques of *Astroscopus* and of the Rajidae. The canalicular network in these electroplaques extends to structures that are analogous with the Z lines (29).

The large extension of the surface of the uninnervated membrane that is made possible by the canalicular network might also account (*cf.* 21) for the very low resistance of the uninnervated surface of torpedine electric fishes (5, 8), *Astroscopus* (6), and *Electrophorus* (1, 24). However, the electroplaques of Rajidae, *Malapterurus*, and probably also the Mormyridae (29) have canaliculi, though both surfaces of the cells have approxi-

FIGURE 4

Upper. A phase contrast micrograph of a cross-section of an accessory organ electroplaque. The gray area extending from the uninnervated membrane (bottom) is the canalicular network. It is seen here enclosing three nuclei. The other dense objects within the electroplaque are mitochondria. Outside are collagenous bundles and interstitial cells. Immediately adjacent to the innervated membrane (top) the larger neural terminals are seen as ovoid dense bodies. The infoldings of the membrane may be seen along its entire length. × 1,000.

Lower. A low power electron micrograph of an electroplaque from the accessory organ. In the central part of the micrograph the canalicular network (bottom) is very thin, but is much thicker at the edges of the section (*C*). A nucleus and several mitochondria are also seen. The innervated membrane (top) shows a few inpocketings (left) and is overlain by presynaptic terminals (*N*) in which may be seen vesicles. × 7,000.

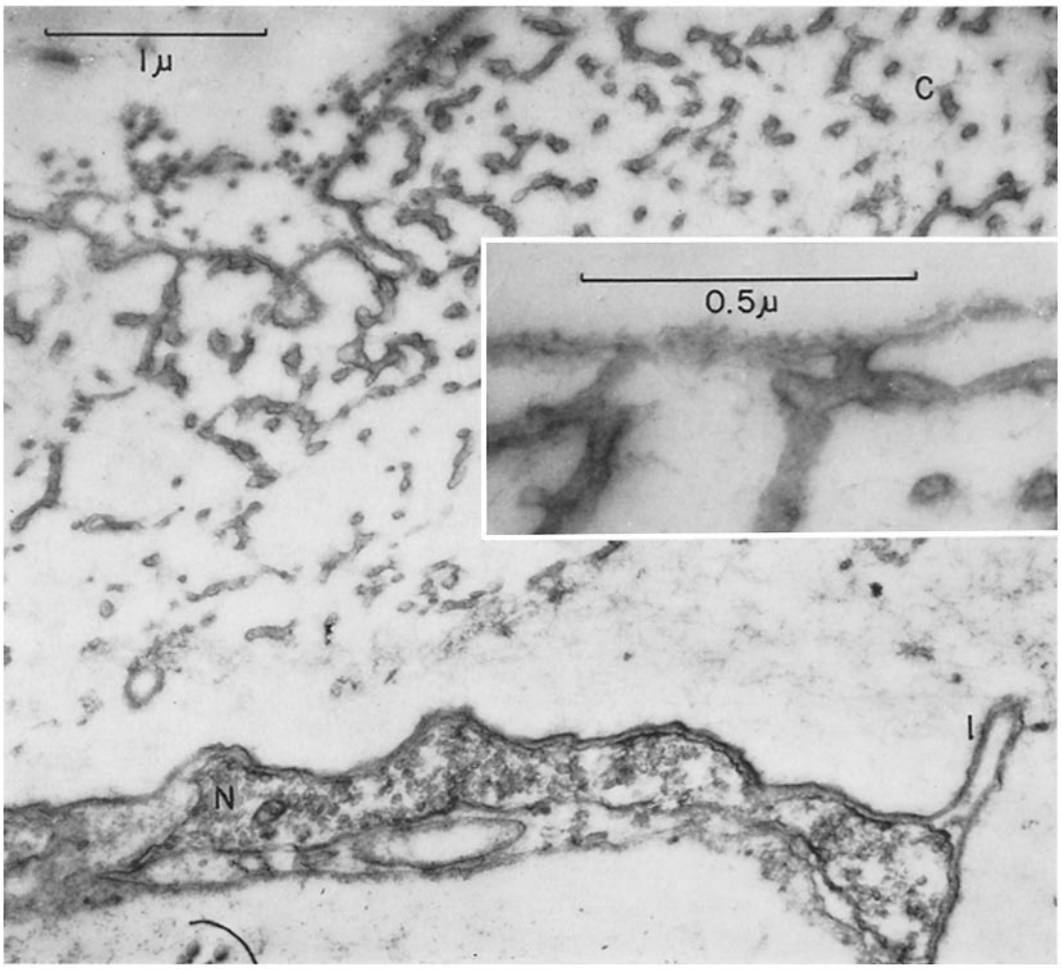


FIGURE 5

An electron micrograph of a moderately thin (*ca.* 0.1 μ) section of an electroplaque from the main organ. The canalicular network takes on the appearance of isolated vesicles, but numerous interconnections are visible in sections of this thickness. Still thinner sections would indicate only vesicles or "caveoli." The indentation of the innervated membrane (*I*, lower right) is empty of any extension of the neural process (*N*), which forms a synapse of considerable length along the membrane. Continuity between the canaliculi (*C*) and the uninnervated membrane is seen on the left side of the micrograph and is further demonstrated at higher magnification in the inset, taken from another section. × 30,000 and × 90,000.

mately equal resistance (3, 7, 21, 23). The degree of canalicular development in different fishes differs to some extent (29), but has not yet been evaluated.

In this connection it is of interest that the most notable difference in fine structure of the electroplaques of the main and accessory organs of *Narcine* is in the thickness of the canalicular network relative to the thickness of the electroplaque. However, this difference arises chiefly from the thicker palisade layer of the electroplaques of the accessory organ. The depth of the canalicular layers appears to be about the same in both kinds of electroplaques, although detailed quantitative data are not available. The uninnervated membrane appears to have about the same very low electrical resistance in both types of electroplaques (5). It is of further interest that both surfaces in

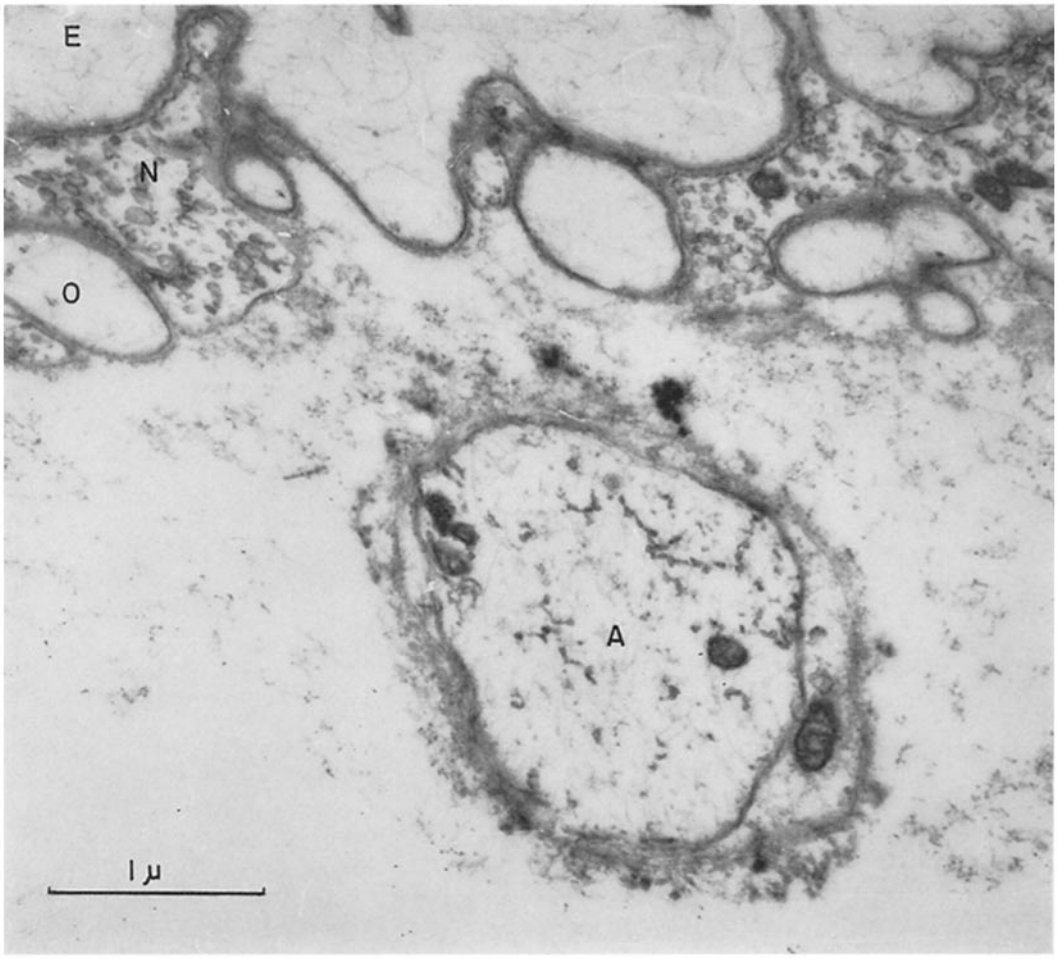


FIGURE 6

An electron micrograph of a section cut obliquely through the innervated membrane of a main organ electroplaque. The oval clear areas (*O*) below the body (*E*) of the electroplaque are extensions of the cell which interdigitate with the interposed vesiculated nerve endings (*N*). The small axon below (*A*), which has numerous fibrillar elements, is enveloped by a Schwann cell. A few vesicles are seen in the axon, indicating that the section is in close proximity to the synaptic terminals. × 30,000.

the electroplaques are remarkably free of connective tissue envelopments. The membrane of the innervated electrogenically active surface, however, offers considerable electrical resistance (5).

The electroplaques of *Narcine* (8) are cholinergic, although they are relatively insensitive to acetylcholine. The insensitivity may be in part due (8) to the high concentration of esterase which is found in these electric organs by chemical tests (2, 9), and also indicated by the present histochemical data.

Of particular interest is a functional correlation provided by the histochemical methods. The innervated sites of other electroplaques are also stained by esterase-localizing methods (12, 13, 29). The uninnervated membrane is not stained in any electroplaques that have been studied, although the electrophysiological properties of the membranes are profoundly different in different fish (21).

The uninnervated membrane of the teleost marine electric fish *Astroscopus y-graecum* is electrogenically unreactive to electrical stimuli (6), resembling in this respect the uninnervated

membrane of the torpedine electroplaques (5, 8). Among the fresh water electric fish, however, there are forms in which neither surface of the electroplaque is innervated, but both do react to electrical stimuli, each generating a spike (7, 23). Histochemical examinations have been made on electroplaques of *Malapterurus* (12, 29) and of several mormyrids (12). The esterase staining appears to be confined to the sites of innervation, which are restricted areas on stalk processes of the cells. The electrically excitable major surfaces are not stained. These data are in agreement with the chemical finding that the *Malapterurus* electric organ has extremely low concentrations of both cholinesterase and acetylcholine (2).

The synaptic contact between the terminals of a single axon and the *Narcine* electroplaque has a considerable area. On the average, the diameter of an electroplaque is about 5 mm and each is supplied by five axons (5). Thus, the area innervated by each axon is about $4 \times 10^6 \mu^2$. Though the entire area is not covered by the presynaptic terminals, nevertheless the numerous interdigitations of axon terminals and postsynaptic membrane must increase the area of contact considerably. Thus, a surface area for the presynaptic terminals of each axon of at least $10^6 \mu^2$ appears to be a reasonable estimate. At their junctures with the innervated membrane the individual axons have diameters not larger than 10 μ (5, Fig. 1 *B*).

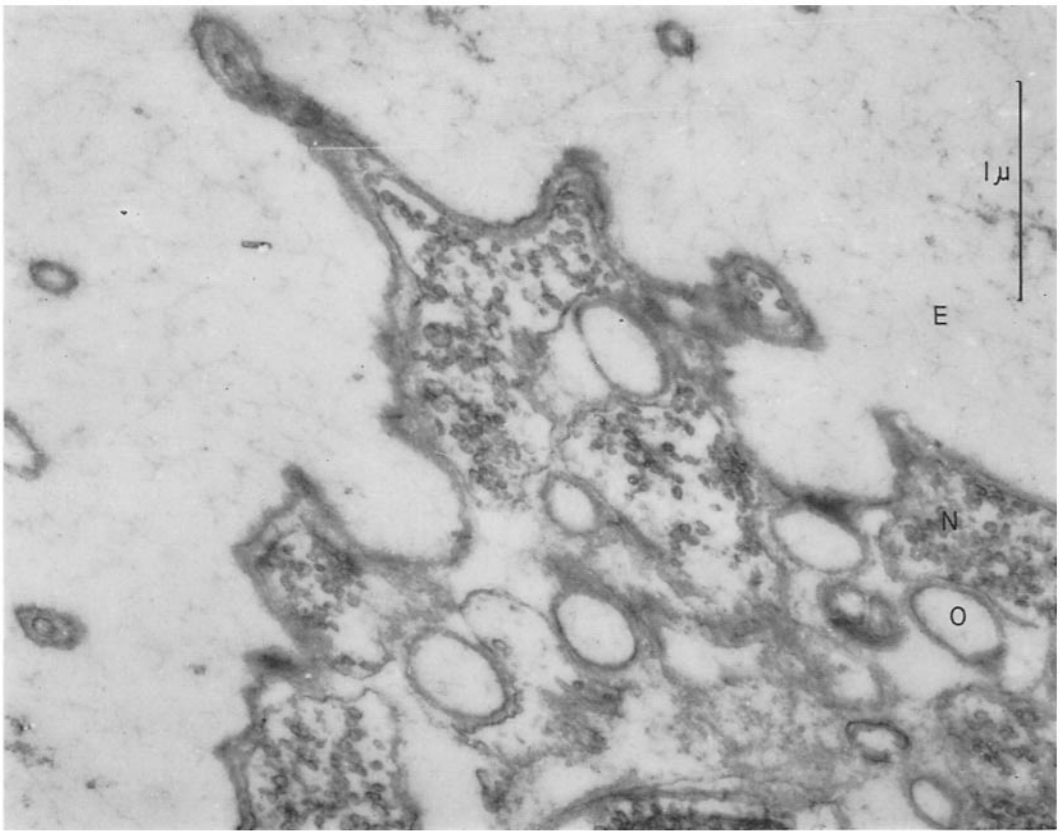


FIGURE 7

Part of a synaptic junction in an electroplaque of the main organ, showing the complex interdigitations of the vesicle-containing presynaptic terminals with the finger-like projections of the electroplaque. The latter are the clear ovoid bodies interspersed among the nerve terminals. Labeling as in Fig. 6. Compare this section with that of Fig. 9, from a preparation showing esterase localization. × 30,000.

Therefore, transmitter substance that must be delivered to the synaptic surface (21) must be funneled through a cross-sectional area some 10^4 times smaller than the surface of outflow. It is likely, furthermore, that a single axon innervates more than one electroplaque. Thus, if the transmitter or its precursors are formed at the cell body of the neuron, the amount of material transported to the synaptic sites must be still larger.

Another type of high demand on synaptic performance occurs in the electric organ of *Malapterurus*. A single axon, some 10 μ in diameter, innervates about four million electroplaques. The area of synaptic contact at each electroplaque is relatively small, being confined to the tip of a stalk process. Nevertheless, at each synapse the axon divides into arborizations which increase the total area of synaptic contact (29). According to

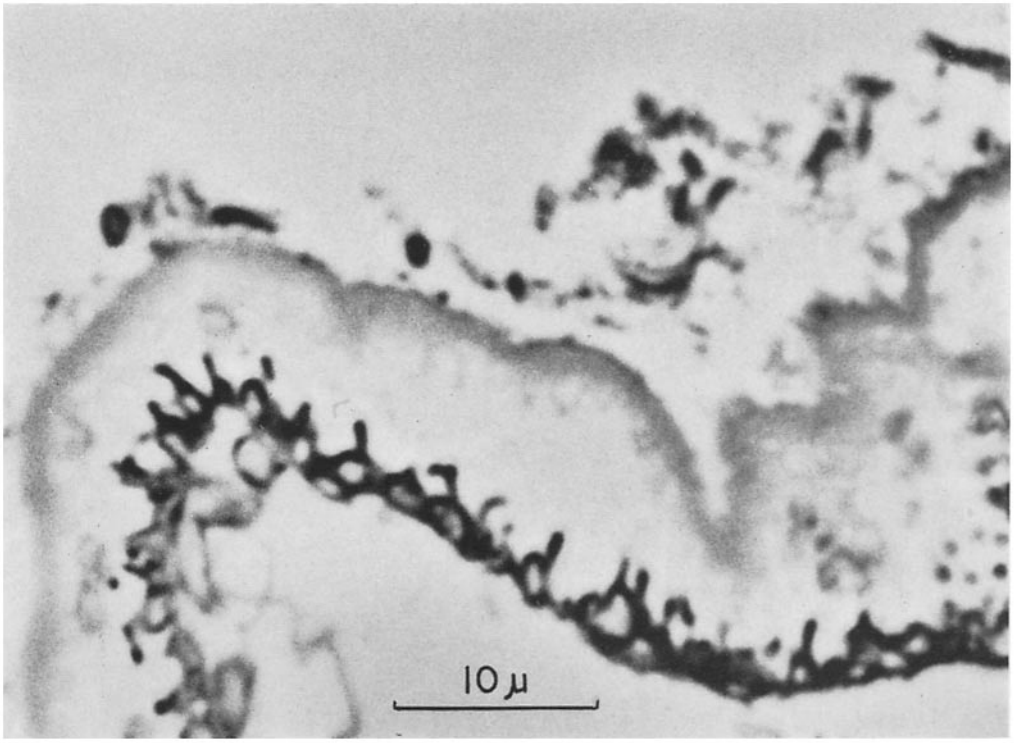


FIGURE 8

A phase contrast micrograph of a section from an electroplaque of the main organ treated for esterase localization. The dense deposit which follows the convolutions of the innervated membrane is seen by ordinary light microscopy to be the only place in which there is dye deposition. The extracellular dense areas are bundles of collagen. × 2,700.

In many gymnotids the anatomical relations of axon to postsynaptic membrane are similar to those that obtain in *Narcine* electroplaques. However, there is an additional factor in that the knifefishes discharge continuously at rates which usually range above 100 per second and in some species the discharge rate is close to or above 1000 per second (18, 19, 21). Since each discharge is initiated by a synaptic excitation, the consumption of transmitter agent would have to be high. Gotch (17) there is an increase of 350,000 times in the cross-sectional area of the main branches of the nerve alone, as compared with the area of the axonal trunk.

Thus, the nerve cells and electroplaques of different electric fish constitute tissues in which several varieties of severe functional demands are met. Since a considerable amount of information is now available regarding these demands, these tissues offer material that should be particularly

advantageous for the study of the morphological correlates of the cellular functions.

CONCLUSIONS

The comparative data on the structure and histochemistry of the two kinds of electroplaques of *Narcine brasiliensis* do not provide clues to the different modes of functioning of the accessory and main electric organs. Thus, they indicate that most of the functional differentiations result from morphological differences that have not been resolved by this study. However, as has been proved to be the case for the electrophysiological

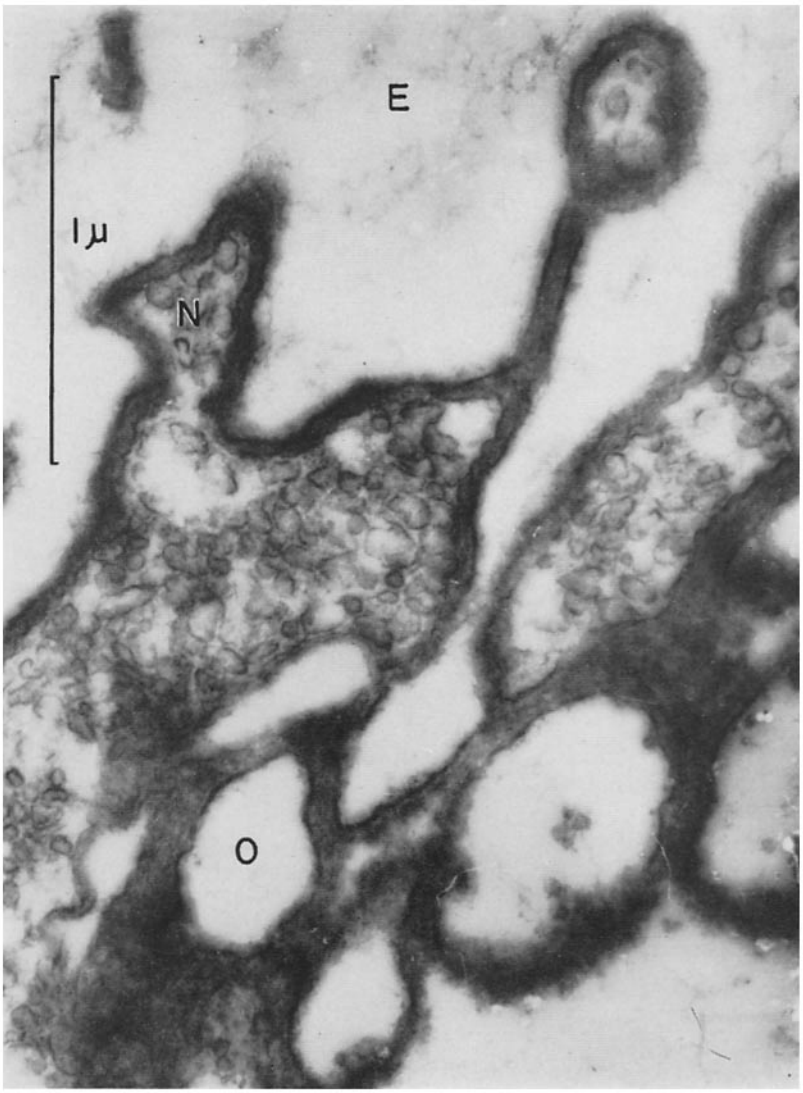


FIGURE 9

An electron micrograph of a part of a synapse in an electroplaque of the main organ, showing a dense deposit, restricted to the synaptic cleft, after staining for esterase localization. The deposit of the dye follows the convolutions of the interdigitated synaptic region. The cross-sectioned digitations of the electroplaque stand out clearly. Labeling as in Fig. 6. Compare with Fig. 7 from tissue which was not treated for esterase localization. × 52,000.

approach, an extensive and intensive comparative study of the morphology of electroplaques in different fish is likely to provide further data and concepts concerning the correlation of structure and function of electrogenic cells.

This work was supported in part by grants from the Muscular Dystrophy Associations of America; the National Institute of Neurological Diseases and Blindness (B-385 C4 and C5) of the National Institutes of Health, United States Public Health Service; The National Science Foundation (NSF G-5665); and the United Cerebral Palsy Educational and Research Foundation.

Received for publication, June 10, 1961.

REFERENCES

1. ALTAMIRANO, M., COATES, C. W., and GRUNDFEST, H., *J. Gen. Physiol.*, 1955, **38**, 319.
2. AUGUSTINSSON, K. B., and JOHNELS, A. G., *J. Physiol.*, 1958, **140**, 498.
3. BENNETT, M. V. L., *Ann. New York Acad. Sc.*, 1961, **94**, 458.
4. BENNETT, M. V. L., and GRUNDFEST, H., *J. Gen. Physiol.*, 1959, **42**, 1067.
5. BENNETT, M. V. L., and GRUNDFEST, H., *J. Gen. Physiol.*, 1961, **44**, 805.
6. BENNETT, M. V. L., and GRUNDFEST, H., *J. Gen. Physiol.*, 1961, **44**, 819.
7. BENNETT, M. V. L., and GRUNDFEST, H., *in* Bioelectrogenesis, (C. Chagas and A. Paes de Carvalho, editors), Amsterdam, Elsevier, 1961, 113.
8. BENNETT, M. V. L., WURZEL, M., and GRUNDFEST, H., *J. Gen. Physiol.*, 1961, **44**, 757.
9. CHAGAS, C., SOLLERO, L., and MIRANDA, M., *Acad. Bras. Cienc.*, 1953, **25**, 319.
10. COHEN, B., BENNETT, M. V. L., and GRUNDFEST, H., *Fed. Proc.*, 1961, **20**, 339.
11. COUTEAUX, R., *in* The Structure and Function of Muscle, (G. H. Bourne, editor), New York, Academic Press, 1960, **1**, 337.
12. COUTEAUX, R., and SZABO, T., *Compt. rend. Acad. sc.*, 1959, **248**, 457.
13. COUTEAUX, R., and TAXI, J., *Arch. anat. micr.*, 1952, **41**, 352.
14. DAVIS, B. J., *Proc. Soc. Exp. Biol. and Med.*, 1959, **101**, 90.
15. FRITSCH, G., Die elektrischen Fische: Die Torpedineen, Leipzig, Veit, 1890.
16. GILBERT, P. W., and WOOD, F. G., JR., *Science*, 1957, **126**, 212.
17. GOTCH, F., *in* Textbook of Physiology, (E. Schafer, editor), London, Macmillan, 1900, **2**, 561.
18. GRUNDFEST, H., *Progr. Biophysics and Biophysic. Chem.*, 1957, **7**, 1.
19. GRUNDFEST, H., *in* The Regional Chemistry, Physiology and Pharmacology of the Nervous System, (S. Kety, editor), London, Pergamon Press, 1961, 378.
20. GRUNDFEST, H., *Ann. New York Acad. Sc.*, 1961, **94**, 405.
21. GRUNDFEST, H., and BENNETT, M. V. L., *in* Bioelectrogenesis, (C. Chagas and A. Paes de Carvalho, editors), Amsterdam, Elsevier, 1961, 57.
22. HUXLEY, A. F., *Progr. Biophysics*, 1957, **7**, 255.
23. KEYNES, R. D., BENNETT, M. V. L., and GRUNDFEST, H. *in* Bioelectrogenesis, (C. Chagas and A. Paes de Carvalho, editors), Amsterdam, Elsevier, 1961, 102.
24. KEYNES, R. D., and MARTINS-FERREIRA, H., *J. Physiol.*, 1953, **119**, 315.
25. LEHRER, G. M., and ORNSTEIN, L., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 399.
26. LUFT, J. H., *Exp. Cell. Research*, 1958, Suppl. 5, 168.
27. MATHEWSON, R., unpublished data.
28. MATHEWSON, R., MAURO, A., AMATNIEK, E., and GRUNDFEST, H., *Biol. Bull.*, 1958, **15**, 126.
29. MATHEWSON, R., WACHTEL, A., and GRUNDFEST, H., *in* Bioelectrogenesis, (C. Chagas and A. Paes de Carvalho, editors), Amsterdam, Elsevier, 1961, 25.
30. NEWMAN, S. B., BORYSKO, E., and SWERDLOW, M., *J. Research Nat. Bur. Standards*, 1949, **43**, 183.
31. ROBERTSON, J. D., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 381.
32. WARD, R. W., *J. Histochem. and Cytochem.*, 1958, **6**, 398.