ELECTRON MICROSCOPE STUDY OF MAMMALIAN CARDIAC MUSCLE CELLS*

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PLATES 77 TO 84

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Since the first attempts to study heart muscle structures with the electron microscope, continuous progress has been made in the development of improved preparation technics and consequently in the knowledge of this tissue (1–6). However, more recent electron micrographs in papers on cardiac histology still reveal technical imperfections (7–14). They are evident in derangement of wide tissue areas and loss of structural details. Controversial interpretations of fibrillar and mitochondrial structures must therefore be due in part to unequal tissue preservation and should be clarified with the improved methods described in an accompanying article (15). Moreover, the morphology of components involved in the conduction of excitation should be studied and compared with skeletal muscle. Conduction, which remains restricted to the stimulated fiber in skeletal muscle, passes from cell to cell in heart muscle. The heart muscle cells show a certain autonomy of impulse production and are regulated by potentials arising from the pacemaker. The question thus arises whether the cell membranes which propagate action potentials along their surfaces and the endoplasmic reticulum regarded as the probable mechanism of conduction to the cell interior (16–19) differ from skeletal muscles.

Materials

Blocks of heart tissue from dogs, rats, and mice were fixed for ½ hour in buffered 1 per cent osmium tetroxide containing sucrose (0.34 total osmolarity), rapidly dehydrated through graded ethyl alcohol solutions and acetone, and were embedded according to the procedure described in the preceding paper (15).[1]

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[1] Except for embedding, the materials were prepared according to procedures currently used in the Cytology Laboratory at The Rockefeller Institute.

OBSERVATIONS

The contractile material of the heart muscle cell is separated by chains of mitochondria and further subdivided by longitudinally arranged elements of the endoplasmic reticulum (Fig. 1). What is usually called the myofibril is part of a synfibrillar system irregularly split into branches composed of 200 to 1,000 myofilaments. Fibril diameters of less than 0.5 to more than 2 μ are observed in longitudinal (Figs. 1, 7, 8, 9 *b*, and 11) and cross-sections (Fig. 2). All classical bands of skeletal muscle are visible in heart muscle and their appearance depends on the state of contraction (Z, I, and A bands in Figs. 1, 5, 7; H in Figs. 1, 8, 11; M in Figs. 4 and 7). In cross-sections the filaments in the A band are arranged in rows, which in many places are separated by a wider distance than are the individual filaments forming the rows. This appears not to result from sectioning since the rows run in different directions in the same section (Figs. 2 and 3). The two-dimensional packing of the filaments is sometimes square and sometimes hexagonal with an average center to center distance after preparation shrinkage of 280 to 300 A. The diameter of filaments in the A band is 100 to 150 A. If the plane of section makes an angle of about 30° with the filaments, subperiods of 230 A distance can be seen (Fig. 4) in the region of the M, H, and A bands, due to the presence of periodically distributed material between the A filaments.

The mitochondria lying between the fibrils are sometimes arranged without apparent respect to the sarcomeres (Figs. 1, 5, 8, and 11) and sometimes in conjugate positions on either side of Z bands (Figs. 7, 8, and 11). They measure from 0.3 to 1.7 μ in length and from 0.2 to 1 μ in width. A double outer membrane is regularly visible, if the angle between the sectioning plane and the mitochondrial surface is close to 90°. The inner double membranes or cristae are either continuous with the inner layer of the outer membrane or separate. In general these inner membranes are oriented at 90° to the direction of the fibrils but deviations are frequent and even concentric courses have been observed (Fig. 11 *a*). Small granules appear in some mitochondria between the inner double membranes; *i.e.*, within the matrix of the organelle (Figs. 8 and 11). One to three rows of mitochondria are frequently seen in the micrographs beneath the scalloped sarcolemma (Figs. 1, 8, and 11). Lipide bodies are occasionally located contiguous to the mitochondria (Figs. 1, 7, 11, and 12).

The dense inner layer of the sarcolemma, the plasma membrane, forms together with the plasma membrane of the adjacent cell and a regular interspace (130 to 150 A), the intercalated disc (Figs. 3 and 12). The outer layer of the sarcolemma is less opaque than the plasma membrane and separated again by an interspace of further decreased opacity. Along intercellular margins of the discs there is a dense substance which seems to fasten the terminating con-

tractile material to each of the two inner sides of the interdigitated plasma membranes (Fig. 5). If the interdigitation is cut transversely, isolated ring-shaped and contacting polygonal double lines are seen (Fig. 6). Mitochondria closely approach the intercalated discs. The endoplasmic reticulum, continuing from the adjacent Z bands, contacts the plasma membranes between the terminating contractile material. No structures pass the discs which are, therefore, considered as cell borders.

The endoplasmic reticulum, which surrounds the sarcomeres at the level of the Z bands and sometimes at the level of the M bands, is attached at these points to the sarcolemma (Figs. 1, 8, and 11). Frequently the reticulum is seen in close peripheral contact with the Z bands, possibly immersing digit-like protrusions into the Z bands between filaments (Fig. 8). In the vicinity of the nucleus, tubular branches of the endoplasmic reticulum approach the outer (cytoplasmic) membrane of the nucleus and even establish continuity with this membrane (Figs. 7, 9, and 10). The zones of attachment appear to form two left-hand spirals around the nucleus at the level of the Z and M bands in Figs. 7 and 9. An accumulation of endoplasmic reticulum at one of the nuclear poles which may represent the Golgi element was occasionally observed (Fig. 7). Pores in the nuclear double membrane are sparse.

DISCUSSION

Opinions concerning the syncytial or cellular architecture of the heart muscle have fluctuated for many decades (for literature see Häggqvist, 1931) and still are controversial (19). The well established electron microscopic observations of the intercalated discs and their interpretation as cell borders by van Breemen (6); Sjöstrand and Andersson (8), Poche and Lindner (12), and Price and coworkers (13), are in favor of a cellular arrangement. Even though cells have not been visualized totally in serial sections and reconstructed in three dimensions, the fact that fibrils and all other structures terminate at the intercalated discs and that these discs appear as adjoining plasma membranes seems to be sufficient proof for the cellular nature of heart muscle. The older concept that fibrils end only at the tips of papillary muscles and at the annulus fibrosus, and that the heart, for this reason, represents a huge syncytium is erroneous. However, another argument in favor of the syncytial nature of the heart muscle is still debated. Häggqvist (19) demonstrated intercalated discs on both sides of endoplasmic regions around nuclei of human papillary muscle and concluded that they could not represent cell borders since the endoplasmic area is common for adjacent segments separated by discs. Whether this conclusion from light micrographs is correct or not remains doubtful if, as in Fig. 7, the sarcolemma passes close to the endoplasmic region or is in contact with the nuclear area.

Dead ends of intercalated discs, which should be expected according to Häggqvist's interpretation, at the borderline of meso- and endoplasma are not found in our material nor are they demonstrated by others. Furthermore, Sjöstrand, as remarked in a footnote in Häggqvist's article, has seen discs passing endoplasmic areas, and Fig. 12 of this paper demonstrates a disc approaching a nucleus, then abruptly changing its direction and turning into the sarcolemmata of adjacent cells. We, therefore, consider the cellular nature of the heart muscle to be well established.

The unusual anatomical intimacy of the cells of heart muscle provides, no doubt, adequate means for the transmission of impulses arising from the pacemaker.

The heart muscle cells, like all others, expose different cell surfaces to adjacent cells of the same type and to the interstitial space. They join, mutually, normal to the direction of the fibrils by contact of two interdigitated plasma membranes while a double layer consisting of this plasma membrane and a "basement membrane" runs longitudinally as sarcolemma and separates cells and interstitium. The interdigitation and the arrangement of the cell interior on both sides of the discs resemble to a high degree the conditions at the muscle tendon junction of skeletal muscle fibers. That is, the fibrils end in a denser material, the inner cementing substance of Poche and Lindner (12). The endoplasmic reticulum continues from the last Z band to the inner surface of the cell border (Edwards and coworkers (16); Ruska (18)). Sections almost parallel with the discs have shown the outlines of the plasma membranes in agreement with the scheme given by Poche and Lindner. The principal differences between cardiac cell junctions and myotendon junctions are that in the latter all sarcolemmic layers are present and the interdigitation is with collagen fibrils.

The cell content resembles very closely the continuously working red skeletal muscles, especially in the abundance of mitochondria, the longitudinal and transverse distribution of the endoplasmic reticulum, its contact with the plasma membrane at the level of the Z and M bands, and its participation in the formation of the Z band (see Porter (17); Bennett (20); and Moore, Ruska, and Copenhaver (21)). The endoplasmic reticulum appears connected with the outer nuclear membrane by numerous branches in a spiral arrangement (Figs. 7 and 9). According to Forster (22, 23) and Heubner (24) the nuclei make left or right turning spiral forms during contraction. Amorim (25) was able to demonstrate a spiral line on the nuclear surface, using the uranium silver technic of Cajal, and his figures show a close correspondence of this line with the muscle striation. The heart muscle nucleus thus has the same connections with the contractile elements of the cell and the cell border by way of the endoplasmic reticulum as the nucleus of skeletal fibers has with a given nuclear area in the

plasmodial fiber. Furthermore, the Z bands of heart muscle (Aurell and Wohlfart (26); and Aurell (27)) like those of skeletal muscles (Aurell and Wohlfart (28); Tiegs (29); and Engelhardt (30)) follow a helicoidal course throughout the cell. This is indicated by the out-of-phase disposition of Z bands and nuclear indentations of Figs. 7 and 9 and is even more convincingly established by the literature cited. The spiral organization therefore seems to be a feature of the total cell which defines the spiral course of the contraction (Matthaei and Tiegs (31)).

The synfibrillar contractile material is split into bundles of very different width (Weinstein (9)) in contrast to fibrillar skeletal muscle and the fibrils of high frequency muscles in insects. The banding of the heart muscle sarcomere corresponds with the banding of skeletal muscle.

Contact with the cytoplasmic matrix is common to one surface of the sarcolemma, intercalated discs, and endoplasmic reticulum. Their other surfaces are exposed to the fluid phase of the sarcolemmic interspace, the disc interspace, and to the fluid phase inside the endoplasmic reticulum, respectively. As a consequence of this morphological arrangement the endoplasmic reticulum probably carries a membrane potential as does the plasma membrane. If the endoplasmic reticulum opened through the plasma membrane and thus had contact to interstitial fluid such a membrane potential would indeed be likely, because in this case the electrolytic properties of the fluid in the endoplasmic reticulum would be the same as those outside the cell. The propagation of a stimulus into the contractile material would then be easily explained, as well as the data of Katz (32) which indicate that the electrical capacity in μfd./cm.^{2} of muscle fibers is some 4 to 6 times greater than that for nerve fibers. The increased membrane surface afforded by the endoplasmic reticulum in the muscle could account for this difference.

SUMMARY

The cellular theory of heart muscle is supported by a detailed description of the intercalary discs. The discs are adjacent plasma membranes separated by an interspace while the sarcolemma appears as plasma membrane, interspace plus basement membrane of the interstitium. The nucleus of the cell is closely associated with the entire cell by way of the endoplasmic reticulum. Transversely it connects the outer nuclear membrane at the level of the Z and M bands with the contractile material and the sarcolemma. Longitudinally it connects the outer nuclear membrane with the plasmalemma at the intercalated discs. The description of the spiral attachment of the endoplasmic reticulum on the outer nuclear membrane supplements earlier observations on the helicoidal structure

of the heart muscle cell. Plasma membranes and endoplasmic reticulum are considered to be carriers of membrane potentials and to conduct excitation.

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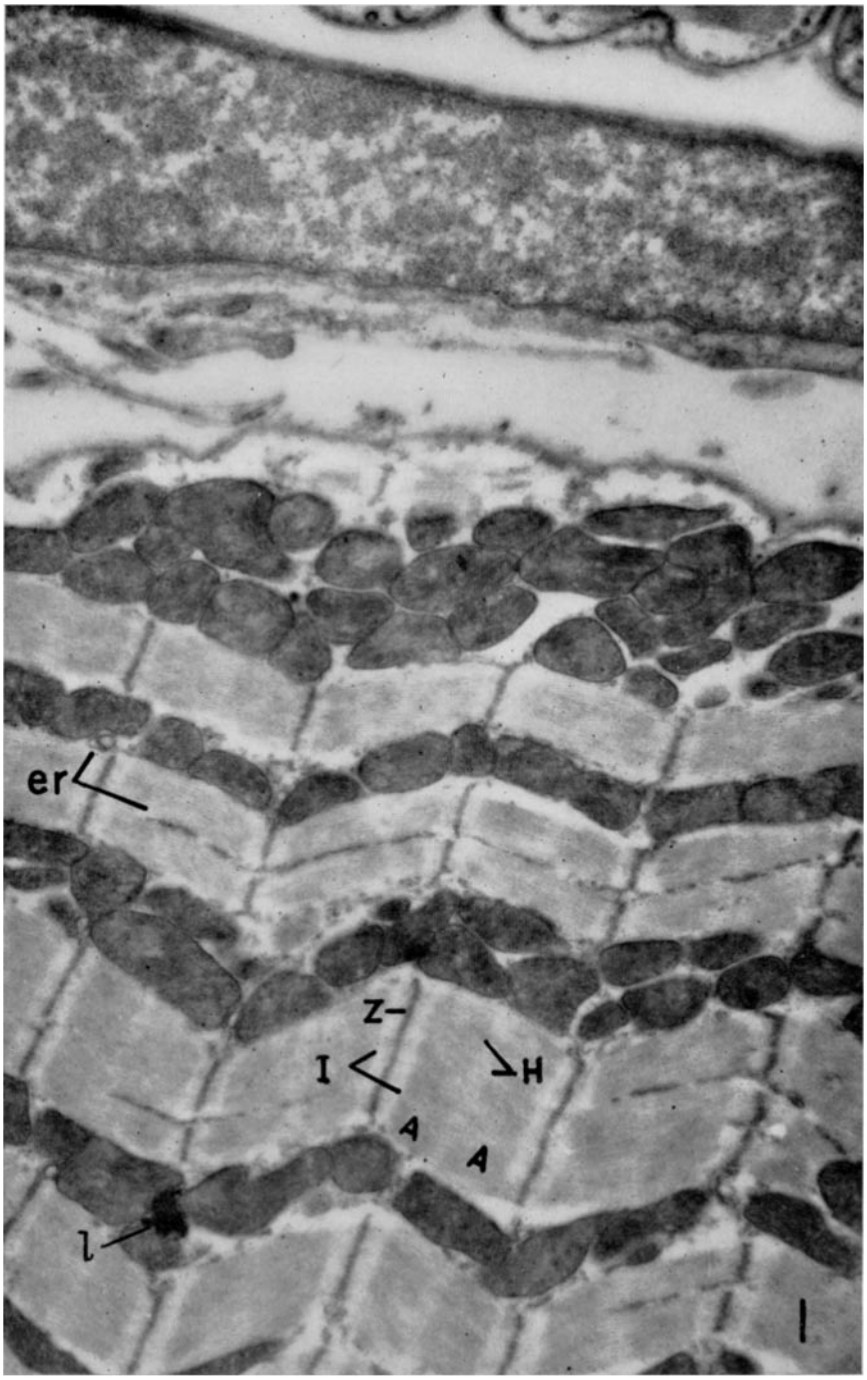
EXPLANATION OF PLATES

PLATE 77

FIG. 1. Ventricular heart muscle of rat sectioned longitudinally. Nucleus of an interstitial cell with traces of surrounding cytoplasm and some connective tissue fibrils is bordered by slightly scalloped sarcolemmata. Numerous mitochondria lie between the fibrils and close to the sarcolemma. The fibrils are further irregularly separated by longitudinally oriented endoplasmic reticulum (*er*). Tubules of endoplasmic reticulum are frequently aligned with the Z band (*z*) and extend to the indentations of the sarcolemma (compare Figs. 7, 8, and 11). The fibrils are not contracted. Isotropic (I) and anisotropic (A) bands can be easily seen and the H band can be differentiated in some places. A dense lipide body (*l*) appears at the lower left between three mitochondria. × 15,500.



(Moore and Ruska: Mammalian cardiac muscle cells)

PLATE 78

FIG. 2. Cross-section of ventricular heart muscle of dog. Notice the different packing of myofilaments. The inner membranes of some of the mitochondria are scarcely visible since cross-sections through the contractile material run mainly parallel with the general course of the inner mitochondrial double membranes. Endoplasmic reticulum is cut transversely beneath the sarcolemma (at top) and between myofibrils. At the lower right it is cut longitudinally. × 40,000.

FIG. 3. Ventricular heart muscle of dog showing continuity of an intercalated disc with the dense inner layer of the sarcolemma. The transversely sectioned disc, composed of two adjacent plasma membranes and their interspace, runs here, longitudinally with the contractile material. × 43,000.

FIG. 4. Oblique section of the same muscle. With a knowledge of the sarcomere length in longitudinal sections this section is calculated to be cut at an angle of about 30° to the course of the myofilaments. Notice the subperiod pattern in A and its absence in I, indicating the absence of a fibrillar component in the isotropic band. × 43,000.

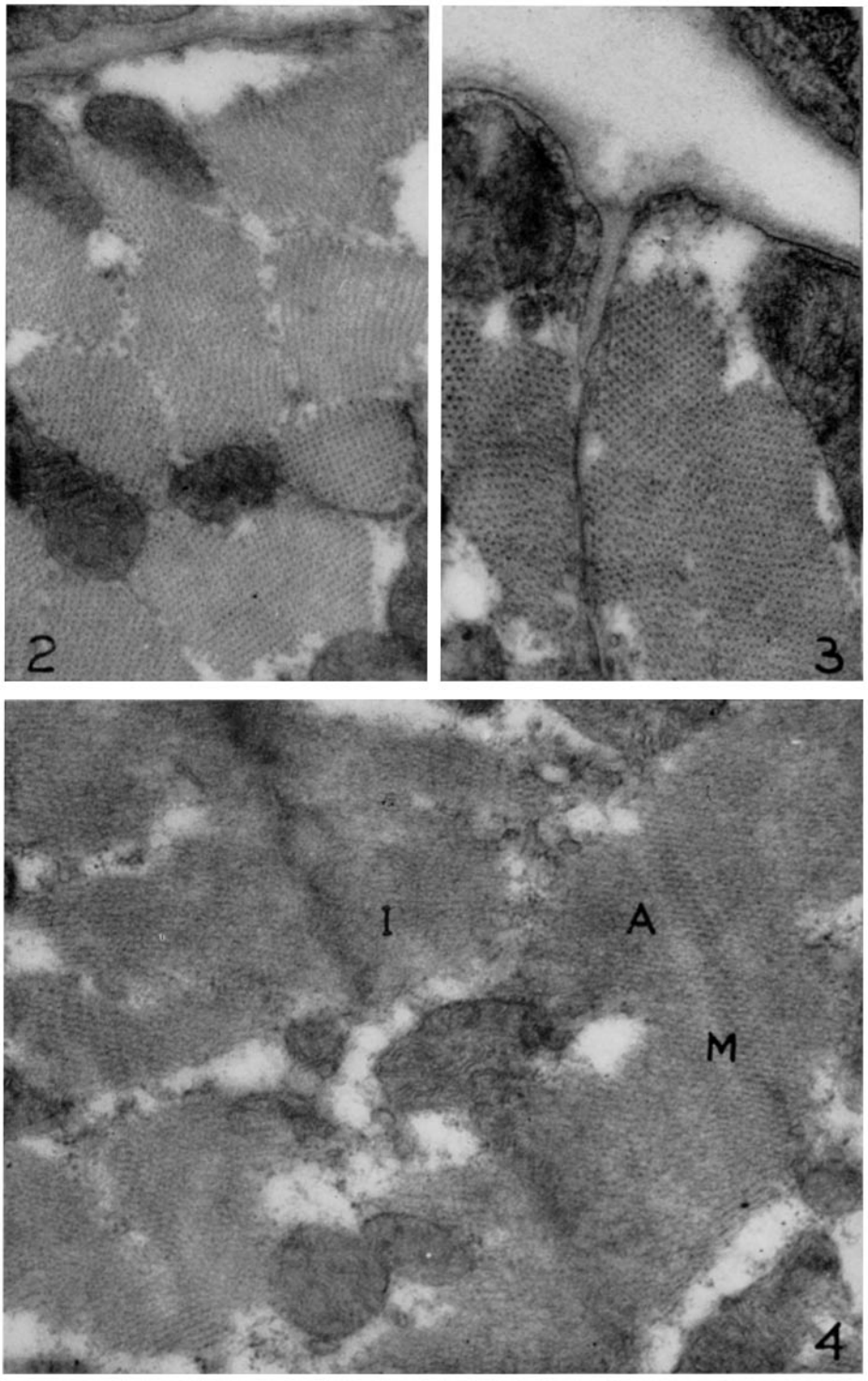


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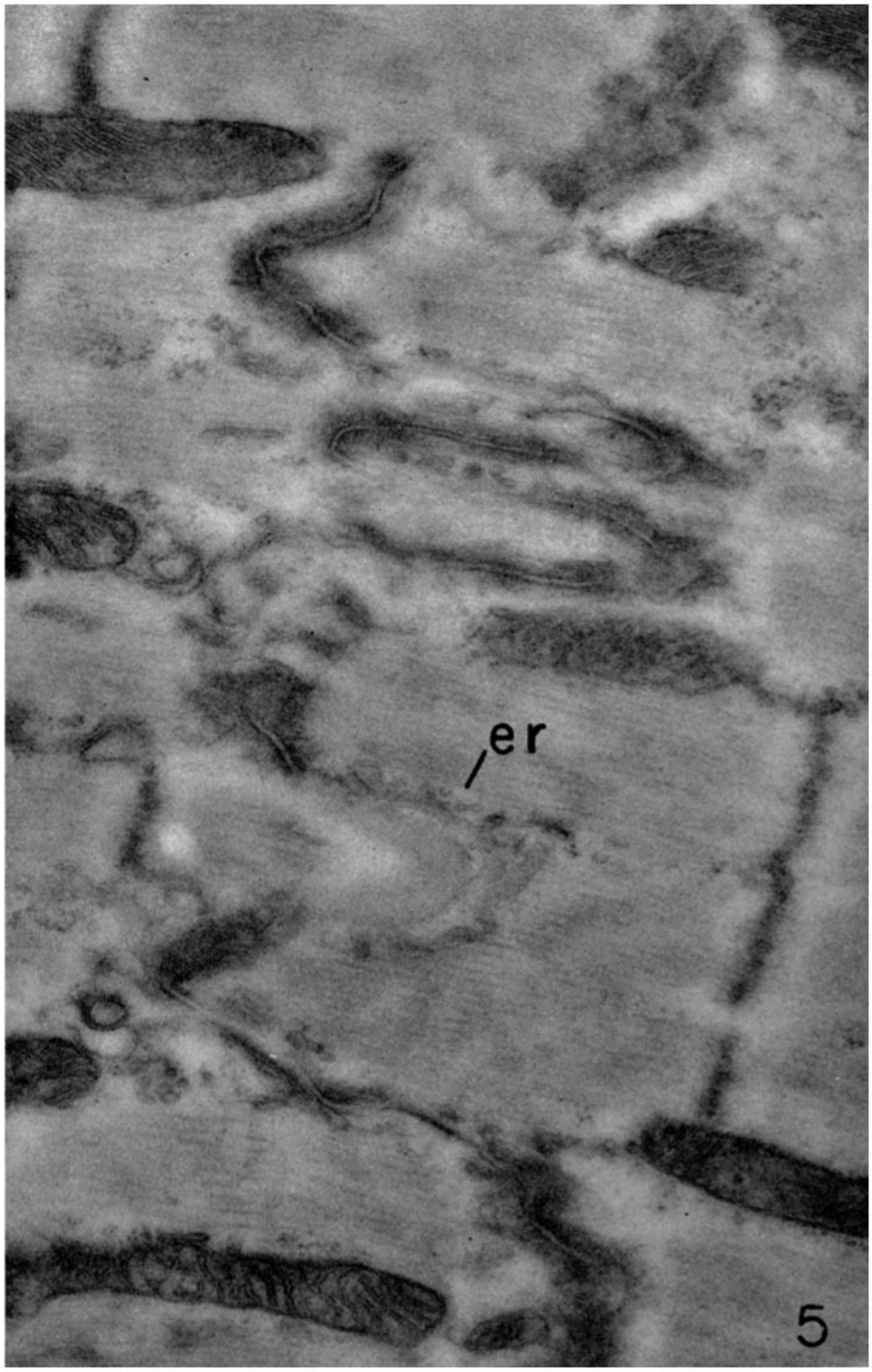
PLATE 79

FIG. 5. Ventricular heart muscle of rat cut longitudinally. An intercalated disc alternates between two Z band levels. Note the clear interspace separating the two interdigitated plasma membranes. Its width and the apparent contrast of the plasma membranes vary with the angle at which the structure is cut. Endoplasmic reticulum (*er*) extends to the cell borders and a dense substance interposes between myofibrils and membranes. × 43,000.

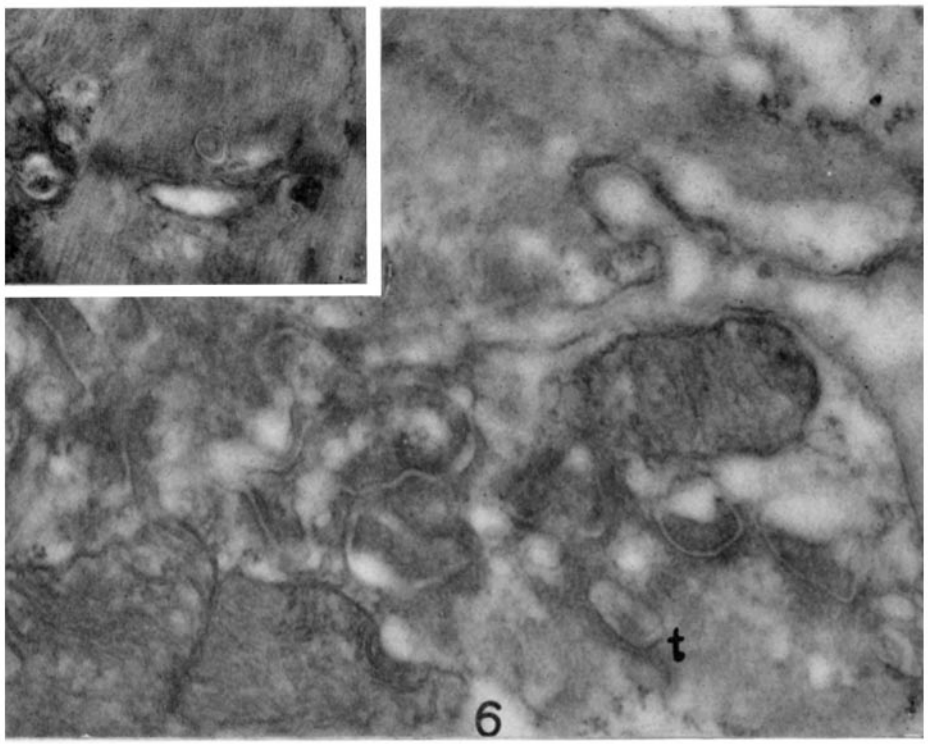


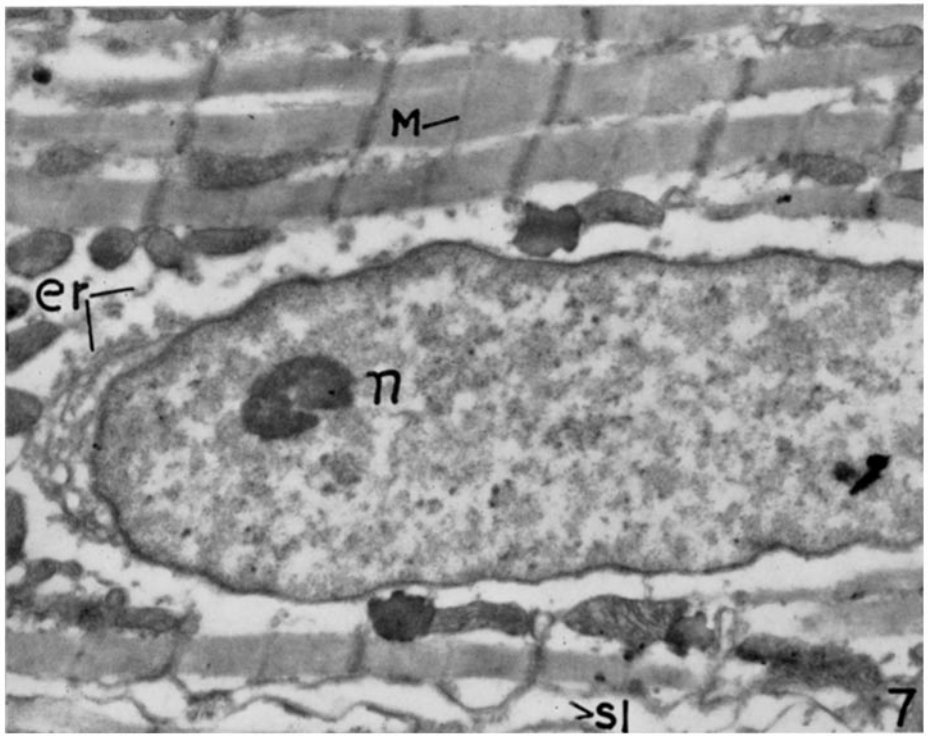
(Moore and Ruska: Mammalian cardiac muscle cells)

PLATE 80

FIG. 6. Ventricular heart muscle of rat showing oblique section of an intercalated disc. Area contains isolated double membraned tips of interdigitations; one tangentially cut shows only one membrane at (*t*). The insert shows another isolated tip. × 43,000.

FIG. 7. Nuclear area of ventricular rat heart muscle. The peripherally located nucleus with nucleolus (*n*) is separated from the sarcolemma (*sl*) by only one myofibril. Endoplasmic reticulum (*er*) is in evidence at the nuclear pole. Two mitochondria and two lipide bodies lie in helocoidal arrangement on both sides of the nucleus. The reticulum approaches the outer nuclear membrane at the level of the M and Z bands. × 15,500.

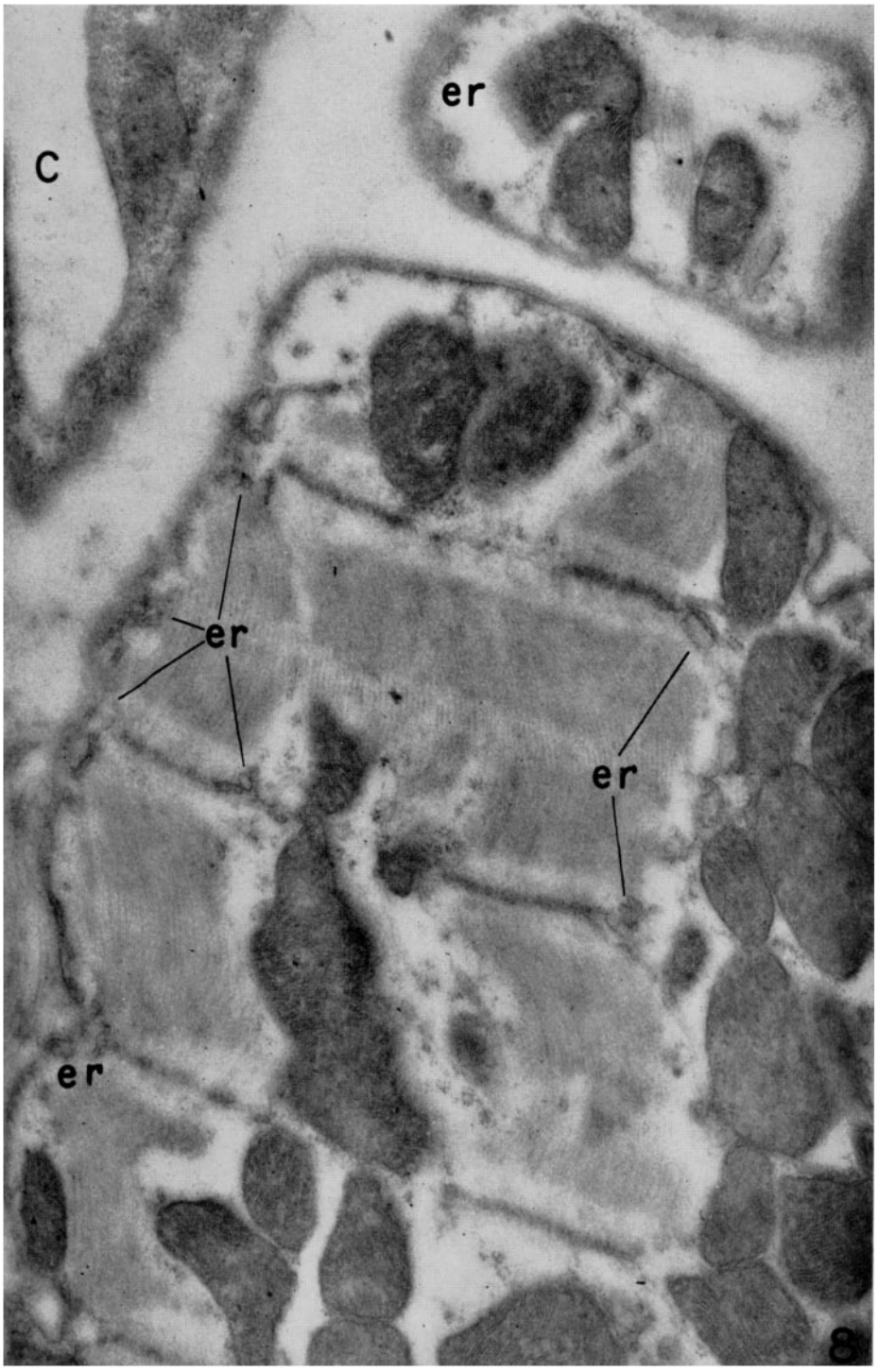




(Moore and Ruska: Mammalian cardiac muscle cells)

PLATE 81

FIG. 8. Ventricular heart muscle of rat cut longitudinally. Note the accumulation of endoplasmic reticulum (*er*) near the sarcolemma at the level of the Z bands and its close relation to the periphery of the Z band. Connective tissue fibrils lie outside the sarcolemma. Many mitochondria are cut at different levels and at different orientations. Some show the inner double membranes very distinctly. Others appear almost without structure since they are cut parallel to the plane of the inner membrane system. The same applies to the outer double membrane. In the mitochondrial matrix are very small dark granules. Capillary (*C*) is at the upper left and a secantly cut extension of the sarcolemma containing two mitochondria, peripheral endoplasmic reticulum, and some myofilaments is at the upper right. × 27,000.

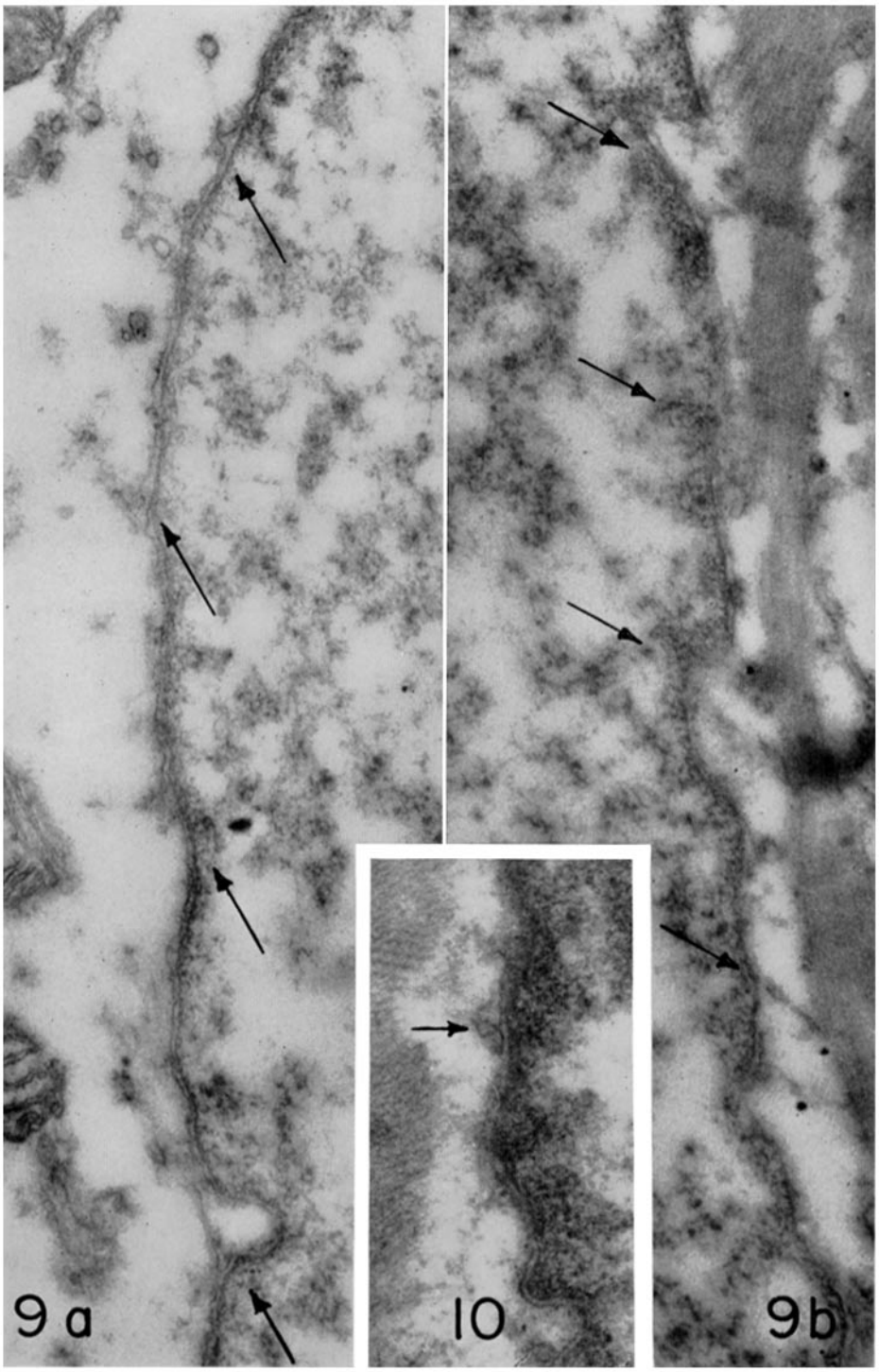


(Moore and Ruska: Mammalian cardiac muscle cells)

PLATE 82

FIG. 9. Rat heart muscle cut longitudinally. Two sides, 9 *a* and 9 *b*, of the same nucleus with double nuclear membrane. The outer membrane seems to be connected with the endoplasmic reticulum. Note the constant distance between points of attachment of half sarcomere lengths (arrows) and the oblique arrangement suggesting a left-hand spiral around the nucleus as in Fig. 7. × 43,000.

FIG. 10. Border area of another heart muscle nucleus. The outer membrane is folded into the cytoplasm (arrow). × 53,500.

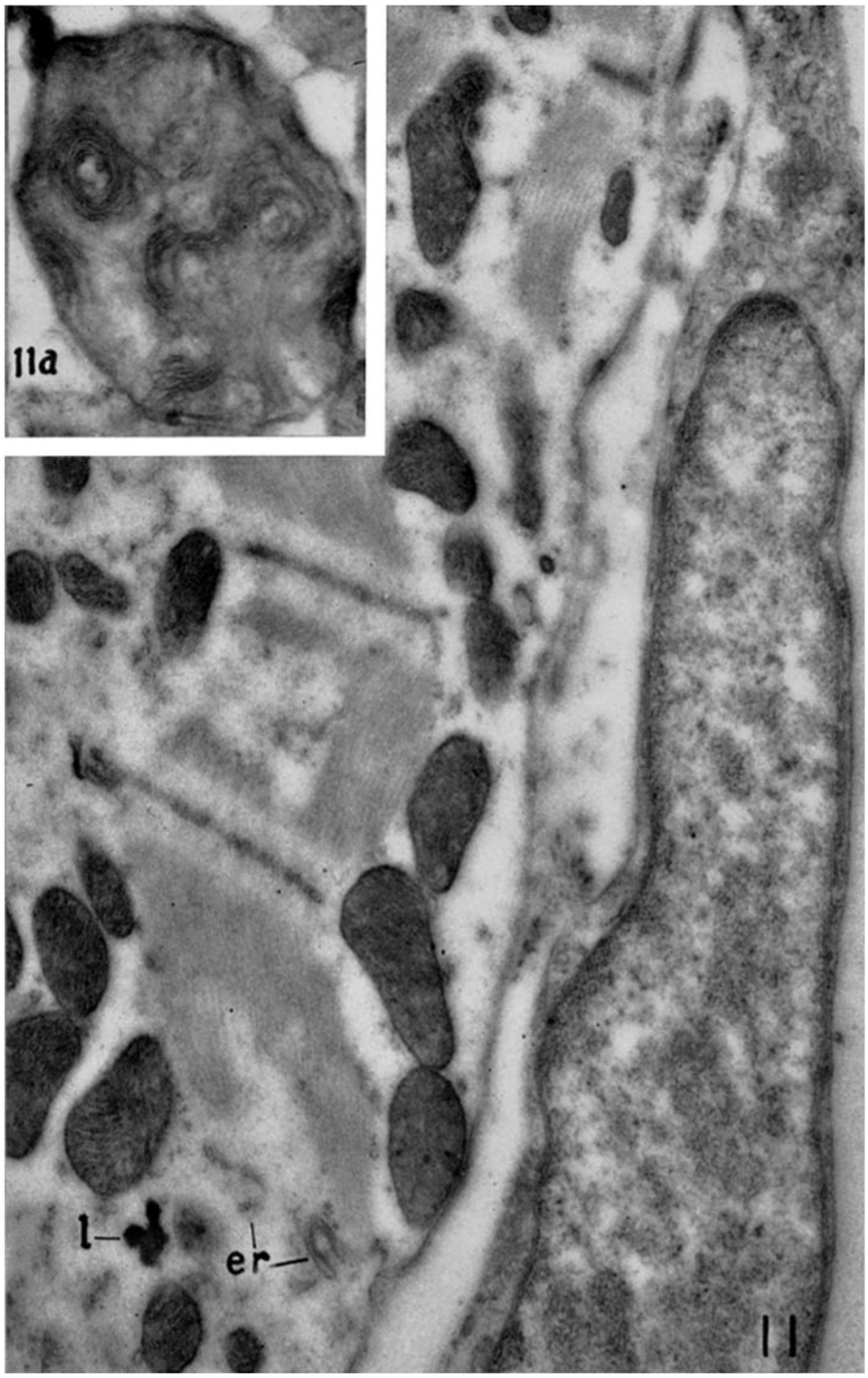


(Moore and Ruska: Mammalian cardiac muscle cells)

PLATE 83

FIG. 11. Rat heart muscle cut longitudinally. Endoplasmic reticulum is seen in close association with the plasma membrane of the sarcolemma. Lipide droplet (*l*) is at the lower left. Connective tissue fibrils and a fibrocyte lie in the interstitial space. Endoplasmic reticulum appears in the fibrocyte cytoplasm at the upper right. Double envelope of nucleus is demonstrated. × 27,000.

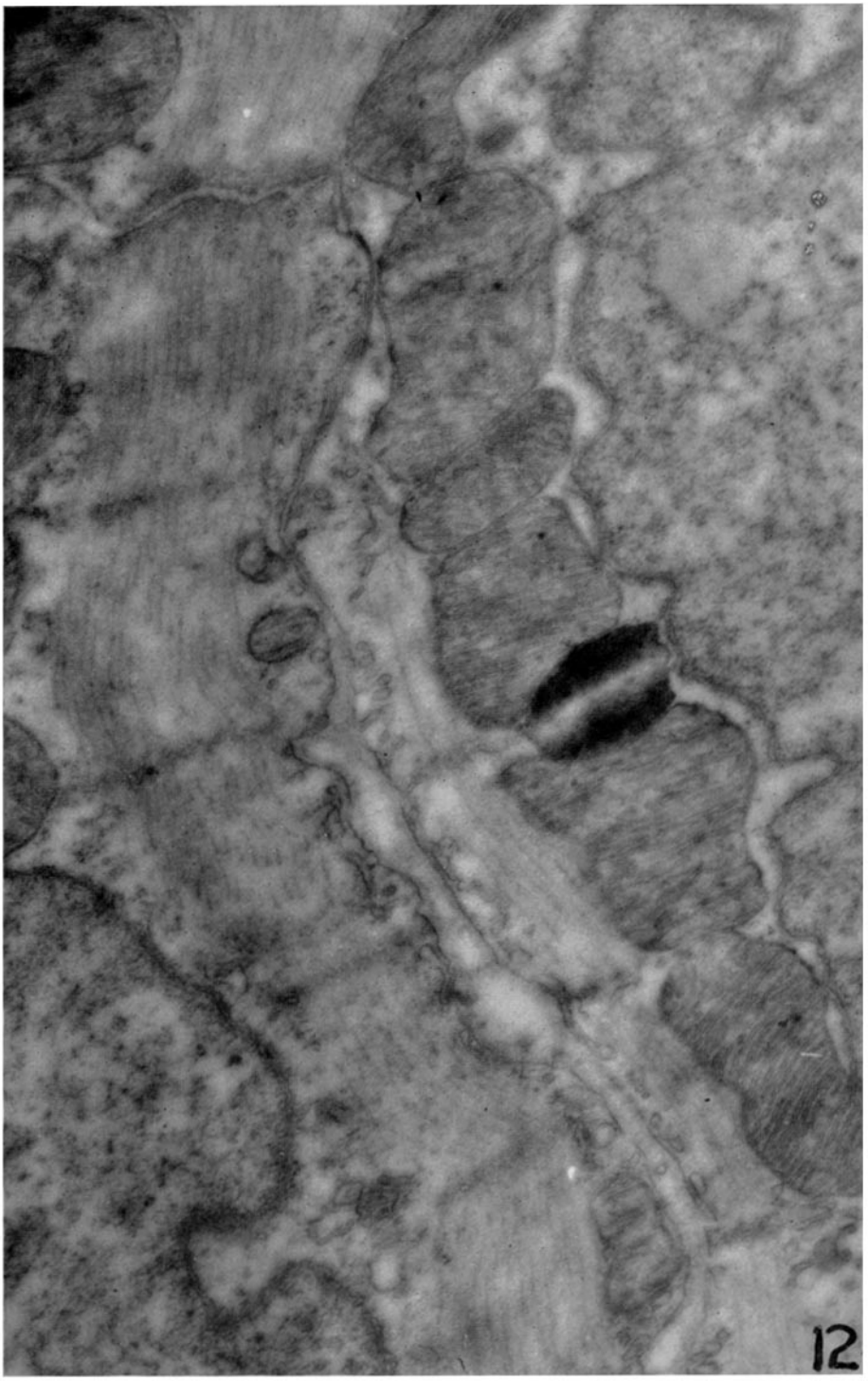
Insert (Fig. 11 *a*) shows mitochondrion of heart muscle cell with concentric inner membranes. × 36,000.



(Moore and Ruska: Mammalian cardiac muscle cells)

PLATE 84

FIG. 12. Portions of two cells from rat heart. Nuclei are at lower left and upper right borders. The transition from sarcolemma to intercalated disc is demonstrated. The relatively wide intercellular space narrows toward the top and cuts across a myofibril forming part of the disc. Pinocytotic vesicles and endoplasmic reticulum are visible near the cell borders. × 41,000.



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