THE STRIATED MUSCULATURE OF BLOOD VESSELS

II · Cell Interconnections and Cell Surface

H. E. KARRER, M.D., with the assistance of J. COX

From the Department of Pathobiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore

ABSTRACT

The interconnections and the surfaces of the striated muscle cells which occur in thoracic and in lung veins of the mouse were studied with the electron microscope. The osmium-fixed tissues were embedded in methacrylate or in araldite and sectioned with a Porter-Blum microtome. Many preparations were stained before embedding with phosphotungstic acid or after sectioning with uranyl acetate. Typical intercalated discs are observed in this muscle. They are similar to the discs found in heart muscle. These intercalated discs represent boundaries between separate muscle cells. Along the discs, cells are joined in planes normal to their myofilaments. The same cells are also joined in planes parallel to the myofilaments by means of lateral interconnections. These lateral cell boundaries are in continuity with the intercalated discs. Three morphologically distinct parts occur within the lateral cell interconnections: One is characterized by small vesicles along the plasma membrane, the second part has the structure of desmosomes, and a third part represents an external compound membrane (formed by the two plasma membranes of the adjoining cells) and is termed "quintuple-layered cell interconnection." Small vesicles and plasma membrane enfoldings along the free surface of muscle cells are interpreted as products of a pinocytosis (phagocytosis) process. Some of them are seen to contain small membrane-bounded bodies or granules. The free cell surface shows a characteristic outer dense layer ("basement membrane") which accompanies the plasma membrane. The topographic relation of this dense layer with the plasma membrane seems to vary in different preparations. The significance of this variation is not well understood. On two occasions a typical arrangement o vesicles and tubules was observed at Z band levels, just beneath the plasma membrane. These structures are believed to represent endoplasmic reticulum. Their possible significance for the conduction of excitation is discussed.

INTRODUCTION

In the previous paper (9) a general description of the striated muscle cells of the pulmonary and thoracic veins of mice was presented. It was concluded that these muscle cells are of the cardiac type.

This report deals primarily with the cell-to-cell interconnections and with the cell surfaces as seen in this muscle. These structures are of interest because of their role in the spread of excitation. This morphological study was undertaken in the hope that it might supplement the physiological data on conduction of excitation.

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MATERIAL AND METHODS

These are the same as in the previous paper (9). Most specimens, especially those used for the study of the cell surface, were stained either with uranyl acetate (32) after sectioning, or with phosphotungstic acid before embedding.

OBSERVATIONS

The Intercalated Discs

Intercalated discs similar to those of cardiac muscle are frequently found in the striated musculature of blood vessels (Figs. 1 to 3). They represent specialized cell interconnections.

In longitudinal sections through muscle fibers the intercalated discs appear in planes at right angles to the course of the myofilaments (*id*, Fig. 1). They are located at Z band levels (Figs. 1, 2). Often they traverse only one to two myofibrils (Figs. 1, 2), whereas more rarely they may cross several adjacent myofibrils.

Within the discs the adjacent plasma membranes usually appear as single dense lines separated by a space about 15 to 25 mμ in width (*pm*, Fig. 3). In some preparations which have been stained with phosphotungstic acid two additional dense strata appear within this space (arrows, Fig. 17). They are thought to be the outer dense leaflets of the two plasma membranes (26). There are no vesicles found along the plasma membranes of intercalated discs, in contrast with the other cell surfaces (see below).

Along the intercalated discs the sarcoplasm of both adjacent muscle cells appears condensed. The total width of this dense zone measures about 250 mμ (*id*, Fig. 1), thus exceeding that of the Z bands. The dense material (*d*, Fig. 3) does not bridge the gap (*gp*, Fig. 3) between the adjacent plasma membranes. It has about the same density as the Z bands (Fig. 1). It appears diffuse and does not show any finer substructure.

Lateral Interconnections

The lateral interconnections between muscle cells (*li*, Figs. 1, 2) are continuous with the intercalated discs (arrow, Fig. 1). Sjöstrand *et al.* (30) designated these interconnections by the term "longitudinal connecting surfaces." The intercalated discs and these longitudinal connecting surfaces together form continuous cell interconnections between adjacent muscle cells. In the muscle described here these lateral interconnections usually have the length of one sarcomere (Figs. 1, 2), less often of more than one.

Within these lateral interconnections three morphologically distinct parts can be recognized. One of these is essentially comparable to the free surfaces of muscle cells in that it shows numerous small vesicles just beneath the plasma membranes. A second part is characterized by the presence of "condensed" cytoplasm similar to that of intercalated discs, and a third part has the structure typical of external compound membranes (26). These three parts of the lateral cell interconnections will be described separately in the following paragraphs.

The first mentioned part of the lateral interconnections of muscle cells is characterized by many small vesicles located just beneath the plasma membrane (*v*, Fig. 2). They measure about 50 to 85 mμ in diameter. Some of them appear to be invaginations of the plasma membrane (arrows, Fig. 2). In one case a slightly larger vesicle was found to contain a small granule (*g*, Fig. 2). These vesicles resemble similar structures which are regularly found along the free surfaces of muscle cells and which will be mentioned again later in this report. The plasma membranes of the adjacent muscle cells appear in sections as two parallel dense "lines" separated by a less dense "gap" (Fig. 1).

A second part of the lateral interconnections has the fine structure of desmosomes as seen also in heart muscle (4). Within these desmosomes the two plasma membranes of the adjoining cells are accompanied by dense zones of cytoplasm (li_2, Fig. 1; *li*, Fig. 17). In this respect the desmosomes resemble intercalated discs; however, they are shorter than the discs (about 300 mμ) and are oriented essentially parallel to myofibrils. In the muscle described here the desmosomes seem to be preferentially located at the level of Z bands (Fig. 1), a fact which also was recognized before in a study of heart muscle (4).

A third part of lateral interconnections which occasionally can be recognized shows a specific organization (*li*, Fig. 4) similar to that described in mouse heart muscle (30). Five distinct layers can be recognized, namely three dense strata and two separating less dense zones (*li*, Fig. 4). Therefore, the term "quintuple-layered cell interconnection" is proposed as an appropriate descriptive designation for this type of structure. The two

outer dense strata are somewhat thicker (~30 A) and denser than the middle dense stratum (~20 A). The total width of the quintuple-layered interconnection is about 150 A. Although no direct evidence was found as to how these quintuple-layered interconnections are formed in this muscle, it is believed that they correspond to similar structures found in cervix epithelium (10). In this epithelium the middle dense stratum of the quintuple-layered interconnection (*ic*, Fig. 5) represents the fused outer dense leaflets of the plasma membranes (*pm*, Fig. 5), whereas the outer two dense strata of the interconnection represent the inner dense leaflets of the individual plasma membranes (Fig. 5). In other words, these quintuple-layered interconnections represent specialized zones of contact between two adjacent cells characterized by the fusion of the plasma membranes and the obliteration of the intercellular space.

Surface of Contact between Muscle and Capillaries

A few capillaries can be found located between the striated muscle cells of these blood vessels. The endothelium of such capillaries is separated from the muscle cell by a rather narrow (~100 mμ) extracellular layer, which does not seem to contain any connective tissue cells, collagen, or elastin. However, it does show two zones of condensed material ("basement membranes") which accompany the endothelium and the muscle cell (arrows, Fig. 11). The capillary endothelium has the same fine structure as that in capillaries of other tissues. In particular it contains the well known small vesicles, some of which are recognized as invaginations of the inner and the outer plasma membrane (v_1, Fig. 11). Similar vesicles occur also beneath the plasma membrane of the muscle cell (Fig. 11).

Even in its thin portions, the capillary endothelium generally appears to be uninterrupted. However, on one occasion two well defined pores were found within a very thin portion of the endothelium (double arrows, Fig. 11). They measure about 65 mμ in diameter and are bridged by a very thin indistinct membrane (Fig. 11). A similar thin membrane is seen to bridge an enfolding of the plasma membrane located at the base of the endothelium (v_2).

The Free Cell Surface

Plasma Membrane and Dense Outer Layer: The muscle cells are bounded by the plasma membrane, which in this osmium-fixed tissue appears as a dark line of an average thickness of 60 to 90 A (*pm*, Figs. 6, 12). Only where the cell surface is sectioned obliquely is the plasma membrane not distinct (*pm*, Fig. 9). On its outside, the membrane is accompanied by a dense outer layer. This layer appears contiguous with the plasma membrane in some preparations (*o*, Figs. 7 to 9), and there it measures 300 to 470 A in width. In other preparations, however, it is only 80 to 100 A wide and is separated from the membrane by a less dense zone (*o*, Figs. 6, 12, 13, 15). This zone measures about 100 to 200 A in width and has no apparent structure. The outer dense layer seems to consist of a mesh containing thin filaments (*o*, Fig. 9).

Collagen fibrils sometimes establish a close contact with the dense outer layer. Single fibrils appear embedded in this layer (c_1 and c_2, Fig. 7). In some preparations a great many fibrils are accumulated in a layer as wide as 700 mμ (c_1, Fig. 8). In other cases long stretches of free muscle cell surface are found devoid of collagen fibrils (Figs. 6, 9).

The muscle cell surface sometimes assumes a scalloped appearance. The indentations at the Z band levels may reach a considerable depth (1 μ in Fig. 10). It is noteworthy that the outer dense layer and collagen fibrils closely follow the indented plasma membrane (*o* and *c*, Fig. 10).

Subsurface Vesicles: Many small vesicles are constantly found just beneath the plasma membrane ("subsurface vesicles"). They are usually separated from the plasma membrane by a narrow layer of sarcoplasm (*v*, Figs. 6, 8, 9). Some enfoldings of the plasma membrane are sometimes found (v_2, Fig. 12), which closely resemble the subsurface vesicles in size and shape. Typically, the outer dense layer does not appear enfolded (v_2, Fig. 12). Such enfoldings may contain a dense granule or membrane-bounded body (*g*, Fig. 15), and similar structures can be found within subsurface vesicles (*g*, Fig. 16).

Endoplasmic Reticulum at Z Band Levels: In accordance with what is generally accepted, the present study does not reveal any continuity between Z bands and plasma membrane. On the other hand, a seemingly specific arrangement of vesicles and tubules exists at Z band levels, between the "ends" of Z bands and the plasma membrane. Fig. 14 shows an enfolding of the plasma membrane (arrow) at the exact level of a

EXPLANATION OF FIGURES

KEY TO ABBREVIATIONS

bi, node of Bizzozero
c, collagen fibril
d, dense layer of sarcoplasm
en, endothelium
f, myofilament
g, granule
gp, intercellular layer ("gap")
h, H band
i, I band
ib, inclusion body
ic, cell interconnection
id, intercalated disc
l, dense layer
li, lateral interconnection
lu, capillary lumen
m, mitochondrion
mc, muscle cell
me, membrane
o, outer dense layer
p, particle resembling Palade particle
pm, plasma membrane
r, endoplasmic reticulum
v, vesicle
z, Z band

FIGURE 1

Longitudinal section through portions of two muscle cells showing intercalated disc and lateral interconnection. The intercalated disc (*id*) is oriented transverse to the myofilaments. Within it the two plasma membranes (*pm*) of the two separate adjoining muscle cells (mc_1, mc_2) appear as two parallel dense lines about 15 mμ apart. The sarcoplasm of both muscle cells appears "condensed" along the disc. The disc is located one sarcomere length from the next Z band (*z*). The lateral interconnection between the two cells (li_1) is oriented parallel to the myofilaments; it continues beyond the Z band (*z*) and thus exceeds the length of one sarcomere. Like the intercalated disc, it represents the boundary between two cells. The arrow marks the spot of transition between the disc and this lateral cell interconnection. Because of oblique sectioning the two parallel plasma membranes do not appear distinct along the whole path of the lateral interconnection. One short portion of the lateral interconnection (li_2) located near the level of a Z band (*z*) is characterized by "condensed" zones of cytoplasm similar to those of the intercalated disc. It is interpreted as a desmosome.

The endoplasmic reticulum consists of membrane-bounded elongated tubules. The longitudinal tubules are arranged roughly parallel to the myofilaments. Some such units are found beneath the plasma membrane of the free cell surface (r_1), and others are located close to the lateral interconnection (r_2). In contrast, endoplasmic reticulum does not appear to be arranged along the intercalated disc. Some units of reticulum stretch between the myofilaments (r_3) where they form an anastomosing system and continue through the whole length of the sarcomere including the H band (*h*). At the Z band (*z*) several transverse tubules are arranged parallel and follow the course of the A band over a short stretch (r_4). The sarcoplasm surrounding the endoplasmic reticulum contains a number of particles (*p*) resembling Palade particles.

One mitochondrion lies just below the free cell surface (m_1), whereas another is surrounded by myofilaments (m_2).

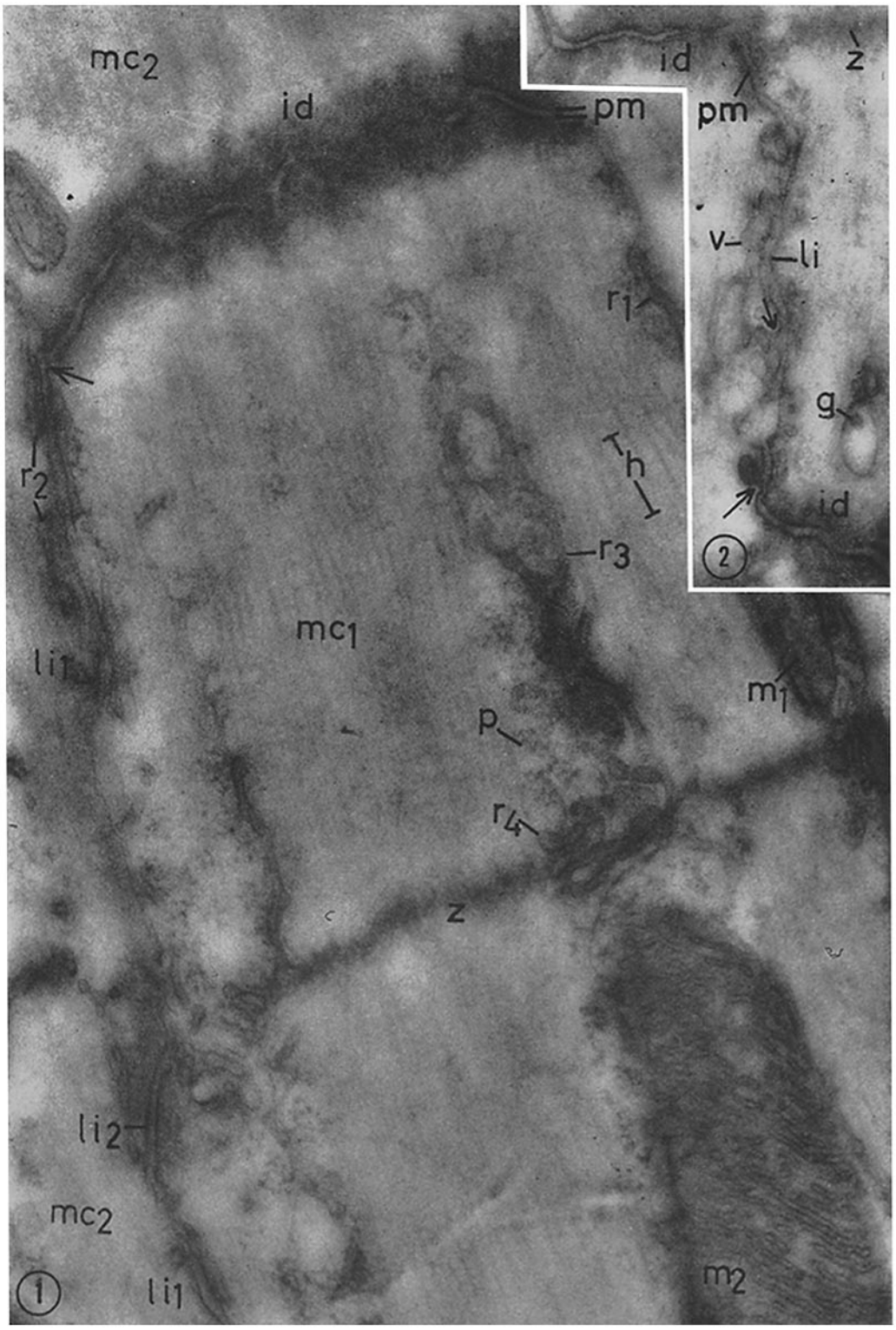
Unstained. × 65,000.

FIGURE 2

Portions of two intercalated discs and of one lateral cell interconnection. The two discs (*id*) are located at the level of Z bands (*z*); the distance between them represents the length of one sarcomere. They are recognized as discs on the basis of the dense cytoplasm accompanying the two parallel plasma membranes (*pm*). Both these discs merge into a lateral cell interconnection (*li*).

A number of small vesicles are recognized along the parallel plasma membranes of the lateral interconnection (*v*). Several of these vesicles are in direct contact with the plasma membrane, and sometimes their bounding membranes are continuous with this membrane (arrows). A larger vesicle lying at some distance from the cell boundary contains a small dense granule (*g*).

Unstained. × 55,000.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Z band, and a chain of vesicles (v) fills the space between this enfolding and the end of a Z band. A similar chain of vesicles accompanies a Z band (v, Fig. 13), but no enfolding is seen in this figure. The chains of vesicles appear to be accompanied by tubules of the endoplasmic reticulum (r_1 and r_2, Fig. 14). These tubules are curved and are apposed to the vesicle chain as well as to the plasma membrane (r_2, Figs. 12, 14). The whole complex of vesicles and tubules is reminiscent of similar structures described by Porter and Palade (21).

DISCUSSION

Intercalated Discs

Intercalated discs were first recognized in electron micrographs of heart muscle by Van Breemen (31) and Sjöstrand and Andersson (29) and since then by various other authors (4, 12, 14, 18). In a recent extensive study of such discs Sjöstrand *et al.* (30) gave a detailed description of the discs as seen in heart muscle of three different species. In the striated musculature of blood vessels, structures are found which resemble the intercalated discs as described by the authors mentioned. These structures are therefore recognized as intercalated discs, and their presence in the musculature here studied confirms the previous conclusion (9) that this musculature is of the cardiac variety.

Intercalated discs had been seen already by Arnstein within the striated musculature of lung veins (1). Arnstein states that "the boundaries" (of the muscle cells) "appear as strongly refractile lines, which traverse the muscular trabeculae in their whole width" (translation from German

FIGURE 3

Detail of an intercalated disc. Within the disc the parallel plasma membranes of the two adjacent muscle cells appear as single dense lines (pm) and are separated by an intercellular layer (gp). The parallel plasma membranes follow a typically winding course. The intercellular layer shows no apparent fine structure. Rather homogeneous zones of dense sarcoplasm (d) accompany the plasma membranes. The myofilaments (f) seem to fuse into these dense zones.

Uranyl acetate–stained. × 155,000.

FIGURE 4

Quintuple-layered cell interconnection. This type of interconnection represents a specialized portion within the lateral cell interconnections (for details, see text). In sections it appears as a complex structure consisting of three dense strata and two less dense interspaces (li). The two outer dense strata are thicker and somewhat denser than the intermediate one. The separate strata are not recognizable where they are sectioned obliquely (arrows). The interconnection represents the boundary between two separate muscle cells.

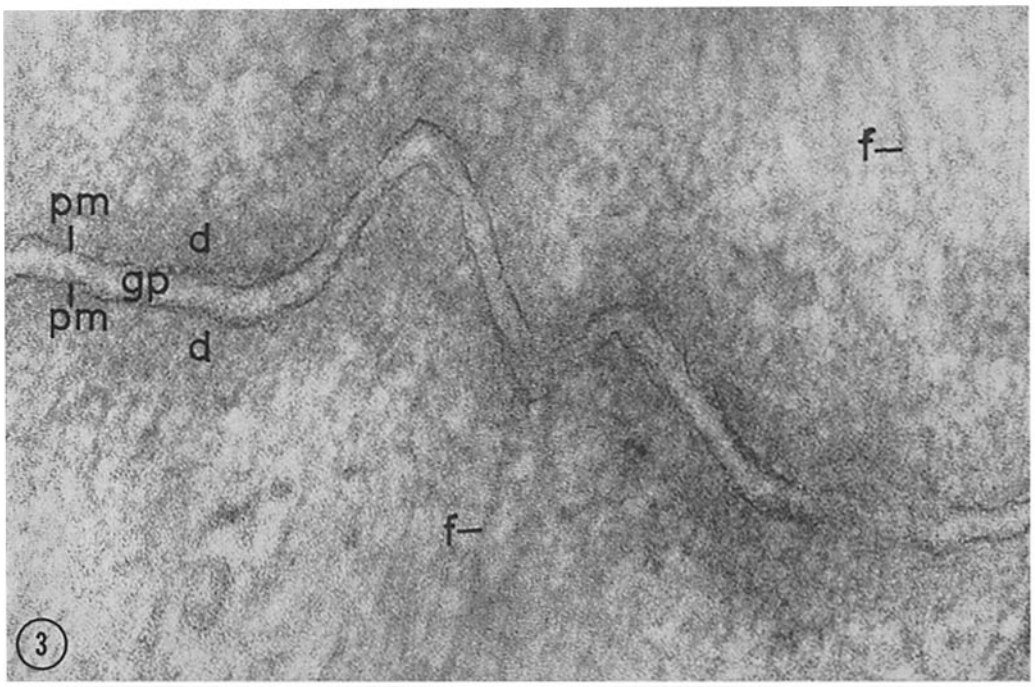
Portions of two mitochondria are seen (m), as well as an elongated tubule of the endoplasmic reticulum (r). There is an indication of secondary myofilaments alternating with primary ones (f).

Uranyl acetate–stained. × 150,000.

FIGURE 5

Cell interconnections in epithelium of human cervix (to be compared with Fig. 4). On the left there are two obliquely sectioned nodes of Bizzozero (bi). Towards the right, an intercellular boundary (ic) shows a fine structure similar to the one shown in Fig. 4. This electron micrograph demonstrates the origin of the different layers within this type boundary. The two plasma membranes (unit membranes, Robertson (25, 26)) appear as pairs of dense strata separated by a less dense zone (pm).At the cell interconnection the outer dense stratum of one unit membrane fuses with the corresponding stratum of the other unit membrane. The quintuple-layered cell interconnection (ic) thus represents an external compound membrane (25, 26).

Uranyl acetate–stained. × 125,000.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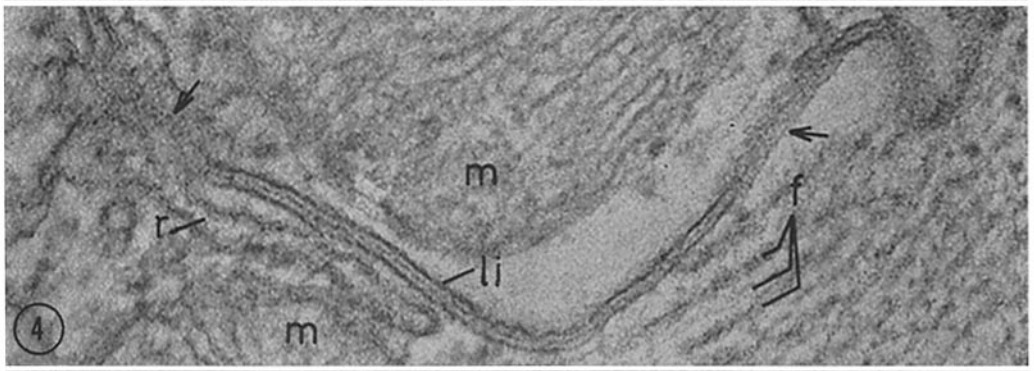
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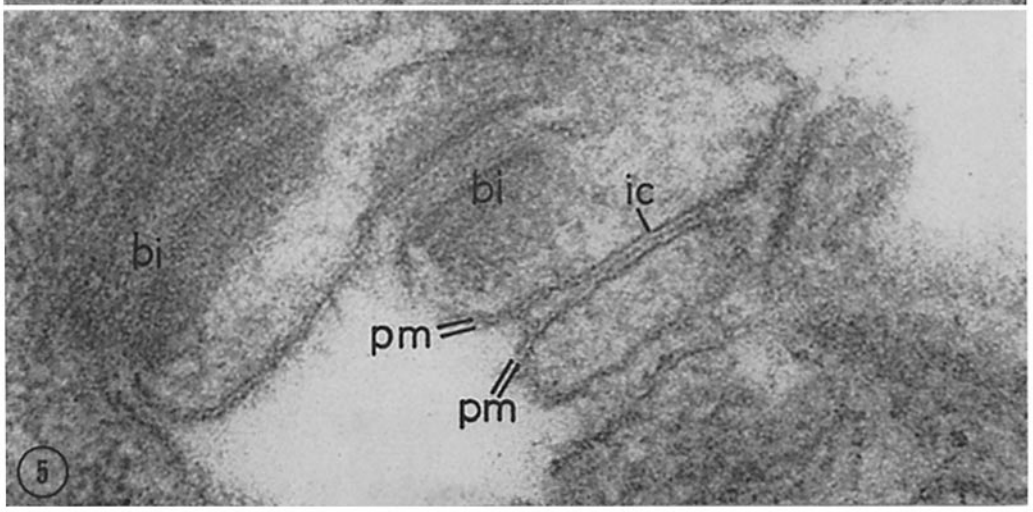
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original). On the other hand, Policard *et al.* have recently stated that this musculature lacks intercalated discs (19). It is believed that the last mentioned authors probably surveyed only a limited number of tissue sections and that this is the reason why they failed to find the discs.

Lateral Interconnections

The three morphologically distinct parts seen within the lateral cell interconnections have previously been described (4, 30). The parts showing parallel plasma membranes and subsurface vesicles are in no way different, morphologically, from the free cell surface, except that dense outer layers ("basement membranes") are absent. The parts characterized by condensed cytoplasm (li_2, Fig. 1) are interpreted, in accordance with Fawcett and Selby (4), as desmosomes (insuring lateral cohesion between the muscle cells), or also as transitional stages between desmosomes and intercalated discs. The latter interpretation is particularly appropriate in the case of the muscle described here because these desmosomes very frequently occur at Z band levels.

The "quintuple-layered" interconnections, which form the third morphologically distinct part within the lateral cell interconnections, have been previously described in heart muscle of the mouse, although no clear illustration was included (30). No clue was found during the present study as to how these interconnections are formed in muscle. However, it is most likely that they arise in the same manner as do morphologically similar

FIGURE 6

Muscle cell surface. The plasma membrane (*pm*) is accompanied by the outer dense layer (*o*). On the left, this appears nearly contiguous with the plasma membrane. On the right it is separated from the membrane by a narrow layer of lower density. Beyond the outer dense layer a few collagen fibrils are recognized in cross-section (*c*).

A number of small vesicles (*v*) and a mitochondrion (*m*) are located within the superficial layer of sarcoplasm. A Z band (*z*) terminates short of the plasma membrane but seems to be connected with it by a thin membrane (*me*). This membrane (*me*) is believed to be the bounding membrane of a poorly preserved sarcotubule similar to the one in Fig. 12 (r_2).

The hexagonal array of cross-sectioned primary myofilaments is apparent on the left side (f_1). On the right the smaller secondary myofilaments (f_2) appear in between the primary ones (f_1). Each secondary myofilament is equidistant from three primary ones (inset).

Phosphotungstic acid–stained. × 90,000; inset, × 170,000.

FIGURE 7

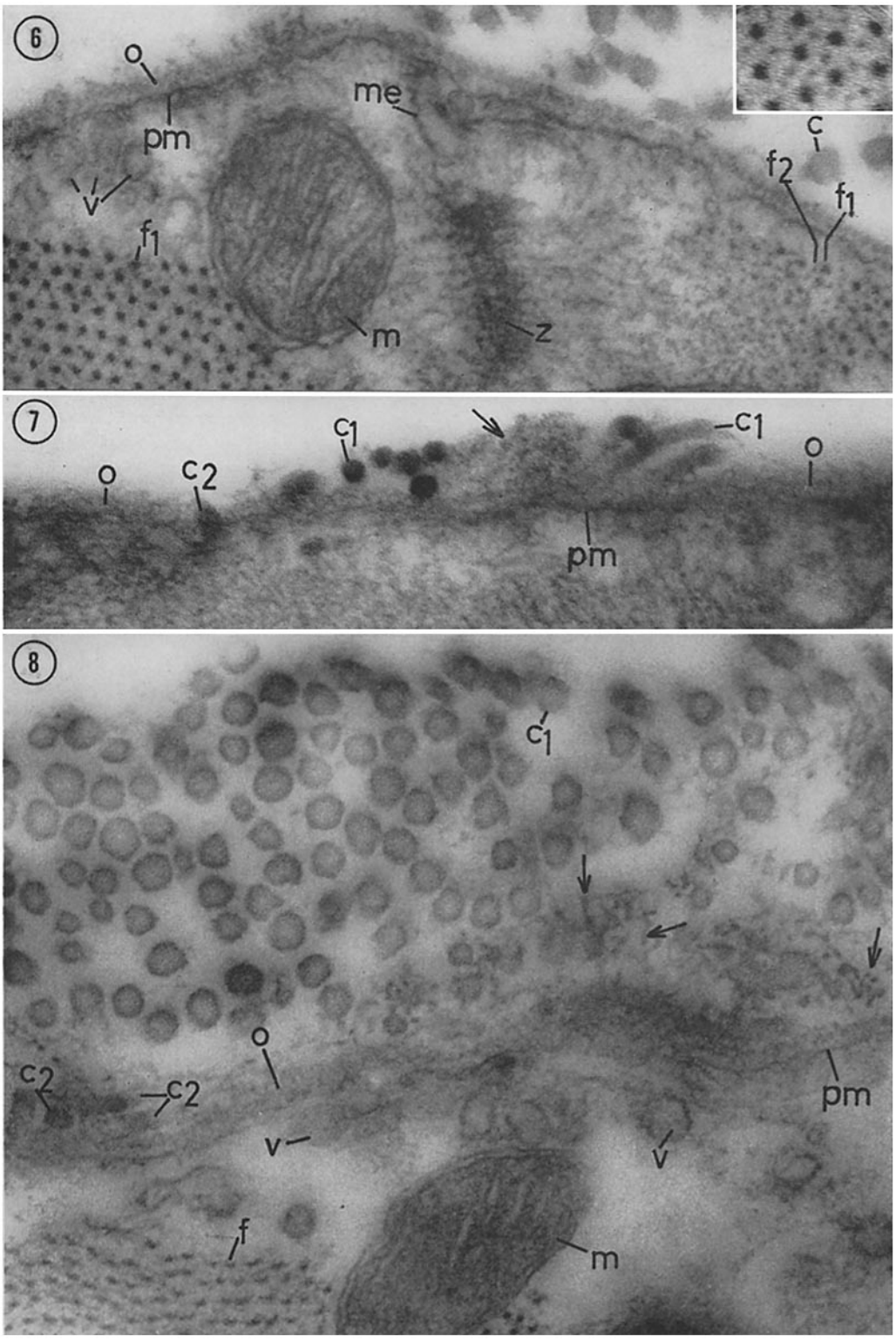
Muscle cell surface. The outer dense layer (*o*) seems everywhere contiguous with the plasma membrane (*pm*), and no separating zone of low density is seen. At one spot the outer dense layer is appreciably thickened (arrow) and is in contact with several collagen fibrils (c_1). Another such fibril appears completely embedded in the layer (c_2).

Phosphotungstic acid–stained. × 80,000.

FIGURE 8

Muscle cell surface. In this not too well preserved preparation the plasma membrane is only faintly visible (*pm*). The rather wide (~40 mμ) outer dense layer appears contiguous with it (*o*). Thin filaments, which in cross-section appear as dense dots, spread outward from this layer (arrows) and infiltrate between collagen fibrils (c_1). These fibrils form a layer of considerable thickness (700 mμ). At left a few such fibrils are seen embedded in the outer dense layer (c_2). Within the sarcoplasm a few subsurface vesicles (*v*) and a mitochondrion (*m*) are seen. Primary myofilaments appear at (*f*).

Phosphotungstic acid-stained. × 80,000.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interconnections in cervix epithelium (10) (*ic*, Fig. 5); namely, through the fusion of the outer dense leaflets of two plasma membranes. Thus, these cell interconnections closely resemble certain mesaxons of nerves. The mode of formation and the significance of such mesaxons have been discussed by Robertson (25, 26). He introduced the term "external compound membrane" for this type of structure, which may be considered as equivalent to one lamella of the myelin sheath (24, 25). However, in contrast to mesaxons, which are folds of one and the same Schwann cell membrane, the quintuple-layered interconnections unite the plasma membranes of two separate cells.

The fact that structures of the "external compound membrane" type are found in at least three different tissues, *viz.* nerve, cervix epithelium, and cardiac muscle, is of the highest interest. Additional studies of still other tissues will be needed to establish whether these compound membrane interconnections are of general occurrence. If so, a study of their functional significance in different tissues would be most interesting.

The quintuple-layered interconnections represent the only spots where separate muscle cells come in contact without any extracellular layer intervening in between the adjacent plasma membranes. Thus they provide for the closest possible contact between separate muscle cells. It therefore appears permissible to speculate that the quintuple-layered interconnections might be of significance in the cell-to-cell conduction of excitation in heart musculature.

Surface of Contact between Muscle and Capillaries

The two pores in the endothelium (arrows, Fig. 11) were the only pores found in the surveyed material. A comparable finding has hitherto

FIGURE 9

Superficial portion of a muscle cell. The outer dense layer (*o*) is contiguous with the plasma membrane (*pm*) and appears to contain thin filaments. It is contacted by a collagen fibril (*c*). Subsurface vesicles (*v*) are found just beneath the plasma membrane. Two inclusion bodies (*ib*) are contacted by several mitochondria (*m*). They are bounded by thin membranes which are distinctly separate at all points from the mitochondrial membranes. Their density varies; the densest portions of their "matrix" have the density of fat. The inclusions also contain ellipsoidal systems of membranes or lamellae (arrows).

Uranyl acteate–stained. × 80,000.

FIGURE 10

Cell surface at a Z band level (*z*). The cell surface shows a deep indentation. Characteristically the outer dense layer (*o*) and several collagen fibrils (*c*) closely follow the indented plasma membrane (*pm*).

Phosphotungstic acid–stained. × 70,000.

FIGURE 11

Muscle cell bounding upon a capillary. The capillary lumen (lu) is bounded by the endothelium (*en*), which on the left becomes quite thin (about 40 mμ). The muscle cell (*mc*) is separated from the endothelium by a layer of low density of a total width of about 90 to 140 mμ within which two ill defined denser strata ("basement membranes") can be recognized (arrows). One of these (below) corresponds to the dense outer layer of the muscle cell surface, the other (above) is considered a portion of the capillary. The capillary endothelium shows the typical vesicles which are recognized as invaginations of the free as well as of the basal cell surface (v_1). Two typical pores are seen within the thin portion of the endothelium (double arrows). These pores are considered to be transitory structures which are perhaps formed at the moment when a vesicle like the one shown at v_2 "breaks through" the whole thickness of the endothelium. The pores as well as the vesicle (v_2) appear bridged by very thin "membranes."

Uranyl acetate–stained. × 70,000.

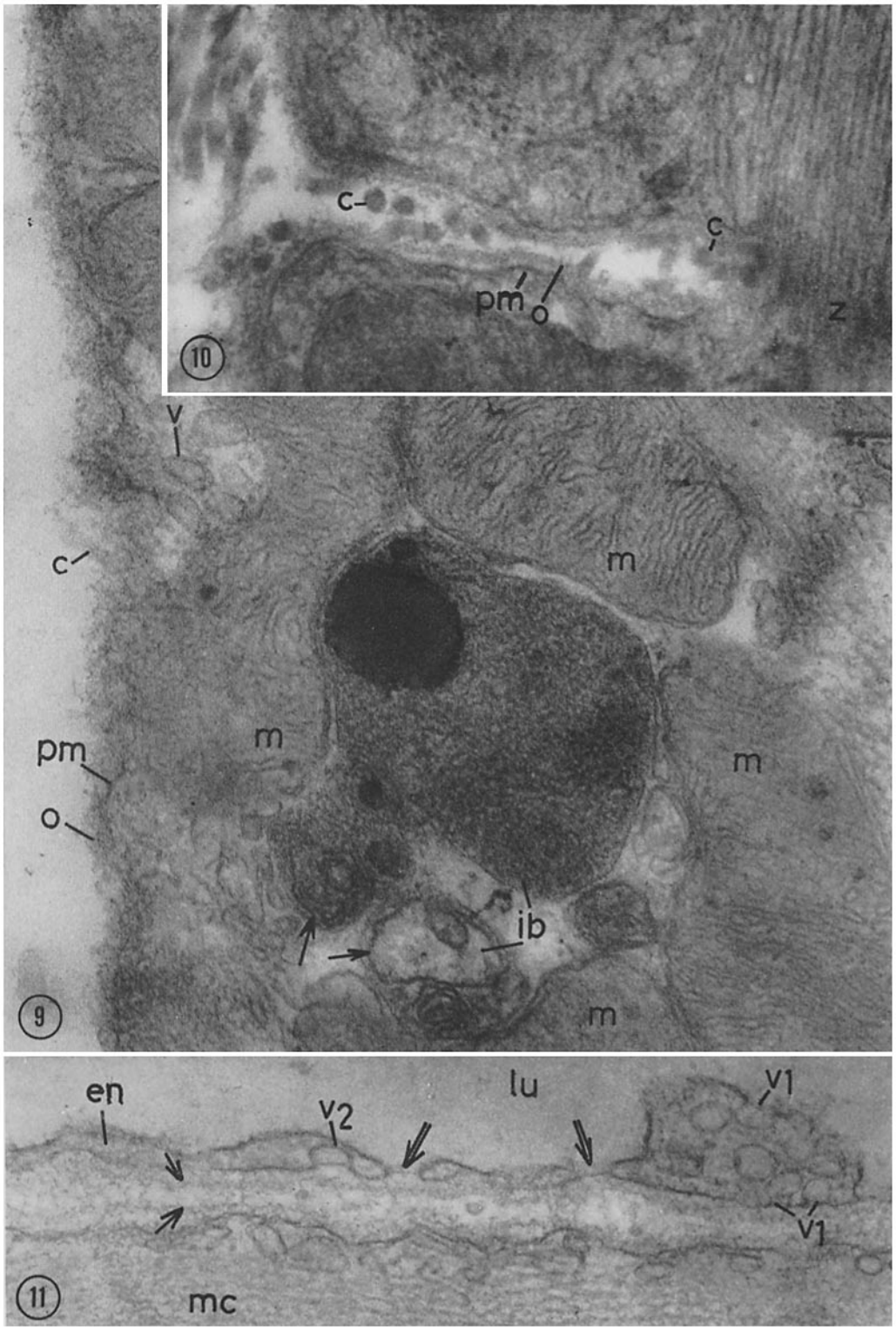
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FIGURE 12

Muscle cell surface. The cell is sectioned longitudinally (parallel with myofilaments, *f*). The outer dense layer (*o*) is separated from the plasma membrane (*pm*) by a less dense zone. Towards the top of the figure the outer dense layer is sectioned obliquely and therefore appears wider (arrows). There it also appears contiguous with the plasma membrane.

A number of small membrane-bounded vesicles (v_{1-3}) and tubules (r_{1-3}) lie just beneath the plasma membrane. A string of vesicles seem to merge into a tubule (r_1) which runs normal to myofilaments. Another tubule (r_2) runs longitudinally below the plasma membrane, then bends at right angle and follows a Z band (*z*). A third tubule (r_3) seems to follow a similar course on the other side of the same Z band. An enfolding of the plasma membrane (v_2) is suggestive of vesicle formation. The outer dense layer does not appear enfolded. Two vesicles (v_3) which are in direct contact with the plasma membrane might just have been formed by such an enfolding process.

Uranyl acetate–stained. × 85,000.

FIGURE 13

Muscle cell surface at a Z band (*z*). Plasma membrane (*pm*), outer dense layer (*o*), and portions of two mitochondria (*m*) are recognized. A string of small vesicles accompanies the Z band (*v*). The outermost vesicle contacts the plasma membrane, but no interconnection can be seen.

Uranyl acetate–stained. × 55,000.

FIGURE 14

Muscle cell surface at a Z band (*z*). Plasma membrane (*pm*), outer dense layer (*o*), and portions of two mitochondria (*m*) are seen. The latter separate the myofilaments (*f*) from the cell surface. A group of vesicles and of tubules is interposed between the "end" of the Z band (*z*) and the cell surface. The vesicles (*v*) are arranged in a row which meets the Z band, and which seems to originate from a plasma membrane enfolding (arrow) at the Z band level. Two tubules (r_1, r_2) flank this vesicle chain on either side. At the cell surface one of these tubules curves away from the vesicle chain and continues beneath the plasma membrane and parallel to the surface (r_2).

Uranyl acetate–stained. × 60,000.

FIGURE 15

Muscle cell surface between two Z bands. An enfolding (v_1) of the plasma membrane (*pm*) contains a small granule or membrane-bounded body (*g*). The outer dense layer (*o*) does not appear enfolded. A small vesicle (v_2) "buds" from the enfolding. Other similar vesicles lie beneath the plasma membrane (v_3). *m* represents portion of a mitochondrion.

Uranyl acetate–stained. × 150,000.

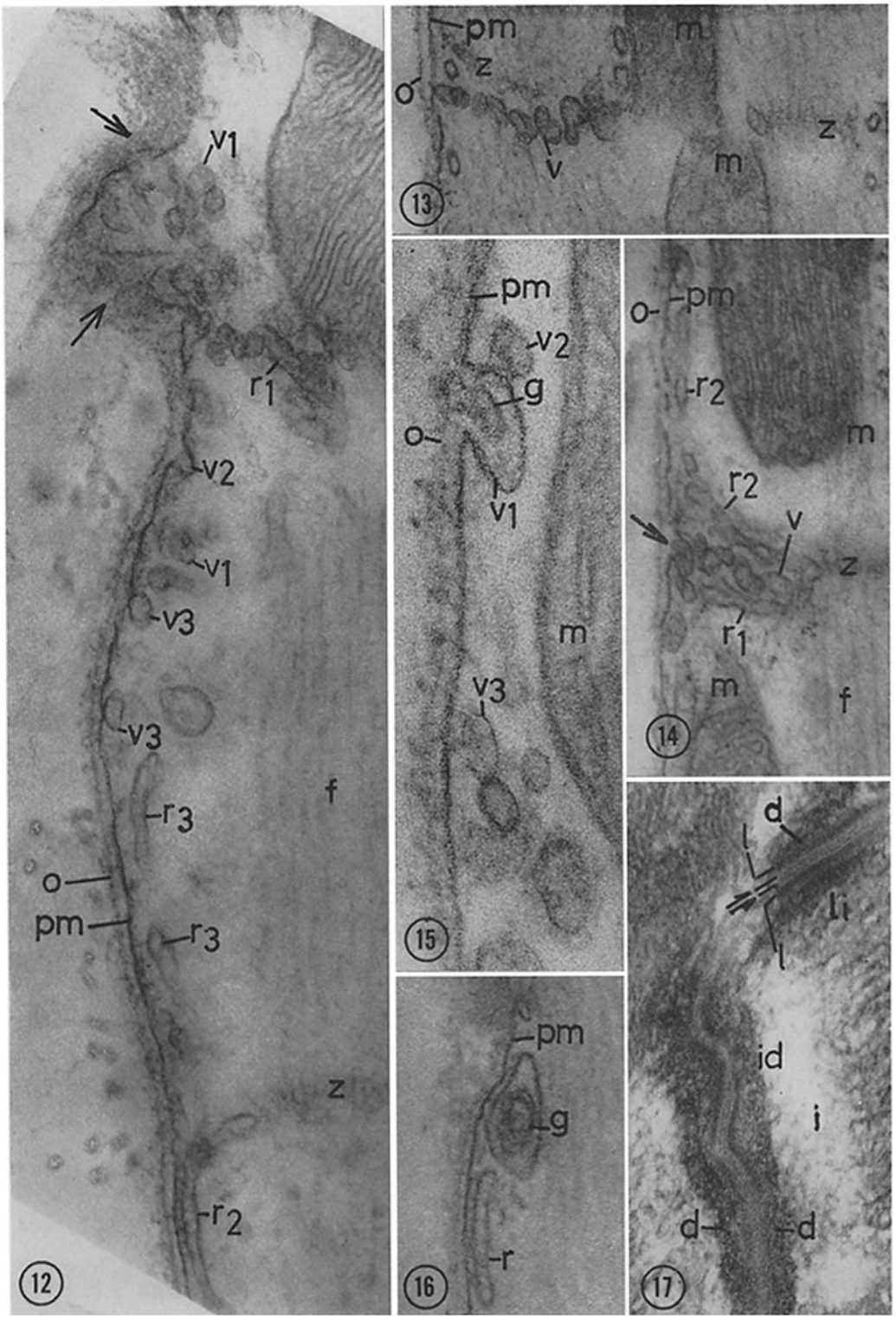
FIGURE 16

Muscle cell surface between two Z bands. Underneath the plasma membrane (*pm*) a small vesicle is seen to contain a dense oval granule or membrane-bounded body (*g*). An adjacent tubular profile (*r*) runs parallel to the cell surface.

Uranyl acetate–stained. × 80,000.

FIGURE 17

Portion of an intercalated disc (*id*), and a desmosome belonging to a lateral interconnection (*li*). The disc and the desmosome show the same fine structure. Several distinct layers are recognized within them. Two diffuse zones of dense sarcoplasm (*d*) lie outermost. The inner portions of these dense zones appear as two well defined layers (*l*). Innermost two parallel dense strata can be recognized (arrows). These are in-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not been reported for the case of muscle capillaries. Regular pores do of course occur in certain capillaries, for instance in the kidney (17, 22) or in the thyroid (3), but the endothelia of many other capillaries are lacking such pores. In the present case, the two pores seen in Fig. 11 are believed to be transitory structures which might be formed at the moment when a plasma membrane enfolding like the one at v_2 (Fig. 11) "breaks through" the whole width of the endothelium. It is not believed that the pores seen here are permanent structures, because if they are, they would probably be found more frequently. If these pores are really formed in the way indicated above an earlier speculation (13) about "cytopempsis" might find its pictorial confirmation in Fig. 11.

The thin membranes which apparently bridge the pores could be interpreted as the tangentially sectioned pore rim. A similar interpretation has been proposed for the case of "membranes" which appear to bridge the pores in the nuclear envelope (33). The width of the pores in the endothelium (about 65 mμ in Fig. 11) is comparable to the section thickness (about 50 to 100 mμ), which makes it likely that a slightly off center section through the pores includes a tangential section through the pore rim. The thin membrane bridging the "neck" of the vesicle (v_2, Fig. 11) could be interpreted in the same way.

The Free Cell Surface

Plasma Membrane and Dense Outer Layer: The free surface of muscle fibers is of particular interest because of its importance for the conduction of excitation. Hence, several electron microscopic studies have included a description of this surface. They established that the plasma membranes of muscle cells are always accompanied on the outside by an ill defined dense layer. Robertson introduced the term "cell surface complex" as a designation for plasma membrane plus outer dense layer and proposed that the term "sarcolemma" be abandoned in discussions of ultrastructure (23). On the other hand, the term "sarcolemma" is applied to the plasma membrane plus outer dense layer by some authors (12, 21), or to the plasma membrane excluding the outer dense layer by others (4). Because of these contradictions and for the sake of clarity, the time-honored term "sarcolemma" has been avoided in the present study.

In some specimens the outer dense layer appears contiguous with the plasma membrane (Figs. 7, 9), whereas in others the well known gap separates the two (Figs. 6, 12, 13, 15). Furthermore, measurement shows that those portions of the layer which are contiguous with the membrane have about the same width as the non-contiguous portions plus the low-density zone together. The reason for this variable appearance of the dense layer is not well understood. Contiguity with the membrane might be only apparent at least in those cases in which these structures are sectioned obliquely (arrow, Fig. 12), but it is probably real in other cases. The zone of low density might be an artifact in some preparations (23), caused by a coagulation or shrinkage of the dense layer. This possibility is suggested by the measurements (see above). On the other hand, a separation of the layer from the membrane must be accepted as real for those cases in which the tissue is generally well preserved. Since the outer dense layer has been observed to vary with the physiological state of the animal (11), it is possible that the variations described here express different functional states of the muscle cells.

It is also interesting to note another variability in the relation between dense layer and plasma membrane. The layer closely follows the membrane in the indentations at Z bands (Fig. 10) which indicates that the two form a structural unit. On the other hand, the dense layer does not follow the plasma membrane at the spots where small enfoldings develop (v_2, Fig. 12; v_1, Fig. 15). This shows that the membrane can actively "disengage" itself from the assumed structural ties which otherwise bind it to the dense layer.

Subsurface Vesicles: The small vesicles arranged below the plasma membrane ("subsurface

terpreted as the outer dense leaflets of the two plasma membranes. The inner dense leaflets of these membranes are not recognizable probably because their density does not differ enough from that of the adjacent layers (*I*).

The intercalated disc is accompanied by a zone of low density (*i*) corresponding to a narrow I band.

Phosphotungstic acid-stained. × 70,000.

vesicles") have been seen in muscle by several other investigators (4, 13, 18, 21). Small enfoldings of the muscle cell membrane, similar to those shown in Figs. 12 and 15, have also been known for some time. Such enfoldings were considered to represent morphological evidence for active pinocytosis occurring at the surface of these muscle cells (27), and it was speculated that the enfoldings would form vesicles which in turn might unite with the endoplasmic reticulum (27). Such a fusion of pinocytotic vesicles with the endoplasmic reticulum has also been discussed by various other authors (2, 15, 21).

The evidence presented here tends to confirm the view that the enfoldings are indicative of pinocytosis or phagocytosis. The granule or membrane-bounded body (*g*, Fig. 15) seen within a cup-shaped enfolding probably represents material being ingested, whereas another similar granule or membrane-bounded body within the lumen of a subsurface vesicle (*g*, Fig. 16) most likely represents material that has been ingested by means of the enfolding process. Furthermore, the close topographic association of some subsurface vesicles with the plasma membrane (v_3, Fig. 12) supports the view that these vesicles are products of the enfolding process.

Endoplasmic Reticulum at Z Band Levels: Various authors have speculated about the possible role of the endoplasmic reticulum in the propagation of impulse laterally across the muscle fiber (12, 16, 20, 21, 27, 28). In addition, A. F. Huxley has demonstrated by means of micro-electrode stimulations of isolated muscle fibers that such stimulations provoke a contraction only if they are applied at certain circumscribed spots. These "sensitive spots" lie—depending on the species—either at Z levels or close to the A-I interface (5–8) and they are located around the fiber perimeter at intervals of about 5 μ (8). It follows that those portions of the endoplasmic reticulum that might play a role in the conduction of impulse across the muscle fiber would have to be located where this conduction occurs; namely, either at Z band levels or close to the A-I interface (depending on the species).

In the course of the present study a special effort was made to find such units of the endoplasmic reticulum which could serve as substrate for the lateral transmission of impulse as demonstrated by A. F. Huxley. A considerable number of Z band levels was surveyed for the presence of such units of reticulum. The A-I interfaces were disregarded, however, because earlier studies of this muscle have shown that I bands are very narrow or are absent altogether (9). It was realized that units of reticulum that might correspond to the "sensitive spots" of the fiber surface would only rarely be found in thin (50 to 100 mμ) sections, since about 50 to 100 such sections would be required in order to cover the distance of 5μ between adjacent "sensitive spots" along each Z band perimeter. Hence it was expected that such units of endoplasmic reticulum would be found only at a few Z band levels within single thin sections. It was also realized that such units of reticulum as well as "sensitive spots" might not be present at all in the cardiac muscle studied here, since impulse conduction within this muscle might occur in a way altogether different from the skeletal muscles studied by A. F. Huxley.

Figs. 13 and 14 represent the only two instances in which a rather typical arrangement of endoplasmic reticulum units was observed at Z band levels. These units resemble similarly arranged structures observed at I band levels of *Ambylstoma* skeletal muscle (21). They appear as rows of separate vesicular profiles that may be flanked by elongated tubules. The outermost vesicle appears as an enfolding of the plasma membrane (Fig. 14), or it at least is in close contact with this membrane (Fig. 13). Possibly these arrangements of endoplasmic reticulum indeed represent the units which conduct the impulse across the muscle fibers—this is suggested by their close association with the plasma membrane, by their location at Z band levels, and by their rare occurrence (corresponding to the widely spaced "sensitive spots"). On the other hand, Figs. 13 and 14 show that the vesicular profiles are probably separate units. These vesicles thus do not provide for a continuous membrane structure along which a depolarization wave could easily be propagated across the muscle cell. Be this as it may, these arrangements of reticulum at Z band levels are typical enough to warrant further exploration of their true significance.

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