THE ULTRASTRUCTURE OF FROG VENTRICULAR CARDIAC MUSCLE AND ITS RELATIONSHIP TO MECHANISMS OF EXCITATION-CONTRACTION COUPLING

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ABSTRACT

Frog ventricular cardiac muscle has structural features which set it apart from frog and mammalian skeletal muscle and mammalian cardiac muscle. In describing these differences, our attention focused chiefly on the distribution of cellular membranes. Abundant inter cellular clefts, the absence of tranverse tubules, and the paucity of sarcotubules, together with exceedingly small cell diameters (less than 5 μ), support the suggestion that the mechanism of excitation-contraction coupling differs in these muscle cells from that now thought to be characteristic of striated muscle such as skeletal muscle and mammalian cardiac muscle. These structural dissimilarities also imply that the mechanism of relaxation in frog ventricular muscle differs from that considered typical of other striated muscles. Additional ultrastructural features of frog ventricular heart muscle include spherical electron-opaque bodies on thin filaments, inconstantly present, forming a rank across the I band about 150 mμ from the Z line, and membrane-bounded dense granules resembling neurosecretory granules. The functional significance of these features is not yet clear.

In frog skeletal muscle, tubular membrane-bounded spaces, continuous with the interstitial space, penetrate the muscle cell transversely at the level of the Z lines of the myofibrils lying in register with one another. The continuity of the membranes of this transverse tubular system with the cell membrane and of its lumen with the interstitial space of muscle has been clearly demonstrated by Huxley (1) using ferritin.

Mammalian heart muscle appears also to possess a transverse tubular system penetrating the muscle cells and having continuities with the cell membrane and interstitial space (2, 3). Rayns et al. (4), using a freeze-etching technique, observed the portals of these transverse tubules on the surface of the cell membrane. The probable role of this internal membrane system in the inward spread of the excitatory signal to the contractile sites in the myofibrils has been elegantly demonstrated by Huxley and Taylor (5) who used microelectrodes to achieve local depolarization of the cell membrane.

From these observations and the ones on the role of calcium in the activation of the contractile mechanism (6, 7), a concept of excitation-contraction coupling has emerged which states that the effects of excitation are borne inward across the muscle fiber by the transverse tubular system releasing calcium ion from the sarcoplasmic reticulum surrounding the myofibrils and thereby activating the contractile elements (8).

In frog heart muscle, however, Niedergerke

(9, 10) found that activation of the contractile mechanism could be explained by diffusion of calcium directly from the cell surface. We thought it would be of interest, therefore, to examine the structure of frog ventricular muscle cells by electron microscopy. We especially wanted to see if there were important structural differences in the cellular membrane systems between frog heart muscle and frog skeletal and mammalian heart muscle.

MATERIALS AND METHODS

Adult frogs (*Rana pipiens*), male or female, obtained at various seasons of the year, were stored for several days at 4°C before use. Each was pithed, the heart was exposed, and the ventricular chamber was injected with a cold fixation fluid (see below). The heart was then quickly excised, and blocks of ventricular muscle, 1 mm^3 or smaller in size, were placed in the fixation fluid and kept at 0–4°C for 2–12 hr.

The fixation fluids used were either 3.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) (11) or a combination of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) (12).

The blocks were then washed for 2 hr in 0.2 M sucrose in 0.05 M cacodylate buffer (pH 7.2), postfixed in 1% osmium tetroxide in Veronal-acetate buffer (pH 7.4) (13), dehydrated in a graded series of alcohol-water mixtures, and embedded in Epon 812 (14).

Thin sections were stained with uranyl acetate and lead citrate (15) and examined in an RCA EMU-3G electron microscope.

RESULTS

Frog ventricle has a very spongelike appearance when seen from the direction of its endocardial surface. This appearance is due to an abundance of small fissures between trabecular strands of muscle. The fissures extend through the wall of the ventricle to the epicardial surface covering. The porous, trabecular structure of the ventricle as seen from the ventricular chamber is illustrated in Fig. 1, a sketch which is taken from Gompertz (16).

Each strand of ventricular muscle, or each trabeculum, is lined by a single layer of endothelial cells with an underlying continuous basement membrane (Fig. 2). This layer of cells and its basement membrane is the endocardial lining of the ventricular chamber. The wall contains no capillaries, and perfusion of the cells apparently takes place from the ventricular cavity by way of the fissures and, thence, through the endothelial lining of each muscle strand or trabeculum. Between the endothelium and the cell membrane of the muscle cells lies an extracellular space which contains bundles of collagen fibers, nerve fibers, and occasional macrophages and fibroblasts.

Nerve fibers, many with no Schwann cell covering, are quite numerous (Fig. 3). They occur singly or in bundles, often in proximity to muscle cells. Along the fibers are found occasional focal dilations containing agranular synaptic vesicles and a few "dense core" granular vesicles; no distinct specialized synaptic junctions with muscle cells were noted.

Each trabeculum with its endothelial covering measures 10–50 μ in diameter and contains from 10 to 15 muscle cells; the long axis of each cell lies parallel to that of its neighbors. The group of muscle cells within the trabeculum is surrounded by a membrane similar to the sarcolemmal membrane of other muscle cells: it is made up of a basement membrane and plasma membrane, with a translucent intermediate layer. The individual

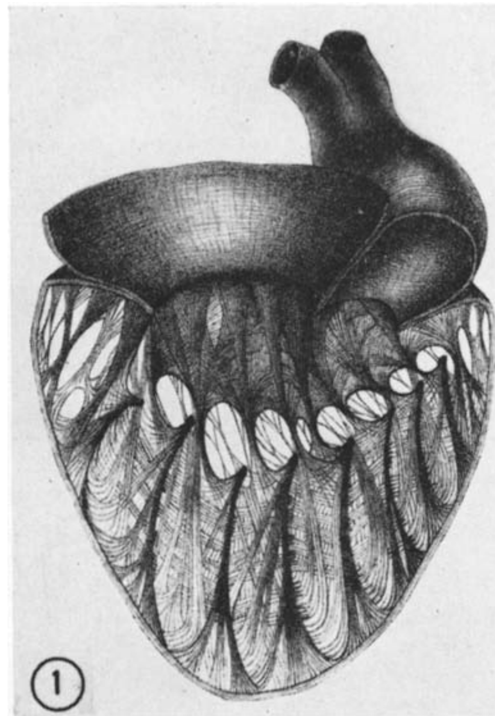


FIGURE 1 A sketch of frog ventricle, viewed from the endocardial surface, illustrating the trabecular structure of the muscle. Redrawn from reference 16.

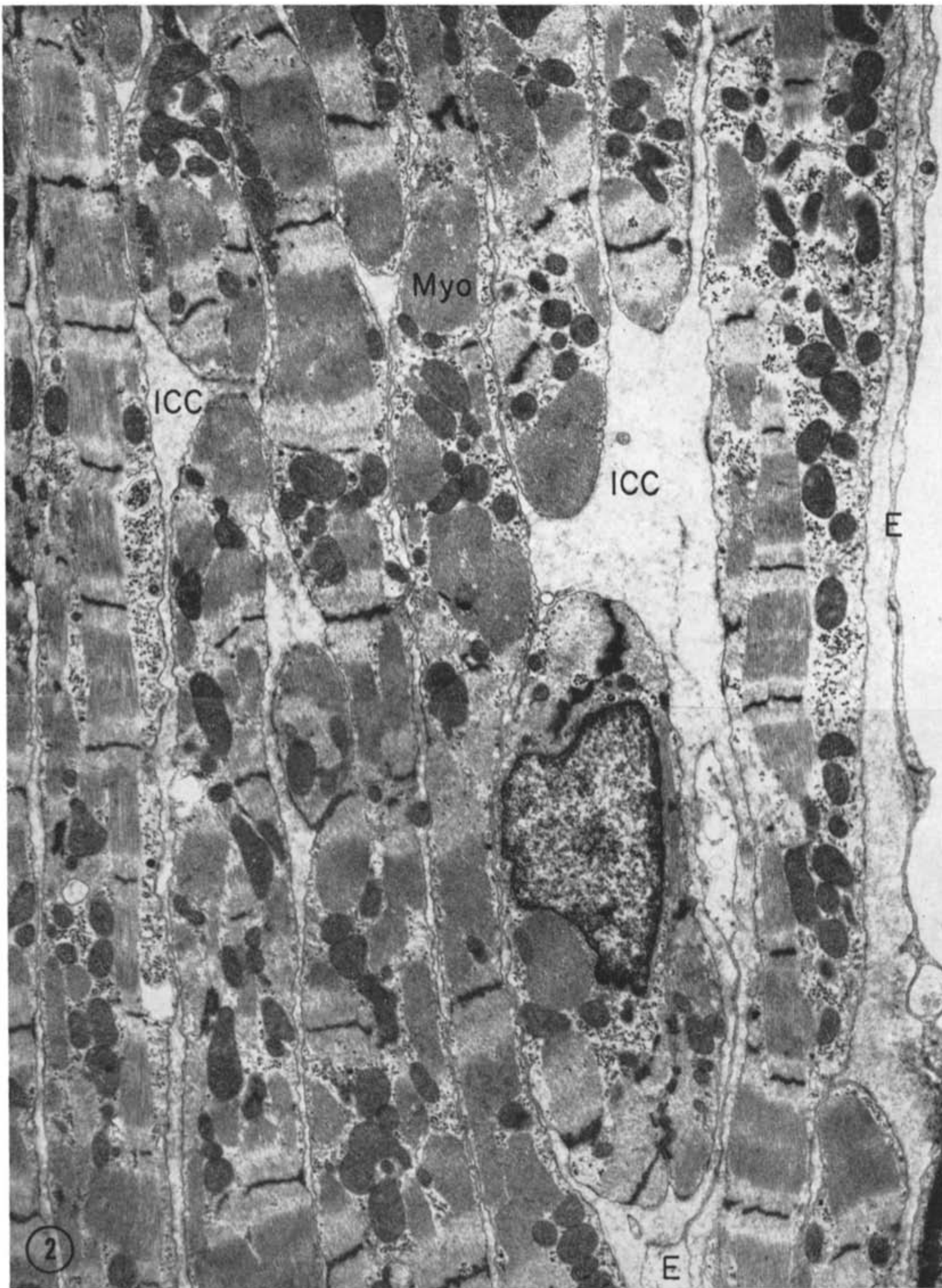


FIGURE 2 Longitudinal section through a portion of one trabeculum shows the surface endothelial layer (*E*), subendothelial extracellular space and muscle cells. Intercellular clefts (*ICC*) separate individual muscle cells, each of which contains one to three parallel myofibrils (*Myo*). × 7,000.

muscle cells are separated from each other by intercellular clefts (Figs. 2 and 4): these are membrane-bounded spaces, the membranous linings of which are continuous with the plasma membrane of the sarcolemma surrounding the bundle of muscle cells in each trabeculum. The basement membrane of the sarcolemma, in general, does not become a part of the lining of the intercellular clefts. At the junction of the clefts with the sarcolemma, the basement membrane of the latter separates the lumen of the intercellular clefts from the extracellular space beneath the endothelial lining of the ventricular cavity (Fig. 5). However, in some of the wider intercellular clefts, the basement membrane dips into the cleft lumen for a short distance.

The intercellular clefts appear to be continuous cell boundaries. In much of their course they are relatively narrow, varying in width from 200 to 400 A. They widen at points at which the curvatures of the surfaces of the adjacent cells diverge (Fig. 4), and in these pockets wisps of basement membrane–like material appear. At frequent but irregular intervals, along the intercellular clefts, fusiform, electron-opaque thickenings of the adjacent plasma membranes occur (Figs. 6 and 7). These thickenings are somewhat granular and have the appearance of the desmosomes seen in the intercalated disc of mammalian cardiac muscle, which occur in the portions of the disc parallel to the long axis of the myofibrils (17), and which in turtle atrial muscle are present at the cell boundaries of adjacent myocardial cells (18). These structures often lie directly opposite the Z lines of the underlying myofibrils of one of the adjacent muscle cells, and the dense material of the desmosome is then continuous with the dense Z-line substance (Fig. 6).

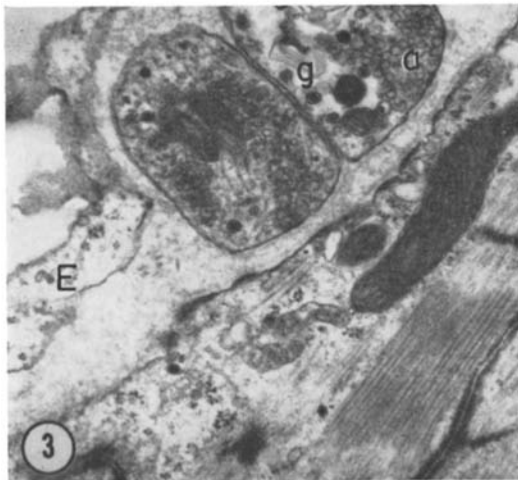


FIGURE 3 Cross-section of a nerve fiber, within the subendothelial extracellular space, which contains agranular (*a*) and granular (*g*) vesicles. No Schwann cell or specialized synaptic junction is present. *E*, endothelial layer. × 18,000.

The abundance of intercellular clefts gives the cells relatively narrow diameters. The cell diameters, from outer cell membrane to cleft or from cleft to cleft, range from 1 to 5 μ. In cross-sections of a cell, one can see three to five myofibrils per cell (Fig. 4). In longitudinal section, one to two myofibrils are seen between one cleft and the next (Figs. 2 and 6). Typically, in scanning across a longitudinal section, one notes an arrangement of this type: cleft, myofibril, a row of mitochondria, myofibril, cleft (Fig. 7).

Intercalated discs are numerous and have a simple structure (Fig. 8). They run transversely across cells from one cleft to the next and cross myofibrils in a plane where the Z line might otherwise be found. They have the typical appearance of what has been termed the "interfibrillar segment" of the disc (17); they are made up of two dense membranes separated by a clear space and surrounded by an irregular band of electron-opaque material. They lack the steplike course of discs seen in mammalian cardiac muscle where longitudinal segments between myofibrils ("intersarcoplasmic segments") alternate with transverse segments intersecting myofibrils (interfibrillar segments) (17). Tight junctions, similar to those of the intercalated discs of mammalian heart muscle, were not seen.

For the most part, the myofibrils have the typical appearance of striated muscle in general, with sarcomeres being made up of interdigitating thick and thin filaments (Figs. 9 and 10). As in mammalian cardiac muscle, the H zone, when present, is indistinct. The M line is seen only occasionally (Fig. 9). A curious, and as yet unexplained, finding in some preparations is the occurrence of spherical electron-opaque bodies, approximately 45 mμ in diameter, on thin filaments. These bodies form a row across the I band, the row being parallel to the Z line and approximately 100–150 mμ from it in sarcomeres at or near rest length (Figs. 9–11).

We have seen no transverse tubules in frog

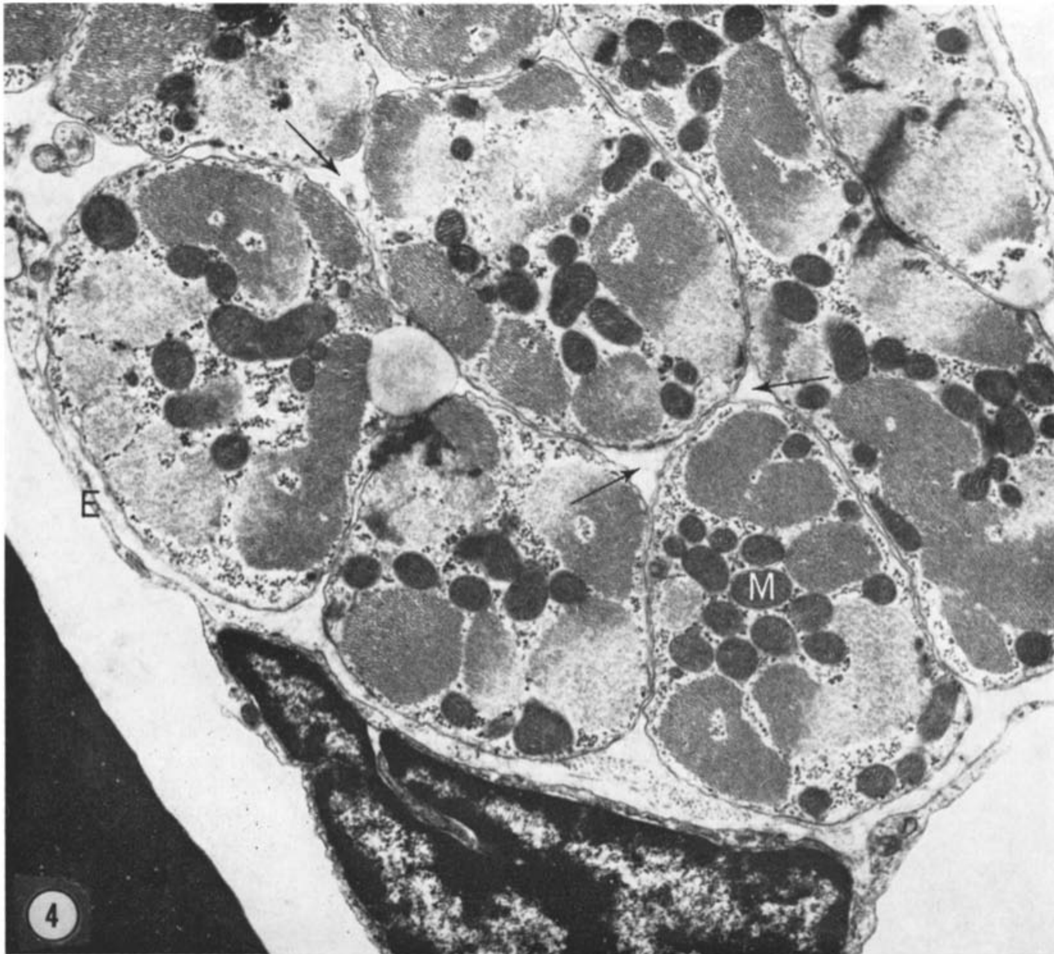


FIGURE 4 Cross-section of one trabeculum. Intercellular clefts (arrows) widen irregularly between muscle cells. Each cell contains three to five myofibrils separated by clusters of mitochondria (*M*) and glycogen. *E*, endothelial layer. × 13,000.

ventricular cardiac muscle. This tubular system which is so prominent in mammalian cardiac muscle (2, 3) appears to be absent from the cardiac muscle of the frog. Furthermore, the sarcoplasmic reticulum, the longitudinal system of membrane-bounded tubules surrounding each myofibril in frog skeletal and mammalian cardiac muscle, is exceedingly sparse in the frog heart muscle we have examined. It appears to consist of scattered small, single, membrane-bounded vesicles bearing no consistent spatial relationship to sarcomere topography (Figs. 6 and 12). In some places, elements of the sarcoplasmic reticulum are closely apposed to the cell membrane and intercellular clefts. Occasionally small vesicles may also be seen in the intermyofibrillar spaces at the Z lines (Fig. 11). These vesicles are lined by a single membrane and do not have the appearance of transverse tubules but resemble the "circumferential Z line tubules" described by Simpson in mammalian cardiac muscle (19). Small pinocytotic vesicles are often seen immediately beneath the cell membrane (Fig. 12).

Nuclei are numerous and contain prominent nucleoli; the nuclear envelope has many pores (Fig. 13). Often in the sarcoplasm near one pole of a nucleus a Golgi apparatus is seen; it is composed of three to four parallel cisternae with numerous adjacent clear vesicles. Vesicles approximately 100 mμ in diameter, and containing spheri-

cal electron-opaque granules, are found in the vicinity of the Golgi apparatus (Figs. 13 and 14) or scattered throughout the sarcoplasm between myofibrils.

The perinuclear spaces contain other cytoplasmic components including multivesicular bodies, dense bodies, occasional cisternae of rough-surfaced endoplasmic reticulum, and clusters of mitochondria (Figs. 13 and 14).

The space between myofibrils is characteristically occupied by elliptical mitochondria which contain many closely packed cristae (Figs. 4 and 6) and are similar in appearance to those of mammalian cardiac muscle (2, 3, 20). Glycogen granules are found in abundance in intermyofibrillar spaces throughout the cell (Fig. 4). Microtubules are occasionally seen in the sarcoplasm between myofibrils (Fig. 9).

DISCUSSION

From the observations described in the foregoing paragraphs, we have constructed a model of the trabeculum of frog ventricular muscle which is reproduced in Fig. 15. The features of this muscle cell bundle may be compared with those of a similar model of mammalian cardiac muscle (3).

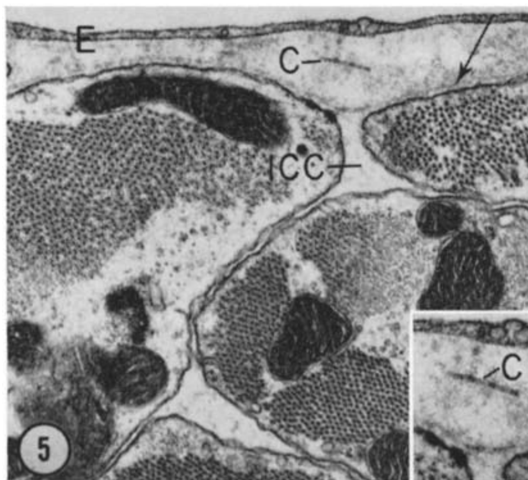


FIGURE 5 The basement membrane (arrow) of the muscle trabeculum extends over the outer surface of the muscle cells and over the junction of the intercellular clefts (*ICC*) with the subendothelial space. A collagen fibril (*C*) within the subendothelial space (insert) can be distinguished from the basement membrane by its cross-banding pattern. *E*, endothelial layer. Fig. 5, × 24,000; insert, × 35,000.

Despite the absence of capillaries, the distance for diffusion from the vascular space, in this case the cavity of the ventricle through the endothelial cell lining and its basement membrane, is not great and probably does not exceed 25 μ. This distance may be compared with the radius of the tissue element around a central capillary in mammalian heart muscle, the maximum path length for diffusion, which has been calculated as 10 μ (21).

The distribution of intercellular membranes, the small diameter of the muscle cells, the absence of transverse tubules, the paucity of sarcoplasmic reticulum, all these structural features of frog ventricular muscle are of unusual interest with respect to current theories of excitation-contraction coupling and the mechanism of relaxation in muscle. These features distinguish frog ventricular heart muscle from frog skeletal muscle and also from mammalian cardiac muscle. Since frog ventricular heart muscle has been used for many important physiological studies, notably ones on excitation-contraction coupling (9, 10), differences in the distribution of membrane systems between frog and mammalian cardiac muscle may be of unusual interest.

Some of these features have been observed previously, notably the small cell diameter (22) and the sparseness of the sarcoplasmic reticulum (23). Furthermore, Kisch (24) has noted a very similar distribution of intercellular membranes in the heart muscle of the torpedo fish. A detailed description of the ultrastructural features of frog ventricular heart muscle, including the distribution of intercellular clefts, to our knowledge, has not previously been made; nor has there been an attempt to relate these structural characteristics to excitation-contraction coupling and relaxation in this muscle.

Huxley and Taylor (5), using microelectrodes to achieve local depolarization of the cell membrane, demonstrated two-dimensional conduction of excitation toward the central axis of the muscle fiber in frog skeletal muscle. The geometry of inward spread of conduction strongly suggested that this conduction took place by way of a transverse structure in the plane of the Z line of the myofibrils. Subsequently, considerable evidence has been put forward that the membranes of the transverse tubular system, at the Z-line level of the myofibrils, are continuous with the cell membrane in fish skeletal muscle (25) and mammalian cardiac

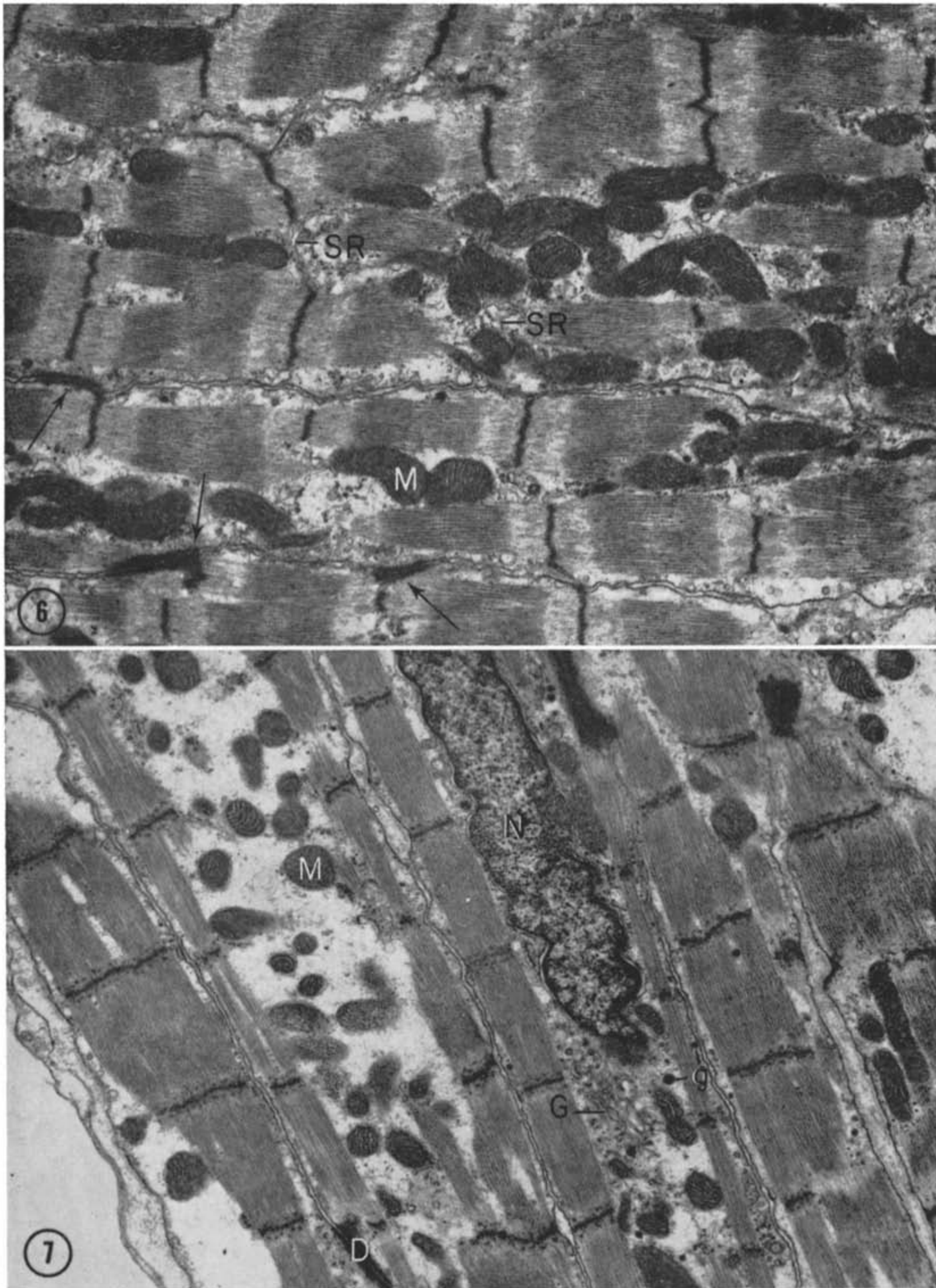


FIGURE 6 Desmosomes (arrows) occur along the intercellular clefts, often at the Z line. Here the electron-opaque material of the desmosome appears continuous with the Z-line material. Sarcoplasmic reticulum (*SR*) is scant and shows no consistent relation to sarcomere structure. *M*, mitochondria. × 13,500.

FIGURE 7 Each muscle cell typically contains two or more myofibrils separated by a sarcoplasmic core containing mitochondria (*M*) or a nucleus (*N*). A Golgi apparatus (*G*), with associated electron-opaque granules (*g*), is located near the nucleus. Desmosomes (*D*) occur along intercellular clefts. × 15,000.

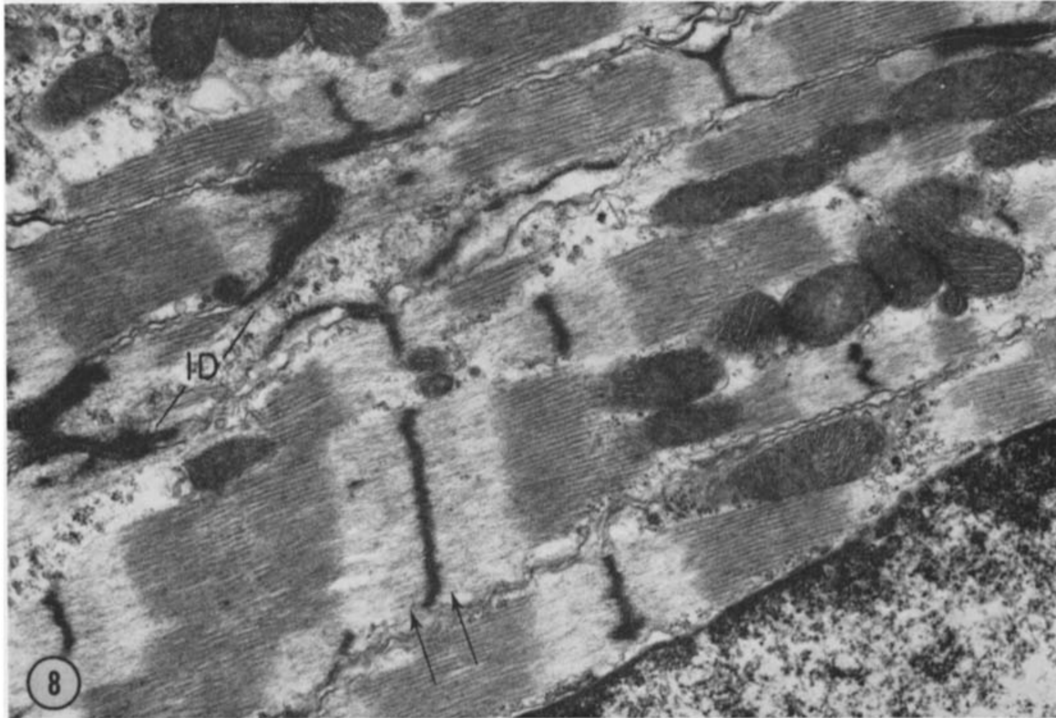


FIGURE 8 Intercalated discs (*ID*) show no associated tight junctions. Within the sarcomere, the I band may show an increased density adjacent to the Z line (arrows). *N*, nucleus. × 19,500.

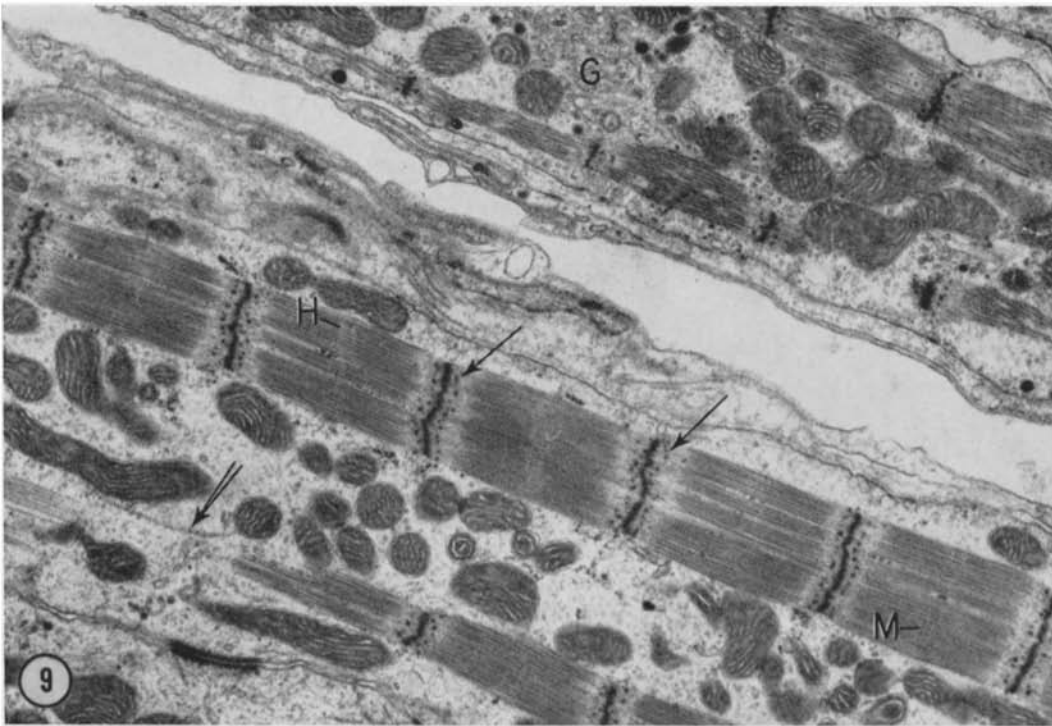
muscle (2, 3). Huxley (1), using ferritin, clearly demonstrated continuity of the lumen of the transverse tubules with the interstitial space in frog skeletal muscle.

These observations recalled earlier ones of Hill (26, 27) who, in noting the speed of transition from rest to full activity after excitation of frog skeletal muscle fibers, suggested that this transition was too rapid to be explained by diffusion of an activating substance from the cell membrane. He predicted that a process, not a substance, must bear the signal for contraction inward to the core of the fiber.

A substantial body of evidence supports the contention that an increase in the concentration of calcium ion in the myofibrillar space is the final step in the activation of contraction. Following the observation of Heilbrunn and Wiercinski (28) that injection of calcium into frog skeletal muscle cells induced contraction, the studies of Ebashi (6) and of Hasselbach and Makinose (29) revealed that the membranes of the sarcoplasmic reticulum were able to accumulate calcium and that this activity was the basis of their relaxing effect on myofibrils. Weber and her collaborators (7) showed that the contractile activity of myofibrillar preparations was controlled by calcium ion in the micromolar range of concentration (10^{-6}–10^{-7} M).

From these observations, the following picture of excitation-contraction coupling in skeletal muscle has been constructed. The effect of membrane excitation is borne inward across the muscle cell diameter by the transverse tubular system which triggers a release of calcium from the membranes of the sarcoplasmic reticulum into the myofibrillar space. Calcium saturates sites on the myofibrils to induce contraction. It is then reaccumulated by the sarcoplasmic reticulum and, as its concentration falls in the myofibrillar space, calcium is withdrawn from the myofibrils and contraction comes to an end (30).

The observations we have described in this paper suggest to us that this picture of the process of excitation-contraction coupling and activation cannot apply in every particular to frog ventricular heart muscle. The absence of transverse tubules and the paucity of sarcoplasmic reticulum in these



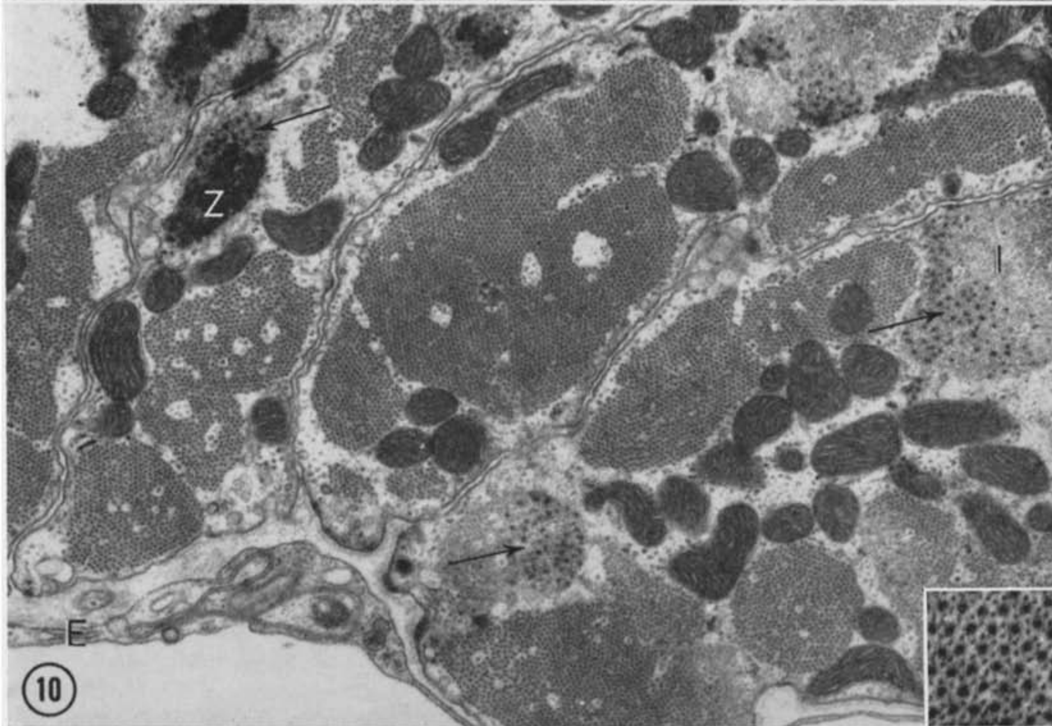


FIGURE 9 Sarcomeres show an indistinct H zone (*H*) and M line (*M*). Spherical bodies (black arrows) attached to thin filaments form rows parallel to the Z lines. Microtubules (double-stemmed arrow) are infrequently seen in the sarcoplasm. *G*, Golgi apparatus. × 13,500.

FIGURE 10 Cross-section through sarcomeres at various levels show spherical bodies (arrows) associated with Z lines (*Z*) and thin filaments in I bands (*I*). Translucent zones within myofibrils represent areas of glycogen storage. Myofibrils in cross-section (insert) show hexagonal array of thick filaments with thin filaments at triagonal points. *E*, endothelial layer. × 23,000; insert, × 82,000.

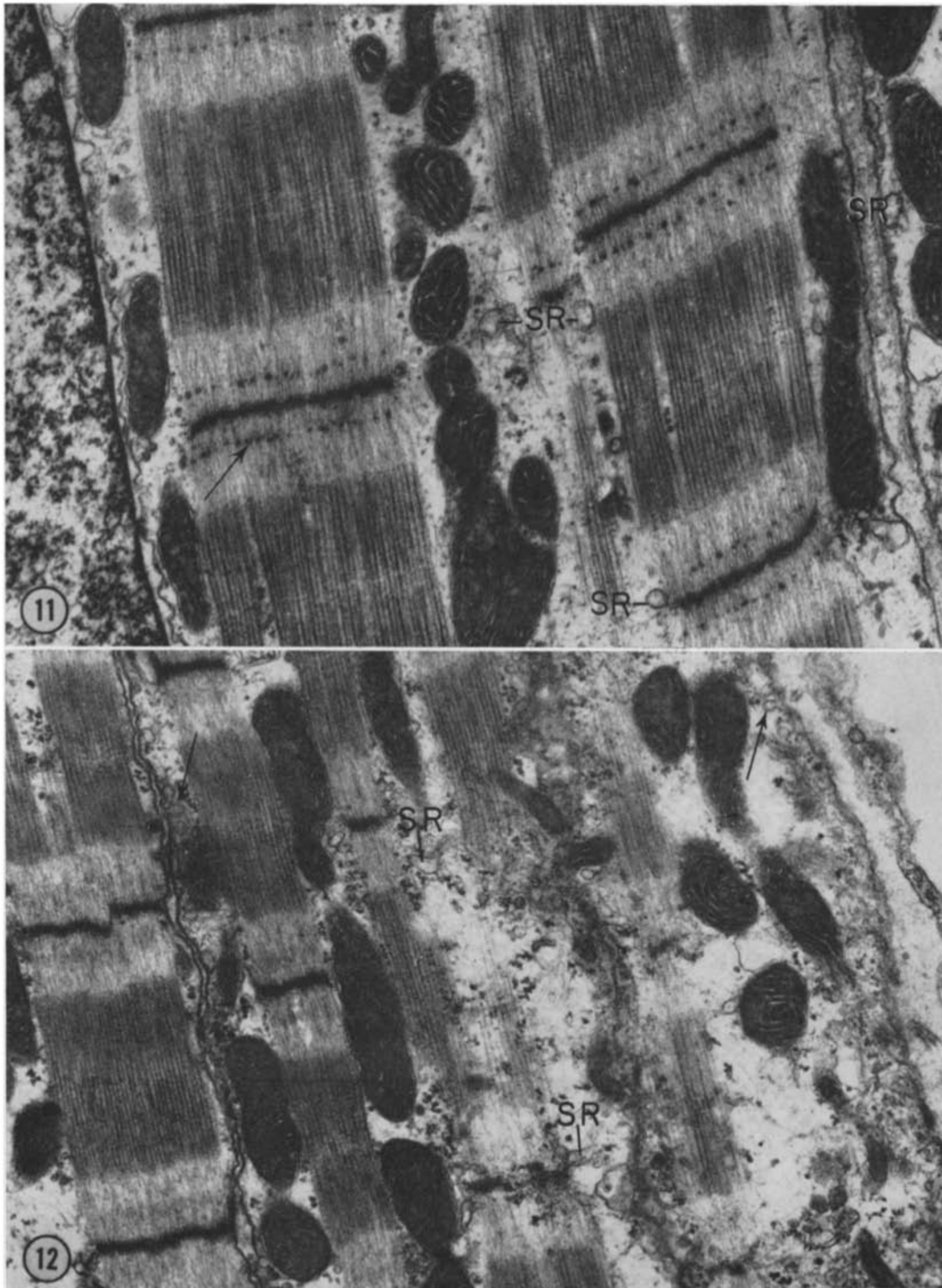
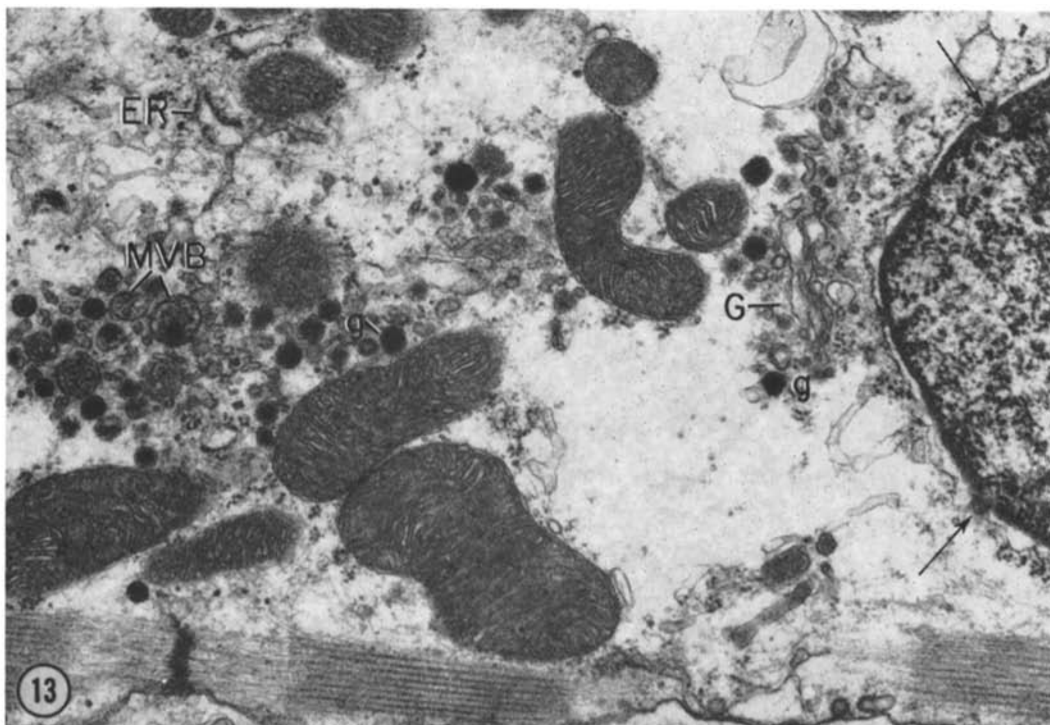


FIGURE 11 Small vesicles of sarcoplasmic reticulum (*SR*) are occasionally seen at or near Z lines or adjacent to the plasma membrane. Spherical bodies (arrow) associated with thin filaments are located 150 mμ from the Z line. The I band shows an increased density within the region between the spherical bodies and the Z line. × 23,500.

FIGURE 12 Sarcoplasmic reticulum (*SR*) is not well developed and consists of single membrane-bounded vesicles. Transverse tubules are absent. Pinocytotic vesicles (arrows) are often numerous. × 20,000.



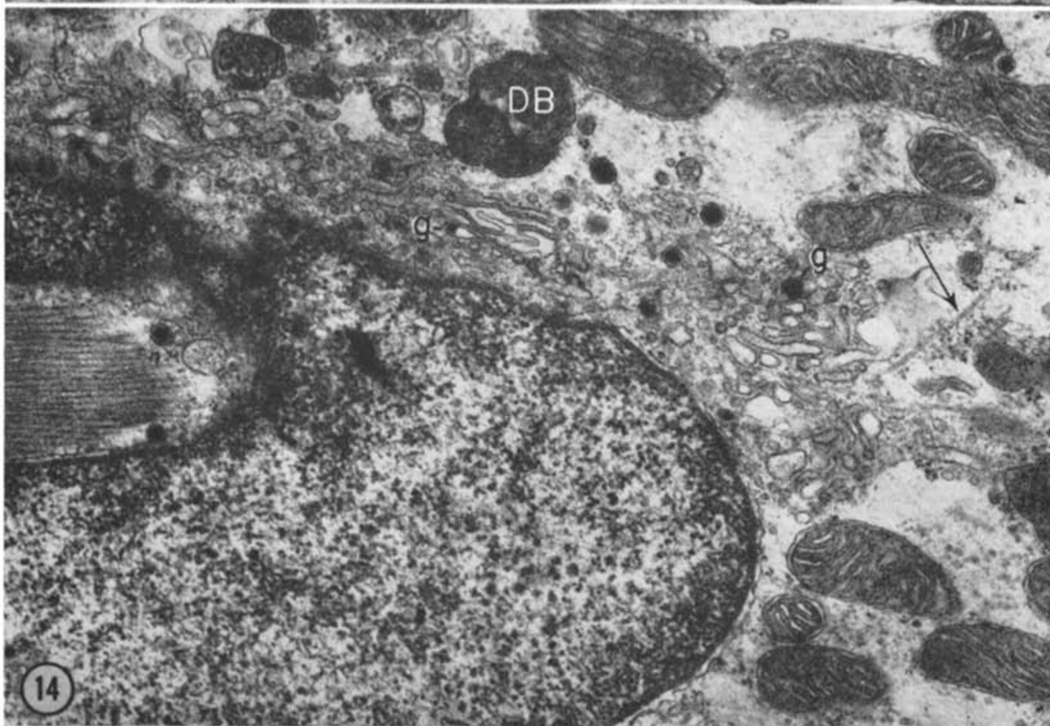


FIGURE 13 The Golgi apparatus (G), located near the nucleus, is formed of two to three parallel cisternae. Adjacent to it are numerous 1000-A electron-opaque granules (g), multivesicular bodies (MVB) and an occasional profile of rough endoplasmic reticulum (ER). Nuclear pores are prominent (arrows). × 28,000.

FIGURE 14 Dense bodies (DB) and microtubules (arrow) are found in the perinuclear area associated with the Golgi apparatus. Electron-opaque granules (g) are seen within Golgi cisternae. × 31,500.

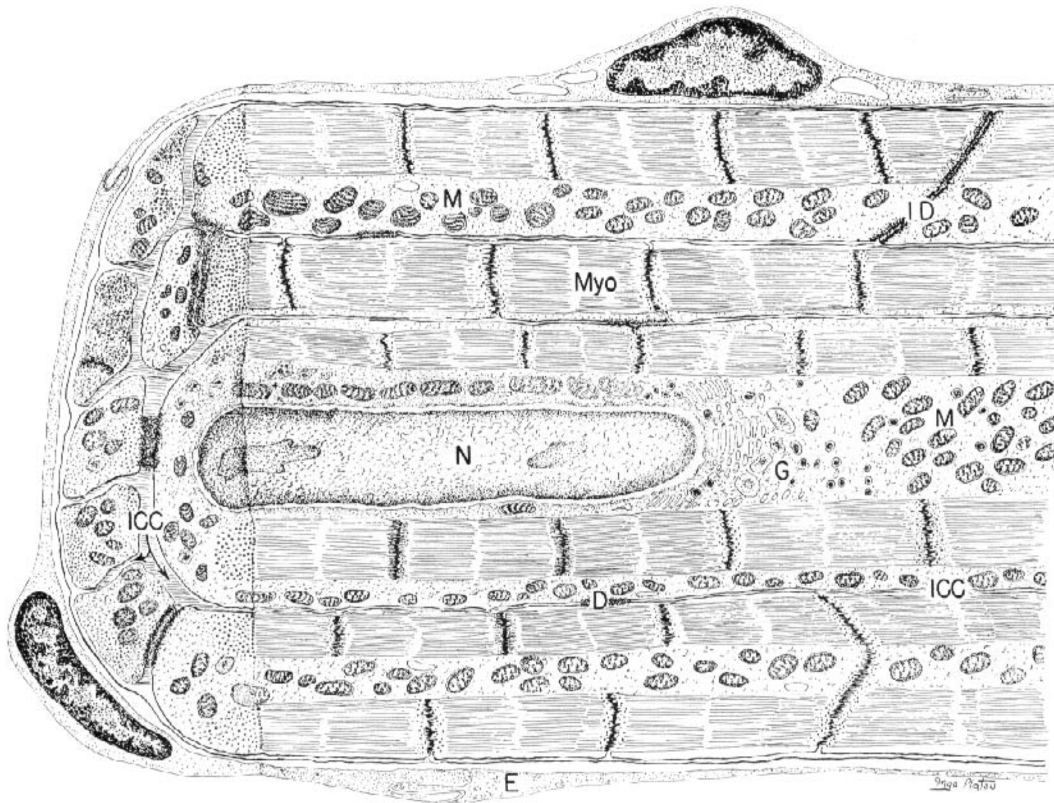


FIGURE 15 A diagrammatic model of one trabeculum of frog ventricular heart muscle with its endothelial covering (*E*) illustrates the relationship of the intercellular clefts (*ICC*) to the muscle cells. The intercellular clefts are narrow extensions of extracellular space that form inteıconnecting channels around muscle cells. Each muscle cell is approximately 5 μ in diameter and contains three to five myofibrils (*Myo*). A Golgi apparatus (*G*), associated electron-opaque granules and mitochondria (*M*) are present adjacent to the nucleus (*N*). Desmosomes (*D*) occur along the intercellular clefts frequently at the Z line.

muscle cells make it necessary to consider alternative mechanisms.

In experiments on frog ventricular muscle in which the movements of ^{45}Ca were studied, Niedergerke (9) noted the effects of contracture-producing solutions (reduced external Na concentration or increased external K concentration) on ^{45}Ca uptake and loss from the muscle cells. These contracture solutions increased calcium uptake by the cells; the strength of the contracture was related to the amount of calcium influx. When these movements were studied in the beating heart, it was found that either increased extracellular K concentration or decreased Na concentration produced both an increase in Ca influx and an increased strength of contraction (10). Both Ca uptake into and release from the cellular space were increased during activity when compared to rest. Niedergerke suggested that entry of calcium into the cell during the action potential initiated contraction in frog ventricular muscle. In mammalian cardiac muscle, on the other hand, Langer (31) has presented evidence that the inotropic effect of low extracellular sodium ion concentration is associated with movement of calcium ion from an intracellular space, presumably the sarcoplasmic reticulum.

Calculations by Sandow (32), using data of Bianchi and Shanes (33) on the amount of calcium entry into skeletal muscle cells during contraction, make it clear that the influx of extracellular calcium in these cells is much too small to account for activation of the contractile elements. Similar observations have been made and conclusions

have been drawn by Winegrad (34) in experiments with guinea pig atrial muscle. Using a different type of calculation, Winegrad concluded that the amount of calcium influx during a twitch was much too small to account for activation of contraction. Recently, on the basis of the effect of contracture-inducing solutions and the magnitude of the calcium influx during membrane depolarization, Edwards and Lorkovic (35) have renewed the suggestion of Niedergerke (10) that contraction in frog ventricle is initiated by entry of extracellular calcium into the muscle cell.

In frog ventricular muscle, Niedergerke (10) found an influx of Ca^{++} per beat of about 3×10^{-6} moles/liter of myocardium. In order to activate the myofilaments and produce contraction, the ionic calcium concentration of the sarcoplasm in the myofibrillar space must rise above that required for threshold activity, and the calcium-binding sites on the myofilaments also must be occupied to an extent greater than that necessary for threshold activity (34). Weber and Herz (36) have shown that threshold activity of skeletal myofibrils occurs at a Ca^{++} concentration of about 1×10^{-7} M and that maximum activity is reached at a concentration of about 1×10^{-6} M. Weber et al. (37) furthermore have found that, over this range of Ca^{++} concentration, bound calcium of myofibrils goes from about 0.9 to 2.2 μmoles/g of actomyosin. Although no data are available concerning the calcium-binding properties of frog cardiac myofibrils, Katz and Repke (38) have found the Ca^{++} sensitivity of dog cardiac actomyosin to be approximately the same as that of rabbit skeletal actomyosin. If we assume, therefore, that the Ca^{++} sensitivity and binding properties of frog cardiac myofibrils are roughly similar to those of skeletal myofibrils and, furthermore, that the actomyosin content of frog heart muscle is approximately the same as that of rabbit heart muscle, an estimate of the amount of added Ca^{++} needed to maximally activate resting frog cardiac myofibrils may be made.

A liter of frog ventricular myocardium contains about 650 ml of intrafiber water (9) and, if the actomyosin concentration is approximately the same as that of rabbit myocardium, 56 g of actomyosin (39). When the myofibrils go from the inactive state to maximum activity, an increase of 0.6 μmoles of free Ca^{++} and 72.8 μmoles of calcium bound to actomyosin per liter of myocardium would be required. The amount of calcium required to induce maximal activity (73.4 μmoles/liter) is far in excess of the amount Niedergerke found to be the maximum influx per contraction (3 μmoles/liter).

These calculations make it seem unlikely that enough calcium enters the frog cardiac muscle cell to activate the contractile elements in each contraction. Alternatively, calcium may be released by membrane depolarization from the inner surface of the cell membrane, including the membranes of the intercellular clefts, may diffuse to the sites of action on the contractile proteins and may return to the cell membrane in relaxation. A mechanism such as this one, indeed, has been suggested by Niedergerke (9).

Hill (26, 27) considered activation on the basis of a process of diffusion from the cell membrane. His calculations, based on the diffusion constant of calcium or a similar substance, the time for diffusion (the time from excitation to the peak of activation) and the radius of diffusion (the radius of the muscle fiber), indicated that in skeletal muscle cells the time-distance relationships ruled out diffusion from the cell membrane as the basis of activation. We have carried out similar calculations on frog ventricular muscle. If activation is as rapid in onset in this muscle as in frog skeletal muscle, then the time for diffusion of an activating substance would be 15 msec (27). At a maximum cell diameter of 5 μ (compared to 100 μ for frog skeletal muscle), ample time for diffusion of calcium from the cell membrane is available for full activation to take place by this mechanism.

It would appear, thus, that important differences exist between the mechanism of activation of contractile elements in frog ventricular cardiac muscle, on the one hand, and that in frog and mammalian skeletal muscle and mammalian cardiac muscle, on the other. While in both groups, undoubtedly, variation in calcium concentration in the myofibrillar space is the immediate means of activation and relaxation of the contractile elements, the site of release and reaccumulation of activating calcium differs between the two groups of muscle types. In frog heart muscle, the activating calcium apparently comes from the cell membrane and returns, in relaxation, to the cell membrane location. In skeletal muscle and in mamalian cardiac muscle, the activating calcium is released from an intracellular compartment and is reaccumulated by this compartment in relaxation. Considerable evidence has been advanced

that this compartment is the sarcoplasmic reticulum (8, 30) and that the stimulus of excitation is borne to it by the transverse tubular system (5, 40).

The absence of transverse tubules in frog ventricular heart muscle, the paucity of sarcoplasmic reticulum, the distribution and continuities of the intercellular clefts, and the small cell diameter all favor a mechanism of activation in which a flux of calcium from a superficial site such as the cell membrane is the activating stimulus for contraction and in which a return of calcium to the cell membrane is followed by relaxation. If this formulation is correct, isolated preparations of cell membrane from frog ventricular muscle might be expected to possess some of the properties of isolated preparations of sarcoplasmic reticulum (vesicular relaxing factor) of skeletal muscle. Notably such preparations may have the ability (*a*) to inhibit contraction of isolated myofibrils, (*b*) to inhibit the ATPase activity of myofibrils, and (*c*) to accumulate calcium in the presence of ATP (30). Studies of such activity may be of some interest.

It should be pointed out that Niedergerke (9) cited unpublished observations made by Huxley and himself, using electron microscopy, in which the absence of a highly developed sarcoplasmic reticulum in frog ventricular muscle was noted.

The intercellular clefts are true cell boundaries. They form uninterrupted boundaries between adjacent cells. The absence of continuous and well-formed basement membranes, the frequent occurrence of desmosomes along their course, and the narrowness of the space within the cleft suggest that these boundaries, like the intercalated discs, may be low resistance spaces across which excitatory impulses readily travel (17).

It must be noted, however, that we have seen no true tight junctions, regions in which the opposing cell membranes are fused along their outer lamellae, in the intercellular clefts of frog heart muscle. Tight junctions (nexuses) have been described in the frog atrium after permanganate fixation (17). These structures are apparently quite labile and easily disrupted by hypertonic solutions (17). Baldwin (41) found very occasional tight junctions in frog atrium after glutaraldehyde fixation; Revel et al. (42) noted 20-A gap junctions in mammalian heart muscle, liver, and smooth muscle, also after glutaraldehyde fixation. It seems unlikely, therefore, that the absence of tight junctions in the frog ventricular muscle we examined in this study is due to a fixation artifact.

As we noted earlier, the heart muscle of torpedo fish (24) bears a striking resemblance to frog ventricular muscle. Boa constrictor heart muscle, described by Leak (43), resembles frog ventricle in that it shows the presence of intercellular clefts, small cell diameters, and a sparsity of sarcoplasmic reticulum. On the other hand, heart muscle cells of the mantis shrimp have well-developed transverse tubules communicating with the interstitial space and an abundant sarcoplasmic reticulum surrounding each myofibril (44). Toad heart muscle, in most respects, also resembles frog heart muscle (45, 46).

We can do no more than speculate on the significance of the dense bodies on the thin filaments (Figs. 9 and 10). These bodies are inconstantly present. Perhaps they are an exaggerated equivalent of the N line irregularly seen in the middle of the I band in skeletal and mammalian cardiac muscle. The significance of the N line is similarly obscure. Gillis and Page (47) noted in skeletal muscle that the position of the N line relative to the Z line varies with sarcomere length and may be caught up in the A band in contracted sarcomeres. In their preparations, I band ATPase activity tended to be localized along the N line. Are these bodies an accumulation of tropomyosin, known to be associated with actin and the thin filaments (48)?

The electron-opaque granules (neurosecretory-like granules) observed in the Golgi region, in these frog ventricular muscle cells closely resemble in structure similar granules found in atrial but not ventricular muscle cells of a variety of mammals (49) and in the ventricular muscle of toads (46) and cyclostomes (50). These granules are very similar in appearance to the large, dense-core vesicles which are found in adrenal medulla (51), peripheral autonomic nerves (52), and in the central nervous system (53) and which are thought to contain norepinephrine. In cyclostome hearts, Bloom has equated these "specific granular bodies" with chromaffin-positive, presumably catecholamine-containing granules seen by light microscopic examination of ventricular muscle (50). In rat atrium (49) similar but larger granules failed to give a positive chromaffin reaction or to incorporate dopamine-^{3}H. The catecholamine content of these granules may be further investi-

gated by studying their uptake of tritium-labeled catecholamine or catecholamine precursors and subsequent depletion of their catecholamine content by administration of reserpine or alphamethyl metatyrosine.

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