THE ULTRASTRUCTURE OF THE CAT MYOCARDIUM

I. Ventricular Papillary Muscle

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ABSTRACT

The ultrastructure of cat papillary muscle was studied with respect to the organization of the contractile material, the structure of the organelles, and the cell junctions. The morphological changes during prolonged work in vitro and some effects of fixation were assessed. The myofilaments are associated in a single coherent bundle extending throughout the fiber cross-section. The absence of discrete "myofibrils" in well preserved cardiac muscle is emphasized. The abundant mitochondria confined in clefts among the myofilaments often have slender prolongations, possibly related to changes in their number or their distribution as energy sources within the contractile mass. The large T tubules that penetrate ventricular cardiac muscle fibers at successive I bands are arranged in rows and are lined with a layer of protein-polysaccharide. Longitudinal connections between T tubules are common. The simple plexiform sarcoplasmic reticulum is continuous across the Z lines, and no circumferential "Z tubules" were identified. Specialized contacts between the reticulum and the sarcolemma are established on the T tubules and the cell periphery via subsarcolemmal saccules or cisterns. At cell junctions, a 20 A gap can be demonstrated between the apposed membranes in those areas commonly interpreted as sites of membrane fusion. In papillary muscles worked in vitro without added substrate, there is a marked depletion of both glycogen and lipid. No morphological evidence for preferential use of glycogen was found.

Since the advent of the electron microscope, a great many significant papers have offered descriptions of limited aspects of the cytology of the ventricular cardiac muscle of various species (54, 67, 71, 85, 87, 99, 101, 108, 110). Considerably less information is available on the specific ultrastructural characteristics of the atrial muscle (28, 50) and, as yet, the nodal and specialized conduction tissues have received little detailed morphological investigation (51, 93, 109). This paper is the first of a series that will present a reasonably comprehensive description of the fine structure of the several regions of the cat myocardium. It is hoped that these papers on the special cytology of the heart will prove to be valuable morphological references for those investigators interested in correlation of the ultrastructure and the physiological and pharmacological properties of cardiac muscle.

The slender ventricular papillary muscles of the cat heart have been extremely useful for physiological studies because they can be removed with minimal trauma and can be stimulated to contract for many hours in a bath of oxygenated

physiological salt solution (7, 73). Therefore, in undertaking a detailed study of the ventricular myocardium, it seemed desirable to devote particular attention to these muscles and to study their ultrastructure under conditions to which they are commonly exposed during physiological experiments. The fact that the fibers are more consistently parallel in papillary muscle than in other parts of the ventricle facilitates its electron microscopic study by permitting orientation of the embedded muscle for transverse or longitudinal sections. For the most part, the structure of the papillary muscle can be considered representative of the cells specialized for contraction in the ventricular cardiac muscle, although very minor regional variations would, no doubt, be disclosed by a systematic study of different regions of the ventricular myocardium.

TABLE I

Physiological Salt Solution

(Buffered at pH 7.4 with 95% O_2-5% CO_2)

Compound	g/liter	Compound	g/liter
NaCl	5.210	Na_2 fumarate	0.80
$NaHCO_3$	2.440	Na pyruvate	0.55
KCl	0.372	NaH glutamate	0.845
$CaCl_2$	0.249	Glucose	1.81
$MgSO_4$	0.120	Insulin	5 units
Na_2PO_4	0.142		

MATERIALS AND METHODS

Hearts were removed from 19 cats of varying age, under chloroform or pentobarbital anaesthesia. During dissection, the hearts were bathed in a bicarbonate-buffered physiological salt solution oxygenated with 95% O_2/5% CO_2 (Table I). Spontaneous rhythmic contractions continued during the excision of the papillary muscles, which required from 3 to 5 min.

The small size of the muscle (0.5–0.7 mm in diameter) favored penetration of the fixative, but there was nevertheless a gradient of preservation within the blocks. Regrettably, the preservation was only fair to poor in the center of the muscle. Therefore, in order to be able to distinguish, insofar as possible, between structural effects of environmental or experimental conditions and artifacts of specimen preparation, the observations were confined to the outer portion where fixation was best. In nine hearts examined in the early phases of the study, the papillary muscle was excised, its ends were tied to a toothpick so as to hold it under moderate stretch during its immersion in the fixative. Later in the study, muscles from ten hearts were fixed in a physiological apparatus in order to permit more accurate control of muscle length and to allow examination of muscles worked under different physiological conditions. In these experiments, the right ventricular papillary muscles were placed on an apparatus permitting measurement of isometric tension with a Statham strain guage transducer (8). The muscles, immersed in the physiological salt solution, were stimulated at 2-sec intervals with electrical pulses of 5 msec duration at just above threshold intensity, delivered to the bathing solution near the base of the muscle. Under these conditions, the driving frequency was greater than any intrinsic rhythmicity so that the muscles were not contracting spontaneously. The origin of the muscle was firmly clamped, and the chorda tendinea was connected to a tension transducer on a moveable micrometer stage mounting. The over-all length of the muscle could, therefore, be varied while the isometric tension was continuously recorded on a Sanborn 150 multichannel recorder. All muscles utilized for in vitro study were slowly stretched to the optimal length for development of isometric tension on stimulation.

Physiological integrity of such preparations was assured by imposing the requirement that all muscles show stable development of 1–2 g isometric tension during a period of 1 hr equilibration in vitro. Muscles meeting this requirement were either utilized for physiological experimentation or fixed for electron microscopy while still held in the apparatus.

Experimental manipulations were of two kinds. The first type of experiment undertook to determine the effects of incubation in vitro, for periods ranging from 2 to 14 hr, on the ultrastructure and the relationship between muscle length and developed tension. In the second kind of experiment, papillary muscles were worked in vitro at optimal length for up to 12 hr in medium free of exogenous energy sources. The fine structure of these muscles was then compared with that of another papillary muscle from the same heart worked in enriched medium for a comparable length of time.

Muscles stretched manually and tied to a toothpick were simply immersed in the fixative for 2 hr. For muscles held in the physiological apparatus at optimal length, electrical stimulation was stopped, and the incubating medium was rapidly replaced with the fixative. For eight of the ten muscles fixed in this way, a continuous recording of the tension was made for the 1st hr of the 2-hr period of fixation.

Four different fixatives were used: (*1*) 6% glutaraldehyde, buffered at pH 7.4 with 0.1 M cacodylate containing 0.05 M $CaCl_2$; (*2*) 6% glutaraldehyde buffered with the physiological salt solution adjusted to pH 7.4 with 95% O_2 and 5% CO_2 ; (*3*) paraformaldehyde and glutaraldehyde (52) in 0.1 M cacodylate

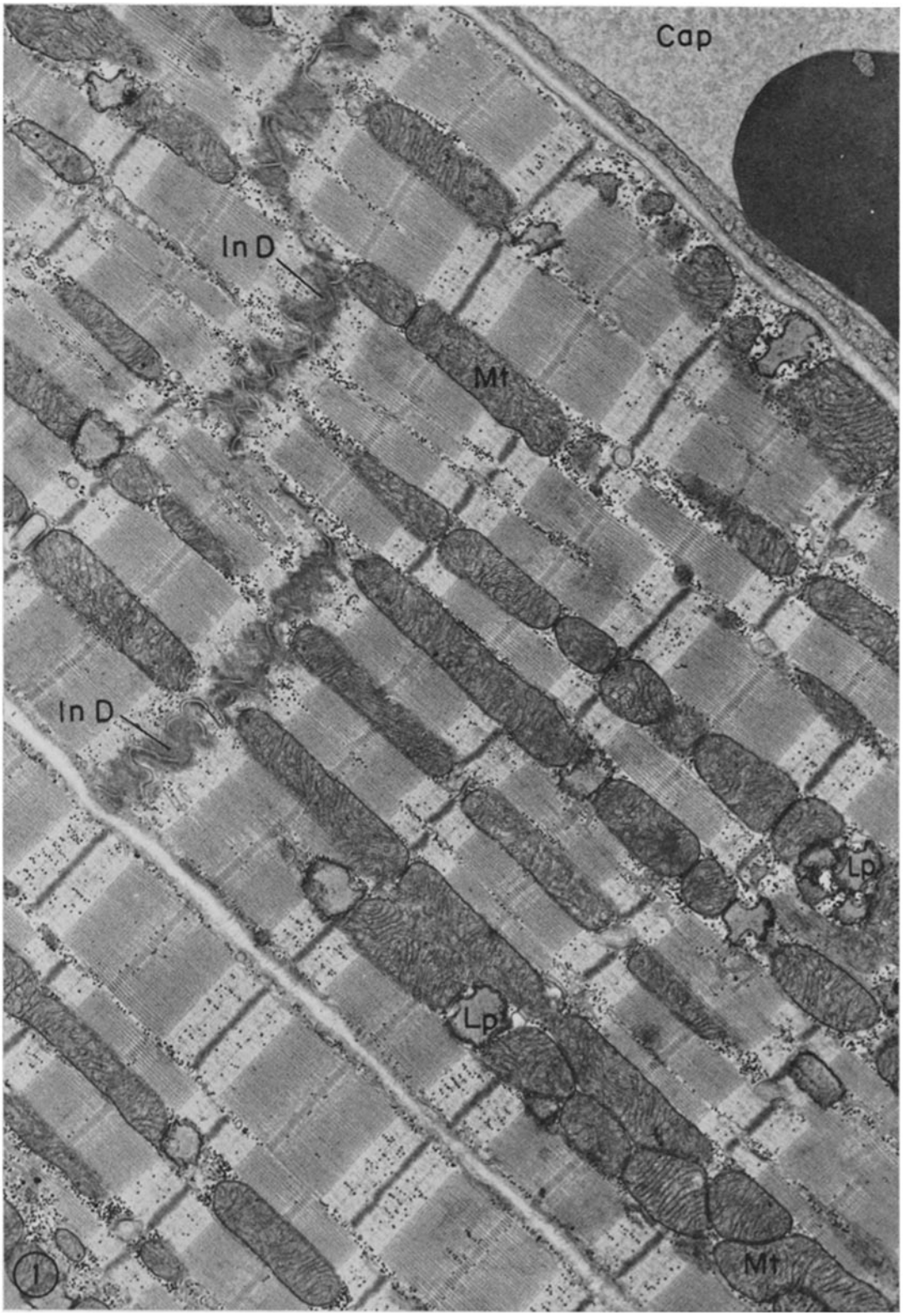


FIGURE 1 Electron micrograph of parts of three cardiac muscle fibers and an adjacent capillary (*Cap*) in longitudinal section. The two upper cells are joined end to end by a typical steplike intercalated disc (*In D*). Rows of mitochondria (*Mt*) appear to divide the contractile substance into myofibril-like units but, unlike the true myofibrils of skeletal muscle, these branch and rejoin and are quite variable in width. Lipid droplets (*Lp*) somewhat distorted in specimen preparation are found between the ends of the mitochondria. × 15,000.

buffer (used either full strength or half-strength); (*4*) 1% OsO_4 in 0.1 M cacodylate buffer at pH 7.4. Muscles fixed in solutions containing glutaraldehyde were rinsed for 2 hr in 0.1 M cacodylate buffer and then postfixed in 1% OsO_4 in 0.1 M cacodylate buffer containing 0.05 M $CaCl_2$.

Dehydration was accomplished in rapid sequential changes of cold (4°C) 50%, 80%, 95%, and absolute ethanol. The dehydration was completed in absolute ethanol at room temperature for 2.5 hr. After two changes (5 min) of propylene oxide at room temperature, infiltration was begun in equal parts of propylene oxide and Epon 812 (2% in DMP-30) for 2–4 hr, and completed in Epon (2% in DMP-30) for 2–12 hr at room temperature. The resin was cured in a 60°C oven.

One of the distinct advantages of the papillary muscle over other areas of ventricular wall for morphological studies is the fact that its fibers are, for the most part, straight and parallel and the block can, therefore, be oriented to provide either transverse or longitudinal sections, avoiding oblique sections that complicate interpretation. Thin transverse or longitudinal sections were cut with glass or diamond knives on a Porter-Blum MT-1 microtome and stained with saturated aqueous uranyl acetate followed by lead citrate (112). The sections were examined with an RCA-3F or Siemens Elmiskop I electron microscope.

OBSERVATIONS

Organization of the Contractile Material

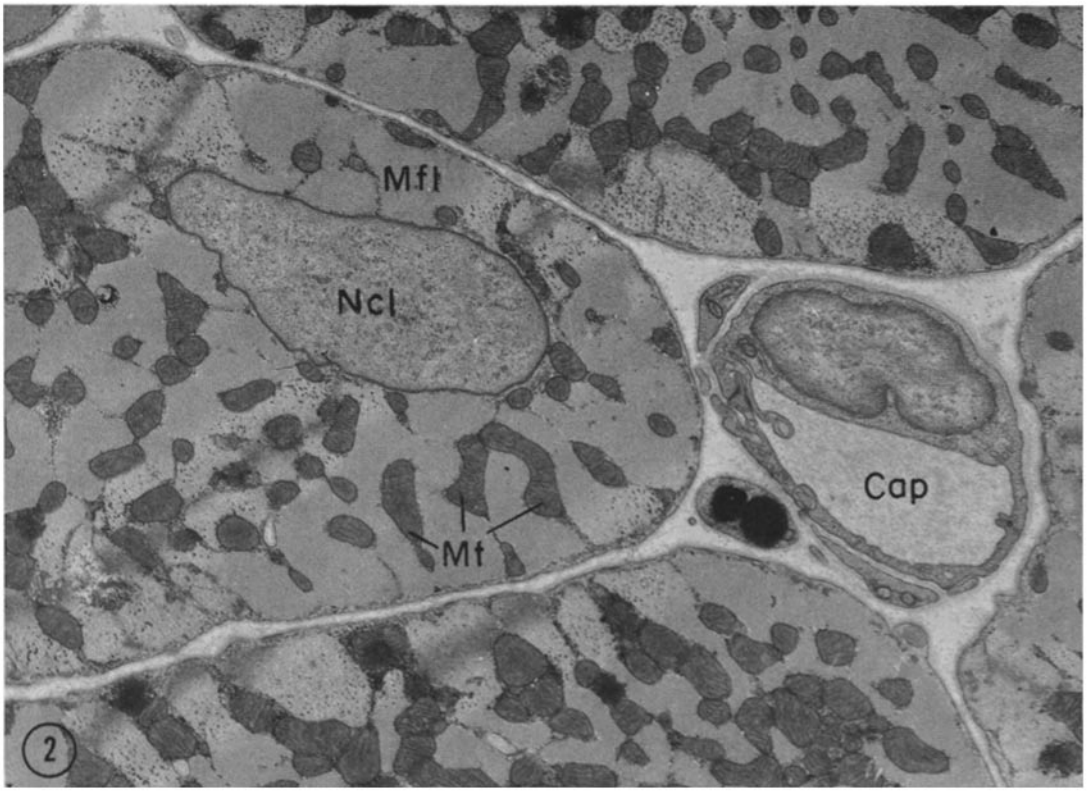
Light microscopists recognized that skeletal muscle fibers are unbranched, multinucleate syncytia of indefinite length containing closely packed myofibrils of rather uniform diameter and polygonal cross-section. The distinctive features of cardiac muscle fibers were their branching pattern, central nuclei, more abundant mitochondria, and the presence of intercalated discs. The electron microscope added to these findings certain points of difference that were not appreciated before. One of the most significant of these differences was the finding that cardiac muscle fibers are not syncytial, but are partitioned into cellular units at the intercalated discs which proved to be specialized cell-to-cell junctions (62, 110). Although the myofilaments, the macromolecular elements responsible for the contractility of both skeletal and cardiac muscle, were found to be the same, their distribution in the sarcoplasm of cardiac muscle differs from that of most skeletal muscles. For technical reasons, this difference has not been widely recognized and is, therefore, deserving of some amplification here.

Some 35 years ago Krüger (59), working with the light microscope, observed that skeletal muscles which gave a prolonged tonic response to acetylcholine contained fibers which differed in cross-sectional appearance from fibers of muscles which responded under similar conditions with a brief twitch. Instead of being subdivided into small myofibrils a micron or less in diameter, the contractile material in these tonic fibers formed much larger units of irregular outline. This latter appearance was described as field-structure (*Felderstruktur*) whereas the more common appearance in fast acting muscles was called fibril-structure (*Fibrillenstruktur*). Electron microscopy has confirmed this structural distinction between twitch and tonus fibers (42, 77, 82) by revealing that in the latter, the irregular myofibril-like units are fused together to form a more or less continuous mass of myofilaments in which isolated areas of sarcoplasm are interspersed.

In longitudinal sections of cardiac muscle, the contractile material is partially subdivided, by rows of mitochondria, into units that bear a superficial resemblance to the myofibrils of mammalian skeletal muscle (Fig. 1) (86). Closer examination, however, reveals that these fascicles of myofilaments vary greatly in width and are not independent throughout their length, but are often confluent at the ends of a row of mitochon-

FIGURE 2 Electron micrograph of portions of four cardiac muscle fibers and a capillary (*Cap*) in transverse section. Myofilaments (*Mfl*) are not grouped in discrete myofibrils, but form a single large bundle interrupted only by the eccentric nucleus (*Ncl*) and the numerous mitochondria. × 6,700.

FIGURE 3 An area of cardiac muscle cell in transverse section, shown at higher magnification. The limits of discrete myofibrils cannot be identified. Instead, the mitochondria (*Mt*) appear evenly distributed in a continuous field of myofilaments (*Mfl*). Small circular profiles occurring singly or in short rows are sections of the sarcotubules (*SR*). × 30,000.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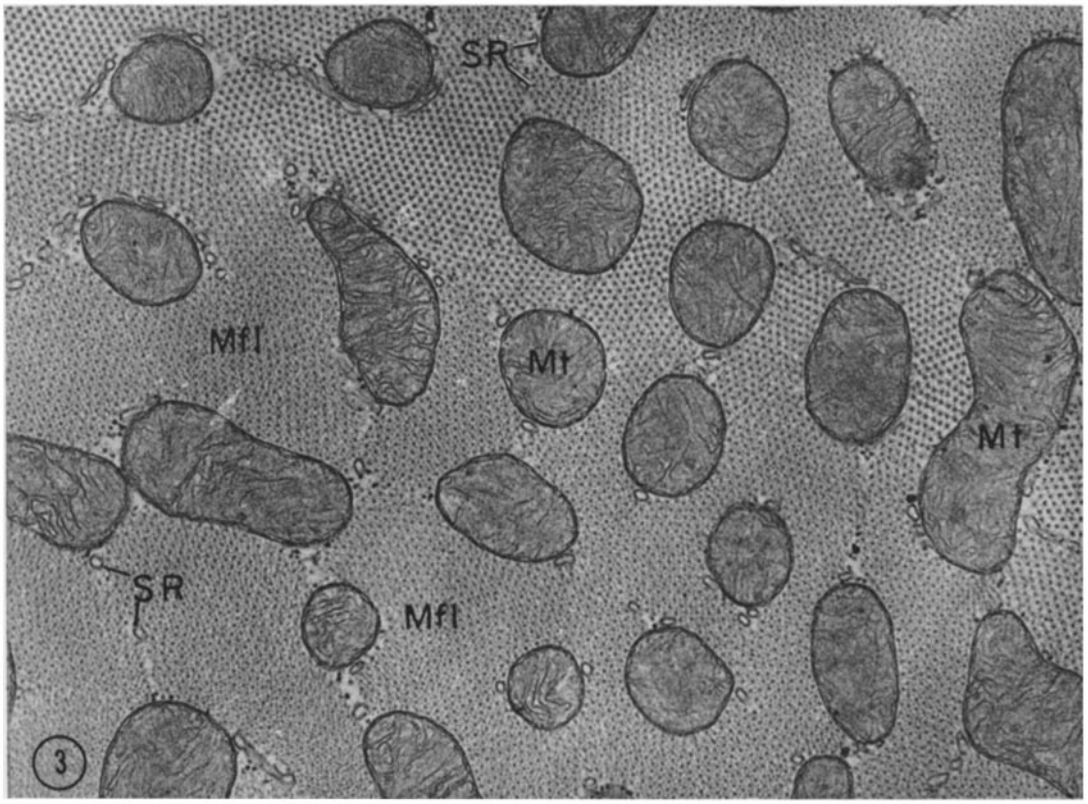
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dria. The characteristic pattern of distribution of the contractile material of cardiac muscle is better understood from examination of transverse sections at low magnification (Fig. 2). Viewed in this plane, the myofilaments are not grouped into clearly circumscribed myofibrils as they are in most skeletal muscles, but instead form a single large bundle that fills most of the fiber except for an axial core of sarcoplasm containing the nucleus. At higher magnification (Fig. 3), the precisely ordered hexagonal pattern of dots representing the transected myofilaments extends throughout the section and is interrupted only by rather uniformly distributed profiles of mitochondria that fill spaces of conforming shape among the myofilaments. In some places in the cross-section, narrow clefts not occupied by myofilaments extend from one mitochondrial profile to another. These clefts contain a few glycogen particles and rows of small circular profiles that represent sections of the tubules comprising the sarcoplasmic reticulum (Figs. 3, 15, and 36). The mitochondria and these narrow connecting sheets or septa of sarcoplasm partially subdivide the myofilament mass, but they do not demarcate independent subunits. The organization of the contractile material of cardiac muscle thus closely resembles that of the contractile muscle of slow skeletal muscle fibers exhibiting *Felderstruktur*. In poorly preserved specimens, however, the sarcoplasmic septa are greatly swollen and widened, thus making the cross-sectional appearance of cardiac muscle approximate more closely that of ordinary skeletal muscle. The frequent references in the literature to the "myofibrils" of cardiac muscle are probably attributable to misinterpretation of such distortions of the normal structure.

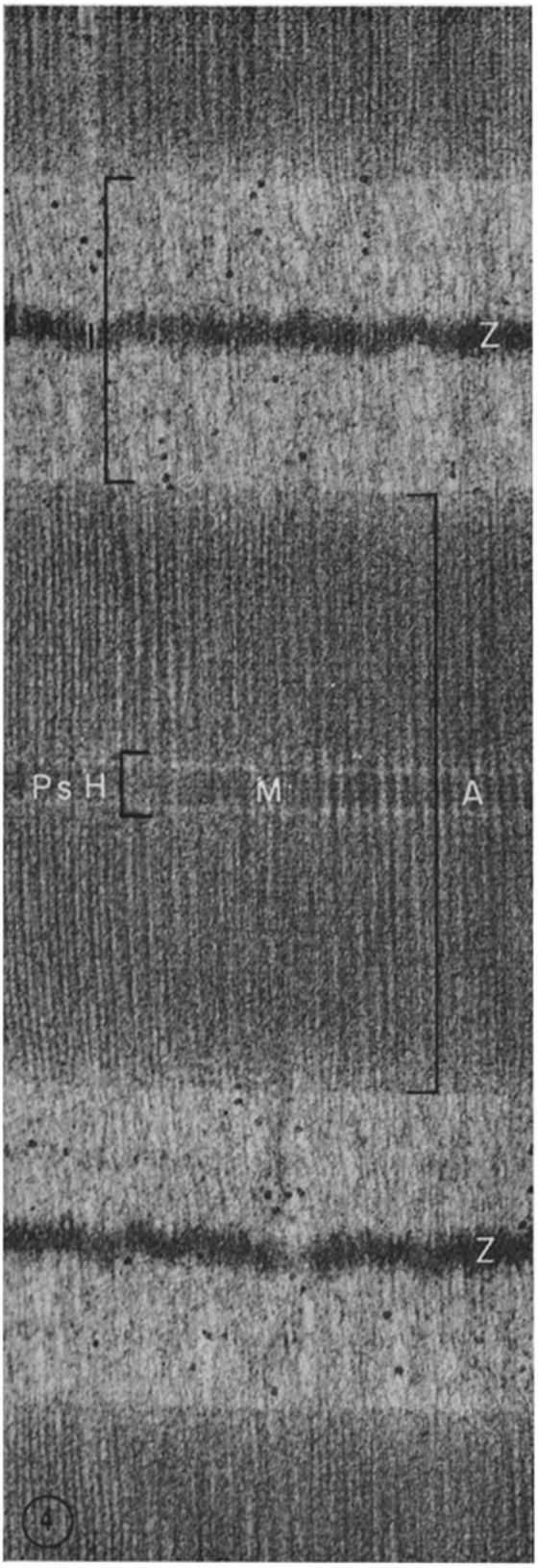
As a consequence of the continuity of the myofilament mass in cardiac muscle, there is a strong tendency for the cross-striations, as viewed in longitudinal sections, to be in register over the full width of the fiber. Some displacement of myofibril-like areas with respect to neighboring areas within the myofilament bundle can take place, however, for transverse discontinuities in the banding pattern are occasionally seen (Fig. 1), and in true transverse sections, irregularly shaped areas may be transected at a level in the sarcomere slightly different from that of adjacent areas. Such misalignments are more commonly observed in papillary muscles stretched manually than in those monitored physiologically and fixed at the optimal point on the length-tension curve. It is quite possible, therefore, that they are produced or accentuated in the stretching.

The functional implications of the association of cardiac myofilaments in a single more or less coherent bundle are not fully understood. However, in view of the similarity of this pattern to that of the slow "tonus muscles" of amphibia which yield a sustained contraction in response to repetitive stimulation, a reasonable inference seems to be that the peculiar geometry of the cardiac contractile mass may also be related to the slow time to peak tension and to the relatively prolonged contractile state of cardiac muscle compared to skeletal twitch muscles.

The dimensions and arrangement of the thick (myosin) and thin (actin) myofilaments of cardiac muscle are in substantial agreement with those given by previous investigators (40, 46, 49, 107). As is now well known, the thick filaments of both cardiac and skeletal muscle are 100–150 A in diameter and 1.5 μ long, while the thin fila-

FIGURE 4 Electron micrograph of somewhat more than one sarcomere length of papillary muscle fixed at the optimum point on the length-tension curve. The sarcomere length is $\sim$2.3 μ. The thin actin filaments can be seen in the I bands, and both thick and thin filaments are discernible in the A band. The prominent M line (*M*) shows a faint striation with a period of 200 A. The narrow pale regions on either side of the M line demarcate the pseudo H zone (*Ps H*). At this sarcomere length the tips of the actin filaments are very near the margins of the pseudo H zone. Therefore no true H zone can be seen. × 53,000.

FIGURE 5 A micrograph of a similar segment from a papillary muscle stretched beyond the optimum length for development of tension. The sarcomere length is noticeably greater (2.6 μ) than in Fig. 4, but the length of the A band remains constant. In stretching, the ends of the thin filaments have been pulled away from the M line so that a true H zone (*H*) is discernible. × 53,000.



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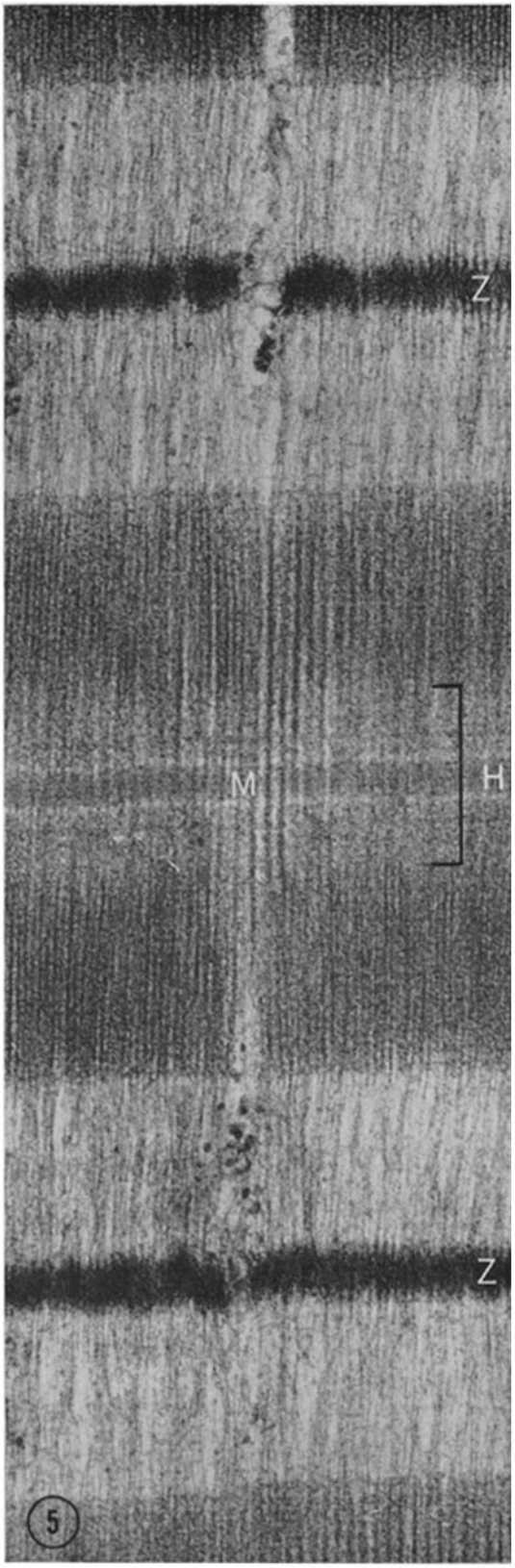

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ments are 50–70 A in diameter and 1.0 μ long. In the cross-banded pattern, the length of the A band is determined by the length of the myosin filaments and is constant. Two sets of actin filaments are connected end to end at each Z line while their opposite ends interdigitate with the myosin filaments of the neighboring A bands. The repeating functional unit of the contractile material, the sarcomere, is the segment between two successive Z lines. The length of the I band corresponds to those portions of the two sets of actin filaments on either side of the Z line that do not extend into the adjacent A bands. It varies, therefore, with the state of contraction. The lighter central region of the A band between the ends of the two interdigitating sets of actin filaments is the H zone. The alignment and cross-bridging of a thickened segment at the midpoint of the myosin filaments gives rise to an M band of constant width bisecting the A band.

In cardiac muscle, the thickened central segments of the myosin filaments comprising the conspicuous M band are 860–900 A long. Five thin lines 30–50 A in thickness and spaced 215–225 A apart can often be resolved within the M band (Fig. 4). Occasionally, the lines at the two edges of the M band are not resolved and only the three central lines are seen. The structural basis for these periodic linear markings is probably the optical superimposition of intermyosin cross-bridges occurring in lateral register at five specific sites along the M band. The exact nature of the specialization of the central region of the thick filaments and of the intermyosin cross-bridges is still not well understood, but has recently been discussed in considerable detail by Pepe (84). Components other than myosin are apparently involved, for this region has special enzymatic properties not commonly attributed to myosin (3, 53), and no such central thickening is present on filaments reconstituted in vitro from purified myosin (14, 47). On either side of the M band for a distance of 180 A, the radial spines or cross-bridges on the thick filaments that interact with the actin filaments to produce longitudinal displacement appear to be absent. This bare region of the myosin filaments results in a slightly lighter band of constant length (180 A) on either side of the M band.

During contraction of skeletal muscle, the ends of the invading actin filaments approach the M band as the ends of the adjacent A bands approach the Z line. In fully contracted skeletal muscle, the sarcomere length is about 1.5 μ, and neither I bands nor H zones are discernible. In relaxed or stretched skeletal muscle, however, the ends of the actin filaments delimit a clearly discernible H zone.

In cardiac muscle fixed at the length at which maximal isometric tension is developed, the sarcomere length is 2.2 μ (Table II), and the ends of the actin filaments are at or very near the lateral margins of the M band and no true H zone is seen (Fig. 4). However, a narrow lighter band on either side of the M band, attributable to the bare region of the myosin filaments, is always visible, and although its dimensions do not vary with the state of contraction, it has been confused with the H zone (103). The constant distance between the lateral margins of these light zones is now commonly referred to as the pseudo H zone of cardiac muscle (10). It is only in fibers stretched beyond the length for optimal isometric contractility (sarcomere length greater than 2.2 μ) that a true H zone can be made out in cardiac muscle (Fig. 5). Even then, the tips of the actin filaments do not seem to result in as sharp a discontinuity in density as they do in skeletal muscle and, at best, the margins of the H zone in cardiac muscle are indistinct.

The relations of two sets of filaments also result

TABLE II

*Lack of Correlation of Per Cent Rise in Resting Tension and Optimal Sarcomere Length or Average Diameter of T Tubules**

Increase in resting tension in response to fixation after 1 hr	Optimal sarcomere length	Average diameter T tubules
%	μ	μ
0	2.30	0.25
18	2.10	0.30
80	2.16	0.23
90	2.10	0.24
100	2.20	0.15
100	2.19	0.29
120	2.22	0.20
250	2.14	0.40
Ave. 95	2.18	0.26

* Note: Incubation of right ventricular papillary muscles in a steady state for 2–6 hr in vitro. Fixation in 6% glutaraldehyde-cacodylate.

in a characteristic cross-sectional appearance for each of the bands. In cells stretched to a sarcomere length of 2.2 μ, micrographs of transverse sections present four different patterns in the punctate cut ends of the myofilaments: thick filaments only (H and M bands); thick and thin filaments (regions of overlap in A band); thin filaments only (I band); and a complex pattern of dense thin filaments (Z band). The distances between the filaments is, to some extent, dependent on the degree of stretch of the muscle at the time of fixation. With a sarcomere length of 2.2 μ, the thick filaments are hexagonally packed with a center-to-center distance of 400–450 A. In the region of overlap of thick and thin filaments, transverse sections show each thick filament surrounded by six thin filaments (Figs. 44 and 45).

The substructure of the Z line of striated muscle is still a subject of dispute and seems to be more difficult to analyze in cardiac muscle owing to the somewhat greater density of an extrafibrillar component that tends to obscure the details of this region. At least three alternative interpretations of Z-line fine structure have been proposed. No evidence was found in the present study that would support the view of Franzini-Armstrong and Porter (34) that the fine zigzag pattern often seen in the Z line is the sectional profile of a thin membrane drawn to points in opposite directions by traction at sites of insertion of two offset, square-packed groups of actin filaments. The various patterns presented in longitudinal and transverse sections of the Z line seem to be best explained in terms of interconnection of the opposing sets of actin filaments by thin filaments traversing the Z line. Our own observations do not permit a clear choice between four diverging Z filaments as described by Knappeis and Carlsen (57) and the interlinked loops of actin strands arising from uncoiling of the ends of actin filament double helices as postulated by Kelly (55). The suggestion that tropomyosin is associated in some manner with the Z line (47) is supported by the finding, in normal cat myocardium, of occasional fibers with abnormally broad Z lines with a repeating period of about 200 A similar to that of crystalline tropomyosin. These anomalous Z lines are similar to the wide Z lines in nemaline myopathy (38), and have deen described and illustrated in a separate communication (27). Their occurrence is not easily explained by any of the current interpretations of Z-line structure.

Other Organelles and Inclusions of the Muscle Cell

SARCOLEMMA: The term sarcolemma was originally defined as an elastic transparent sheath of the muscle fiber that could be demonstrated in teased preparations examined with the light microscope. With electron microscopy, it has become apparent that the sheath visualized with the light microscope is not a single entity but a multicomponent complex made up of (*1*) the plasma membrane of the muscle cell, (*2*) an extracellular protein-polysaccharide coating corresponding to the basal lamina or "basement membrane" of epithelial cells, and (*3*) an associated reticulum of fine collagen fibrils. These three components seen in electron micrographs are now described separately. The term sarcolemma has been retained, but is now restricted in its use to the plasma membrane of the muscle cell. It is a typical 90 A trilaminar unit membrane not visibly different from the limiting membrane of other cells though it possesses certain physiological properties that are not common to all cell membranes. The protein-polysaccharide layer (88) coating the sarcolemma is of rather uniform thickness (~500 A) and is composed of a dense mat or feltwork of exceedingly fine filaments (Fig. 8). In some instances it appears layered, having a less dense inner zone (~200 A) adjacent to the sarcolemma and a slightly denser outer zone (~300 A). At higher magnification the filamentous components extend from one zone to the other without any sharp line of demarcation, and the greater density of the outer zone seems attributable to its components' being condensed and more closely interwoven. This investment of the sarcolemma is referred to in the literature by various names—basal lamina, external lamina, basement membrane, boundary layer, or glycocalyx. The outermost layer of the traditional sarcolemmal sheath is a reticulum of small bundles of collagen fibrils at the outer surface of the protein-polysaccharide coating or partially embedded within it. It is not notably different from the reticulum surrounding smooth and skeletal muscle fibers or that supporting the basal lamina of many epithelia.

The unit membrane or sarcolemma proper is presently considered to be the morphological outer limit of the cell, but the properties of the various coatings of this and other cell types have

been too little investigated to establish to what extent they may influence ion fluxes and other properties commonly attributed to the plasma membrane. The sarcolemma has occasional small invaginations ~800 A in diameter (Figs. 8 and 9) resembling the so-called micropinocytosis vesicles that are seen in great numbers at the surface of capillary endothelial cells and smooth muscle cells. In addition, there is a second type of vesicular invagination that occurs in smaller numbers. These vesicles are somewhat larger (800–1,000 A) and have a limiting membrane with a distinctive fuzzy coating on its outer and its sarcoplasmic surfaces (12, 36, 95, 96). The physiological significance of these two types of vesiculation of the plasmalemma is still not fully established for any cell type. There is evidence that the smooth-surfaced form in endothelium is involved in transcellular vesicular transport (13) while the alveolate or spiny vesicles have been implicated in a more selective uptake of protein (1, 36, 95, 96).

In longitudinal sections of contracted cardiac muscle, the outline of the fiber is often scalloped, with sarcomere length bulges alternating with narrow grooves at each Z line. This appearance fostered the belief among classical cytologists that the Z line was a membrane (Krause's membrane) continuous with the sarcolemma. There is no evidence for such a membrane in electron micrographs, but a special relationship of the sarcolemma to the Z lines is incontestable. There appears to be a dual basis for this relationship. The extrafibrillar dense substance of the Z line sometimes extends beyond the outer limit of the myofibril to the sarcolemma and may, in part, be responsible for the tendency of the two to adhere (28). In addition, the tubular invaginations of the sarcolemma that comprise the T system (vide infra) originate at the Z lines and tend to fix the sarcolemma at that level to deeper lying structures.

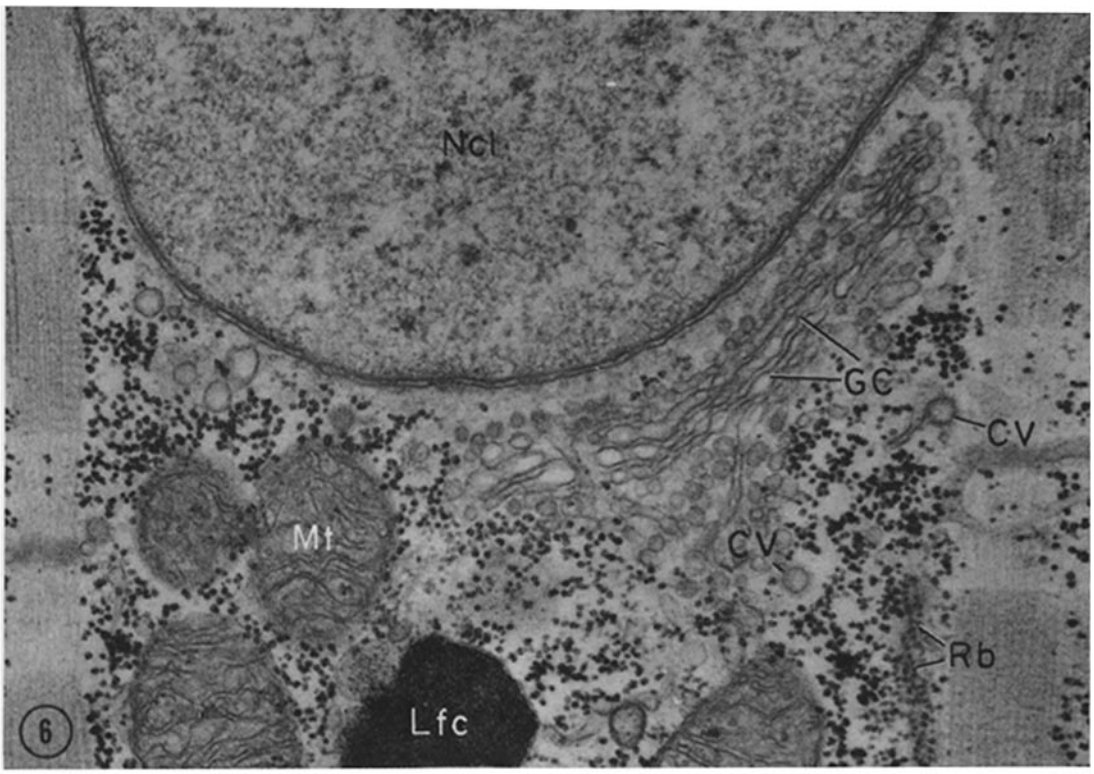
The local specializations of the sarcolemma at sites of cell-to-cell attachment and in regions of interaction with the sarcoplasmic reticulum will be discussed below.

NUCLEUS AND JUXTANUCLEAR ORGANELLES: The single greatly elongated nucleus is usually deep in the interior of the cell, but often eccentric in position (Fig. 2). Rarely, it may be situated immediately beneath the sarcolemma. The nucleus is generally fusiform but may be deeply infolded in contracted muscle. It has a typical nucleolus (Fig. 13) and a moderate amount of chromatin distributed mainly around the periphery (Fig. 7). The nuclear envelope is traversed by a small number of nuclear pores and has a few ribosomes on its cytoplasmic surface.

A small Golgi apparatus is situated at one pole of the nucleus, often in close relation to the nuclear envelope (Fig. 6). It consists of three to five fenestrated, flat saccules or cisternae, 200–300 A across throughout most of their length but often expanded at their ends to 400–500 A. The so-called forming face of the Golgi apparatus is directed toward the nucleus and is associated with large numbers of small vesicles (Fig. 6). The outer leaf of the nuclear envelope adjacent to the Golgi apparatus is usually devoid of ribosomes and often shows small vesicular outpocketings (Figs. 10–12) reminiscent of those seen on cisternae of the endoplasmic reticulum adjacent to the forming face of the Golgi apparatus in secretory cells (35). Evidence of several kinds supports the view that the nuclear envelope is essentially a perinuclear cisterna of the endoplasmic reticu-

FIGURE 6 Micrograph of the end of the nucleus (*Nel*) and the adjacent sarcoplasm containing the Golgi complex (*GC*), mitochondria (*Mt*), and lipofuscin pigment (*Lfc*). The Golgi complex has many small smooth-surfaced vesicles associated with it and a few larger coated vesicles (*CV*). At the lower right are several chains of ribosomes (*Rb*) probably associated with the surface of a sarcotubule just deep to the plane of section. The other dense granules of more variable size not associated in chains are glycogen. × 40,000.

FIGURE 7 A portion of the nucleus of a cardiac muscle cell showing the peripheral distribution of the chromatin typical of glutaraldehyde-fixed muscle. Adjacent to the nucleus is a small portion of the Golgi complex and three centrioles (*Ce*). Since centrioles generally occur in pairs, it is assumed that a fourth centriole is present but out of the plane of section. × 42,000.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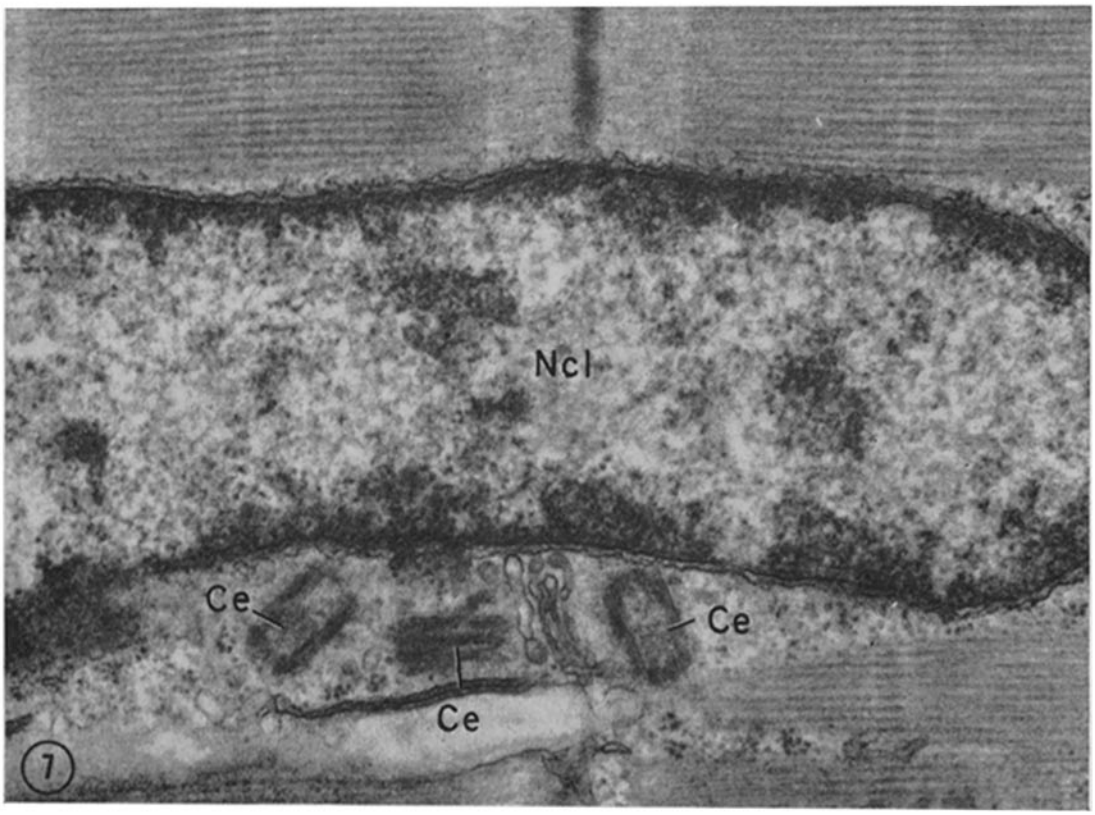
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lum, but to our knowledge this is the first report of the formation from the nuclear envelope of vesicles that are probably destined to be incorporated in the Golgi cisternae.

In addition to the numerous small smooth-surfaced vesicles associated with the Golgi complex, there are small numbers of coated or spiny vesicles of slightly larger size (500–800 A) (Figs. 6 and 8). Their functional relationship to the Golgi apparatus is unclear. Also common in this region are multivesicular bodies ~1200 A in diameter enclosing many small vesicles (200 A) within a single membrane.

Centrioles are very rarely encountered in cardiac muscle, but this may simply be caused by the small probability that two structures representing such a small fraction of the cell volume will be included in the sections examined. Centrioles have been seen in two instances in the Golgi region: in one instance there was a pair, which is probably the normal complement. In the other, three were included in the plane of section and the presence of a fourth may be assumed (Fig. 7). It was not possible to determine whether this cell was binucleate, but binucleation seems likely.

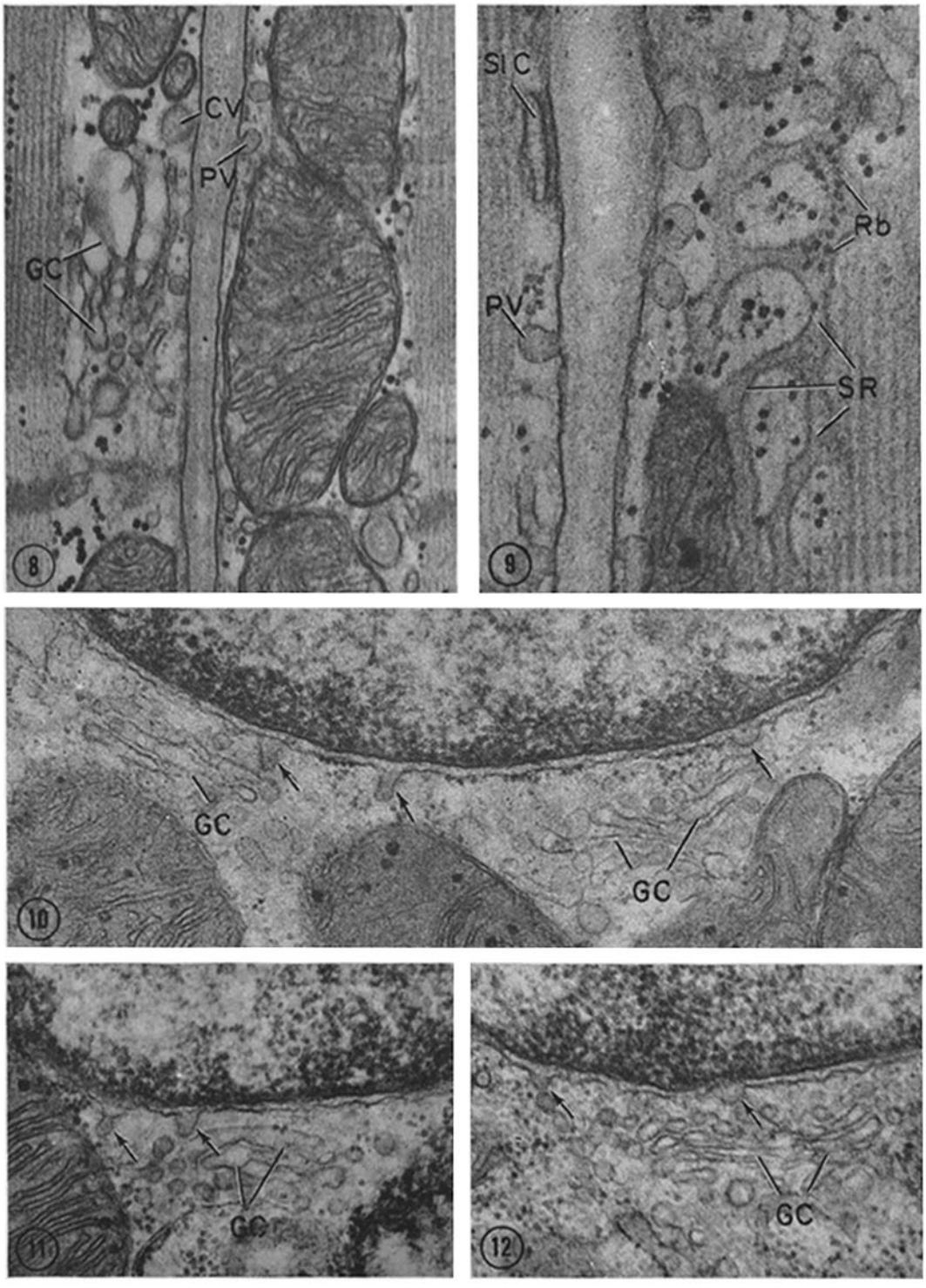
MITOCHONDRIA: The mitochondria of cardiac muscle are more numerous and more pleomorphic than are those of skeletal muscle. They occur throughout the cell but are particularly abundant in the conical regions of sarcoplasm at either end of the nucleus (Fig. 13). There they tend to be randomly oriented, 2–3 μ in length and cylindrical in form, but they may branch, bend back upon themselves, or have two lobes connected by narrower segments. Those grouped end to end in slender clefts among the myofilaments are perhaps even more variable in size and shape. They are oriented parallel to the long axis of the cell, and are usually about one sarcomere (2 μ) long with their ends near the Z lines (Fig. 1), but they may range up to 9 μ in length spanning several sarcomeres. In transverse sections of cardiac muscle, the predominant cylindrical form of the mitochondria is evident in the frequency of round profiles, particularly near the center of the fiber (Fig. 3). Nearer the periphery, many of the mitochondria are larger and are flattened so that they present an elliptical cross-sectional profile with the long dimension radial to the fiber axis (Fig. 15).

The internal structure of the mitochondria is noteworthy for the abundance of long foliate cristae that often exhibit periodic angulations along their length (91, 102). Angles of about 120° are formed alternately in one membrane of the crista and then in the other membrane at regular intervals of 500–600 A along their length, resulting in a characteristic zigzag configuration (Figs. 16–18). The angulations on adjacent cristae are usually conforming and in register, but occasionally the angles of neighboring cristae appear to have fused forming an hexagonal system of anastomosing cristae that delimit intercristal spaces resembling the cells of a honeycomb (Fig. 16). In some mitochondria, this tendency for

FIGURE 8 Portions of two neighboring cardiac muscle fibers, each showing a protein-polysaccharide coating on its sarcolemma. Also of interest in this figure is the unusual location of a Golgi complex (*GC*) immediately beneath the cell surface. The coated vesicles (*CV*) associated with the Golgi complex can be compared with the smooth pinocytosis vesicle associated with the sarcolemma (*PV*). At the upper left are sections of two slender mitochondrial processes adjacent to a mitochondrial profile of normal size. × 50,000.

FIGURE 9 A small section of the surface of two neighboring cardiac muscle cells showing smooth-surfaced micropinocytosis vesicles (*PV*), a subsarcolemmal cistern (*SlC*), and, at the right, an area of sarcoplasmic reticulum with polyribosomes (*Rb*) on one of its tubular elements. The larger particles free in the sarcoplasm are glycogen. × 84,000.

FIGURES 10–12 Juxtanuclear areas of sarcoplasm illustrating, at the arrows, the formation of vesicles by evagination of the outer membrane of the nuclear envelope. In cardiac muscle the "forming face" of the Golgi complex evidently receives vesicles from the perinuclear cisterna in much the same manner as the Golgi complex of some secretory cell types receives vesicles budded off from smooth-surfaced areas on adjacent cisternae of the endoplasmic reticulum. × 64,000; × 64,000; × 58,000.


CV
PV
GC
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SlC
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PV
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GC
GC
10
GC
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GC
12

angulation of the internal membranes is very pronounced; in others, it is scarcely detectable except as a rather square outline of the tips of the cristae (Fig. 20).

The mitochondrial matrix is moderately dense and contains conspicuous dense granules 300–400 A in diameter. In some specimens the matrix granules are very abundant (Figs. 19 and 20); in others, they are relatively few (Figs. 14 and 15). Although the number of these granules in mitochondria of other tissues has been reported to vary with the state of physiological activity (80), no clear correlation with physiological state or previous experimental treatment could be established for the various specimens of myocardium examined in the present study.

An unusual feature of mitochondrial form in cardiac muscle is the very common occurrence of slender lateral or longitudinal prolongations about 0.1 μ in diameter and of variable length. In occasional favorably oriented sections in which the continuity of these projections with the main body of the organelle is included, the mitochondrial profiles have a tadpole or tennis-racket outline (Figs. 21 and 22). In other planes of section, the presence of such projections is recognized in the frequent finding of juxtaposed mitochondrial profiles of widely discrepant diameters (Figs. 8, 23, 24).

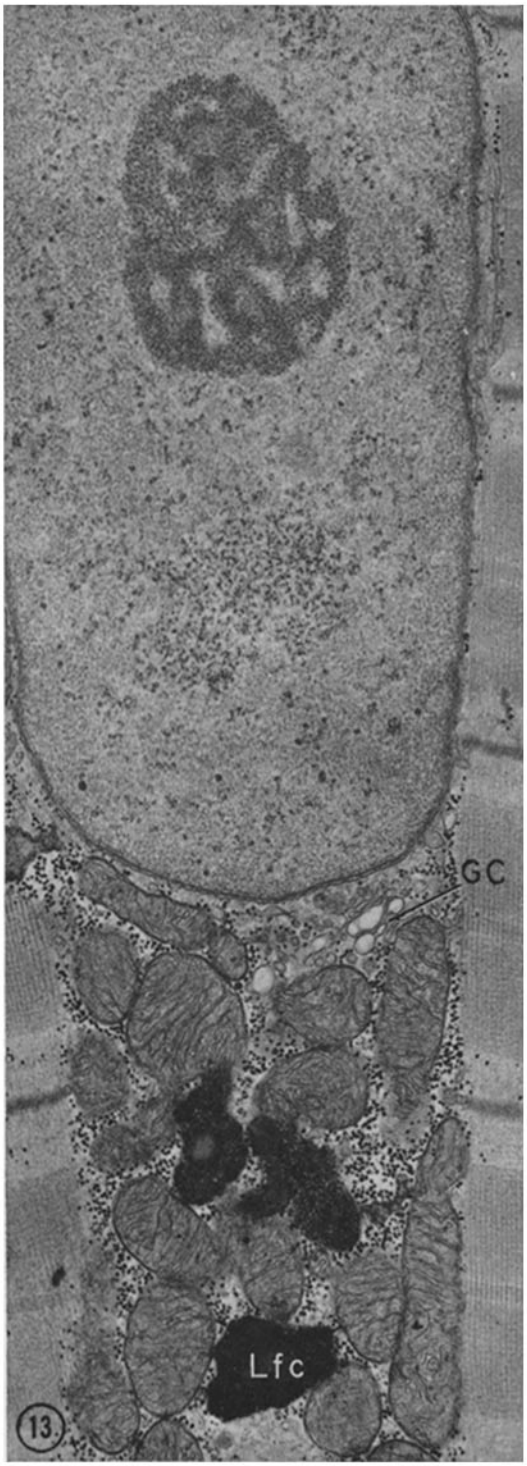
Characteristic of these mitochondrial projections is the presence of very narrow or tubular cristae oriented along the long axis of the projection (Fig. 23, 24). The significance of these slender finger-like prolongations is not entirely clear. It is evident, however, from examination of cross-sections of heart muscle that mitochondria are not randomly distributed but are so evenly spaced (Figs. 3 and 15) as to suggest that each mitochondrion serves only a very limited area of the myofilament mass immediately surrounding it. Accumulation of myofilaments beyond a certain critical diffusion distance would probably require redistribution of the existing mitochondrial mass or formation of new mitochondria. It is possible that the mitochondrial processes represent a means of increasing surface and extending a mitochondrion to shorten the diffusion distance to an area of the myofilament mass beyond the domain served by the main body of the organelle. Alternatively, in a muscle cell undergoing hypertrophy or continually renewing and reordering its contractile material, the projections may represent a stage in a process of new formation of mitochondria by which mitochondrial buds are produced that extend into neighboring areas of the myofilament mass and ultimately separate to form independent mitochondria.

PIGMENT: The myocardium of young cats is relatively free of lipofuscin pigment, but in older animals such pigment is abundant (Fig. 13). This pigment is located almost exclusively in the axial sarcoplasm at the poles of the nucleus and occurs in membrane-limited masses of irregular shape, 0.5–2 μ in diameter. These masses have a highly variable fine structure, but they seem to be agglomerations of exceedingly dense granules of varying size embedded in a slightly less dense matrix. If the prevailing interpretation of such deposits (26) as end stages of lysosomal activity—the insoluble residues of degraded organelles—is correct, their localization in the core of the cell raises the interesting question as to how effete or damaged mitochondria isolated in clefts among the myofilaments make their way to the juxtanuclear cytoplasm.

GLYCOGEN AND LIPID: The sarcoplasm is rich in dense granules ranging from 150 to 300 A in diameter. Small 150 A granules occurring in

FIGURE 13 The elongated nucleus of the cardiac muscle cell is centrally situated in a fusiform axial core of sarcoplasm free of myofilaments, but rich in other organelles and inclusions. The nucleolus in the upper part of the figure displays the usual structure of this organelle. A small Golgi complex (*GC*) is adjacent to the end of the nucleus. In old cats, deposits of lipofuscin pigment are very common in this region but are seldom found in other parts of the cell. × 23,000.

FIGURE 14 A comparable area of a cardiac muscle cell from a kitten showing the absence of lipofuscin pigment and the abundance of mitochondria in the conical core of sarcoplasm extending from the ends of the nucleus (*Nel*). The mitochondria are quite pleomorphic and often have slender lateral or terminal processes some of which are shown here in various planes of section (at the arrows). × 25,000.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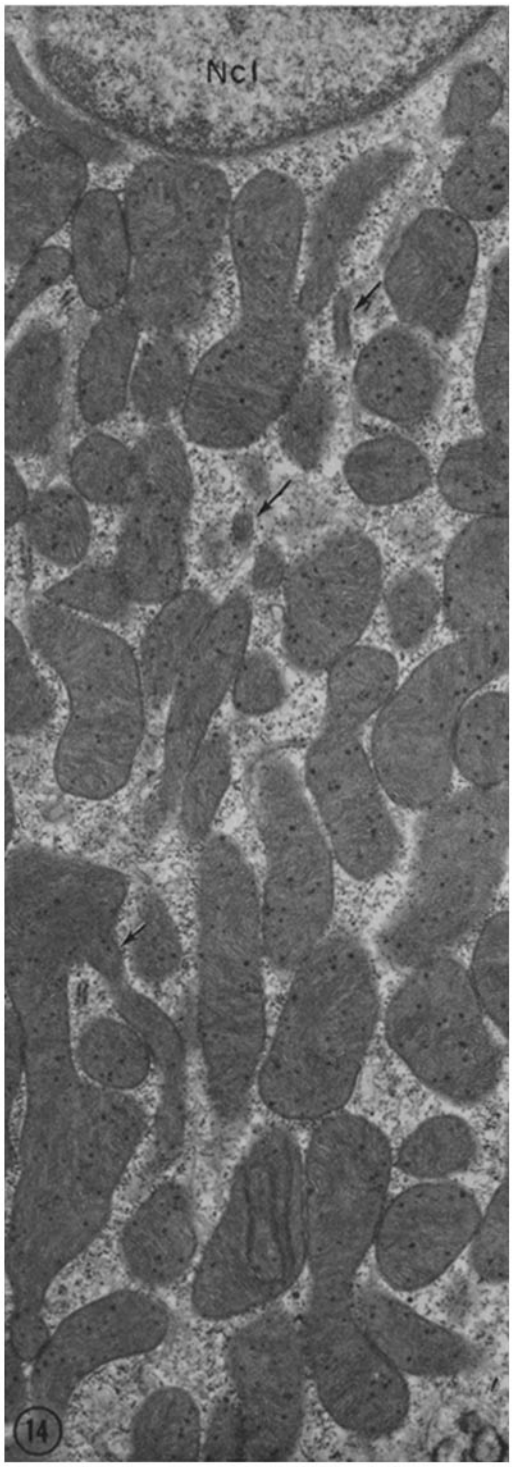
Ncl
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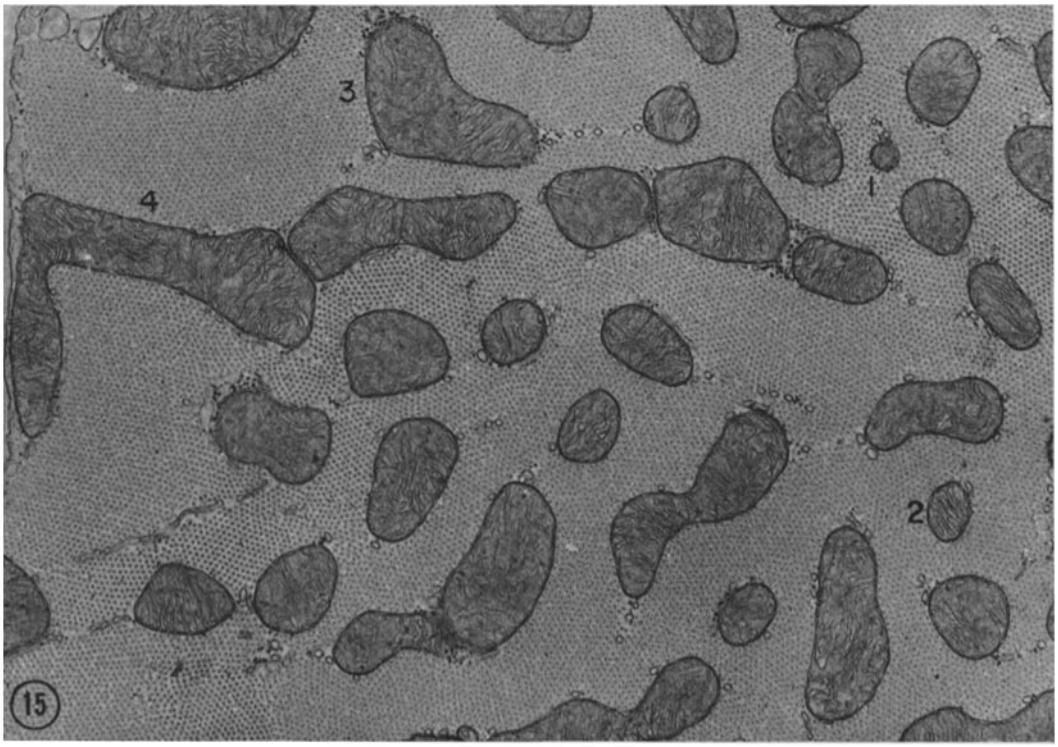
rows and associated with the surface of tubular elements of the sarcoplasmic reticulum are polyribosomes (Figs. 9, 40). Ribosomes may also exist free in the sarcoplasmic matrix, but are difficult to distinguish from the smallest glycogen particles because of the overlap in the size range of these two populations of particles. The larger particles 250–300 A in diameter which stain intensely with lead and uranyl ions are readily identifiable as glycogen in routine preparations. The carbohydrate nature of these larger particles was further established by the selective enhancement of their contrast when treated by a histochemical procedure devised by Revel and Karnovsky (personal communication) for localization of carbohydrates.

Glycogen of cardiac muscle usually occurs as individual beta particles rather than in the form of the larger aggregates called alpha particles which are characteristic of liver cells. In rare instances, however, aggregates of ten or more beta particles are observed. The bulk of the glycogen is found in the cones of sarcoplasm at the poles of the nucleus (Fig. 6) and in the interfibrillar clefts in the contractile substance occupied by mitochondria and sarcoplasmic reticulum (Fig. 38). It is also present, however, in small amounts among the myofilaments. The distribution of glycogen with respect to the cross-striations is not random. It is preferentially localized as single particles or as particles aligned in short rows between the thin filaments of the I band (Figs. 25 and 26). A few particles are also found in the H band immediately adjacent to the M line, but only very rarely in other regions of the A band. This localization of glycogen particles is probably not related to specific metabolic events of the contractile cycle occurring at these particular sites, but is probably merely a reflection of the fact that these sites are the places in the sarcomere in which the spaces between myofilaments are largest. Clearly, there is insufficient room for particles of this size in the region of interdigitation of thin and thick filaments at the ends of the A band. One wonders whether their occurrence along the margins of the M band does not offer a mechanical obstacle to the insertion of the actin filaments in contraction.

Lipid droplets are a normal constituent of cells in the ventricular myocardium (65). They are not common in the axial sarcoplasm or at the periphery of the cell, but are usually found between the ends of the successive mitochondria aligned in clefts within the myofilament mass (Fig. 27). After glutaraldehyde fixation, the droplets are regular in outline and very lightly stained, appearing uniformly pale grey throughout. After primary fixation in osmium tetroxide, they are blackened (Figs. 28 and 29) and may either be round and smooth-contoured or shrunken and irregular in outline (Figs. 1 and 35) depending upon the buffer and dehydration procedure used. The droplets are 0.3 to 1.0 μ in diameter, devoid of a limiting membrane, and are in very close association with the mitochondrial surface, often occupying deep indentations in the ends or sides of mitochondria (65, 79). Where transverse sections of cardiac muscle pass through the I band, lipid droplets seem more abundant than where the section cuts across the A bands. In longitudinal sections, the explanation for this is evident. Since lipid droplets occur mainly between the ends of the mitochondria, their tendency to be located at the I band is probably secondary to the preferential localization of the mitochondria adjacent to the A bands. The intimate relation of the lipid droplets to the mitochondrial surface is no doubt related to

FIGURE 15 A micrograph of a cardiac muscle cell in transverse section illustrating the considerable range of size and cross-sectional shape of the mitochondria. Compare, for example, profiles *1* and *2* with *3* and *4*. Those profiles in the center of the fiber tend to be more or less cylindrical (*1* and *2*), whereas many of those near the surface are larger and flattened so that both their radial and their longitudinal axes are quite long (*3* and *4*). This micrograph illustrates again the continuous nature of the myofilament mass. × 24,000.

FIGURES 16–18 Examples of the periodic angulation of the membranes of the mitochondrial cristae which is very commonly seen in heart muscle. The tips of the angles on successive cristae may coalesce (as at arrow in Fig. 16), giving rise to hexagonal patterns resembling honeycomb. × 75,000.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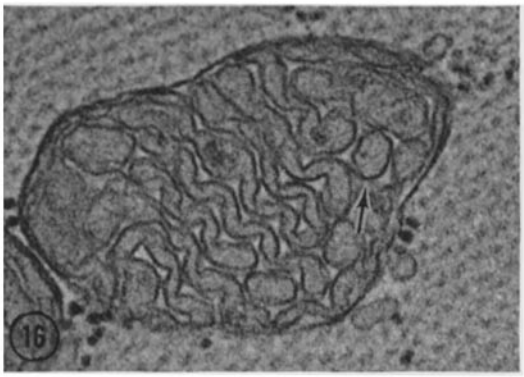
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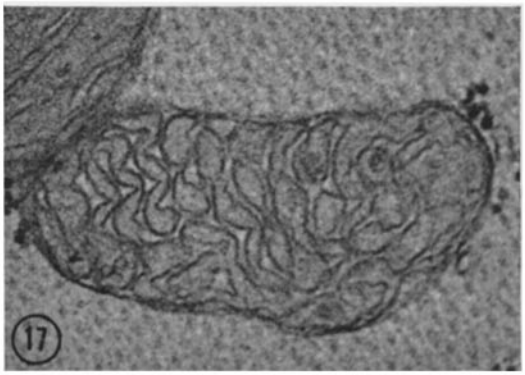
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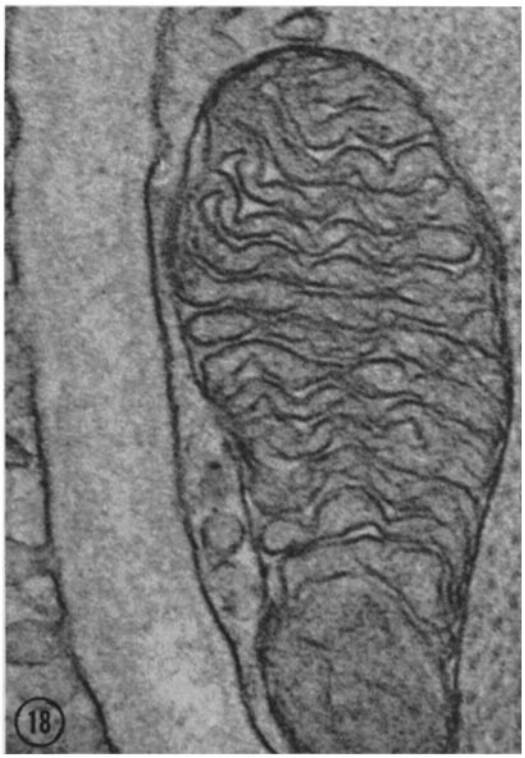
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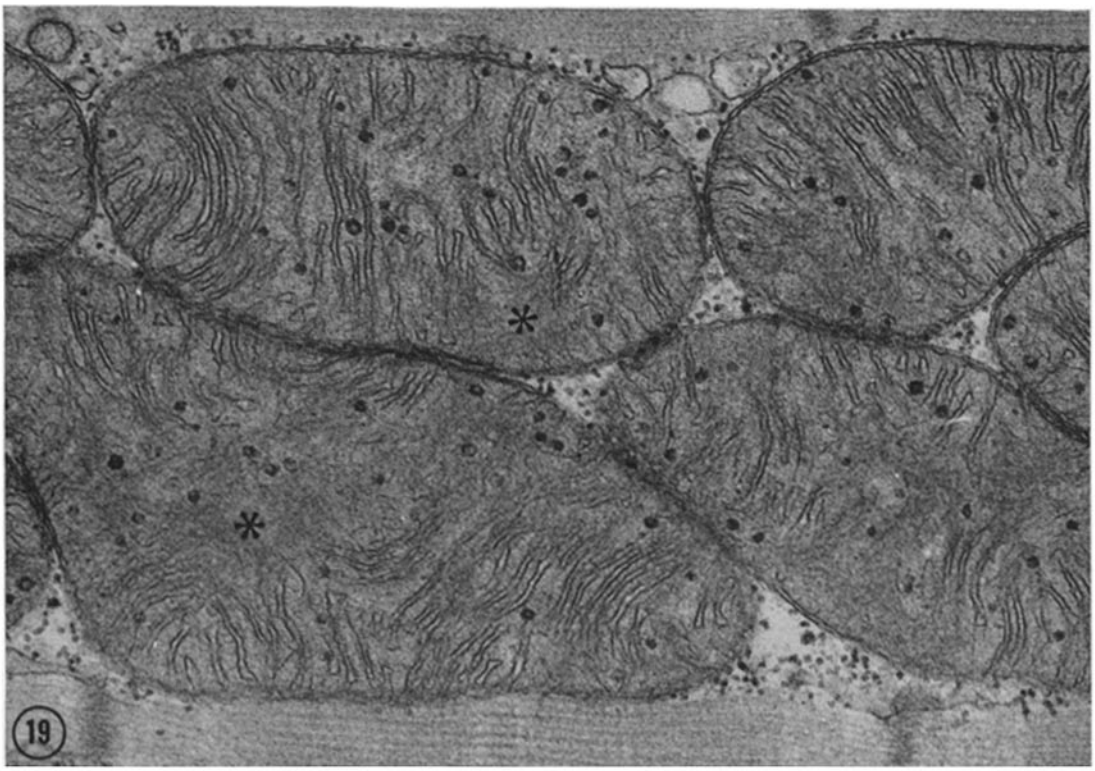
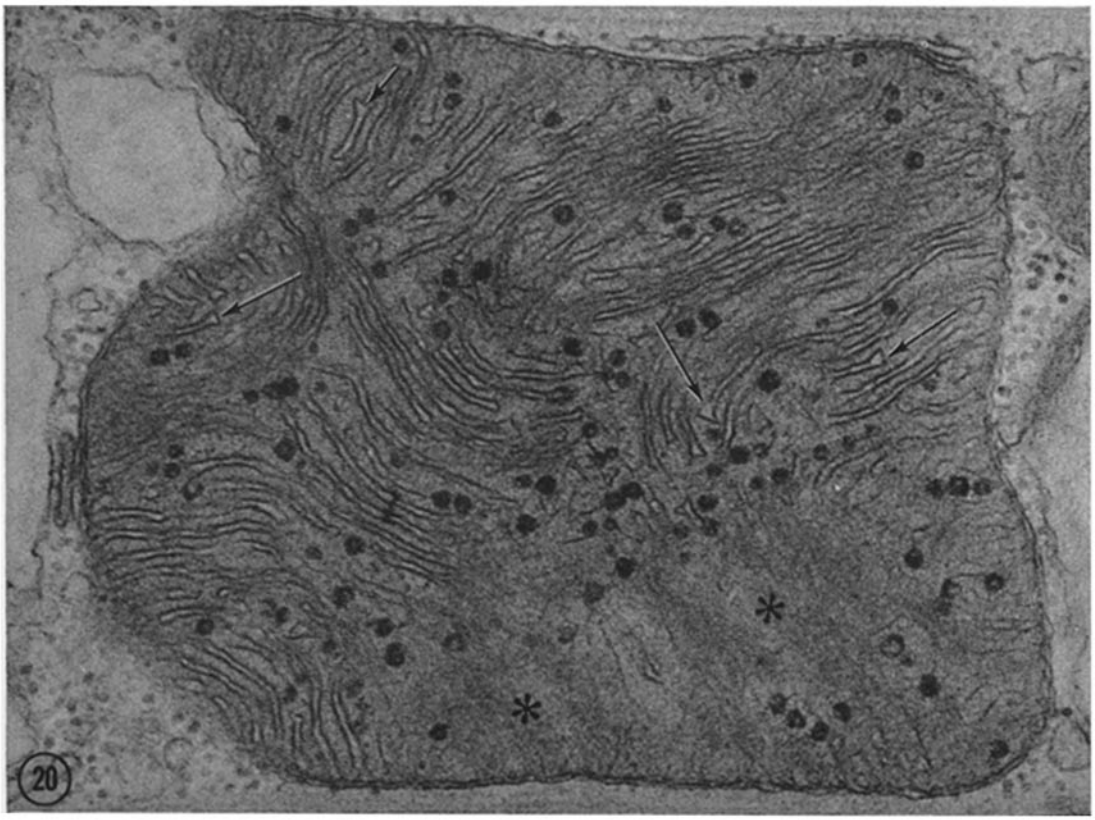
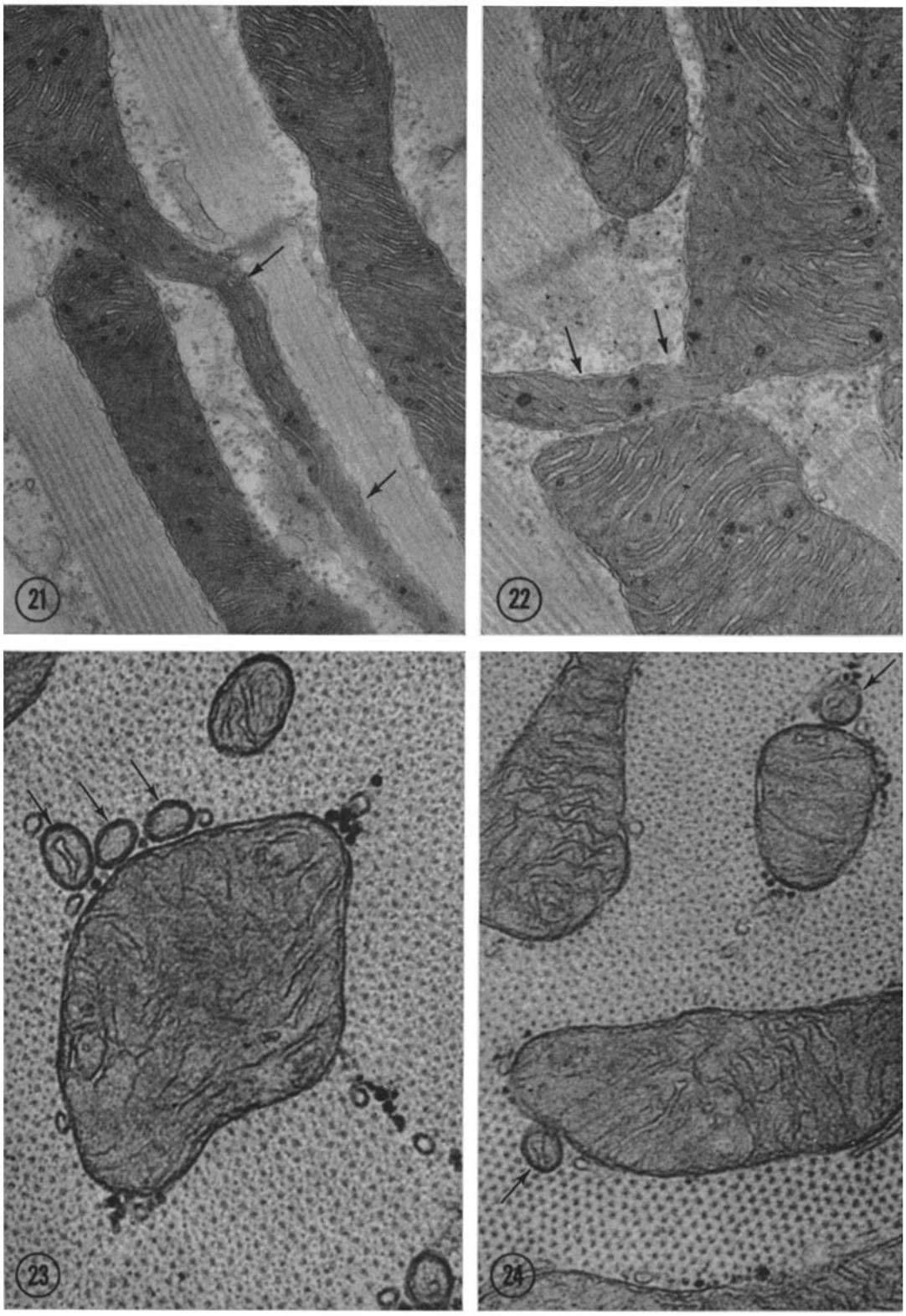


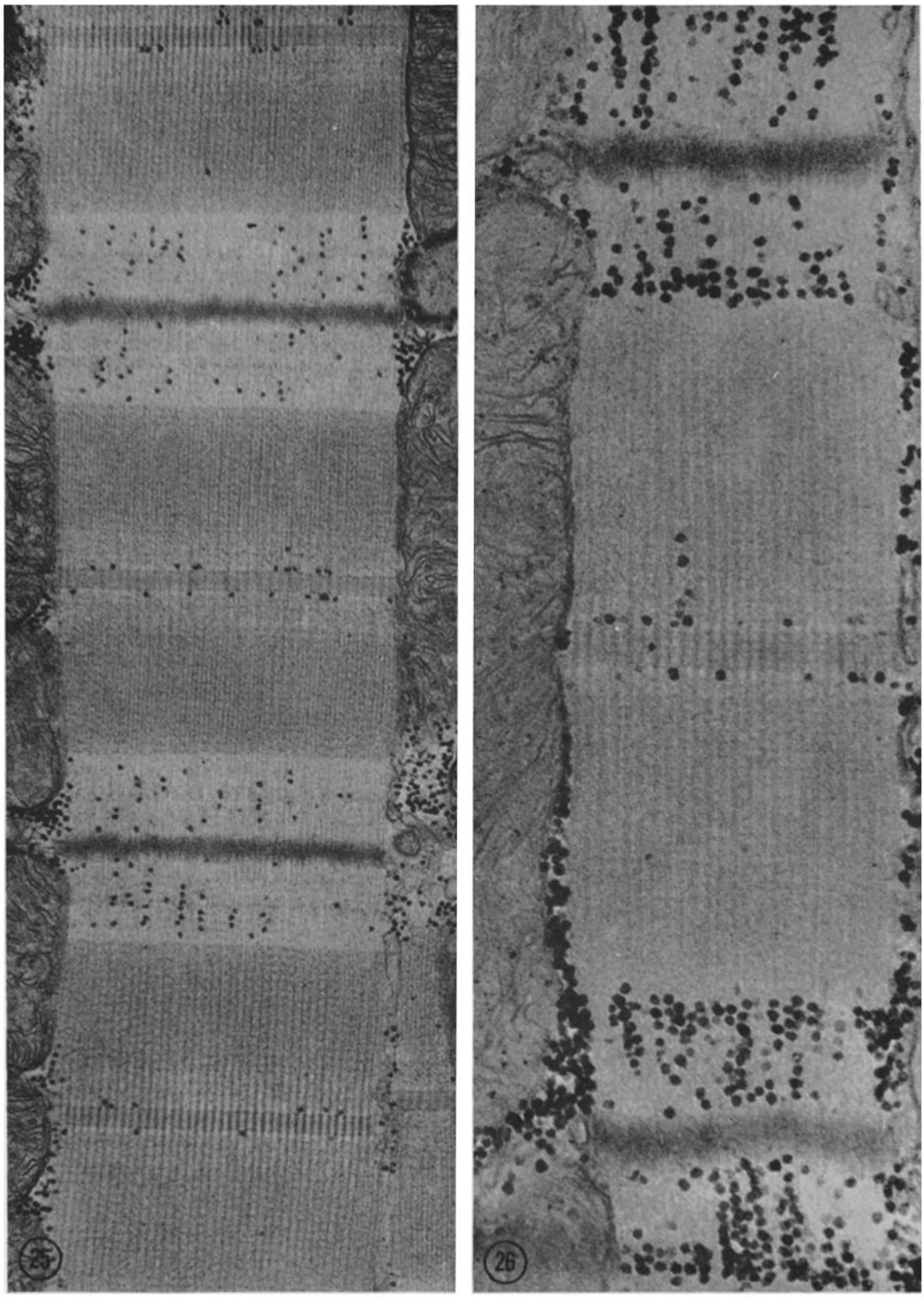


FIGURES 19–20 Typical appearance of cardiac muscle mitochondria. Groups of cristae run parallel, but their orientation is not consistent and, as a consequence of their curving course, they are tangential or parallel to the plane of section in some areas. Such areas (asterisks) give the erroneous impression that they are devoid of cristae. Notice that even though the cristae do not show a zigzag course in these mitochondria, this tendency to angulation is manifested by square ends on the cristae (arrows). In the specimens illustrated here, dense matrix granules are abundant whereas in other specimens (viz. Figs. 3, 15, and 18) they are few or absent. × 50,000; × 59,000.



FIGURES 21–22 Not infrequently in longitudinal sections mitochondria of cardiac muscle are seen to possess long slender processes that extend longitudinally or laterally from the main body of the organelle. (arrows). × 30,000; × 35,000.

FIGURES 23–24 In transverse sections, the slender mitochondrial processes often appear as miniature mitochondrial profiles (arrows) adjacent to profiles of normal size. They have a typical double limiting membrane and one longitudinally oriented crista or sometimes none. × 80,000; × 65,000.



FIGURES 25–26 Glycogen is abundant in the axial core of sarcoplasm, and among the mitochondria in the clefts in the myofilament bundle. Glycogen particles also occur between the myofilaments. They are found most often between the thin filaments in the I band and adjacent to the M line of the A band. This localization is presumed to be related to the fact that there is slightly more space at these sites than in regions where the two sets of filaments interdigitate. × 39,000; × 75,000.

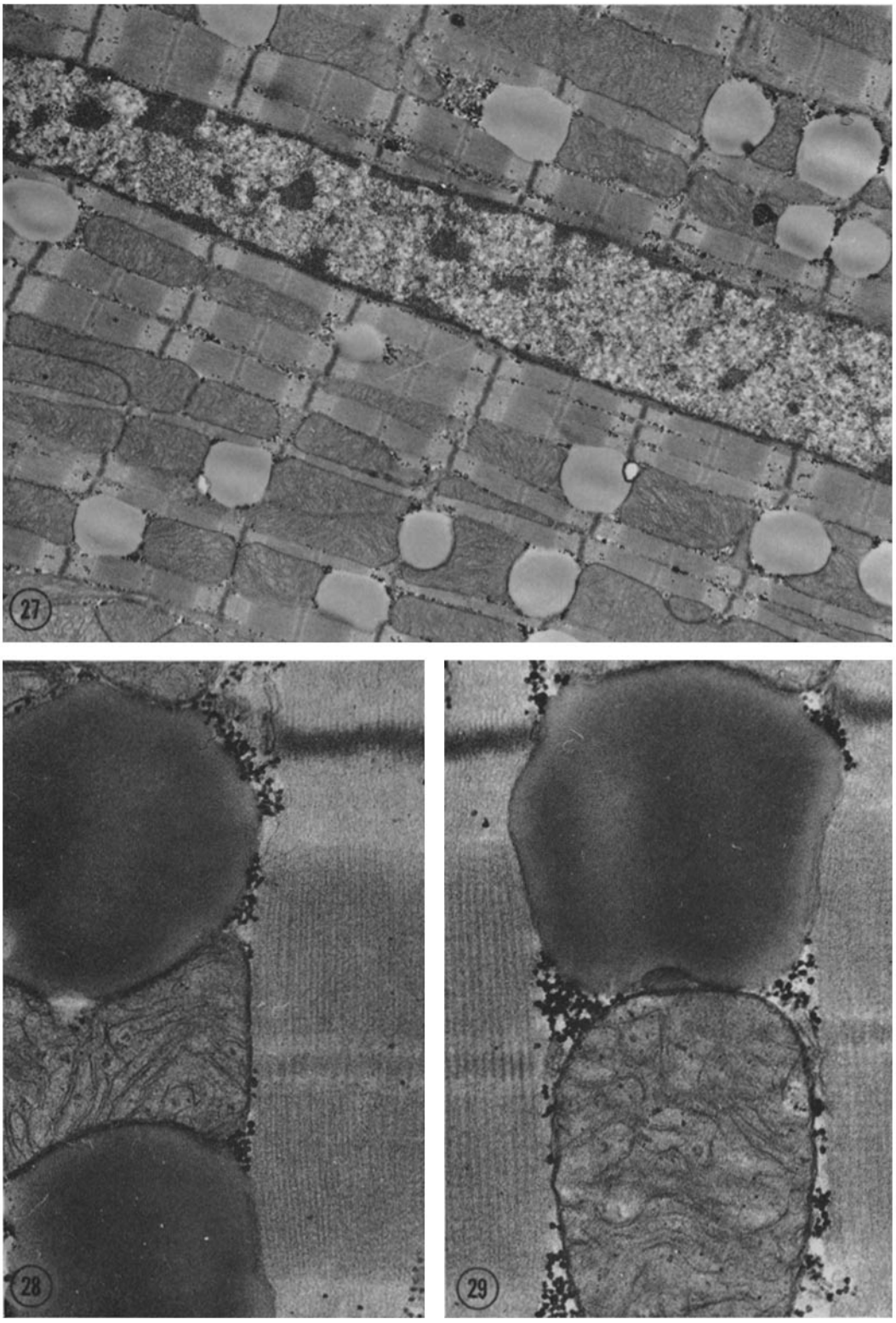


FIGURE 27 A low-magnification micrograph of papillary muscle showing an elongated nucleus and numerous lipid droplets among the mitochondria. After primary glutaraldehyde fixation, the lipid is generally quite pale. × 12,000.

FIGURES 28–29 After OsO_4 fixation the lipid droplets are blackened. These two figures show the intimate relation of the lipid droplets to the mitochondria and the absence of a limiting membrane around the lipid. × 48, 000; × 50,000.

the known participation of mitochondria in fatty acid synthesis and oxidation (106).

The Sarcoplasmic Reticulum and the T System

To have a basis for comparison, it will be advantageous to review briefly the evolution of our present concept of the relationship of the sarcolemma to the internal membrane systems of the sarcoplasm in skeletal muscle.

Each myofibril of skeletal muscle was shown by Bennett and Porter (6) in 1953 to be surrounded by a lacelike network of tubular elements comprising the sarcoplasmic reticulum. The predominant orientation of the component sarcotubules was longitudinal; in later work on amphibian muscle, it was found that these tubules were confluent with conspicuous transverse elements of larger caliber called terminal cisternae (87), situated on either side of the Z line. Between the parallel terminal cisternae was a row of small tubular and vesicular profiles. The apparent discontinuity of these membrane-limited intermediate elements later proved to be artifactitious (86) and, with improved fixation, a slender continuous tubule ~40 mμ in diameter could regularly be demonstrated between the pairs of terminal cisternae (90). The lumen of this tubule was subsequently shown to be in open communication with the extracellular space (20, 32, 33, 48, 75, 76, 77, 81). Individually the slender transverse elements which are continuous with the sarcolemma are called *T tubules* and collectively they constitute the *T system* of skeletal muscle. Although they were originally considered to be a part of the sarcoplasmic reticulum, they are now regarded a separate system of invaginations of the sarcolemma. A transverse tubule of the sarcolemma and the two associated terminal cisternae of the sarcoplasmic reticulum comprise the so-called *triad* of skeletal muscle (Fig. 30). In fish and amphibians, the triads are at the level of the Z

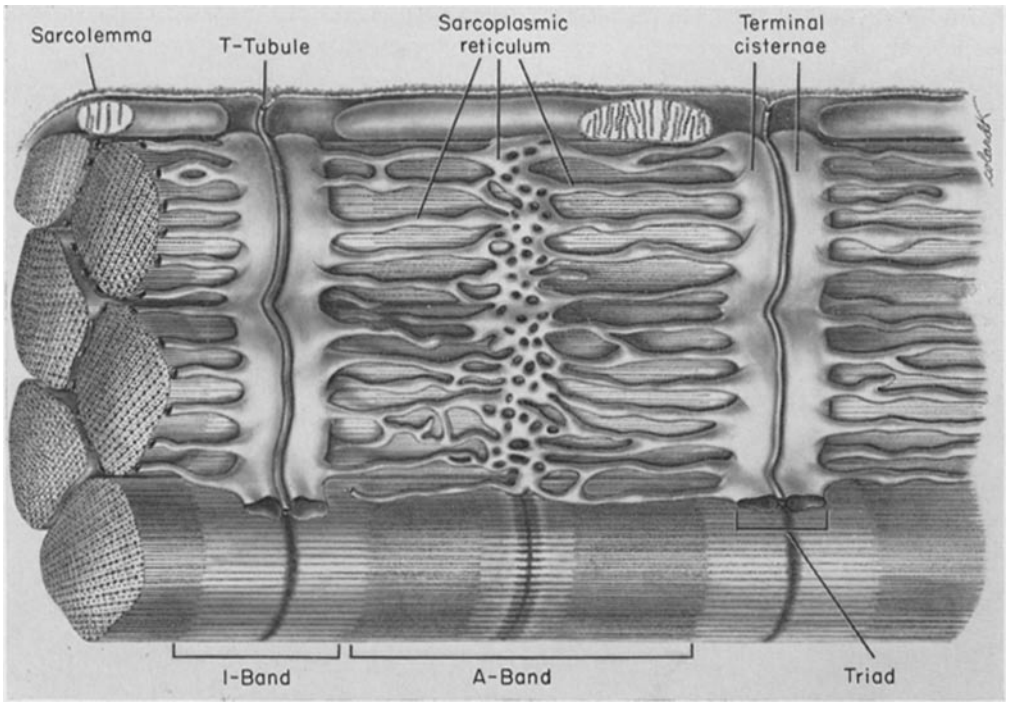


FIGURE 30 A drawing depicting the T tubules and sarcoplasmic reticulum relation to several myofibrils of amphibian skeletal muscle. There are discrete myofibrils of uniform size, each ensheathed by sarcoplasmic reticulum, with pairs of terminal cisternae associated at each Z line with a slender T tubule to form a "triad." The localization of the triads at the Z line of amphibian muscle makes it more comparable to mammalian cardiac muscle. (Redrawn from L. Peachey. 1965. *J. Cell Biol.* 25:209).

lines but in mammals they occur, two to a sarcomere, at or near the A-I junctions. It is now widely accepted that the T tubules of the triads provide for spread of electrical activity from the surface of the muscle fiber into its interior (37, 45, 83), while the terminal cisternae and the contiguous longitudinal sarcotubules of the reticulum are probably involved in release and recapture of calcium ions in the contraction-relaxation cycle (15, 18, 41).

Early investigators of the fine structure of the heart found none of the triads characteristic of skeletal muscle, but recognized, in thin sections, pairs of membrane-limited structures consisting of a narrow elongate profile closely apposed to the periphery of a larger circular opening. These structures were termed "dyads" in the belief that they corresponded to the triads of cardiac muscle (87). Some investigators erroneously interpreted the larger profile as corresponding to a single terminal cisterna and the smaller, to the intermediate element of the striated muscle triad (24). Continuity between a system of transverse tubular structures and the extracellular space of striated muscle was actually first suggested by Lindner (62) on the basis of electron microscopic studies of cardiac muscle, but this observation was largely overlooked. Subsequent investigations of heart muscle of several species have now clearly established that it is the larger element of the so called dyads of cardiac muscle that corresponds to the intermediate tubule of the triads of skeletal muscle. The continuity of these relatively large transverse tubules with the cell surface has now been repeatedly demonstrated (4, 25, 30, 71, 99). The diameter of T tubules of cardiac muscle varies somewhat with the method of specimen preparation, but it is of the order of 1500–2000 A as compared to ~400 A for the T tubules of skeletal muscle. Owing to the straighter course and larger size of T tubules, their openings at the cell surface are encountered in electron micrographs of cardiac muscle more frequently than in electron micrographs of skeletal muscle. Occasionally, the *openings* are found at several successive Z lines in the same section (Figs. 32 and 33), and the inference can be made that they tend to occur in rows. This is borne out in recent observations by the freeze-etch method which show that the apertures of the transverse tubules appear on the cell surface in approximately parallel rows (89). These rows are interpreted as corresponding longitudinally to the clefts in the myofilaments occupied by the sarcoplasmic reticulum, and transversely, to the in-register Z-band regions.

At the mouths of the transverse tubules, the protein-polysaccharide coating of the sarcolemma can be traced without interruption into the tubules (Fig. 32), and its presence is helpful in identifying profiles of this system deep in the interior of the fiber (Figs. 36 and 37). As noted by Simpson and Oertelis (98, 99), additional evidence that the sarcolemma and the membrane lining the T system are identical is to be found in the frequent occurrence, on both structures, of small vesicular invaginations of the kind commonly interpreted as a form of micropinocytosis. The depth of penetration of the T tubules into the cell is, no doubt, variable, but it is not uncommon to find sections of them near the nuclear envelope or in the axial core of sarcoplasm. Thus, the length of T tubules may be as much as half the diameter of the fiber. The term "transverse tubule" accurately describes their prevailing direction, but it is somewhat misleading in that it tends to obscure the fact that there are also branches of this system that run longitudinally and connect successive T tubules (Fig. 35). In longitudinal sections, these longitudinal T tubules are not easily distinguished from extracellular clefts between adjacent muscle cells, but in transverse sections of heart muscle their circular profiles are easily identified and they are found to be quite numerous (Fig. 36). Longitudinal connections between the transverse tubules of different sarcomeres were described for skeletal muscle by Veratti (113) and Revel (90). Their occurrence in cardiac muscle was noted by Nelson and Benson (71) and Simpson and Rayns (100), but they have not received the attention that they deserve.

The sarcoplasmic reticulum of cardiac muscle is less extensive than that of skeletal muscle, and its plexiform pattern is not locally modified in relation to particular bands of the repeating cross-striations (Fig. 31). The pairs of transverse cisternae located at the Z bands or at the A-I junctions of skeletal muscle are absent, and there is no region of especially abundant lateral anastomosis of the sarcotubules at the middle of the A band (compare Figs. 31 and 32). Instead, the sarcoplasmic reticulum of cardiac muscle is a simple plexus of tubules of rather uniform caliber freely anastomosing at all levels of the cross-banded pattern (Figs. 39 and 40). In the ab-

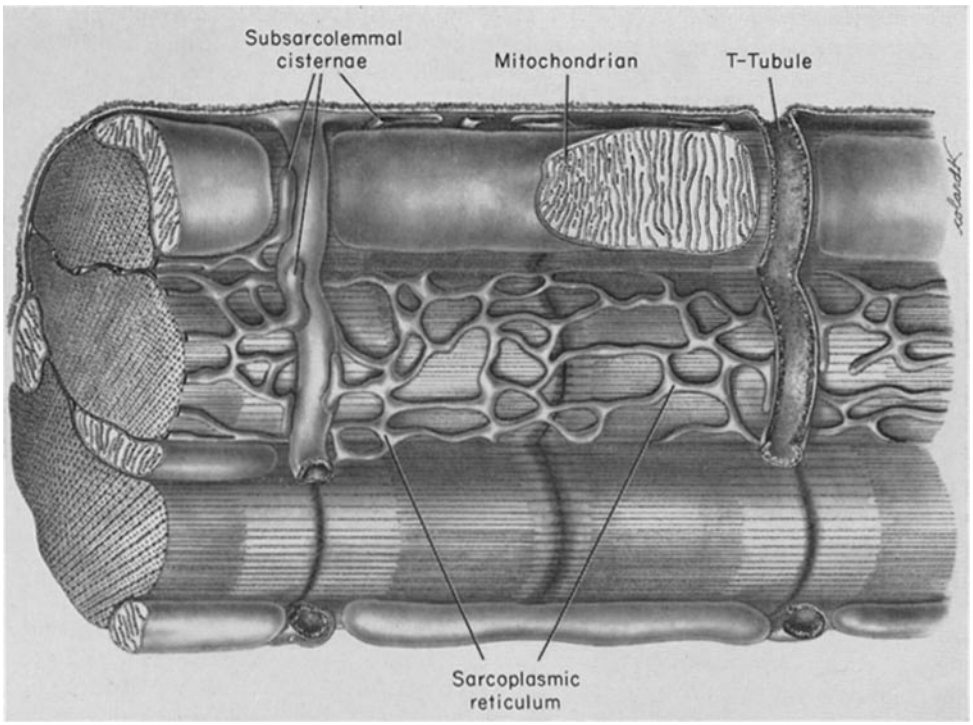
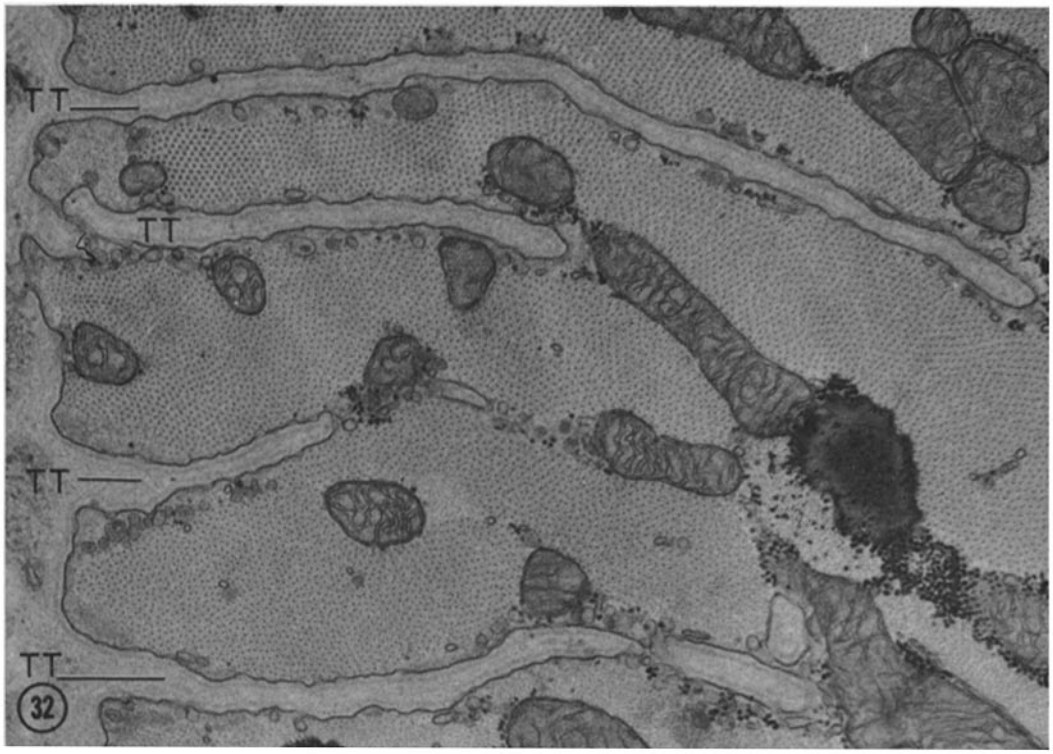


FIGURE 31 A drawing of the T tubules and sarcoplasmic reticulum of mammalian cardiac muscle. Notice the large size of the T tubules, the simpler pattern of the reticulum, and the absence of terminal cisternae. Instead of terminal cisternae, small saccular expansions of the reticulum, called subsarcolemmal cisternae, are in close contact with the T tubes or with the sarcolemma at the periphery of the fiber. The myofilament mass is partially subdivided into irregular myofibril-like areas by clefts that are penetrated by T tubules. (Drawing prepared for this study, but previously published in a Textbook of Histology. 1969. W. Bloom and D. W. Fawcett, W. B. Saunders, Philadelphia).

sence of discrete myofibrils, it ramifies over the surface of the large coherent bundle of myofilaments and extends deep into narrow clefts that partially subdivide this mass of myofilaments into irregular myofibril-like areas. Owing to the meandering course of these clefts, extensive surface views of the reticulum are uncommon in cardiac muscle. It is only when the plane of a thin section coincides for some distance with a surface of the myofilament bundle that an area of the reticu-

FIGURE 32 Micrograph of a transverse section of cardiac muscle showing four T tubules (*TT*) extending inward from the periphery of the fiber. This is an uncommon picture for the reason that the plane of a thin section seldom happens to coincide with one of the rows of T tubes opening onto the surface. The upper three T tubules are somewhat unusual in being located at the level of the A band, but the lower one is at the level of the I band as expected. × 32,000.

FIGURE 33 A transverse section at the periphery of a cardiac muscle fiber showing the origin of three T tubules at the level of the I band. This section also coincides with one of the rows of T tubule openings (*TT*) at the periphery of the fiber. Both the sarcolemma and its protein polysaccharide coat are invaginated to form the T system which is clearly a direct extension of the extracellular space into the "interior" of the cell. Three subsarcolemmal cisterns associated with T tubules are indicated by arrows and asterisks. × 35,000.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TT
TT
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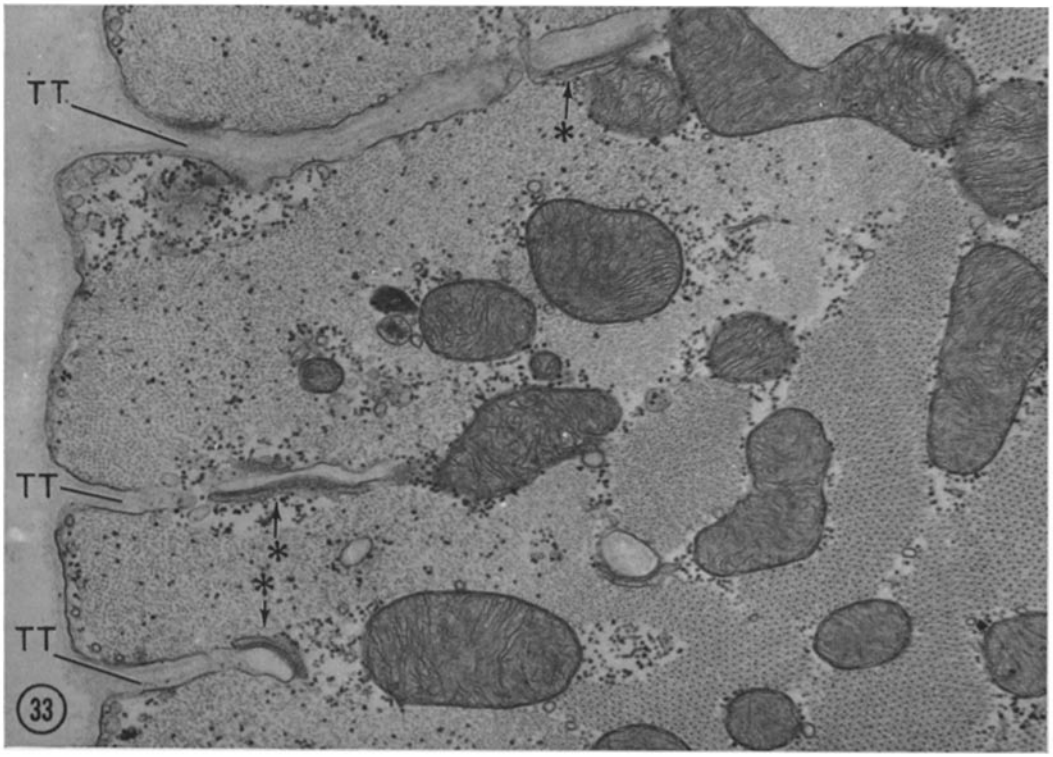
TT
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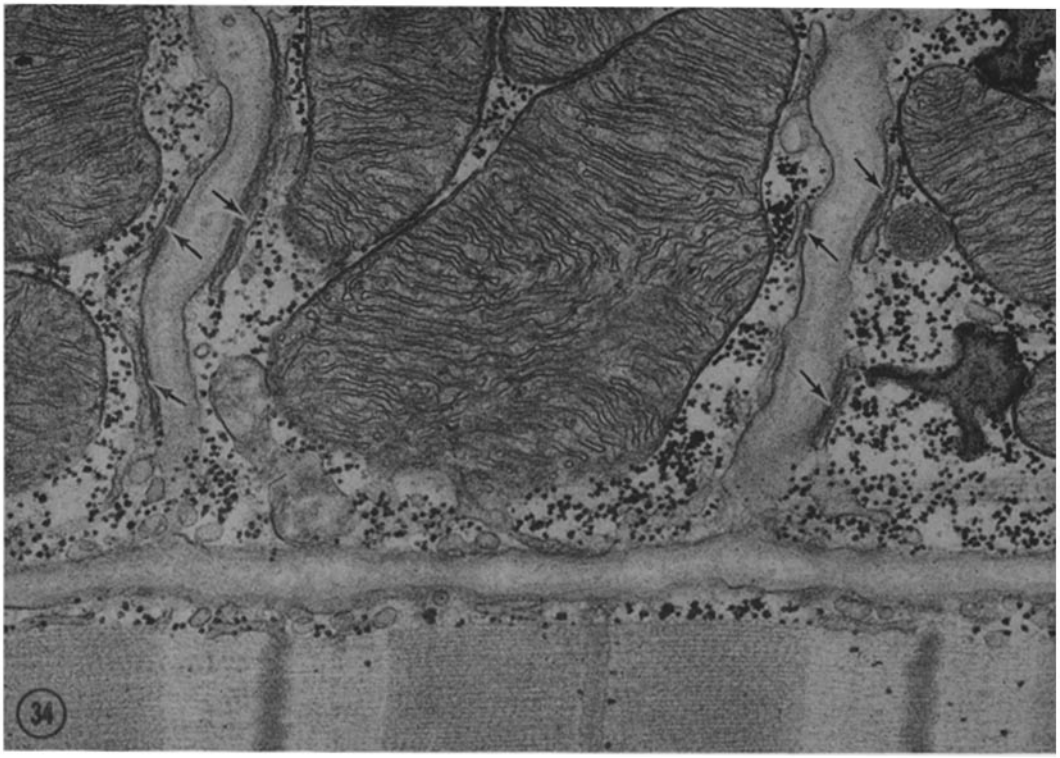


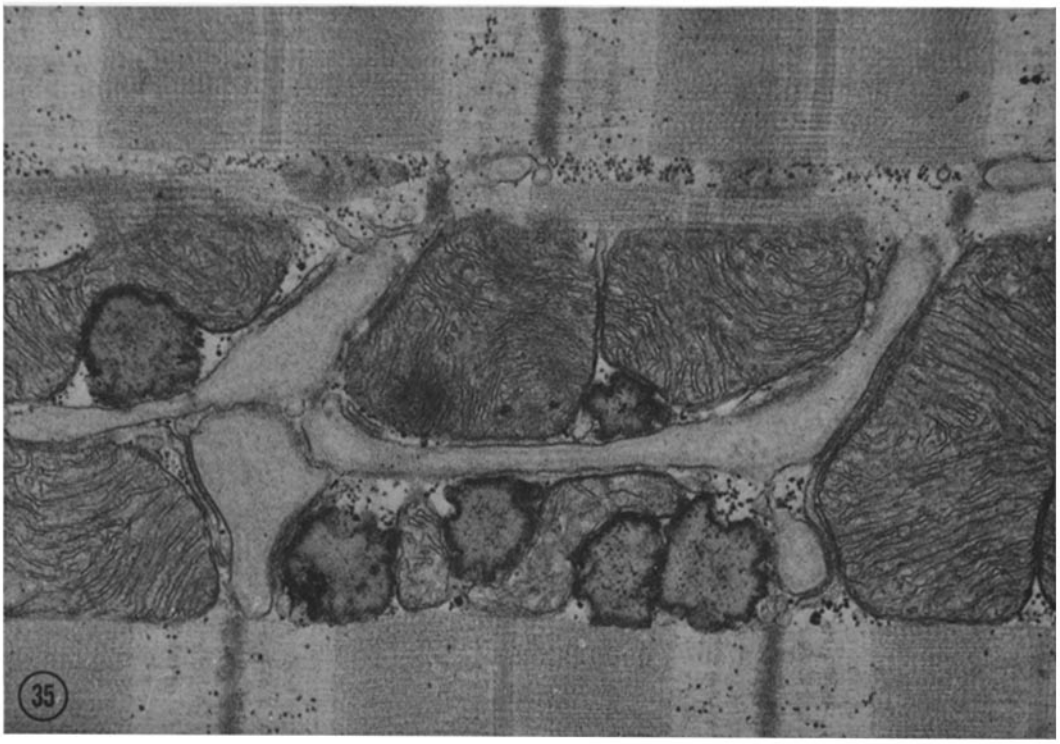


FIGURE 34 Longitudinal section of papillary muscle showing two T tubules and, at the arrows, several sites of contact with subsarcolemmal cisternae of the reticulum. The T tubules curve out of the plane of section, and their openings onto the surface are not shown here, but are in the neighboring section. × 40,000.

FIGURE 35 Longitudinal section of the central region of a muscle cell illustrating two longitudinal interconnections between successive T tubules. Although as the name implies, T tubules generally run transversely, longitudinal branches such as are shown here occur quite frequently. × 33,000.

lum is seen *en face* (Figs. 40–42). A study of such areas reveals that there are no transverse cisternae and no other interruptions in the longitudinal continuity of the sarcotubular network either at the Z line or at the A-I junctions.

At the I-band region, however, flattened saccular expansions of the sarcotubules are closely applied to the sarcoplasmic surface of the T-tubule (Figs. 31, 34, 36–38). These flattened saccules, or "foot plates" of some authors (70), are the smaller element in the "dyads" of earlier descriptions of cardiac muscle. They are also applied to the inner aspect of the sarcolemma at the periphery of the fibers and occasionally to small areas of the surface of contact between successive muscle cells. Although of much more limited content, these local expansions of the sarcotubules are the anatomical, and presumably the physiological, counterpart of the terminal cisternae of skeletal muscle. Their relationship to the sarcolemma is identical whether they occur at the periphery of the fiber or at the T tubules in its interior. The term *subsarcolemmal cistern* is perhaps the most useful term for these structures, for it is equally descriptive of those associated with the T system and those at the periphery of the fiber. The flattened saccule or cistern is parallel to the sarcolemma for a distance of from 0.1 to 1.0 μ and is separated from it by a 150–200 A space which has a beaded or serrated appearance owing to the presence of globular densities $\sim$150 A in diameter, regularly spaced about 200 A apart (Fig. 45). These ill defined densities between the sarcolemma and the adjacent surface of the reticulum cannot be said to be more closely associated with one membrane than the other. The subsarcolemmal cistern has a content which often appears as a 30–50 A dense line in the center of its 150 A flattened lumen (Figs. 38 and 45).

The sarcoplasmic reticulum of cardiac muscle is very largely composed of smooth-surfaced membranes and is considered to be a special form of the agranular endoplasmic reticulum. However, short segments of sarcotubules may bear polyribosomes on their surface (71) (Figs. 6, 9, 40). These ribosome-studded tubules appear as rather randomly distributed segments in the reticulum. If the membranes are continually being renewed, as appears to be the case in the smooth reticulum of other cell types (16), then these rough-surfaced segments intercalated in the system of smooth sarcotubules may play an important role in the biogenesis of the new smooth membrane. These segments are probably more prevalent than is commonly appreciated, since glycogen particles of similar size occurring in abundance in the same areas tend to interfere with their visual recognition in electron micrographs.

In cardiac muscle of the ox (97) and the ferret (100), a slender circumferential tubule 220–400 A in diameter has been described as closely encircling the "myofibrils" at the level of the Z disc. This structure has been called the *Z tubule* and is said to communicate with the longitudinal sarcotubules of the reticulum and laterally with the Z tubules of other "myofibrils." Although both occur at the level of the Z band, the Z-tubule is said to be easily distinguishable from the T tubules because of its smaller size. According to Simpson and Rayns (100), the Z tubules in the species they have studied form the primary connection of the sarcoplasmic reticulum to the flattened saccules that are in apposition to the T tubules. In the optimally stretched cat myocardium studied here, Z tubules could not be clearly identified. In longitudinal sections providing *en face* views of the reticulum, its meshes are seen to continue uninterruptedly from one sarcomere to the next (Figs. 39–42). No continuous tubule has been found running parallel to the Z line such as depicted by Simpson and Rayns (100). Moreover, in transverse sections, slender circumferentially oriented tubules were not observed at the level of the Z band more frequently than at other levels. We conclude, therefore, that in the cat myocardium there are no regularly occurring circumferential tubules at the Z bands and hence no category of sarcotubules that deserves the separate designation *Z tubule*.

Previously undescribed components commonly found in the vicinity of the Z bands are spherical, coated vesicles 400–600 A in diameter (Figs. 40 and 42). In the character of their limiting membranes, these vesicles resemble the coated vesicles found in the Golgi region and at the cell surface. They usually appear free in the sarcoplasm, but not infrequently they connect by narrow necks to tubular elements of the sarcoplasmic reticulum near the Z lines (Fig. 42). Occasionally, short segments of sarcotubules of the reticulum near the Z band have a limiting membrane that displays a similar finely fibrillar coating (Fig. 40). The frequent localization of coated vesicles at or near the Z bands remains

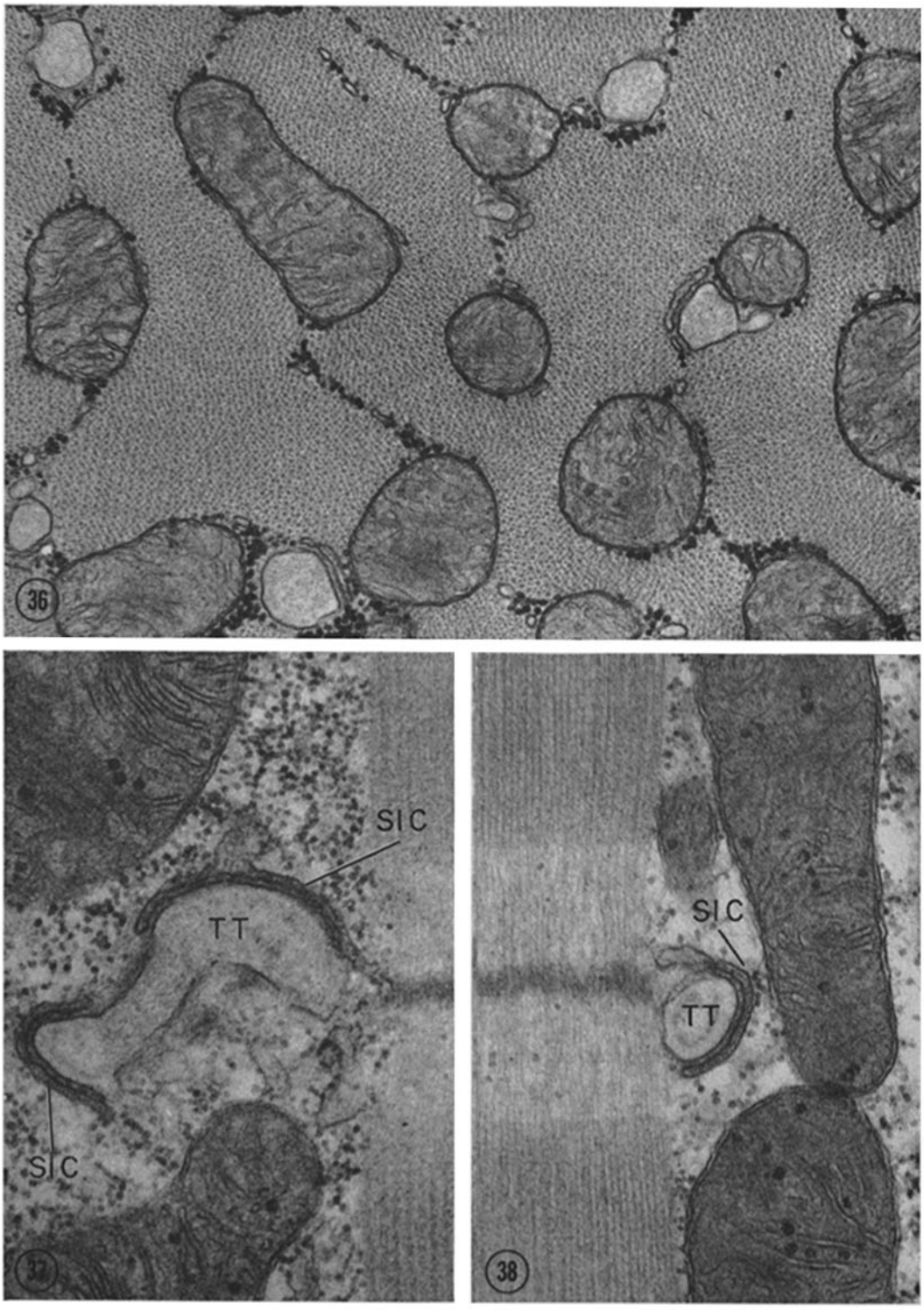


FIGURE 36 Transverse section of papillary muscle fiber showing five rounded profiles of longitudinal branches of T tubules in the relatively small area encompassed by this figure. Two of the branches have subsarcolemmal cisternae associated with them. Longitudinally oriented branches of the T tubules are, therefore, more common than is generally realized. × 54,000.

FIGURES 37–38 Longitudinal sections of papillary muscle showing typical examples of flattened saccules or cisternae of the reticulum (*SIC*) closely applied to T tubules (*TT*). With glutaraldehyde primary fixation the cisternae are rather flattened and appear to have a dense content. × 57,000.

FIGURES 39–40 Two views of the sarcoplasmic reticulum which consists of a simple network of anastomosing tubules, continuous across the Z line and with no local specialization there or over the center of the A band. The circumferential "Z tubule" described by some authors as encircling myofibrils at the level of the Z disc is not a regular occurrence in the cat. In Fig. 40 are three examples of the coated vesicles (*CV*) that are very often located at the Z line. At the upper part of the figure (arrows and asterisks) the membrane limiting a portion of the reticulum has a similar density and surface coat, suggesting that one or more coated vesicles have become incorporated into the wall of the reticulum. × 47,000; × 51,000.

unexplained. The occasional continuity of these vesicles with the sarcotubules in this region suggests, however, that they either arise from or coalesce with the reticulum. Their very common occurrence at the Z band suggests the possibility that these coated vesicles may have contributed to the impression of other authors that circumferential tubules (Z tubules) are common at this site. Since they are a striking finding in the atrial myocardium, these vesicles are described in more detail in the companion paper (66).

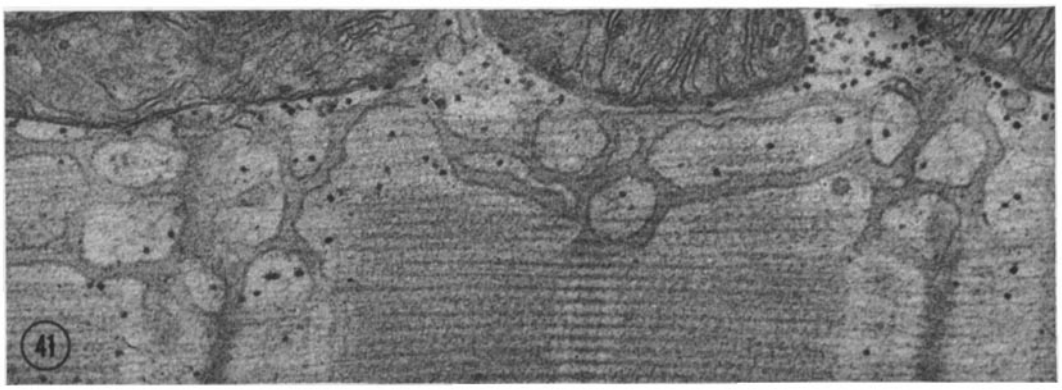
Intercalated Discs

In classical descriptions of the cytology of cardiac muscle, the intercalated discs were variously interpreted as sites of formation of new sarcomeres; irreversible contraction bands; special devices for coordination of contraction; or tendon-like junctions between cellular units. All controversy as to their nature was resolved by the early electron microscopic studies which clearly showed that they are specialized junctions between separate cellular units (28, 68, 69, 85, 100, 110). As a rule, the discs do not extend across the full width of the cell at the same level, but are offset so that the junction as a whole has a steplike course with alternating transversely and longitudinally oriented portions corresponding respectively, to the treads and risers of a staircase (Fig. 1). The cell surfaces in the transverse portions are deeply interdigitated and elaborately specialized whereas in the longitudinal portions the membranes are relatively straight and uncomplicated. The term "intercalated disc" originally referred only to the highly specialized transverse portions which were easily visible with the light microscope. It is now clear that if it is to be perpetuated, the term should be redefined to include the entire continuous steplike cell boundary. It is in this sense that we use the term intercalated disc in this paper. For convenience of description, we distinguish the *transverse segments* of the disc, where rectilinear cell processes meet end to end, and the *longitudinal segments* where these same processes join side to side.

The interdigitated transverse portions of the discs exhibit three distinct types of junctional specializations corresponding in general appearance, but not in extent, to the zonula adherens, zonula occludens, and macula adherens of epithelial junctional complexes (23). Over the greater part of the area of end-to-end cell attachment, the specialization resembles the zonula adherens. The confronted membranes are separated by an intercellular cleft ~200 A in width, and the sarcoplasmic surface of the membranes is reinforced by a thick layer of densely staining interwoven fine filaments. It is into this dense fibrous mat that the actin filaments of the terminal sarcomere penetrate and inset on the end of the cell. Because this kind of specialization extends over areas of variable size, the term *fascia adherens* has been suggested as more appropriate than zonula adherens which implies a beltlike distribution (26). In segments of the junction between the insertions of bundles of myofilaments, typical *maculae adherentes* (desmosomes) are often found. In these, the opposing cell surfaces are ~300 A apart and there is a conspicuous specialization of the membranes consisting of a very dense layer of uniform thickness applied to the inner leaf of the sarcolemma and an associated tuft of fine filaments such as is found at desmosomes in other tissues. Elsewhere along the transverse portions of the intercalated discs, between sites of insertion of myofilaments, are areas 0.1 μ or more in extent in which the cell membranes come into such close apposition that, in low-magnification micrographs, the intercellular gap appears to be entirely obliterated. At these sites, there are few

FIGURES 41–42 Two additional examples illustrating the continuity of the reticulum across the Z line, with no specialized circumferential element that could be designated a Z tubule. Fig. 42 shows, at the asterisks and arrows, examples of coated vesicles with dense content associated with the reticulum at the Z line. The two vesicles at the right appear to be continuous with tubular elements of the reticulum. × 50,000; × 56,000.

FIGURE 43 A portion of lateral junction of two cardiac muscle cells. At the left of the figure, the two sarcolemmae are coated with protein polysaccharide and are separated by a narrow recess of the extracellular space. In the right half of the figure, the membranes are closely apposed forming what is commonly called a nexus or tight junction. × 55,000.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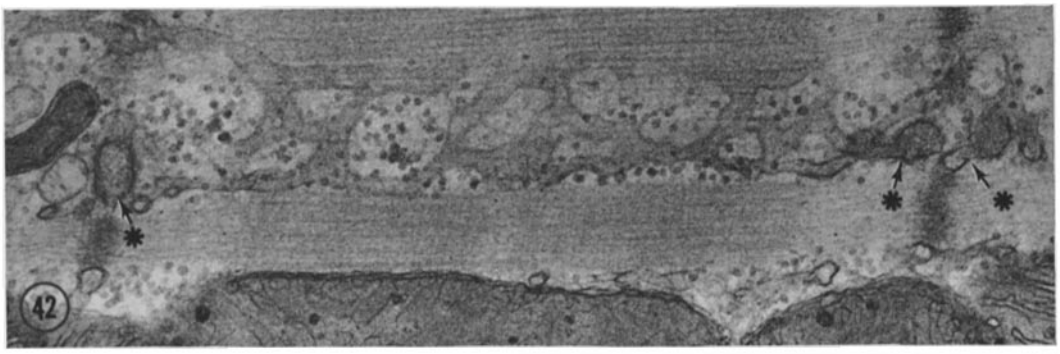
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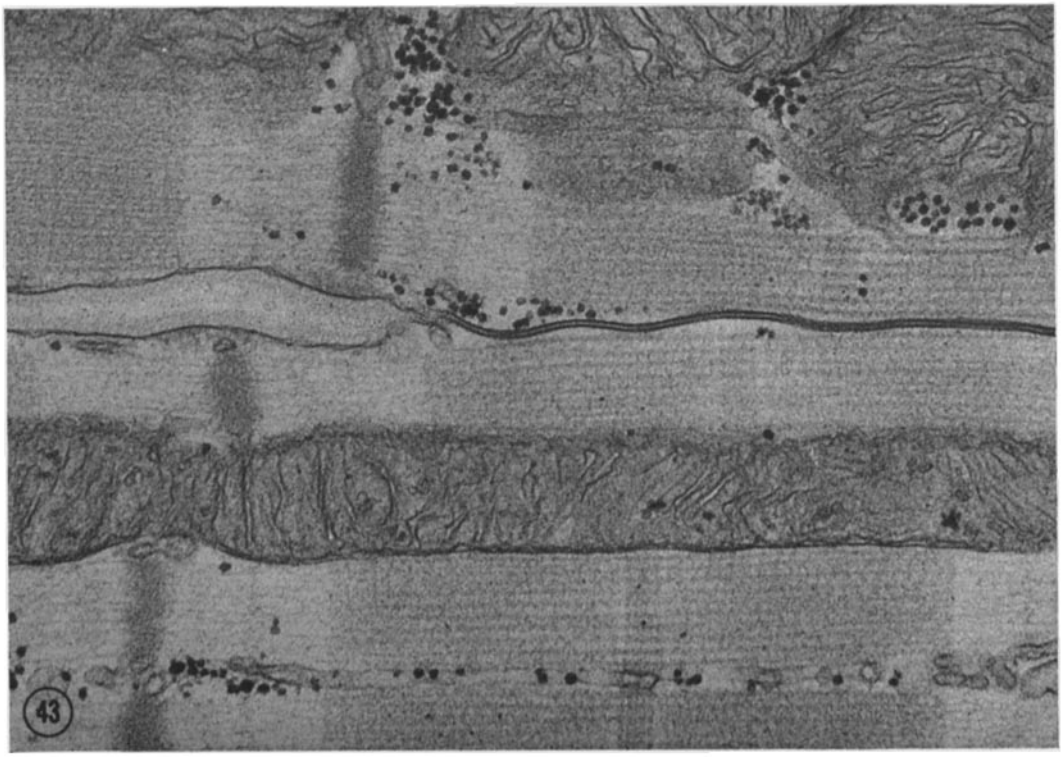
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or no filaments in the subjacent sarcoplasm. The relationships of the membranes in these areas have generally been interpreted as basically the same as at the zonula occludens of an epithelial junctional complex. Because of their limited extent and plaquelike configuration, the descriptive term *macula occludens* has been applied to these small areas of intimate contact within the transverse portions of the discs (26). On the longitudinal portions of the intercalated discs, there are areas of close membrane apposition identical with these but far more extensive (Fig. 43). Their area is variable and their shape in the plane of the junction is not known, but in the absence of any evidence that they girdle the process or the cell as a whole, the term zonula occludens seems inappropriate, and *fascia occludens* has been proposed instead (26).

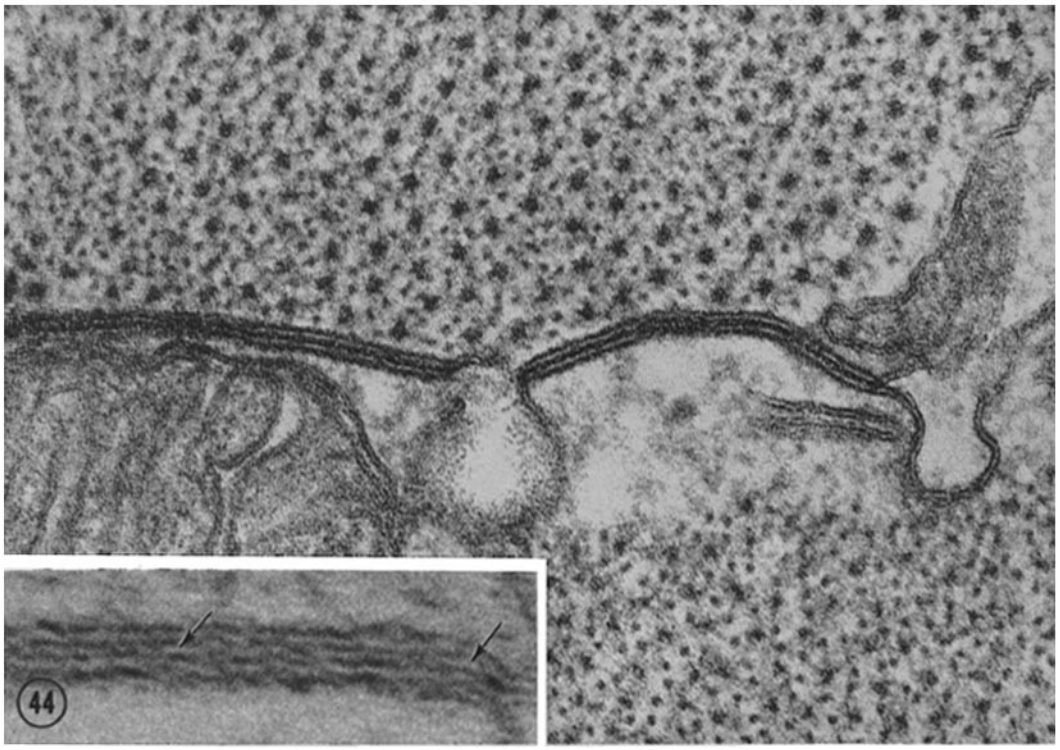
From studies of high magnification electron micrographs, these areas of close membrane apposition have been interpreted as typical quintuple-layered junctions (54, 70, 101). The outer leaflets of the apposed unit membranes have generally been assumed to be fused, completely obliterating the intercellular space, as is reported to be the case at zonulae occludentes of many epithelia (23).

Employing new methods of study, Revel and Karnovsky (92) have now shown that the electron microscopic image of these specializations depends, to an unexpected extent, upon the method of specimen preparation. The change in our concept of the membrane relationships at these close junctions which has resulted from their studies will now require a further change in terminology. In electron micrographs of cardiac muscle fixed in OsO_4 containing solutions and stained with uranyl acetate and lead citrate, a typical five-layered junction is observed in which the outer leaflets of the membranes appear to be fused in a single intermediate dense line (Figs. 43 and 44). However, as Revel and Karnovsky (92) have shown, if cardiac muscle first fixed in a formaldehyde-glutaraldehyde mixture and then postfixed in osmium tetroxide solution is then stained in block with uranium (23) and viewed at high magnification, a narrow gap of about 18 A can be seen between the outer leaflets of the apposed membranes. Moreover, if cardiac muscle fixed in the same manner is immersed in a solution of lanthanum nitrate, the lanthanum is found to permeate the intercellular cleft of the entire intercalated disc. Although the interspace is greatly narrowed in the areas of close junction, the ~18 A gap is readily penetrated by lanthanum (92). In tangential sections of such areas, the membranes exhibit closely packed, subunits with a center-to-center spacing of 90 A separated by channels 30–40 A wide filled with lanthanum, forming a very regular hexagonal pattern. Although the common interpretation of the areas of close membrane apposition as "tight junctions" characterized by fusion of the membranes now seems to be erroneous, this does not necessarily require any modification of the prevailing view that these junctions are probably sites of low electrical resistance permitting rapid spread of excitation throughout the myocardium. A similar hexagonal pattern has been demonstrated in the membranes at electrical synapses in the goldfish brain (94). It has also been found at the junctions between astrocytes and ependymal cells in mouse brain (11) and can be demonstrated by negative staining in some areas of close cell-to-cell contact between liver cells (19).

A detail of the region of insertion of the myo-

FIGURE 44 A micrograph of one of the lateral cell junctions of two cardiac muscle fibers These junctions were formerly considered to be "tight junctions," with fusion of the outer leaflets of the apposed membranes. In very lightly stained sections viewed at high magnification, the outer leaflets of the two unit membranes can be shown to be separated by a narrow gap of 18–20 A (see at arrows on inset). × 300,000.

FIGURE 45 High-magnification micrograph of the sarcolemmal unit membrane (*Sl*) and a subsarcolemmal cisterna (*SlC*). The limiting membrane of the latter is separated from the sarcolemma by a distance of 150–200 A. Periodic densities in this interspace (arrows) give it a beaded or serrated appearance. The content of the saccule or cisterna is often condensed to form a central linear density. The inset presents another example showing to better advantage the periodic densities (arrows) between the subsarcolemmal cistern and the sarcolemma. × 300,000.



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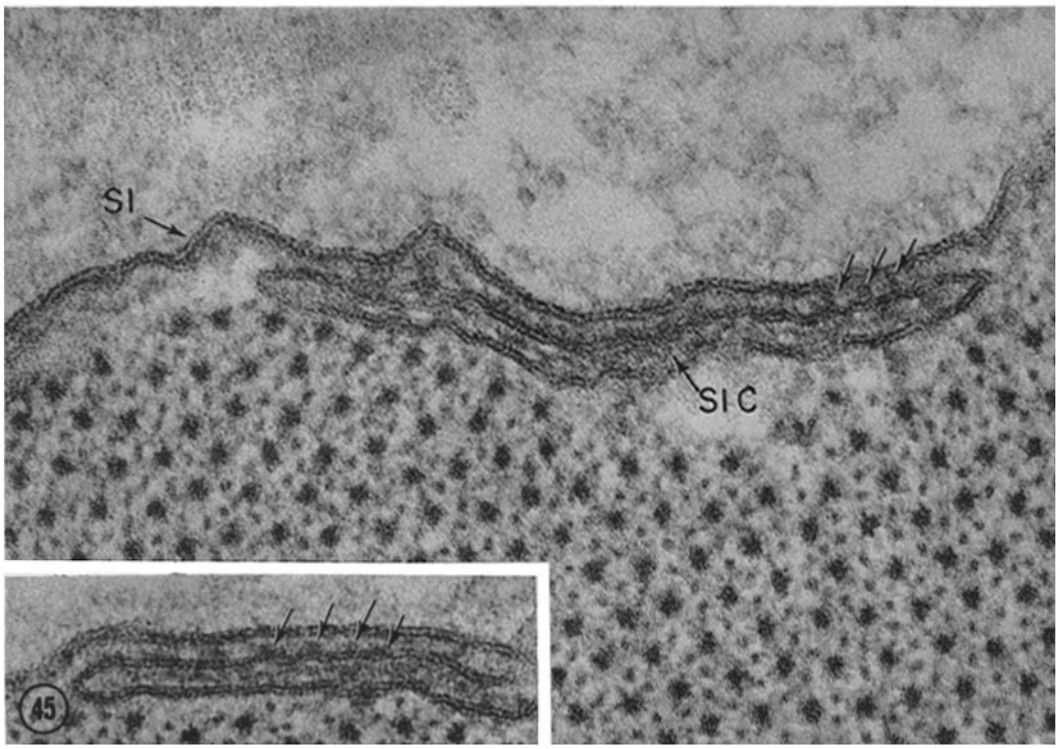
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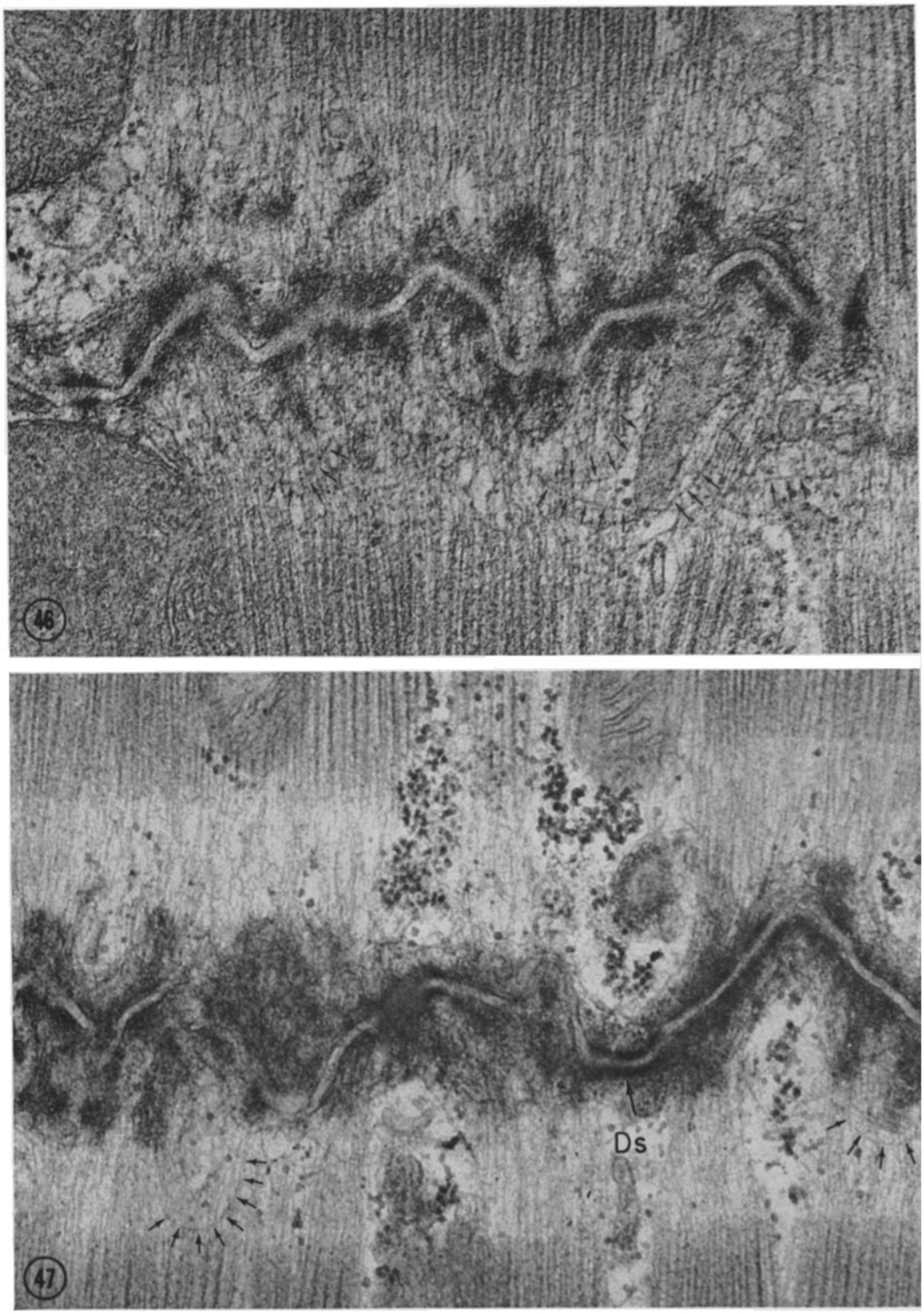

FIGURE 46 Micrograph of a restricted region of the transverse portion of an intercalated disc. The cell surfaces are, for the most part, 200–300 A apart. The actin filaments can be seen entering accumulations of dense material in the sarcoplasm immediately subjacent to the cell membrane. Other filaments near the disc, apparently also actin, are seen coursing transverse or oblique to the prevailing direction of the myofilaments (at arrows). × 70,000.

FIGURE 47 Another example of a transverse segment of the intercalated disc. The portion of the cell junction in which the myofilaments terminate resembles the zonulae adherentes of epithelia, but between bundles of myofilaments typical desmosomes or maculae adherentes are found (*Ds*). In this figure individual thin filaments also can be seen describing arcs transverse to the axis of the myofilament bundles (at arrows). × 68,000.

filaments on the intercalated discs which does not seem to have been noted before is the occurrence of occasional filaments that pursue a curving course transverse to the axis of the bulk of the myofilaments (Figs. 46 and 47). These filaments have the same appearance as the thin filaments of the terminal sarcomere and are presumed to be actin. They have been observed only near the intercalated discs.

Effects of Incubation in Vitro and Changes Associated with Fixation

In addition to establishing the normal fine structure of the right ventricular papillary muscle, one of the objectives of the present study was to determine the cytological effects of incubation of cardiac muscle in vitro under conditions commonly employed by physiologists and pharmacologists (58). A number of the papillary muscles described here were, therefore, fixed after varying periods of time in vitro. The experiments were terminated by immersion of the whole papillary muscle in the fixative. Even though the muscles were very thin, there was an obvious gradient in quality of fixation from the surface to the interior, with the best preservation being found in the outer 0.2 mm. Because of inadequate penetration of the fixative to greater depths, no conclusions could be drawn as to the adequacy of nutrition or oxygenation in the center of the papillary muscles under the conditions of incubation. The observations were, therefore, confined to the outermost 0.2 mm. Incubation in vitro for as long as 11 hr at 35°C in the medium described in Table I had surprisingly little effect upon the fine structure of this outer region of the muscle.

After 1 hr of isometric work in vitro, there was an increase in the width of the spaces between the lateral surfaces of the cells, except where the surfaces were adherent in special junctions. This widening of the intercellular spaces is probably attributable to increased hydration of the extracellular space and is consistent with the finding by Page and Solomon (75) of a slight increase, during incubation in vitro, of the per cent of total water in the space available to inulin. The increased volume of the extracellular space did not seem to be reflected in an enlargement of the diameter of the transverse tubular system.

Equilibration in vitro affected the distribution of sarcomere lengths within the muscle. Papillary muscles cut free of their connections and allowed to float in oxygenated physiological salt solution go into a fully contracted state which in some ways resembles rigor. This state differs from true rigor, however, in that it is completely reversed by stretching. Initial stretching produces high resting tensions which relax rapidly. The basis of this initial resistance to passive stretch is unknown. If a muscle in this rigor-like state is fixed for electron microscopy, one observes in the micrographs fully contracted sarcomeres 1.5 μ in length. If the muscle is suddenly stretched 30–50% of its resting length, tied to a toothpick, and fixed immediately, the sarcomere length varied somewhat even in adjacent cells. In papillary muscles slowly stretched to their optimal over-all length for contractility while working in vitro, the sarcomeres were remarkably uniform in length.

After a period of physiological observation in vitro, papillary muscles were fixed and an effort was made to assess some of the changes induced during fixation. Eight papillary muscles were stretched to optimal length, and their tension was monitored after sudden immersion in the fixative. The fixative first triggered a contractile response. The force of this induced contraction was variable but usually less than the response to electrical stimulation. The duration and the number of contractile responses varied. After these responses ceased, there was a slow rise in resting tension over the ensuing hour. The amount of increase in tension ranged from relatively little up to a 250% increase over the initial resting tension. There was no significant correlation between the per cent increase in tension and the average sarcomere length or average diameter of the tubules of the transverse tubular system subsequently measured in electron micrographs (Table II). No other morphological features appeared to be correlated with the observed variations in degree of rise in resting tension. The basis of this variable response to fixation remains obscure.

Studies of the mechanical activities of muscles in vitro give no indication of the adequacy of exogenous energy sources when oxygenation of the medium is optimal. Medium containing substrate may be replaced with substrate-free medium without any detectable diminution in the force of contraction. Preliminary experiments were carried out to determine whether under these conditions there was any morphologically detectable change in the amount of glycogen or lipid which might serve as endogenous substrates. After an initial

2-hr incubation period in substrate-containing medium, three papillary muscles were worked in substrate-free medium for 12 hr with no marked diminution in contractile strength. Fixation for electron microscopy after this time revealed depletion of all lipid droplets in two of the three muscles. The third muscle showed a significant depletion of lipid, retaining only a small amount compared to a muscle from the same heart fixed before exposure to substrate-free medium. Glycogen was also depleted to some extent so that the large accumulations of particles usually present in the sarcoplasm could no longer be found after work of this duration in substrate-free medium. All other structures appeared to be well preserved and normal in appearance after depletion of endogenous energy supplies. Papillary muscles from other cats, worked in substrate-containing medium for eleven hours, retained abundant lipid and glycogen. It is concluded, therefore, that, provided oxygenation is optimal, endogenous lipid utilization might serve as a useful index of the adequacy of the supply of exogenous substrate.

DISCUSSION

The present study has confirmed previous descriptions of the dimensions and spatial relations of the actin and myosin filaments which account for the characteristic pattern of cross-striations of cardiac muscle, and which constitute the structural basis for its sliding filament mechanism of contraction. We have emphasized, more than have most other investigators, that cardiac muscle fibers tend to have a single coherent bundle of myofilaments extending throughout the fiber cross-section instead of discrete myofibrils of uniform size. It was pointed out that when the tissue is well fixed the distribution of the contractile material in cardiac muscle resembles that of the contractile material in skeletal muscles of the slow, tonus type (i.e. *Felderstruktur*) rather than that of the contractile material in fast, twitch muscles (*Fibrillenstruktur*). This particular geometry of the contractile mass is thought to be related to cardiac muscle's slower time to peak tension and its relatively prolonged contractile state. It also helps to explain the tendency of the cross-striations of cardiac muscle to be in register across the entire width of the fiber.

Attention was drawn here to an unusual feature of the structure of the mitochondria in cardiac muscle. The mitochondria very often have slender prolongations which may be several microns long, but only a fraction of the diameter of the main body of the organelle and which may contain only one or two short cristae. Johnson and Sommer (51) evidently saw miniature mitochondrial profiles in the trabeculae carneae of the rabbit ventricle, but, failing to observe any connections between these profiles and other mitochondria of normal size, they interpreted the profiles as a second population of mitochondria. They believed that these profiles were peculiar to the trabeculae and were not present in papillary muscle. In our experience, the mitochondrial projections are abundant in the cat papillary muscle and it seems likely that they are a general feature of cardiac muscle. Although their significance remains uncertain, it seems reasonable to speculate that in muscle, where they are impounded in clefts within the myofilament mass, the mitochondria are not free to move about as they are in other cells. One may conjecture that these slender prolongations either represent a stage in the new formation of mitochondria by budding, or may be a means of permeating narrow interstices to effect a redistribution of mitochondrial mass so as to shorten the diffusion distance to certain areas.

The recent advances in electron microscopic analysis of striated muscle have brought us much closer to an understanding of the structural basis of contraction and of excitation-contraction coupling. The sliding filament mechanism applies to both skeletal (47) and cardiac muscle, but certain important differences in the physiological behavior of these two muscles remain to be explained. It is the prevailing view that, during activation of skeletal muscle, membrane depolarization initiated at the myoneural junction spreads inward along the T tubules of the triads (45), somehow releasing stored calcium from the neighboring terminal cisternae (15, 115). The calcium released diffuses to the myofilaments and triggers the contractile process. During relaxation, the longitudinal sarcotubules are then presumed to reaccumulate calcium released from actomyosin (18, 41). Calcium seems to play the same key role in excitation-contraction coupling in both skeletal and cardiac muscle but the intermediate source of the calcium probably differs in the two. When extracellular calcium is replaced by magnesium, skeletal muscle is still capable of hundreds of isometric contractions (63, 64), and this seems to be relatively independent of extracellular calcium. This is apparently related to the capacity

of skeletal muscle to accumulate and to store calcium intracellularly. There is now abundant evidence that the sarcoplasmic reticulum is involved in intracellular calcium storage in skeletal muscle (18, 41). Cardiac muscle, on the other hand, is much more dependent on extracellular calcium; when this ion is no longer available in the extracellular fluid, the heart soon weakens and finally stops. The difference between skeletal and cardiac muscle with respect to calcium metabolism may reside, in part, in the differences in their sarcoplasmic reticulum.

The characteristic distribution of the contractile material in the two types of muscle would, in itself, account for a substantial quantitative difference in the extent of their sarcoplasmic reticulum. The subdivision of the contractile substance of skeletal muscle into large numbers of discrete myofibrils, each ensheathed by reticulum, makes the total surface very extensive compared to that of cardiac muscle, in which sheets of reticulum incompletely subdivide the continuous mass of myofilaments into relatively large myofibril-like areas. Perhaps even more meaningful than the total extent of reticulum is the relative simplicity of its pattern in cardiac muscle. Periodic specializations of the reticulum at specific sites in the repeating pattern of cross-striations are largely lacking. Instead, a simple plexiform arrangement of anastomosing tubules extends uninterruptedly from sarcomere to sarcomere.

Since it is the terminal cisternae of the reticulum in skeletal muscle that are believed to be the principal sites of calcium storage (15, 115), the explanation for the lower capacity of cardiac muscle for calcium storage probably resides mainly in the fact that its *subsarcolemmal cisternae* are far less capacious and are in apposition to the sarcolemma over a much more limited area than are the terminal cisternae of skeletal muscle. The marked differences in the T tubules themselves may also be of importance. The T tubules of cardiac muscle are fewer but larger and are lined with a layer of protein-polysaccharide that is not evident in those of skeletal muscle. It is tempting to speculate that some or all of these differences in the reticulum and its association with the sarcolemma are related to the greater dependence of cardiac muscle upon extracellular calcium. The wider lumen of the T tubes in cardiac muscle would seem to favor more rapid equilibration between the lumen of this system and the general extracellular space than would be the case in skeletal muscle. However, in studying the cat papillary muscle with the "osmotic gradient" technique, Page (74) found that its extracellular space was not homogeneous. Inulin equilibrated slowly with a portion of the space. The suggestion was made that this slowly equilibrating portion, although extracellular, was within the limits of the fiber rather than between fibers and that it probably corresponded to the T system. Since morphological studies show that the diameter of these tubules is many times the dimensions of the inulin molecule ($\sim$30 A), the slow equilibration of this portion of the extracellular space cannot be explained from steric considerations alone. In this relation, we clearly need to know more about the protein-polysaccharide layer lining the T tubules of cardiac muscle. It is conceivable that this layer might impede diffusion of inulin. Quantitative electron microscopic, histochemical, or radioautographic methods for localizing extracellular tracer substances need to be applied to this problem before any reasonably meaningful statements are possible concerning the contribution of the T system to the total extracellular space.

Bennett (5) has suggested that the protein-polysaccharide coat on cell membranes might modify the ionic environment of the plasma membrane and of the cell itself. Certainly if the polyelectrolyte properties of the material made possible the accumulation of a high concentration of calcium in a rapidly equilibrating store on the surface of the sarcolemma, then such a calcium store would favor a mechanism of muscle activation dependent upon influx of extracellular calcium. Such a mechanism also requires a means for lowering calcium concentration in the sarcoplasm and for exporting calcium during relaxation. The sarcoplasmic reticulum and its expansions that are in contact with the T tubules or with the peripheral sarcolemma are strategically situated to carry out this function.

Relevant to this discussion is the finding of Fanburg et al. (22) that isolated fragments of the reticulum from cardiac muscle possess only about a hundredth the capacity for calcium accumulation (per milligram protein in the fraction) that is exhibited by similar preparations from skeletal muscle. Mindful of the abundance of mitochondria in cardiac muscle and their close topographical relation to the T tubules, Forss-

mann and Girardier (31) have suggested that the mitochondria may play an important role in uptake of calcium from the sarcoplasm of cardiac muscle during relaxation and in export of calcium to the lumen of the T system. However, there is as yet no compelling evidence for involvement of the mitochondria. It is clear that much remains to be learned about the mechanisms of calcium movement in cardiac muscle.

Investigators are in agreement in assigning important physiological significance to the points of close apposition of the subsarcolemmal cisterns of the reticulum to the sarcolemma, whether these points are located on the T system or at the periphery of the cell. The detailed structure of these junctions, however, has been variously described. This is due, in part, to the fact that their appearance in electron micrographs is quite dependent upon the method of specimen preparation. Revel (90) found that the limiting membrane of the terminal cisterna in bat skeletal muscle had a scalloped appearance, with the outer surface of the membrane seeming to be studded with uniformly spaced, small projections about 100 A across. It was not clear to him whether these projections were separate entities or represented extensions of the limiting membrane of the cisternae. In sections of osmium tetroxide–fixed skeletal muscle stained with potassium permanganate and lead citrate, Fahrenbach (21) consistently found a dark line between the terminal cisterna and the T tubule of the triads and interpreted this line as an "intermediate membrane" comparable to the middle dense line of a five-layered intercellular junction. On this basis, he suggested that the association of the terminal cisternae with the sarcolemma limiting the T tubule is similar to a "tight junction" or "nexus." Such a low resistance path, it was pointed out, would permit the depolarization of the sarcolemma to spread along the T tubule and into the terminal cisternae, thus directly coupling calcium release from the reticulum to the electrical activity in the sarcolemma. Other investigators of skeletal muscle have been unable to fully substantiate Fahrenbach's observations. In frog skeletal muscle, Peachey (81) found 200-A densities bridging the gap between the terminal cisternae and the T tubule. Kelly (56) described this junction in amphibian muscle as consisting of the apposition of membranes at an over-all distance of 100–150 A, with "dimples" in the membrane of the terminal cisterna occurring at regular intervals and bringing that membrane into closer proximity to the T tubule membrane. He suggested that these minute dimples might be considered small focal "tight junctions." On the other hand in cardiac muscle, Forssmann and Girardier (31) have emphasized that the apposed membranes at the dyads or subsarcolemmal saccules differ from those of the triads of skeletal muscle in the absence of thickenings or other specializations reminiscent of a synaptic relationship. With the methods of specimen preparation used in the present study, there was a junctional specialization consisting of an intermediate dense layer between the apposed membranes. This density seemed not to be a part of either membrane and, depending upon the quality of preservation, it appeared either as a continuous intermediate line or as a row of more or less uniformly spaced densities crossing the intermembranous gap. We concur with Forssmann and Girardier, however, in that in all specimens examined with appropriate staining and at high enough magnification both dense layers of the participating unit membranes could be resolved throughout the length of the junction and no examples of either focal or more extensive membrane fusion were observed (Fig. 45).

The conduction of the action potential in nerve and in skeletal muscle fibers has long been interpreted on the basis of core conductor theory—the fiber being likened to a long cable (44). The discovery that the myocardium is made up of separate cellular units (67, 68, 101) at first raised some doubt as to the applicability of this principle to cardiac muscle. Some investigators believed that the action potential in the myocardium was conducted from cell to cell by a specialized mechanism peculiar to cardiac muscle (105). However, the fact remained that cardiac muscle behaved electrically as though it were a syncytium, and physiological studies soon demonstrated that the shape, time course, and conduction velocity of the action potential of ventricular muscle were in accord with predictions from cable theory. These findings argued strongly for the existence of low electrical resistance at the junctions between cells (111). Electron microscopic observation of areas of *apparent* membrane fusion in the transverse and longitudinal portions of the intercalated discs led to the supposition that these junctions were the sites of electrotonic coupling

(2, 26, 39, 54). The circumstantial evidence implicating these junctions in cell-to-cell conduction has now received strong experimental support from the work of Barr, Dewey, and Berger (2) and of Dreifuss et al. (17). By increasing the osmolality of the medium bathing cardiac muscle, a gradual dissociation of cell surfaces at the intercalated discs was obtained in which only the close junctions remained coherent. The propagation velocity in these preparations was not reduced, indicating that these regions of the disc alone are adequate to maintain conduction.

It has now been shown for the mouse heart by Revel and Karnovsky (92) and confirmed in the present study on the cat that the cell-to-cell junctions previously described as "tight junctions," "pentalaminar junctions," or "nexuses" are not sites of complete obliteration of the intercellular space. No sites of true fusion of the leaflets of the unit membranes are observed between cardiac muscle cells in specimens stained *en bloc* with uranyl ions or treated with the lanthanum tracer technique. The evidence, therefore, still points strongly to the so called "close junctions" or "gap junctions" as the low resistance pathways for spread of excitation throughout the myocardium. Thus, contrary to earlier impressions, fusion of the outer leaflets of opposing membranes is not a *sine qua non* for a low resistance pathway between cells.

The principal function of the fasciae adherentes and maculae adherentes of the intercalated discs is considered to be maintenance of intercellular adhesion. It is of interest, however, that the experiments of Muir (70) and of Dreifuss et al. (17) show that the close junction ("gap junction" of Revel and Karnovsky) is also a site of very firm adherence that does not depend for its integrity upon the presence of calcium ions. Isolated perfused hearts allowed to beat in a calcium-free medium until they become quiescent show separation of the cellular units except at the close junctions where the two elements remain so firmly adherent that, when the cells are drawn apart, the membrane pulls off of one cell instead of separating along the narrow intercellular gap (70). Though the primary function of these junctions may be to maintain electrical coupling of the cellular units of the myocardium, it is evident that these junctions also possess considerable mechanical stability.

Close scrutiny of the intercalated disc region in the present study has revealed thin filaments of aberrant orientation for which no satisfactory interpretation can be offered at present. It is commonly assumed that actin filaments, like the myosin filaments, are of constant length. If this is true, there is a problem at the ends of the cells in cardiac muscle. The intercalated disc always occurs where a Z band would be expected in the repeating pattern of cross-striations. Whereas the terminal A–I junctions run straight across the contractile material, the ends of the cells are highly irregular in contour. It follows, therefore, that the actin filaments inserting on the ends of the cells must either vary in their length, or a portion of the length must turn back forming a hairpin turn or loop in the extrafibrillar dense substance of the disc in much the same manner as Kelly (55) has described for the insertion of tonofilaments at the desmosomes of amphibian epidermis. A similar structural problem exists at the terminal sarcomeres associated with the highly irregular myotendinous junctions of skeletal muscle. If the actin filaments are of constant length, then in both of these situations it would not be surprising to find short, recurrent free ends of actin filaments in close proximity to the deeper invaginations on the ends of the muscle fiber. The filaments described here as forming broad loops or pursuing a curving transverse course near the intercalated discs appear to be much too long to be simply redundant portions of those actin filaments that insert upon the cell surface at sites closer to the terminal A band than half the normal width of an I band. There is a need for detailed study of this region of the cardiac muscle cell.

The attachment of the sarcolemma at the periphery of the cardiac muscle fibers to the Z bands of the underlying contractile material was first noted by light microscopists and was attributed by some authors to continuity of the plasmalemma with a transverse Z membrane (Krause's membrane). This interpretation was not substantiated by electron microscopy. The periodic scalloping of the lateral surfaces has been attributed instead to an extrafibrillar dense component of the Z bands that extends to the inner aspect of the sarcolemma and binds it to the subjacent myofilaments (28, 43). In the adult cats studied here, we find no reason to accept the interpretation of Johnson and Sommer (51) that subsarcolemmal accumulations of this dense material are related to the addition of new myo-

filaments at the periphery of the fiber. We draw attention to this component once again in the hope that it may stimulate new efforts to learn more about its chemical nature and structural significance. It is less abundant in the mammalian heart than in the reptilian heart (28, 60). If its principal role is to fix the sarcolemma, it may be less important in the mammal where the well developed T system with invaginations of the sarcolemma at each Z band would also serve to fix the membrane to more deeply lying structures at this level.

In recent years, the progressive elimination of gross artifact in specimen preparation has made it possible to obtain reasonably dependable measurements from electron micrographs in studies correlating structure and function. It has become increasingly important, therefore, to assess the dimensional changes and other effects of application of fixatives to living muscles. Page and Huxley (78) reported that both sets of filaments shorten during fixation in osmium tetroxide, if free to do so. The shortening in glutaraldehyde was less than in osmium tetroxide and seemed only to involve the I filaments, but it was, nevertheless, appreciable. Sonnenblick et al. (104) recorded a rise in tension in heart muscle during glutaraldehyde fixation but did not discuss its basis or its possible effects upon their measurements. In our study, as in theirs, the optimal muscle length at which developed tension was maximal and resting tension was minimal corresponded to a sarcomere length of 2.2 μ. This value agrees well with the values given by Huxley (47, 49) for skeletal muscle. This sarcomere length did not appear to be affected by the duration of incubation in vitro or to be changed with the rise of tension associated with exposure to the fixative. The diameter of the transverse tubules was not markedly increased, and there was no other visual evidence of gross shrinkage of the cells that might explain the observed rise in tension. It has been reported (9) that although glutaraldehyde appears to be superior to other fixatives in a number of respects, glutaraldehyde fixation reduces the volume of the myofilament lattice in skeletal muscle to 75% of the in vivo volume. The increase in isometric tension recorded here during fixation may well be a consequence of a change in lattice volumes which does not result in a detectable change in the sarcomere length of the muscle in electron micrographs.

The results of the present study demonstrate that even in a muscle as small as the right ventricular papillary muscle, the penetration of the fixatives now in general use is not sufficient to insure preservation of uniform quality throughout the muscle. There is always a gradient within the block, with normal cytological appearance in the superficial zone and varying degrees of structural distortion in the deeper regions. While it is impossible to exclude the possibility that the micrographs of deeper lying fibers are accurately representing regressive changes due to inadequate oxygenation, the stable contraction strength of the muscle for long periods makes it seem more reasonable to attribute the structural alteration in the interior of the muscle to poor or delayed penetration of the fixative. The findings, therefore, emphasize that for studies attempting structural-functional correlations, it is important to compare zones at equivalent depths in this gradient of preservation. Alternatively, the papillary muscle arterial perfusion technique may be attempted (61).

Numerous physiological studies have established that the isolated perfused rat heart can contract vigorously for 1 or 2 hr in the absence of exogenous substrate (29). The energy reserves within the muscle cells are, therefore, considerable. It has been calculated, however, that the glycogen of the perfused heart would be used up in 14–19 min. It has been reported from biochemical analysis that the glycogen is, in fact, depleted in about 10 min. Inasmuch as amino acids contribute little to the energy metabolism of the heart (114), it has been assumed that tissue lipids play an important role in sustaining the contractility of the perfused heart after exhaustion of the carbohydrate stores. This assumption was borne out by the recent biochemical studies of Olson and Hoeschen (72), which showed that when normal rat hearts were perfused with a nutrient-free medium until exhaustion, the triglyceride content of the heart declined from 43 to 13 μmoles/g dry weight. To our knowledge, the observations reported in the present study are the first in which the diminution of morphologically demonstrable glycogen and lipid has been followed in cardiac muscle beating in the absence of exogenous substrate. The findings are in agreement with biochemical analyses of other investigators showing a progressive depletion of triglyceride under these conditions. However, the assumption that glyco-

gen stores are rapidly and preferentially utilized and that lipid is the energy source for the continuing contractile activity beyond the first 10–15 min is not substantiated. Although it was more difficult to assess in the micrographs quantitative changes of glycogen than changes in lipid, a substantial amount of glycogen was still present after several hours of work in nutrient-free medium and at a time when almost all of the lipid droplets were gone. Thus, there was no morphological evidence for a preferential use of glycogen, but it appeared that both carbohydrate and lipid were utilized concurrently during work at high oxygen tensions.

Because the myocardium can switch from utilization of exogenous substrate to use of endogenous substrate without even a temporary decrease in contractility, its mechanical performance in in vitro experiments of a few hours' duration cannot be relied upon to give any indication of the adequacy of the supply of exogenous substrate. The results described here show that while the papillary muscle may contain energy stores sufficient to sustain contraction aerobically for many hours, these reserves are not drawn upon to any significant extent when the muscle is working in a nutrient medium. Thus, in physiological experiments carried out with morphological controls, the degree of utilization of endogenous lipid may serve as sensitive measure of the adequacy of the supply of exogenous substrate.

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