THE ULTRASTRUCTURE OF A REPTILIAN MYONEURAL JUNCTION* †

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The description of motor end-plates by Doyère (15) in 1840 initiated a series of light microscope investigations leading to the cytological concepts of the structure of the motor end-plate described in the papers of Kühne (26), Tiegs (44), Couteaux (6-10), and Gutman and Young (22). The hundredfold or more higher resolution of the electron microscope (EM) clearly offers promise of distinct advances in this field. The first attempt to study this problem with the EM (1) resulted in little if any new information because of limitations imposed by the techniques of specimen preparation. Three concurrent independent studies of this problem at higher resolution have been reported in abstract form by Palade (31), Reger (33), and Robertson (36, 37). The complete report of one of these has recently been published by Reger (34). However, again those specimen preparatory techniques now considered satisfactory were not applied. As a result some of the grosser features of the junctions were observed but the details of the membrane structures involved were obscured. The complete report of the other concurrent study of this problem by Palade has not yet appeared.

Two recent reviews of the cytological literature on myoneural junctions are available (10, 45). With respect to finer cytological details it is known that the myelin sheath is lost at the terminals. The endoneurial sheath (sheath of Key and Retzius or sheath of Henle) appears to fuse with the endomysium and the unmyelinated terminal twigs often appear to penetrate the muscle surface to ramify and end blindly in an accumulation of sole plate sarcoplasm (Doyère eminence). Within the endings, Tiegs (44), Couteaux (8), Tello (42), and others observed a clear zone between the...
neurofibrils and sarcoplasm. The clear zone sometimes appeared to be separated from sarcoplasm by a very thin (about 1 micron) cross-striated border. This clear zone and striated border were observed by Kühlne and referred to as the "stroma." Kühlne regarded the stroma as a component of the neuroplasm of the ending, since it disappeared after denervation. Tiegs (44), on the other hand, studying preparations either impregnated by gold and silver or stained with methylene blue, stated that the clear zone and the striated border did not degenerate after denervation, the former becoming continuous with the cords of Büngner. Therefore, regarded the clear zone and its striated border as a Schwannian investment of the axon ending and designated it by the term "perilemma." Couteaux observed similar structures after Janus green and methyl violet postvital staining techniques and applied the term "teloglia" to the clear zone next to the neurofibrillar mass of the axon endings. On the basis of embryological studies he considered this layer to represent an investment of Schwann cytoplasm. He demonstrated the striated border quite clearly and designated it as the "subneural apparatus." He considered the latter to be of muscle origin, and hence the nerve endings to be epimembranous, a view concurred in by Gutman and Young (22) but not by Tiegs (44, 45). Recently, the cholinesterase staining technique of Koelle (25) has been applied by Couteaux and Taxi (11) and by Harris (23) with selective staining of Couteaux' subneural apparatus. Holt (24) using a different technique for cholinesterase has obtained similar results. These more recent light microscope studies, while clearly delineating the subneural apparatus, have failed to show Couteaux' teloglia layer clearly deep in the endings.

Several observers have found granules concentrated in both sarcoplasm and axoplasm at myoneural junctions and Boeke and Noël (4) and Noël (28) concluded that these were mitochondria. Couteaux (8) has demonstrated that these granules are Janus green-positive in support of this conclusion.

Foettinger (18), von Thanhoffer (43), and Tiegs (44) have observed a connection between the Z bands of the myofibrils and the axon endings of motor end-plates. Tiegs's light micrograph is more convincing in this respect than the electron micrographs of Beams and Evans (1) which purport to show such a connection. The study of Reger (33, 34) failed to demonstrate such Z band connections but it is stated that the Z bands extend from the myofibrils into sole plate sarcoplasm. Unfortunately, however, the validity of this observation is open to considerable question because of the distortions resulting from the preparatory techniques. No such Z band connections have been observed in the present study.

The main findings reported here concern the nature of the membrane structures intervening between axoplasm and sarcoplasm, the characteristics of terminal axoplasm and sarcoplasm, and the disposition of the terminal Schwann cells. The characteristics of the muscle surface have been reported in a preceding paper (40). The characteristics of the Schwann cell surface and other features of Anolis nerve fibers have been reported previously (39).

**Materials and Methods**

Small muscles from the hind legs of freshly decapitated chameleon lizards (Anolis carolinensis) were rapidly dissected and tied at their rest length to applicator sticks. These sticks were then immersed in small test tubes containing 1 per cent OsO₄ at 25°C. or 0°C. buffered at pH 7.4-7.6 by the method of Palade (29). In some instances intercostal muscles were used.
Fixation was often begun in situ. The material was usually allowed to remain in the fixative for 2 or 3 hours, the longer time applying in the cold. Some of the best results were obtained by precooling the animals on ice and fixing at a pH of 7.4 at 0°C in the dark for 1½ to 4 hours. Many of the preparations were stained in 1 per cent phosphotungstic acid (PTA) in phthallate buffer at pH 5.4 for periods varying from 12 to 48 hours. The preparations were removed from the fixative, given a brief rinse in distilled water, and placed directly into PTA at room temperature. They were then dehydrated in alcohol in the usual manner and embedded in a mixture of 85 per cent butyl methacrylate and 15 per cent methyl methacrylate. Sections were cut at an estimated thickness of 200 to 300 Å with an international Minor rotary microtome modified according to the method of Geren and McCulloch (21) with glass knives prepared according to the method of Latta and Hartman (27). The plastic embedding medium was not removed from the sections since this treatment produces drastic distortions. The sections were examined with an RCA model EMU-2B electron microscope equipped with a reduced condenser aperture and an externally centerdale objective aperture as designed by Farrant and Hodge (16). Apertures were prepared by puncturing a hole in a two mill thick platinum disc with an acid-etched steel needle or an oxidized, KOI-Lcleaned tungsten needle. It was found necessary to replace the apertures frequently because of evidence of astigmatism produced in the image by the apertures after a few hours of use.

The myoneural junctions were located by phase contrast microscopy prior to thin sectioning. Sections about 0.5 μ in thickness were cut with a glass knife. They were transferred directly to a glass slide, covered with oil, and examined with an oil immersion lens without removal of the plastic embedding medium. Successive sections were examined in this manner until a myoneural junction was found. The block face was then trimmed to include the desired area and thin sections prepared from it for electron microscopy.

OBSERVATIONS

The Endoneurial Sheath and Juxtaterminal Nerve Fibers

Near myoneural junctions the endoneurial sheath consists of a thin sheet of cells with scattered collagen fibrils around it forming a complete tube enveloping small nerve fibers. Such a tube one cell thick, is shown in Fig. 3 (endo.). A portion of the wall of another one with three nerve fibers is enlarged in Fig. 4. Both the inside and outside boundaries of these endoneurial sheath cells consist of two dense lines bordering a light central zone. It is not clear in Fig. 4 whether the cellular layer of the endoneurial sheath is syncytial since the presence of a cell boundary interrupting the cytoplasm of the single circumferential cell layer cannot be excluded; nor have sufficiently clear longitudinal sections been studied. In other regions, further removed from myoneural junctions, this tubular sheath has been seen to consist of more than one layer of cells (39).

Myelinated nerve fibers are frequently seen in small bundles passing between muscle fibers (Figs. 1, 3, 4). The structure of these fibers is considered in more detail in a separate paper (39). The Schwann cell boundary is a double membrane complex resembling in many details the endoneurial sheath cell boundaries and the muscle surface membrane complex described in the preceding paper. The Schwann cell surface is connected with the myelin by a double membrane (unlabelled arrow in Fig. 4).

Four nerve fibers which are presumed to be near a motor end-plate are
seen in Fig. 2. A Schwann nucleus is present in one fiber. A thin layer of myelin (~0.1μ) is present about one of the axons but is absent in the others. Two of the unmyelinated axons are greatly reduced in size (<1μ). It seems likely that the reduced diameter of these axons is the result of branching near the myoneural junctions with separations of the branches. Evidently the myelin is lost in association with this branching. Two such unmyelinated axons are seen in a single Schwann cell overlying a motor end-plate in Fig. 6. These may represent branches which have not yet separated. The axon-Schwann double membrane may be seen clearly between axoplasm and Schwann cytoplasm in the latter instance. At the myoneural junction these small unmyelinated axons partially leave their Schwann cells and enter depressions or troughs in the surface of the muscle fiber (Fig. 5). Fig. 5 shows a Schwann cell closely associated with but not connected with a terminal axon twig. The Schwann cell here no longer contains an axon and may be a principal constituent of Kühne's telolemma.

The Myoneural Junction

A phase contrast micrograph of a section of myelinated nerve fibers and a nerve ending in Anolis skeletal muscle is shown in Fig. 1. Nerve fibers have been traced into such endings by examination of serial sections with the phase microscope. It has sometimes been possible to compare directly phase and electron micrographs by this method. Since some of the endings have thus been definitely identified on structural grounds as motor endings it seems reasonable to assume tentatively that all the endings shown represent some variety of motor nerve ending. No degeneration experiments to prove this point have yet been undertaken.

At the myoneural junctions the surface of the muscle fibers is depressed to form troughs, which partially envelope the terminal axons. The structure of the muscle surface within the troughs, while essentially similar to that described in the preceding paper, differs in a few respects. First, collagen fibrils are absent. The outer dense line and the middle clear zone of the surface membrane complex are reduced in thickness and measure about 200 A and 100 to 200 A respectively. The 300 to 500 A granular bodies observed in sarcoplasm near the inner dense line of the surface membrane complex have not been observed definitely within the synaptic troughs. There are, however, irregular densities in the appropriate location which could represent this component in a poorly fixed state. For example, in Fig. 7 there is a rather diffuse accumulation of dense material in the sarcoplasm immediately bordering the sarcoplasmic dense line of the synaptic membrane complex. In some regions this material has a coarse granular character which suggests a relationship with the granular component.

Near and within the synaptic troughs the entire three-layered muscle mem-
brane complex is thrown into deep folds, which branch and anastomose in a complex fashion (Figs. 7 to 15, arrows j.f.). These folds will be referred to hereafter as junctional folds. The appearance presented by the folds in sections cut parallel to the surface of the end-plate (Fig. 9) might be considered crudely analogous to the human finger-print. It should be noted that while such folds as these appear to be peculiar to myoneural junctions they are not strictly confined to the synaptic troughs. They may occur in the muscle surface just outside of the troughs, as may be seen best in Figs. 6 and 9. Also in Fig. 11 a rather distorted fold appears below the arrow marked m.s.c. The junctional folds are rather loosely packed side by side, surrounding the axon like the petals of a flower (Figs. 11 and 12). They measure about 0.5 to 1.0 μ in length and about 500 to 1500 A in width, being more narrow toward the outer (axonal) surface. They are usually separated by a distance about equal to twice their width. These junctional folds of the synaptic troughs would appear in cross-sections in the light microscope as lines arranged parallel to one another leading to a picket fence-like effect or striated border such as that referred to above (Couteaux' subneural apparatus). The three layers of the muscle surface membrane complex persist clearly in the folds. The outer dense zones are, however, brought together near the origin of the folds and fuse. As may be seen in Text-fig. 1, this fusion near the origin of the folds results in the appearance of a single dense line between the two light zones and sarcoplasmic dense lines of the apposed surface membrane complexes. Near the tips of the folds these outer dense zones are usually separated to leave an unstained zone between them (Fig. 7). The synaptic troughs and junctional folds in some planes of section surround the axons completely (Figs. 10 and 12).

Within the synaptic troughs in which the terminal axons lie, a compound membrane consisting of five distinct layers separates sarcoplasm from axoplasm. These five layers may be seen best at the arrow (syn.m.c.) and in the inset in Fig. 12. Proceeding from sarcoplasm to axoplasm they are as follows: (1) a very dense zone, less than 100 A thick, next to sarcoplasm; (2) a light zone about 100 to 200 A thick; (3) a dense zone about 200 A thick; (4) another light zone about 100 to 200 A thick; and (5) a very dense zone under 100 A thick next to axoplasm. These layers may be seen in diagrammatic form in Text-fig. 1. All these structures constitute a composite bounding layer about 500 to 700 A thick between sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm. It is assumed that the terminal axoplasm in the junctional troughs is bounded by a surface membrane complex similar to that bounding sarcoplasm and axoplasm.
Text-Fig. 1. An interpretative diagram of a myoneural junction in Anolis. The double surface membrane complexes of the Schwann cells (S.c.), muscle fiber, and endoneurial sheath cells (endo.) are shown. The manner in which the muscle surface complex is thrown into the junctional folds of the subneural apparatus is indicated. The five layers of the compound synaptic membrane complex which separates terminal axoplasm from sarcoplasm may be seen. The main features of terminal axoplasm and sarcoplasm are included. The continuities of the synaptic, muscle, and Schwann cell surface complexes shown in the diagram represent in part interpretations rather than direct observations. Furthermore, the inclusion of the surface connecting membranes (mesaxons) in the juxtaterminal nerve fibers is partly interpretative.

The region marked by the circle is enlarged to show more detail. The continuities between the membrane structures in the region of arrow is interpretative. The five layers of the compound synaptic membrane complex shown between the arrows sym.m.c. are discussed in the text. $\times \sim 10,000$. Inset $\times \sim 60,000$. 
and zones 3, 4, and 5 the axon surface membrane complex. The outer layer of the Schwann cell surface complex meets the outer layer of the muscle surface complex and that of the axon surface at a triple junction at the edges of the synaptic troughs. In this region the middle light zone of the axon surface complex is probably continuous with the middle light zone of the axon–Schwann membrane and with that of the Schwann cell surface membrane. However, this is not to be considered as proven by present evidence.

In those regions which appear to be best fixed, the dense inner edge of the axon membrane complex does not seem to dip into the junctional folds but to cover over the entrance to them (Fig. 12 inset). It should be noted, however, that in many regions the dense line next to axoplasm is fragmented or absent. (Figs. 11 and 12). Also the dense lines of the junctional folds are in some regions quite indistinct. In regions such as these it sometimes appears that axoplasm dips into the folds. Whether such indistinct regions represent a different physiological state from the more distinct regions described above or are the result of fixation or plastic polymerization artifacts (5) or specimen orientation effects (46) remains to be determined by further study.

In those nerve fibers that are incompletely embedded in the junctional troughs and those that are still connected with their investing Schwann cells there appear one or two oval sections of cytoplasm which differs distinctly from terminal axoplasm in appearance (Figs. 5 to 7 and 10 to 12). This difference is particularly evident in Fig. 11. This cytoplasm is separated from axoplasm by a dense membrane, which appears clearly double in Figs. 6 and 12. In Fig. 11 the line next to axoplasm is partially broken but in some regions appears double. These extra-axoplasmic cytoplasmic masses usually occur on the side of the axon close to the Schwann cells of the telolemma. They are seen only in the regions where the axon twigs are incompletely embedded in the synaptic troughs. Hence it seems logical to relate them tentatively to the Schwann cell. Such a cytoplasmic layer is seen quite clearly in Reger’s Figs. 4 to 8 (34). He refers to this as a neuroglial layer. This agrees with the view presented here.

Terminal Sarcoplasm and Axoplasm

The sarcoplasm and axoplasm in the region of the end-plate are both characterized by concentrations of large numbers of mitochondria (4, 8). These vary somewhat in shape and measure from about 0.25 to 0.5 μ in diameter and up to 1 μ or more in length. They sometimes appear round, sometimes oval, and sometimes elongated and slightly folded in sections. They exhibit in some regions the surface and internal double membranes or tubules which have repeatedly been described in vertebrate mitochondria (30, 41). The mitochondria of junctional axoplasm and sarcoplasm do not differ in any obvious structural way; nor do they appear to be differentially concentrated on either side of the junction.
Mitochondria and nuclei of the sole plate sarcoplasm as well as a distinct layer of sarcoplasm often separate the junctional folds from the myofibrils of the muscle fibers (Figs. 10, 12, 13). In other regions the junctional folds are more closely related to the myofibrils. No definite evidence of attachment of any myofibrillar constituent, such as the Z bands, to the junctional folds has been observed (1, 44) nor do the Z bands appear to penetrate the sole plate sarcoplasm (34) in this material.

Numerous rather large nuclei with prominent nucleoli are seen concentrated about the endings in sole sarcoplasm. These nuclei do not differ obviously from other sarcoplasmic nuclei but are larger and less dense than the telolemmal Schwann cell nuclei. They are also less dense and larger than the endoneurial cell nuclei. The density of the latter is intermediate between that of the muscle and Schwannian nuclei.

It is perhaps worth while to mention that no differentiation of sole sarcoplasm that can definitely be related to the controversial periterminal network of Boeke (2, 3) has been observed.

The axoplasm of the terminal axons of the end-plates exhibits another peculiar feature in addition to accumulations of mitochondria. The axoplasmic filaments are notably absent and the axoplasm contains large numbers of often closely packed bodies measuring about 300 to 500 A in diameter (a. and t. in Figs. 11 to 16). These bodies are usually oval or round and their centers appear less dense than their peripheries. Hence they might appropriately be designated as "vesicles." However, in some instances they are quite elongated (e. v. in Fig. 16). Occasionally elongated profiles are seen in which the over-all width is approximately equal to the diameter of the round bodies (t. in Figs. 11, 14, and 16). It thus appears likely that some of these round or oval bodies should be referred to as tubular rather than vesicular.

No convincing regions are seen in which these vesicular or tubular appearing structures seem to be penetrating the junctional membrane complex. However, such a process of penetration as that described by De Robertis and Bennett (14) cannot be excluded. Indeed similar appearing bodies are sometimes seen in sarcoplasm between the junctional folds (Fig. 9 and inset). Furthermore, at the unlabelled arrow in Fig. 11 a vesicular body seems to lie within the synaptic membrane complex. But this region does not seem well enough fixed to justify any conclusions at present.

DISCUSSION

At the myoneural junction the terminal axon twigs are partly freed of Schwann cytoplasm. However, the axon so divested of Schwann cytoplasm is closely enveloped by the structures composing the synaptic trough. This situation in which the axon is freed of its usual covering of Schwann cytoplasm is observed very rarely away from nerve endings. Fawcett's micro-
graph of a Remak fiber in a paper by Gasser (19) contains the only example known to the author of an axon without an investing layer of Schwann cytoplasm. In this micrograph of Fawcett's the axon, unlike the axons of the endings observed here, has no such covering as is provided by the synaptic trough. It is of importance to note that in this situation, observed in the Fawcett micrograph, the axoplasm is separated from extracellular space by a double membrane complex that is structurally indistinguishable from the Schwann cell surface membrane complex or the muscle surface membrane complex. On this basis it seems reasonable to assume that within the synaptic troughs the axon is bounded by a double surface membrane complex like these other surface membrane complexes. Furthermore, it seems that a thin layer of Schwann cytoplasm is interposed between axoplasm and extracellular space as the axon enters the synaptic troughs and that the double surface membrane complex of the Schwann cell in this region is continuous with the similar axon double surface membrane complex.

It thus appears that the half of the synaptic membrane complex within the synaptic troughs next to axoplasm can be considered as belonging to the axon. The other half belongs to the muscle fiber and is continuous with the muscle surface membrane complex. It seems fruitless to discuss where the dividing line should be placed in the middle dense line of the synaptic membrane complex since this layer evidently is shared by both the axon and the muscle and probably represents a fusion of the outer dense layers of the double surface membrane complexes of both the axon and the muscle. Despite this apparent fusion when the muscle surface complex is thrown into a junctional fold its outer dense layer leaves its axonal counterpart and goes into the fold with the other two layers of the muscle surface complex. Near the origin of a fold this outer dense layer is brought into contact with itself and again a fusion occurs simulating that occurring when it contacts the outer axonal dense layer. As the tip of the fold is approached, however, the dense layers separate and two distinct outer dense lines with a light region between them again become visible. These relations may all be seen in the enlarged portion of Text-fig. 1.

The synaptic trough appears to be formed by a primary invagination of the muscle surface membrane complex which is then thrown into numerous secondary folds pointing into the sarcoplasm. The axon lies within the primary invagination. As may be seen in the diagram (Text-fig. 1) the position is adopted here that the axon surface membrane complex does not dip down into the junctional folds of the synaptic trough. This view-point is based largely on the inset enlargement in Fig. 12 and parts of Figs. 11 and 13. In the inset in Fig. 12 there can be no doubt that this interpretation is correct. However, it must be borne in mind that other regions exist in which alternative interpretations may be made. But in every such region the membranes
do not appear clearly resolved. Similarly complete breaks are very often encountered in all these membrane structures and it might be said that they represent physiologically significant discontinuities. But the position is taken that here again one is more likely to be dealing with fixation artifact, specimen orientation factors (46), or plastic polymerization artifacts (5) than with a significant feature. Therefore, more weight is attached to the region seen in the inset of Fig. 12 than to the other observations. Only additional evidence can be expected to decide this important point with certainty.

The question of the constant presence of a layer of Schwann cytoplasm between axoplasm and extracellular space in those regions in which the axon is not completely enveloped by the synaptic trough is again debatable. It might appear that in some of the micrographs presented here there is no definite layer of extra-axonal cytoplasm in this position. However, in each of these instances the preparation is locally defective and should not be used to decide this point. It seems that the evidence, while inconclusive, is in favor of the presence in Anolis of a thin layer of non-axonal cytoplasm separating axoplasm from extracellular space in those regions not covered by the synaptic trough. If this concept of protection of terminal axoplasm from direct contact with extracellular fluid is correct then the freedom of the axon from Schwann cytoplasm within the synaptic trough becomes of greater interest for it would then be here only that the axoplasm could interact even indirectly with extracellular fluid. The properties of the compound synaptic membrane structures in this region with respect to ionic permeabilities are then obviously of the greatest importance.

The question of the epilemmal or hypolemmal position of the terminal nerve fibers of the endings has received considerable attention in the past. Krause, Kolliker, and Retzius are said to have considered the endings to be epilemmal (45) while Kühne (26) considered them to be hypolemmal. The more recent work of Gutman and Young (22) and of Couteaux (6–10) seems to support the former point of view. Tiegs (44, 45), on the other hand, still seems to consider the endings to be hypolemmal. The endings do ramify beneath the surface of the muscle fiber in the sense that they are located in a deep inpocketing of its surface, which may indeed completely envelope the terminal axons throughout part of their course. However, the inpocketed muscle surface membrane complex seems to remain intact providing an apparently uninterrupted membranous barrier between sarcoplasm and the axoplasm at the present level of observation.

The fact that the light zone of the muscle surface membrane complex is so distinctly narrowed in the synaptic troughs is of interest. Thus while the light zone is often as wide as 300 A when seen at the muscle surface away from the myoneural junctions, within the synaptic troughs it is quite frequently as thin as 100 A. The narrowing of these layers is comparable to
the distinct narrowing of the layers of the surface-connecting membrane (mesaxon) (19, 20) which has been found in myelinated nerve fibers as the myelin is entered (39). Perhaps the most interesting feature of this narrowing is that the light zone is merely reduced but does not disappear. While this apparent lability suggests that one of the components of the light zone may be a quite variable hydration layer, the fact that it does not disappear here or in nerve myelina suggests the presence of another component.

The vesicular bodies of the terminal axoplasm are of considerable interest. Such profiles as these have been observed in axoplasm before and thought to be characteristic of nerve endings (13, 14, 29, 31, 32). The term “vesicle” has been generally used to describe them. Similar vesicular bodies have been observed in crayfish postsynaptic axoplasm concentrated in a region in which axoplasmic filaments are known to be concentrated (35, 38). Such bodies in the crayfish have been clearly observed in this region to have a direct connection with the axoplasmic filaments and the hypothesis was advanced that they represented bead-like vesicular swellings of axoplasmic filaments, which collapsed upon removal of the plastic embedding medium (38). In the terminal axoplasm of motor end-plates, where such bodies are greatly concentrated, there is a striking absence of axoplasmic filaments. It may be that this apparent absence of axoplasmic filaments is an artifact due to the fragility of these structures. But the filaments are consistently seen in the same preparations in nearby myelinated nerve fibers (Fig. 4) where the vesicular bodies are absent. This finding is clearly open to several alternative interpretations but it is intriguing to consider the possibility that the filaments are absent in the terminals because they have been converted into tubular bodies that appear vesicular in cross-section. Admittedly the apparently tubular structures seen in the sections could represent a separate membranous component. But the rarity of their appearance, the fact that they may be followed for only short distances, and the presence of the oval transitional forms (Figs. 14 and 16) suggest that they are actually tubular. Only serial sections can settle these points adequately and it must be emphasized that these ideas are presented here only in a speculative manner.

Current physiological concepts suggest that nerve fibers secrete discrete packets of acetylcholine at the myoneural junction and thereby bring about a depolarization of the muscle surface membrane (12, 17). The folds of the junctions are admirably constructed to bring about a very great increase in the total area of the muscle surface membrane complex in contact with the nerve endings. Since it seems likely that acetylcholine is secreted in discrete packets or quanta at the endings one might expect to see something analogous to secretion granules of acetylcholine either in axoplasm, sarcoplasm, or within the junctional membranes. It seems reasonable to speculate that the tubular or vesicular bodies of terminal axoplasm might represent such pack-
ets of acetylcholine. If this be the case it may be possible to alter their number or size by stimulation of the endings. The isolated observation of vesicular appearing bodies (Fig. 11) within the light zone of the axon surface membrane complex is of potential interest in relation to secretory processes at the endings but must be studied further before any conclusions can be drawn.

**SUMMARY**

Myoneural junctions in *Anolis* are characterized by the formation of troughs in the surface of the muscle fibers in which small branches of the terminal axon lie. The muscle surface membrane lining the troughs is thrown into complex branching and anastomosing folds, which compose the sub-neural apparatus of Couteaux. A compound membrane 500 to 700 A thick separates axoplasm from sarcoplasm at the endings. This consists of five distinct layers and is described in detail. A thin layer of cytoplasm (probably Schwann) separates terminal axoplasm from extracellular space at the surfaces of the junctional troughs. Terminal axoplasm lacks axoplasmic filaments and contains numerous vesicular or tubular appearing structures about 300 to 500 A in diameter. Both terminal axoplasm and sarcoplasm contain numerous mitochondria.

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*Note Added in Proof.*—The dense line (zone 5 of the synaptic membrane complex) next to axoplasm in the inset enlargement of Fig. 12 itself appears double. This was not discussed in the text because of the isolated nature of such observations. But similar observations have been made during high resolution studies conducted since this manuscript was prepared and this matter will be considered in detail in another publication. This does not, however, fundamentally alter the interpretations given since they were made with this possibility in mind.

**BIBLIOGRAPHY**

EXPLANATION OF PLATES

List of Symbols Used in Figures

ax., axoplasm.
a.x.S.m., axon Schwann membrane.
c.f., collagen fibrils.
c.t., connective tissue fibrils.
ec.s., extracellular space.
endo., endoneurial cell.
e.v., elongate vesicle.
j.f., junctional fold.
m., mitochondria.
m.f., myofibril.
m.m.c., m.s.c., muscle (surface) membrane complex.
mnj., myoneural junction.
myl., myelin.
sare., sarcolemma.
sare.n., sarcoplasmic nucleus.
s.c., Schwann cell or Schwann cytoplasm.
s.c.m., surface-connecting membrane (mesaxon).
s.n., Schwann nucleus.
s.m.c., Schwann (cell surface) membrane complex.
syn.m.c., synaptic membrane complex.
t., tubule.
v., vesicle.

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Fig. 1. Phase micrograph of 0.5 μ methacrylate-embedded section of skeletal muscle fibers showing a myoneural junction (arrow mnj.). Two myelinated nerve fibers are seen in cross-section to the upper left. A Schwann nucleus is visible in the lower fiber and the endoneurial sheath is visible around both fibers. The subneural apparatus is faintly visible around the unmyelinated terminal axons in sole plate sarcoplasm. OsO₄-fixed; PTA-stained. × 1,900.

Fig. 2. Electron micrograph of transverse section of nerve fibers near a myoneural junction. Four axons (ax.) are visible. One of these has a myelin sheath but the other three are very small and not myelinated. S.n., Schwann nucleus. OsO₄, 3 hours at 0°C.; PTA 24 hours. × 14,000.

Fig. 3. Myelinated nerve fiber (ax.) and its endoneurial sheath (endo.) with a nucleus. OsO₄, 3 hours at 0°C.; PTA 24 hours. × 15,000.
(Robertson: Ultrastructure of reptilian myoneural junction)
Fig. 4. Myelinated nerve fibers in skeletal muscle in an endoneurial sheath. Note the double character of the surface membrane complexes of the endoneurial sheath cell (endo.) and the Schwann cell (S.c.). Numerous transected connective tissue fibrils (c.t.) are inside and outside the endoneurial sheath cell layer. The unlabelled arrow points to a surface-connecting membrane (mesaxon). OsO₄ 4 hours; PTA 48 hours. X 16,500.

Fig. 5. Electron micrograph of small myoneural junction in which the Schwann cell is not connected with but is still closely associated with a terminal axon (ax.). A thin layer of Schwann cytoplasm (S.c.) is visible about the Schwann nucleus (S.n.). An oval zone of cytoplasm which may be Schwannian (?S.c.) is visible where the axon is not surrounded by the synaptic trough. OsO₄ 4 hours. X 14,500.

Fig. 6. A myofibril with its hexagonally arrayed myofilaments is seen to the lower right. A terminal axon containing numerous vesicular appearing bodies extends diagonally up from the lower left (ax.). A few indistinct junctional folds protruding into sarcoplasm may be seen to the right below the axon. To the left is a zone of cytoplasm bordering axoplasm which may represent terminal Schwann cytoplasm (?S.c.). This cytoplasm is separated from axoplasm by a double membrane and is bounded on the side toward extracellular space by a double membrane. Immediately above the junction a terminal Schwann cell (S.c.) containing two small axons (ax.) about 0.5 μ in diameter is seen. The double character of the axon–Schwann membrane bounding these axons is evident but no definite surface-connecting membranes (mesaxons) are visible. OsO₄ 2 hours; PTA 48 hours. X 38,500.
(Robertson: Ultrastructure of reptilian myoneural junction)
Fig. 7. Electron micrograph of the edge of a myoneural junction. A terminal axon extends diagonally across the field bounded to the right and bottom by the junctional folds which line the synaptic trough. The muscle surface membrane complex (m.s.c.) may be seen at the upper right extending from the muscle surface into one of the junctional folds. Toward the tip of this fold there is a relatively wide separation of the outer dense lines of the surface complex. The outer dense lines are more closely approximated in the fold marked in the lower center. In this instance there is seen an anastomosis of this fold with the adjacent one leading to an H-shaped figure. The terminal axoplasm contains vesicular appearing bodies except for a peculiar "washed out" round area in the upper center which is considered to represent an artifact. OsO₄ at 0°C.; PTA-stained. × 13,500.

Fig. 8. Electron micrograph of the edge of a myoneural junction showing a terminal axon (ax.), sarcoplasm (sarc.), a sarcoplasmic nucleus (sarc.n.), and several junctional folds (j.f.). Extracellular space (e.c.s.) may be seen to the upper left. The enfolding of the double muscle surface membrane complex to form the junctional folds may be seen at the joined arrows. OsO₄ 2 hours; PTA-stained. × 22,000.

Fig. 9. Electron micrograph of section parallel to the surface of a myoneural junction which includes a portion of a terminal axon (ax.) with its associated junctional folds (j.f.). Between the junctional fold marked by the arrow and the one just above it two round vesicular appearing bodies of about the same size and appearance as those seen in axoplasm are seen. This area is enlarged in the inset. Such bodies are not infrequently seen in sarcoplasm about the junctional folds. The origin of the junctional folds from the surface of the muscle fiber bordering extracellular space (e.c.s.) may be seen here. OsO₄ at 0°C.; PTA 24 hours. × 19,000.

Fig. 10. A section adjacent to the junction shown in Fig. 12. Note the layer of ?Schwann cytoplasm (?S.C.). × 19,500.
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Fig. 11. Electron micrograph of terminal axon embedded in a synaptic gutter. A junctional fold (j.f.) in which the light zone of the muscle surface membrane complex is clearly evident is seen to the upper right. The close approximation of the light zones near the origin of the fold is seen here. In this location the dense outer surface material is greatly reduced in amount. The five layers of the synaptic membrane complex (syn.m.c.) may be seen at the arrows. The vesicular appearing bodies of the terminal axoplasm and the absence of the axoplasmic filaments are particularly clearly shown here. Note the tubular appearing bodies (t.) in terminal axoplasm. At the unlabelled arrow a structure is seen within the synaptic membrane complex which resembles somewhat the axoplasmic vesicular bodies. A layer of cytoplasm (?S.c.) which is clearly different from axoplasm is seen between axoplasm and extracellular space (e.c.s.). OsO₄ 4 hours. X 43,000.

¹This t looks like f in the reproduction because of a fortuitous black spot on the original plate.
(Robertson: Ultrastructure of reptilian myoneural junction)
FIG. 12. Electron micrograph of two terminal axons partially surrounded by synaptic troughs. Note the prominent junctional folds (j.f.), mitochondria (m.), and vesicular appearing bodies (v.). The five layers of the synaptic membrane complex (syn.m.c.) may be seen clearly between the aligned arrows. This region is enlarged in the inset to the left. In this particular region the dense line of the synaptic membrane complex next to axoplasm definitely does not dip into the junctional fold which it overlies. In other instances this region is not so clearly defined and this disposition is not so easily discerned. The region marked ?S.c. is clearly bounded both toward axoplasm and extracellular space by a double membrane structure. Unfortunately, the continuity, if any, between these membrane structures and those of the synaptic membrane complex cannot be seen. To the lower left connective tissue fibrils (c.f.) may be seen in extracellular space (e.c.s.). A layer of sarcoplasm less than 1 micron thick containing junctional folds lies between these connective tissue fibrils and axoplasm. It is not clear in this preparation whether or not the tips of these folds open to extracellular space. In the inset the dense line of the synaptic membrane complex next to axoplasm itself appears double. OsO₄ 2 hours. × 30,000. Inset × 63,000.
Robertson: Ultrastructure of reptilian myoneural junction
FIG. 13. Another section of the same myoneural junction shown in Fig. 12 but somewhat deeper in the block. OsO₄ 2 hours. × 30,000.

FIG. 14. Electron micrograph of terminal axon (ax.) showing the branching and anastomosing junctional folds. The vesicular appearing bodies (v.) of terminal axoplasm are prominent. In the central region of this axon a network of tubular appearing structures (t.) of the same dimensions as the vesicular appearing structures is seen. OsO₄ 2 hours. × 34,000.

FIG. 15. Electron micrograph of terminal axon (ax.) showing its associated junctional folds (j.f.). The vesicular appearing bodies (v.) are particularly clearly shown here. OsO₄ 3 hours at 0°C.; PTA 24 hours. × 23,000.

FIG. 16. Enlargement of the area including the arrow marked v. in Fig. 15. Note that the vesicular appearing bodies sometimes appear round, sometimes oval (e.v.), and sometimes tubular as at t. OsO₄ 3 hours at 0°C.; PTA 24 hours. × 87,000.
(Robertson: Ultrastructure of reptilian myoneural junction)