SOME FEATURES OF THE ULTRASTRUCTURE OF REPTILIAN SKELETAL MUSCLE*,$⁺

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(Received for publication, May 9, 1955)

This paper is concerned mainly with some features of the surface layers of muscle fibers observed during a study of motor end-plates in the chameleon lizard Anolis carolinensis (37, 39). Certain other new observations on components within the muscle fibers are also described.

There is still much confusion as to the surface layers detectable by electron microscopy and their relation to the sarcolemma as seen with the light microscope. At the myoneural junction two parallel dense lines separated by a light interzone are seen (37, 39). It will here be shown that this membrane complex is also present over the remainder of the muscle fiber surface (41); see also review by Bennett (5). The relations of these layers to the more external sheaths of the fibers will be discussed.

Material and Methods

Live Anolis previously equilibrated to either 25° or 0°C. were decapitated. In some instances hind legs or intercostal muscles were exposed and immediately flooded during dissection with 1 per cent OsO4 buffered by the method of Palade (29), usually at pH 7.4-7.6. In other experiments the muscle fibers were rapidly removed before fixation was begun. The tendons or ribs were tied and the muscle fibers stretched to rest length on applicator sticks. The sticks were then immersed in fixative kept at 0°C. or about 25°C. in the dark for periods varying from

* This investigation was supported in part by research grant No. B365 from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service, by an Institutional Grant from the American Cancer Society, Inc., and by a grant from the Kansas Division of the American Cancer Society, Inc.

$ The findings presented here were reported in part at the 1954 meeting of the American Association of Anatomists (37) and in part at the 1955 meeting of the International Association of Medical Museums (now International Academy of Pathology).


⁺ Porter (34) presented preliminary observations of the myotendon junction in Amblystoma larvae at the 1954 meeting of the American Association of Anatomists in which the double appearance of the muscle surface membrane complex was observed. In this report the inner dense line was interpreted as the complete plasma membrane and the light zone as a "cuticular layer." Some of the results reported here were presented at that meeting (37).

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11/6 to 4 hours. The specimens were then removed either to distilled water or to phosphate-buffered (pH 5.4) 1 per cent phosphotungstic acid (PTA) at 25°C. for 12 to 18 hours. Sometimes staining was continued in PTA for 24 to 48 hours. The specimens were then dehydrated in ethanol and embedded in a solution of 15 per cent methyl methacrylate and 85 per cent n-butyl methacrylate. Polymerization was catalyzed by benzoic peroxide and dry heat or, more often, ultraviolet light. Sections were cut with a glass knife and an international rotary (Minot) microtome (16). An RCA model EMU-2B electron microscope with an externally centerable objective aperture (14) and reduced condenser and projector apertures was used.

**OBSERVATIONS**

1. **The Outer Investing Layers.**—In suitably preserved fibers the sarcoplasmic surface is consistently bounded by two dense lines with a lighter zone between. Outside these layers there are more loosely packed and irregular elements that are less easy to analyze. Usually there is a loose layer of widely separated round or oval dense profiles measuring about 250 to 350 Å in diameter (Figs. 3, 4, 6, 8, and 9). In Fig. 4 a local aggregation of such profiles (c.f.) is seen. Such an aggregation as this would be visible with the light microscope as a small connective tissue fiber, while the smaller fibrils would be unresolved. A longitudinal repeat period of about 700 Å has been demonstrated in some of these fibrils and it appears that they are collagen fibrils cut at various orientations. The appearance of the surface collagen fibrils is not inconsistent with the double helical arrangement of the collagen fibrils of other kinds of vertebrate skeletal muscle observed with indirect light microscopy by Bairati (1) and by electron microscopy of fragmented muscle preparations by Reed and Rudall (35) and Draper and Hodge (10).

In Figs. 6, 8, and 9 a second delicate irregularly arranged fibrillar component (arrows f.) consisting of filaments well under 100 Å in diameter is seen interspersed among the larger fibrils. The nature of these smaller filaments is at present unknown. Jakus observed a similar smaller fibrillary component interspersed between the 200 to 300 Å collagen fibrils of the stroma of rat cornea (26). She considered these fibrils to be present also in the basement membrane. Rhodin and Dalhamn (36) have observed delicate filaments of similar dimensions in the lamina propria of the tracheal mucosa of the rat. They have considered them to be components of elastic tissue.

In Figs. 6, 8, and 9 the small fibrils are rather closely associated with the outer dense layer of the muscle surface membrane complex and may indeed become part of it, although this point cannot be decided on present evidence. The collagen fibrils are also often closely applied to the outer dense surface layer but could hardly be considered a component of this layer since their diameter greatly exceeds the thickness of the outer dense surface zone. Neither of the two fibrous components of the surface has been identified within the light interzone of the surface membrane complex.

2. **The Limiting Layers of the Sarcolemma.**—The muscle surface membrane
complex is seen consistently in well fixed preparations to consist of three distinct zones (Figs. 1 to 9). The outer dense layer is quite variable but of the order of 100 to 300 Å in thickness. It sometimes appears relatively dense and compact as in Figs. 3, 6, 8, and 9 and sometimes lighter and more diffuse as in Figs. 1, 2, 4, 5, and 7. Zones of discontinuity in this layer are sometimes observed as in the central portion of Fig. 3 and parts of Figs. 7 to 9. Such discontinuities are often seen in these preparations and it is considered likely that they represent deviations of the membranes from a strict perpendicularity to the plane of the section such as that recently described by Williams and Kallman (48) or some artifact induced by the polymerization of the plastic embedding medium (7).

The outer dense zone is fairly sharply demarcated from a light interzone about 200 to 300 Å in thickness, in which no distinct regular structure has been observed. This light interzone is again sharply separated from an inner dense zone, which is often well under 100 Å in thickness. This zone is often somewhat more sharply defined and more dense than the outer zone. While the outer dense zone sometimes appears distinctly granular (Fig. 3 at $SM$) the inner dense zone appears more homogeneous (arrows $SM$ in Figs. 2 and 3 and Fig. 7). Sometimes there are discontinuities in the inner dense zone as in the outer. Such apparent discontinuities again may represent a specimen orientation effect such as that mentioned above.

In some regions (top and bottom of Fig. 4) the three zones of the muscle surface membrane complex disappear and a single poorly defined line is seen. The light zone is sometimes distinctly widened (Figs. 2, 3, and 5). In some less favorably fixed preparations it may be greatly swollen, measuring more than a thousand angstrom units in thickness (39). Sometimes only the dense inner line can be seen with complete absence of the outer dense line. In such instances as these it seems more likely that fixation effects are responsible for its absence than the factors mentioned above (7, 48).

At the sarcoplasmic surface of the inner zone are numerous round or oval, somewhat irregular, dense bodies usually about 300 to 500 Å in diameter ($G$ in Figs. 2, 3, 7, and 9). These may well correspond to the 300 Å corpuscular bodies observed in replicas of the inner surface of muscle surface membrane fragments by Reed and Rudall (35) and the similar round 400 to 1000 Å bodies observed by Jones and Barer on fragmented muscle membranes (27).

3. Relation of Surface Layers to Z Bands.—The inner dense layer of the surface membrane complex appears to be attached to the Z bands of the underlying muscle fibrils (unlabelled arrows in Figs. 1 and 2). This produces a scalloped effect such as that observed with the electron microscope by others (23) in rat cardiac muscle. At these loci of attachment there is an accumulation of dense amorphous material, which sometimes contrasts distinctly with surrounding sarcoplasm. This material may be identical with the
amorphous material observed by Draper and Hodge (10) on their flattened sheets of “sarcolemma” and referred to as Z band remnants. This finding is consistent with the old histological concept of Z band attachment to the surface membrane, which appears to have originated with Dwight (12). On the contrary, some others (6, 41), while sometimes observing dense material between Z bands and the surface membrane in other kinds of vertebrate striated muscle, did not regard this as evidence of Z band attachment. These investigators also did not observe the scalloped appearance. Rather dense amorphous material is sometimes seen in sarcoplasm between adjacent myofibrillar Z bands (Fig. 10, SZ). This material perhaps is related to the dense material observed in a similar location by Bennett and Porter (6) and referred to as sarcoplasmic reticulum. It certainly is not collagenous (18).

4. Myofibrils.—Longitudinal sections display the usual bands of the uncontracted sarcomere with the exception of the N band (Fig. 10) or of the contracted sarcomere (Fig. 1). In addition the 400 A axial periodicity observed in different kinds of vertebrate muscle by others (10, 11, 23, 40) is clearly evident in the A and I bands. In these fibrils it averages about 375 A in both these regions. This is within the range of 350 to 420 A deduced by Bear (4) from X-ray diffraction studies of amphibian and mammalian skeletal muscle. It is also close to the period of 400 A observed by Philpott and Szent-Gyorgi in light meromyosin extracted from skeletal muscle (32). Draper and Hodge (10) state that this small axial periodicity in toad muscle is directly related to sarcomere length. Hoffmann-Berling and Kausche (24) observed an axial period of 200 to 300 A in fragmented frog muscle and also stated that the values were dependent on segment length. Bennett and Porter (6) observed a 225 A axial periodicity in fowl muscle but did not find this correlated with degrees of contraction. Hodge (22) observed a 250 A axial period in contracted insect muscle but did not establish a correlation between this period and degrees of contraction. In Fig. 1 it is possible to measure a periodicity of about 200 to 250 A along the axis of some of the myofilaments of the fully contracted myofibrils. It might then appear that the 400 A period shortens during contraction. But this period is not considered sufficiently regular in Fig. 1 to establish this important point.

The Z bands in Fig. 10 are about 600 A wide. They consist of a filamentous component parallel to the myofibril axis terminating on either side of a central membranous component perpendicular to the myofibril axis. The filaments are of about the same density and diameter as the myofilaments in the H bands. If they are the ends of myofilaments then it appears that they do not completely traverse the Z band in this animal as has been suggested by others for other vertebrates (20) and one invertebrate (22). The central membranous component is about 100 A thick. In some regions this fairly dense membrane appears to have more dense edges. One Z band showing these
components is enlarged in the inset. The present preparations are not ade-
quate to decide whether or not this central membranous component of the
Z bands is continuous across the sarcoplasm between adjacent myofibrils.
The importance of this point is emphasized by the recent observations of
Huxley and Taylor (25) which have revived interest in the old idea that some
kind of physical element probably exists to convey the influence of surface
membrane depolarization across the muscle fiber to the myofibrils (21, 45).

5. Double Membranes between Myofibrils.—A curiously regular array of
double membranes is often seen in the sarcoplasm between the myofibrils (M in Fig. 10). The over-all thickness of these structures is about 300 A and
their light zones measure about 150 to 200 A in thickness. The plane of sec-
tion, in a part of Fig. 10, passed through the thin layer of sarcoplasm border-
ing the myofibrils in the plane designated b in Text-fig. 1, so that a relatively

![Text-Fig. 1. Diagram of a cross-section of a few myofibrils within a skeletal muscle fiber.](image)

Line a represents a plane of longitudinal section in which the sarcoplasm would appear as relatively narrow zones. This is the usual plane of section in Fig. 10. Line b represents another plane of longitudinal section in which the sarcoplasm would appear as a wide zone. The central portion of Fig. 10 shows such a relatively wide zone of sarcoplasm in which double membranes or tubules lie.

wide expanse of sarcoplasm between myofibrils is shown. The section grazed
one myofibril and just enough of its filaments are included to permit its H
bands (arrows H) and two of its Z bands (arrows Z) to be identified. The H
bands so identified lie in a position midway between those of the out of regis-
ter myofibrils on either side. Using the identifiable H bands and the
portions of the Z bands that are also visible it is possible to orient the double
membranes in the sarcoplasm with respect to the bands of the tangentially
sectioned myofibril with which they are closely related. It is evident that
three successive sets of double membranes are associated with this myofibril,
roughly at the junctions of the A and I bands. One set is enlarged in Fig. 11.

The remaining parts of Fig. 10 often show very short segments of similar
double membranes (arrows M) cut along their shortest axis between myo-

2 The term "double membrane" as used here means a sheet-like structure which in cross-
section consists of a central zone relatively transparent to electrons bordered by denser edges
that are more opaque to electrons. It is used in the same sense as the terms "dense edged"
and "double contoured" that have been used previously.
fibrils (plane a in Text-fig. 1) and these may often be seen to delimit the I band regions. Similar double membranes in sections tangential to myofibrils are seen in Figs. 13 and 14. A set of four of these membranes cut along their shortest axis (plane a in Text-fig. 1) are shown in Fig. 12. In this instance the sarcomeres of the adjacent myofibrils are in good register and the membranes are clearly located at the edges of the I bands. The appearance of the double membranes in such sections sometimes suggests a tubular form, but the preparations are not conclusive. Others (5, 6, 31) have observed membranes of similar structure in sarcoplasm, regarding them as components of the endoplasmic reticulum, but no organization of these membranes in the regular manner shown here has previously been published. Whether or not this relatively high degree of order is fortuitous can only be determined by further study.

DISCUSSION

1. Sarcolemma.—The term sarcolemma has long been a source of confusion to light microscopists since it has never been quite clear whether or not the term includes connective tissue fibrils as an integral part of the plasma membrane. Gutman and Young (17) have even suggested that the term be restricted to the connective tissue layer outside of the plasma membrane of the muscle cell and that the latter be called the “sarcoplasmatic” membrane. Some electron microscopists (10, 40) have used the term as if the plasma membrane and a thin layer of connective tissue fibrils were intimately associated into a single specialized kind of surface membrane. The implication is given by this usage that a very thin, probably “sublightmicroscopic,” layer of connective tissue is an integral part of the surface membrane of muscle fibers. More recently electron microscopists (5, 6, 23, 47) have begun to use the term to designate a single dense line of at most not over a few hundred angstrom units in thickness which they have seen in thin sections bounding sarcoplasm. The connective tissue is excluded from the term by this usage and the suggestion of Gutman and Young (17) that the term be used only for the connective tissue is thus completely reversed. The lack of unanimity in the use of the term sarcolemma which has bedevilled histologists for many years is thus in danger of continuing into discussions of ultrastructure.

If for historical reasons it is desirable to continue to use the term sarcolemma then it would seem most appropriate to use it for the structure for which it was invented; namely, the clear translucent membrane which can be seen by light microscopy bridging the gap in muscle fibers produced by retraction clots (2). This is the membrane which Bowman (8) discovered and for which he invented the term “sarcolemma.” When viewed by modern light microscopy (2) this membrane still appears structureless and membranous as it did to Bowman. That it is different from many plasma membranes
is suggested by the fact that it maintains its shape after the formation of retraction clots (2), and even after the complete extrusion of the cytoplasmic contents. Thus Jordan (28) described it as a “relatively robust and resistant lamina.”

Bairati (1) by the use of more refined optical methods, including darkfield and polarization microscopy, concluded that this apparently structureless membrane actually consisted of a thin layer of connective tissue fibrils and an interfibrillary “colloid” substance. Barer (2, 3) on the other hand, from his studies of the empty translucent sarclemnic sacs left after the formation of retraction clots in skeletal muscle, concluded that no fibrillar element was present. Some of the earlier electron microscope investigations of fragmented muscle preparations were more compatible with Bairati’s conception. Thus in fragmented muscle preparations some investigators (10, 35, 40) have found apparent membranes consisting chiefly of quite thin layers of collagen fibrils 250 to 400 A in diameter. On the other hand, Jones and Barer (27) by similar methods found a thin (400 to 1000 A) membrane which had no fibrillar component. Considering all these results it seems probable that a very thin “sub-lightmicroscopic” layer of connective tissue fibrils was included in the membrane originally described by Bowman and that this membrane can be separated by fragmentation techniques into a fibrillar and membranous component. Thus, in a historical sense it is probably more accurate to apply the term “sarcolemma” to both the membranous and fibrillar components together as was done by Draper and Hodge (10). But it would probably be even better to drop the term altogether now that the components can be described separately.

The very delicate filamentous strands of material seen between the collagen fibrils at the muscle surface are of undetermined nature and may, of course, be artifacts. However, occasional 250 to 350 A collagen fibrils have been seen in cross-section in this material having a fairly regular array of densities of about the same diameter as the smaller filaments observed between the collagen fibrils at the muscle surface. The possibility then exists that this delicate filamentous material represents still smaller units of collagen. No proof of this has, however, been obtained. Nor is it known whether or not this component is involved in the formation of the outer dense line of the surface membrane complex.

2. The Surface Membrane Complex.—The significance of the three densities observed in the surface membrane complex cannot at present be stated in molecular or chemical terms. The outer dense material resembles strongly the material which in other tissues such as the kidney and lung has been often referred to by electron microscopists as basement membrane material. It should also be noted that the structure of this three layered surface complex is very similar to that of the surface membrane complexes of endoneurial
sheath cells and Schwann cells (38, 39). In the latter the inner dense line and
the light zone have been shown respectively to be continuous with the dense
lines and light zones of nerve myelin (15, 38). Whether or not any such in-
foldings of the muscle surface membrane complex establishing continuities
with the membranes of the Z bands or with the sarcoplasmic double mem-
branes exist remains to be determined.

It has become common among electron microscopists to refer to a thin
dense line next to cytoplasm as “the” plasma membrane and to adopt other
terms for any additional structures such as the outer dense line and light
zone observed here at the surfaces of muscle cells. Such a point of view might
be based physiologically on such facts as measurements of membrane capacity
which suggest a thickness of about 100 Å or less for some cell membranes
and morphologically on the finding of only a single dense line about 100 Å thick
at certain cell surfaces. Notable among these are the surfaces of blood vas-
cular cells, fibroblasts and the luminal surfaces of endothelial cells, pancreatic
acinar cells, renal epithelial cells, etc. But it is a striking fact that all these
cells (excluding, of course, blood cells and fibroblasts) when side by side in
an immobile tissue structure exhibit a definite light zone between the two
dense lines next to cytoplasm. Furthermore, their free surfaces not in contact
with some rapidly changing medium such as blood or secretions also con-
sistently exhibit a definite light zone with an associated outer dense line.
When the single dense line bordering cytoplasm at the cell surface is infolded
to form intracellular double membrane structures such as some of the renal
cell double membranes (43) or the surface connecting membranes (mesaxons)
of Schwann cells (15, 38) again the light zone persists as a definite structure.
Another example of this is provided by the persistence of a light zone between
the dense lines next to cytoplasm in the intercalated discs of cardiac muscle
(42, 46). Disregarding fruitless discussions of what materials, forces, or lack
of forces might be responsible for this characteristic of many cell surfaces
the fact remains that the light zone regardless of its failure to reduce OsO₄
is of considerable interest and until further information is available should
be included as a part of the surface membrane complex. A more difficult
point arises with respect to the inclusion of the outer dense line as a part of
the surface membrane complex. It is perhaps logical to consider it as a base-
ment membrane which is simply added to the surface complex. But it seems
equally logical to state that a basement membrane is simply a greatly thick-
ened outer layer of a surface membrane complex. It seems that this matter
can only be decided when it is known how this layer originates and what part
it plays. Since this matter is at present unsettled it seems reasonable tenta-
tively to include the outer dense layer as a part of the surface membrane
complex of muscle and nerve fibers. A decision with respect to the designation
of any one or combination of these components as the physiologically identi-
fiable plasma membrane is thus left open until more evidence accumulates.
3. The Surface Granular or Corpuscular Component.—The dense round or oval bodies lying in the layer of sarcoplasm immediately adjacent to the inner dense line of the surface membrane complex have been seen with sufficient frequency to suggest that they are a significant component. The densities of these bodies seem fairly homogeneous and no apparent membrane invests them. Thus it seems unlikely that they represent vesicular invaginations of the inner surface density such as that seen by Palade in endothelial cells (30). No evidence has been found that these bodies are cylindrical structures and their resemblance to the roughly round corpuscular bodies seen on fragmented muscle membranes by both Reed and Rudall (35) and Jones and Barer (27) seems to make this unlikely.

4. Sarcoplasmic Double Membrane Structures.—The presence of a highly orientated system of double membranes or tubules within the sarcoplasm of these muscles deserves some discussion. These have not been sufficiently studied at high resolution to allow any conclusions to be drawn about the exact manner in which they are structurally related to the myofibrils nor whether they may be related to the surface membrane complex. No definite tendency for these structures to remain attached to displaced myofibrils has been observed. Furthermore, mitochondria sometimes lie across the boundaries of the A and I bands. Hence it seems likely that no firm attachment exists.

It is interesting that regular, presumably lipoprotein, structures have been previously observed by light microscopy in the regions occupied by these double membranes or tubules. Thus Dempsey, Wislocki, and Singer (9) demonstrated by light microscopy some minute argyrophilic sarcoplasmic granules between myofibrils of skeletal muscle. These are seen in their Fig. 8 (9) as dense granules lying quite regularly at the junctions of A and I bands in about the location of the membranes described here. Furthermore, Speidel (44) has observed in living skeletal muscle regularly arranged “dark refractive” granules of about the same size and in the same position as those seen by the above authors (9). While the 300 A thick transverse sarcoplasmic membranes observed here could not be directly resolved by light microscopy they might be expected to be seen as apparent granules when observed in profile through a layer of muscle several microns thick. Deposition of silver on the structures would, of course, greatly accentuate this effect. Either these investigators actually observed the same structures discussed here or these several observations represent a striking coincidence. Double membranes or tubules have been observed by others in sarcoplasm (5, 6, 13, 31) and considered to be components of the endoplasmic reticulum (33) but the arrangement of these membranous structures has been reported to be more or less random and an analogy has been drawn between them and the disorganized reticulum observed long ago by Heidenhain (19). The concept of an irregularly arranged system of anastomosing membranes and tubules envelop-
ing the myofibrils has thus arisen. The present findings, however, suggest that this system may not always be random but highly ordered as a system of regularly arranged transverse membranes or tubules. It would be premature to discuss this system further until it has been investigated more since it may prove that the sampling has been inadequate to be of general significance even in this species.

SUMMARY

The ultrastructure of reptilian skeletal muscle is described with particular emphasis on the surface structures. The surface is found to be enveloped intimately by a very delicate layer of collagen fibrils of diameters below the resolving power of the light microscope. This layer, in some instances in regions removed from the ends of the muscle fibers, is quite thin, consisting of only a few scattered fibrils. Interspersed between the collagen fibrils a delicate irregularly arranged network of fibrils less than 100 A in diameter is seen. The fibrillar layer is separated from the sarcoplasm at the surfaces of the muscle fibers by a surface membrane complex consisting of two dense lines separated by a light interzone. The light interzone measures about 200 to 300 A in thickness. The over-all thickness of the entire complex varies between 400 and 700 A. On the inner (sarcoplasmic) surface of the membrane complex moderate numbers of round or oval bodies are seen which measure about 300 to 500 A in diameter.

A preliminary description is given of a peculiarly ordered arrangement of transverse sarcoplasmic double membranes or tubules of about 300 A over-all thickness which lie between the myofibrils. A membranous component within the Z bands is described.

The author wishes to express his appreciation to Professor J. Z. Young, Professor B. Katz, and Professor F. O. Schmitt for their critical reading of this manuscript. The technical assistance of Mrs. Hanna Mary Warren is gratefully acknowledged.

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Fig. 1. Longitudinal section of a contracted intercostal muscle fiber including the surface membrane. The surface membrane complex (arrows SM) is attached to the underlying myofibril in the lower left hand corner, by irregular amorphous material (unlabelled arrows). The three zones of the surface membrane complex are shown in the inset enlargement. OsO$_4$ 4 hours at 0°C.; PTA 48 hours. × 24,000. Inset × 50,000.

Fig. 2. Longitudinal section of an intercostal muscle fiber including the surface membrane complex (arrows SM). Below is a marginal portion of one sarcomere of a contracted myofibril and the Z bands. Some fairly dense amorphous material (unlabelled arrows) lies between the Z bands and the surface membrane complex at the loci of attachment. Scattered large granules (G) are closely associated with the inner dense edge of the surface membrane complex. OsO$_4$ 4 hours at 0°C.; PTA 48 hours. × 60,000.
(Robertson: Ultrastructure of reptilian skeletal muscle)
FIG. 3. Surface of a transected leg muscle fiber. The double surface membrane complex (arrows SM) is shown. Irregular widening of the light central zone of the membrane is evident in places. Irregular round or oval dense granules (G) lie just beneath the inner dense edge of the surface membrane complex. The outer dense edge of the surface complex is unusually compact here and the transected surface connective tissue fibrils (c.f.) appear clearly as dense 250 to 350 A profiles. The surface of an endoneurial cell (end. c.) is visible. OsO₄ 4 hours at 25°C.; PTA 48 hours. × 60,000.

FIG. 4. Cross-section of a small leg muscle fiber. The sarcoplasm contains only one myofibril (mf) and a sarcoplasmic nucleus. The double surface membrane complex (SM) is clearly visible and a few transected connective tissue fibrils may be seen scattered about close to the outer edge of the surface membrane complex. These are aggregated into a small (<1 μ) connective tissue fiber to the lower right. OsO₄ 2 hours at 25°C.; PTA 48 hours. × 30,000.

FIG. 5. A slightly oblique section of an intercostal muscle fiber near a myoneural junction. The double surface membrane complex (SM) is folded inward between the two nuclei in the center of the field. A moderate number of transected connective tissue fibrils are seen in and about this fold. Obliquely sectioned myofibrils (mf) are seen. OsO₄ 4 hours at 0°C.; PTA 48 hours. × 25,000.

FIG. 6. Segment of the central region of Fig. 9, with more of the connective tissue near the surface membrane complex (SM) included. Note the very delicate filamentous component (f) between the larger connective tissue fibrils (c.f.). OsO₄ 3 hours at 0°C.; PTA 24 hours. × 105,000.
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Fig. 7. Oblique section of the surface membrane complex of a muscle fiber. Sarco- 
plasm to the left. The dense line of the surface complex bordering sarcoplasm is clearly 
shown, with round or oval dense bodies (G) associated with it. The irregular very 
dense bodies about 150 Å or less in size scattered about are considered to be artifacts. 
OsO₄ 3 hours at 0°C.; PTA 24 hours. × 96,000.

Fig. 8. Oblique section of the surface membrane complex, with a portion of a myo- 
fibril and a small amount of sarcoplasm to the left. The three layers of the surface 
membrane complex (SM) are well defined and a few 250 to 350 Å connective tissue 
fibrils (c.f.) may be seen to the right of the outer dense line of the surface complex. A 
very delicate network of filaments (f.) less than 100 Å in diameter is present between 
the larger fibrils. OsO₄ 3 hours at 0°C.; PTA 24 hours. × 120,000.

Fig. 9. Oblique section similar to that shown in Fig. 8. Myofibril to the left. Both 
large connective tissue fibrils (c.f.) and smaller filaments lie outside the three layered 
surface membrane complex (SM). A few of the large granular bodies (G) in Fig. 7 may 
be seen less distinctly. OsO₄ 3 hours at 0°C.; PTA 24 hours. × 115,000.
(Robertson: Ultrastructure of reptilian skeletal muscle)
Fig. 10. Longitudinal section of a leg muscle fiber. The out of register bands of the myofibrils are clearly shown. The 375 Å period is clearly evident in both A and I bands. The myofibril running diagonally from the upper left corner of the field has been barely grazed by the plane of the section (plane b in Text-fig. 1). Note the pairs of double membranes (arrows M) or tubules lying in the sarcoplasm immediately adjacent to this grazed myofibril associated roughly with the edges of the A bands. In one favorable spot in which the Z bands of two adjacent myofibrils are in register some dense material can be seen in sarcoplasm between the Z bands (arrow SZ). In several regions including that enlarged in the inset a membranous component can be seen in the Z bands. OsO₄ 2 hours at 25°C.; PTA 48 hours. × 33,000. Inset × 69,000.
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Fig. 11. Enlargement of the middle set of double membranes or tubules shown in Fig. 10. The edge of the Z band of the associated myofibril is marked (arrow Z). OsO₄ 2 hours at 25°C.; PTA 48 hours. × 69,500.

Fig. 12. Portion of a longitudinal section of a leg muscle fiber showing segments of three myofibrils with their I bands bisected by the dense Z bands in the central portion of the field. The A bands appear to the right and left. The dense edges of the short sarcoplasmic membranes (M) or tubules running between myofibrils at the edges of the A bands are shown. OsO₄ at 0°C.; PTA 24 hours. × 71,000.

Fig. 13. Portion of a longitudinal section of a leg muscle fiber including several myofibrils in fairly good register. At the top and the bottom the plane of section has barely grazed two myofibrils. The sarcoplasmic double membranes (M) or tubules may be seen in these regions approximately at the edges of the A bands. OsO₄ 2 hours at 25°C.; PTA 48 hours. × 39,500.

Fig. 14. Longitudinal section of a leg muscle fiber including three myofibrils in good register. The Z bands (Z) in the central portion of the field identify the I bands. The edges of the A bands lie to the right and left. The bottom myofibril has been grazed by the plane of section so that the myofilaments in its A bands can barely be discerned. At the edges of the A bands the sarcoplasmic double membranes (M) may be seen. OsO₄ 3 hours at 0°C.; PTA 24 hours. × 53,000.
(Robertson: Ultrastructure of reptilian skeletal muscle)