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Ultrastructure of Heart Muscle

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The ultrastructure of cardiac muscle is described. In the course of the descriptions advantage has been taken of comparative anatomy, in order to elucidate the relationships between structure and function. Physiologic parameters are discussed in a comparative manner including information from skeletal muscle fibers that often provide a well-studied point of departure. The descriptions of the ultrastructure of cardiac muscle are illustrated by a few electron photomicrographs to give a general overview.

Cardiac muscle and skeletal muscle are striated muscles (Figs. 1–3). The striations which run at right angles to the longitudinal axis of the muscle fibers (cells) are brought about by the peculiar organization of the contractile material into sarcomeres. The presence of contractile material organized in this manner distinguishes the muscle cell from all other cells (e.g., secretory or connective tissue cells), although most cells contain small amounts of various contractile proteins (*1*). The contractile material shares the cell space with all kinds of organelles, e.g. mitochondria, endoplasmic reticulum (sarcoplasmic reticulum, SR, in muscle) a nucleus etc., which all are obligatory cell components in eucaryotes.

Electron microscopy has vastly advanced our knowledge of the structure and function of striated muscle and has significantly contributed to the definition of the morphologic differences between cardiac and skeletal muscle. One of the earliest and most important contributions of electron microscopy to cardiac muscle research was the demonstration that each of the rather small (about 15 × 80 μm on the average) cardiac muscle cells is surrounded by a plasmalemma of unit membrane configuration (*2*). This observation eliminated the possibility that the heart is an anatomical syncytium. But it also created the problem of explaining the fact that, physiologically, the heart acts like a syncytium: an electrical stimulus given one cell spreads by resistive coupling into all cells of the entire muscle mass of the heart. Electron microscopy in combination with electrophysiological studies subsequently succeeded in identifying the anatomic structure responsible for the low resistance pathways between cells (*3*). It is called the nexus (fascia communicans) and consists of a structural specialization of the plasma membranes joining adjacent cells. Skeletal muscle, which is composed of very long fibers that result from the progressive fusion of hundreds of cells during embryogenesis, has no nexuses. In contrast to cardiac muscle, each skeletal muscle cell is supplied by a nerve fiber and each cell contracts individually in response to stimulation through its own neuromuscular junction. Nerve fibers, especially of the autonomic variety, are found also throughout the heart, but they do not serve the purpose of stimulating the muscle cells to contract. Rather, they seem to regulate the overall activity of the heart.

Heart muscle cells are apposed to form bundles which, in turn, make contact with adjacent bundles. The result is a layered muscle mass which forms roughly two main layers that are skewed to one another at an angle in a fanlike fashion, the vortex being at the tip of the heart. The mammalian heart has an overall conical shape which favors the extrusion of its contents from the tip toward the base where the great vessels are located. But the four chamber heart with its one-way valves demands that different regions of the heart contract in a certain sequence. Contraction of the antechambers, the atria, must precede the contraction of the ventricles, and the contraction at the base of the heart must follow contraction at the apex. This sequence is programmed by a system of specialized heart muscle cells, the conduction fibers. Most of these fibers are clearly distinguishable from the larger

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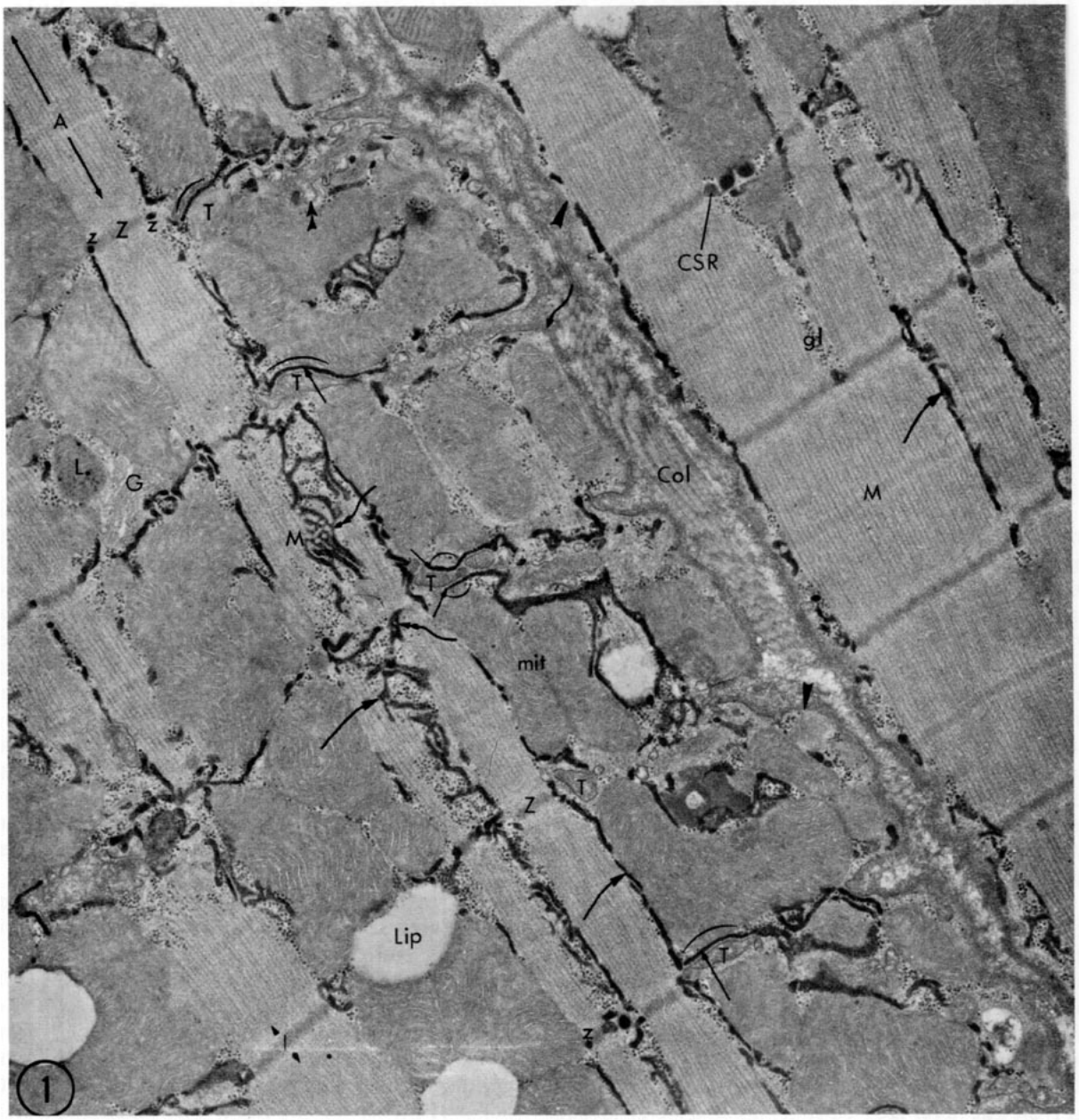

FIGURE 1. Hamster cardiac muscle. Parts of two fibers that are separated by extracellular space containing collagen fibers (Col). Plasmalemma is scalloped, forming grooves at the Z lines from which transverse tubules (T) take their origin. The laminar coat (curved arrow) of the peripheral plasmalemma (large arrow head) extends into the transverse tubules in contrast to skeletal muscle in which it does not. The perpheral plasmalemma and the interior plasmalemma (that of the transverse tubules) has many caveolae (double arrow head) in contrast to skeletal muscle in which caveolae are seen only in the peripheral plasmalemma. The sarcoplasmic reticulum is stained dark and is composed of several parts. The free SR (large curved arrow) is differentiated into the M and Z retes (small double headed arrows) and into junctional SR (straight arrows) at the transverse tubules (T) forming couplings (curved brackets). Part of the Z rete is the z tubule (z) which is attached to the Z line. Two couplings at a transverse tubule make a triad (small round brackets), one makes a dyad (large round brackets). The A band (A) is composed of thick myosin filaments, the I band (I) of thin actin filaments that on one side are anchored at the Z line (Z). CSR refers to corbular SR which is another specialization of sarcoplasmic reticulum at the Z lines homologous to the junctional SR (see reference 32). L, Lysosome; G, Golgi apparatus; mit, Mitochondrion, Lip, Lipid; M, M Line; Gl, Glycogen. × 23,874. (courtesy of Am. J. Pathology).

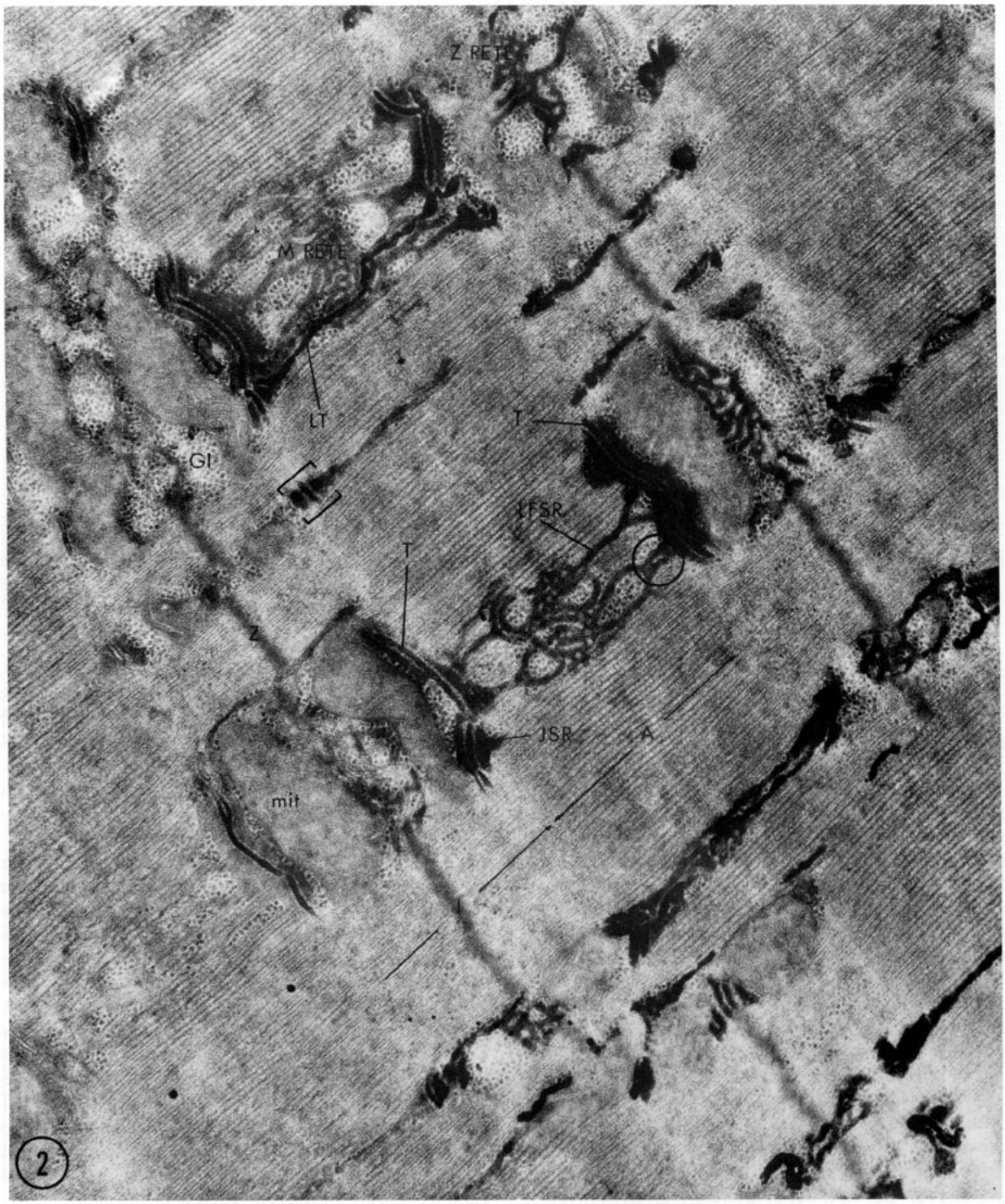

FIGURE 2. Mouse diaphragm muscle. The triads in many skeletal muscles are located at the junction between the A and I band (brackets). They are made up of junctional SR (JSR) and transverse tubules (T). The free SR between the junctional SR on either side of the sarcomers, the distance between two Z lines, forms the M and Z retes. The retes are connected to the junctional SR by few longitudinally oriented SR tubules (LFSR), the very junctional segment being called intermediate cisterna (circle). The junctional SR is attached to the transverse tubules by periodic densities, the junctional processes or feet. The transverse tubules have branches that run in longitudinal direction (LT). mit, mitochondrion; Gl, glycogen; Z, Z line; A, A band; I, I band. × 59,649 (courtesy of J. Cell Biol.).

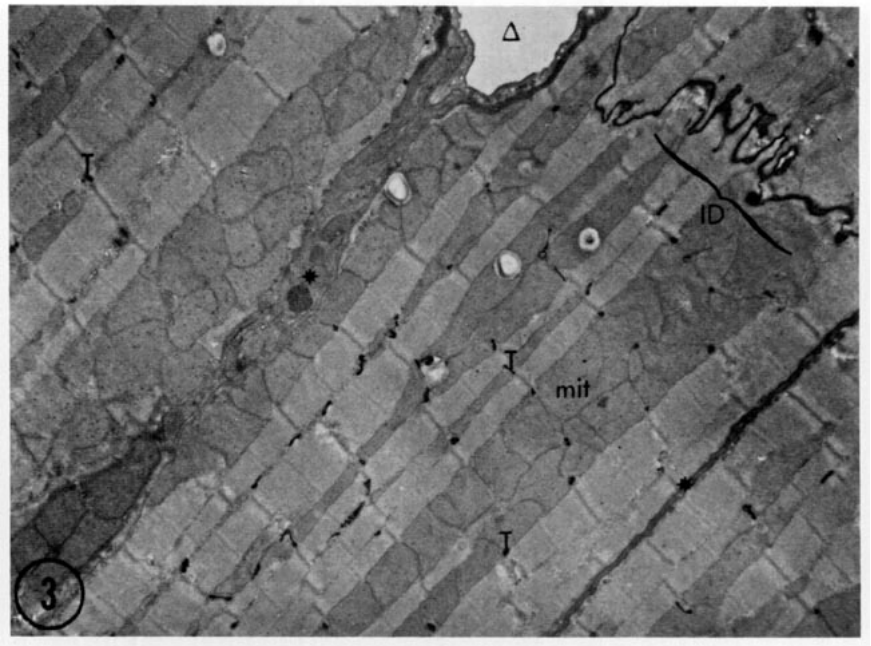

FIGURE 3. Mouse cardiac muscle. Parts of three fibers that are separated by extracellular space (asterisks). The extracellular space is contrasted by lanthanum hydroxide which is electron-dense. By diffusing throughout the extracellular space it outlines especially the intercalated disc (ID) and the transverse tubules (T) which are extensions of that space. mit, mitochondrion; triangle, blood vessel. × 10,000 (courtesy of Am. J. Pathol.).

population of working fibers by their structure.

The Conduction System

The conduction system has three main components: The sinoatrial (SA) node and the atrioventricular (AV) node, both in the right atrium, and the His bundle with its ventricular ramifications. The existence of continuity between the distal conduction fibers, which were first described by Purkinje, and the bundle of His was established by Tawara (*4*). The SA node is composed of a bunch of cells that have little contractile material and few small nexuses. The SA node is the site in which electrical stimulation of the heart takes its origin through unknown mechanisms. Excitation spreads in a broad front through atrial muscle from the SA to the AV node, whence it continues on, confined in the bundle of His and its ventricular branches, until it enters the working fibers of the myocardium. The ultrastructures of the AV and SA nodes (*5, 6*) are quite similar, but the cells of the SA node are typically tightly packed into more loose bundles as compared with the AV node. The cytoplasm of the nodal cells has little contractile material and there are few nexuses between the cells. The cells are also a bit smaller than the common atrial fibers. In spite of rather sophisticated and tedious efforts (*7–9*) it has not been possible to correlate, directly, the ultrastructure of the various regions of the SA or AV nodes with known functional parameters, such as for example, the impulse generation in the SA node and the impulse delay in the AV node. However, some progress has been made in relating the structure of the conduction fibers of the ventricles to at least some of the observed properties.

The fibers of the ventricular conduction system are located primarily in the subendocardium and are characterized by two morphologic features of importance: one relates to the geometry of the individual fiber, the other to the geometry of the way the fibers are combined to form bundles. Concerning the geometry of the individual fiber, the conduction fibers, unlike the working fibers, have no transverse tubules (*10, 11*). Since transverse tubules are tubular invaginations of plasmalemma, the surface area of a cell with transverse tubules, given all other parameters being equal, is significantly greater than that of a cell without transverse tubules. Thus it follows that the total membrane capacitance of a conduction fiber must be less than that of a working fiber and that, according to the inverse relationship between membrane capacitance and conduction velocity, the conduction velocity of a conduction fiber must be greater than that of a working fiber (*11*). Conduction velocity can be further modulated to meet particular require-

ments by changing cell sizes, since the conductio velocity in a cell with a larger diameter is greate than in a cell with a small diameter. Indeed, great variations in cell diameter of conduction fibers are actually observed. Classical Purkinje fibers with clear cytoplasm and little peripherally located contractile material can have a diameter as large as 100 μm and are predominantly seen in the ventricles of large mammals, such as the ungulates, elephants and whales. In contrast, in the smaller mammals, such as the mouse, cat, or rabbit, the conduction fibers are morphologically indistinguishable from the working fibers except for the absence of transverse tubules. The other important morphologic feature characteristic of conduction fibers relates to the manner in which the cells are apposed to form bundles. In the small mammals the bundles of conduction fibers are loosely assembled with large intercellular spaces (greater than 1 mμ) separating the component fibers, except where they meet to become attached to one another over short distances. In contrast, in larger mammals, the usually large Purkinje fibers are tightly packed into bundles with the intercellular space having been reduced to narrow clefts. The clefts separate the cells over considerable distances and are of the order of 25–35 nm in width. The width of these clefts seems to be maintained by numerous desmosomes looking like spot-welds, as it were. One consequence of such an arrangement is that in a circular bundle a considerable proportion of the total membrane capacitance (about 80%) would be isolated by a series resistance residing in the clefts (*11*). As a result, during periods of high conductance the remaining membranes covering the very periphery of the whole bundle would depolarize much more rapidly. In that case, the propagation velocity of the action potential would approach that of a single fiber with a diameter equal to the overall diameter of the bundle. Conforming with these theoretical considerations, the geometry of conduction fibers and bundles actually encountered in hearts of different sizes appears quite suitable to accommodate the distances to be negotiated by the excitatory impulse.

The geometry of cell apposition within a bundle of cells is also crucial to an understanding of the overall current distribution within such a network of electrically connected components. Cardiac muscle cells are several times longer than they are wide. They are attached to one another at the intercalated discs which are located at the ends of the cells. The surface of the cells is rabbeted, creating steps in the outer contour, each step representing an intercalated disc making contact with a neighboring cell. By comparison, lateral adhesions between cells are few, except between fibers of the conduction system which are often held together by numerous desmosomes. The low resistance pathways, the nexuses, are predominantly associated with the intercalated discs and are, thus, at the ends of the cells. As a result, current flowing from one cell to another must first travel the length of the fiber before it encounters a nexus leading to the next fiber. Therefore, current flowing in longitudinal direction through a bundle of cells can take a direct course, whereas current traveling transversely to the longitudinal axis of a bundle must follow a zigzag course, since direct low resistance connections between the long sides of adjacent cardiac cells are infrequent. The retardation of current spreading transversely (as opposed to longitudinally) to the axis of a bundle of fibers can readily be explained by this geometry.

It will be recalled that the impulse for cardiac excitation is generated in the SA node of the right atrium, whence it travels in a broad front throughout the atrium to the AV node (*12*). At this point a delay in the propagation of the impulse is generated prior to its further transmission through the bundle of His and the ventricular conduction system. Interestingly, in the atrium a conduction system comparable to that in the ventricles has not been identified morphologically in spite of a spirited search in several laboratories (*13, 14*). Nevertheless, conduction fibers may exist in the atrium in the form of a very large population of fibers without transverse tubules which is randomly distributed mainly toward the endocardium (*11*). These fibers do not occur in identifiable isolated tracts, but are more diffusely integrated, electrically and morphologically, into the atrial myocardium. They may be but remnants of the embryological anlagen of the conduction system. In birds, unequivocal conduction fibers of the type described by Purkinje are present in the atrium but without direct connections between the SA and AV node ever having been observed.

The Working Fibers

In working fibers of the heart (mouse, rat, finch) the contractile material and mitochondria occupy about 50% and 35%, respectively, of the cell volume. Corresponding values in frog skeletal muscle are 80% and 2%, respectively. The relative quantity of these organelles in skeletal versus cardiac muscle is in harmony with the different tasks that the two kinds of striated muscle are adapted to perform. Cardiac muscle must provide moderate forces continuously over years, skeletal muscle is called upon to deliver shorter bursts of activity over a wide range of forces, including large forces. The con-

tractile material in cardiac and skeletal muscle is composed of thick myosin filaments (1.65 μm in length) forming the A bands, and thin actin filaments (1 μm in length) forming the I bands (*15*). The actin filaments are anchored at one end of the Z line. The distance between two adjacent Z lines is called the sarcomere length. The contractile process consists of the sliding of the free ends of the actin filaments into the free spaces between the myosin filaments. Consequently, the Z lines are pulled toward each other and, thus, the sarcomere length becomes shorter. The amount of shortening is a measure of the state of contraction and, at least in skeletal muscle, can be correlated with force development (*16*). No striking differences between the contractile filaments of cardiac versus skeletal muscle are apparent with the electron microscope, although the mechanical behavior of cardiac muscle bundles is quite different from the well studied behavior of isolated single skeletal muscle fibers. At the moment it is not known whether this is due to differences in the properties of the contractile proteins and their interactions, or whether this is due to differences in the geometry of the preparations employed for study. The mechanical properties of cardiac muscle are usually studied in bundles of fibers, which are composed of numerous cells connected with one another longitudinally at the intercalated discs and in some cases via lateral attachments. The mechanical properties of a bundle of cardiac fibers are further influenced by the large amount of collagen fibrils in the interstitium, as well as by numerous microfibrils that insert into the plasmalemma and intertwine with the collagen fibrils suspended in the extracellular matrix. Indeed, it has been suggested that the compliance of cardiac and skeletal muscle, the compliance of cardiac muscle being considerably larger than that of skeletal muscle, may ultimately reside in the interplay between microfibrils that are attached to the cells at one end and wrapped around collagen fibrils at the other (*17*).

Very little is known about the structural substrates of the process or processes that couple excitation to contraction. There is no doubt, however, that Ca^{2+} activates the muscle for contraction by appropriately interfering with the geometry of a regulatory protein associated with the actin filaments (*18, 19*). There is also little doubt that Ca^{2+} is released into the myofibrillar compartment for the purpose of contraction, and removed again for complete relaxation. Skeletal and cardiac muscle show different behavior with respect to extracellular Ca^{2+}. Skeletal muscle is independent of the extracellular Ca^{2+} concentration, whereas cardiac muscle is highly dependent on it. It should also be pointed out, however, that according to available figures, calcium entering cardiac cells during periods of high conductance does not seem to be sufficient to elicit contractions (*20*). The intracellular compartment that contains a pool of Ca^{2+} which might be suitable for release for contraction is the sarcoplasmic reticulum (SR).

The Sarcoplasmic Reticulum

The SR is an intricate network of tubules akin to the endoplasmic reticulum in other cells. It comprises from 0.6 to 1.5% of the total cell volume in cardiac muscles, depending on the species (e.g. finch, mouse, rat). In skeletal muscle (frog sartorius) it has been determined to be 8%. The SR is wrapped around the contractile material of both cardiac and skeletal muscle in a highly directional and ordered fashion. The SR tubules measure from 20 to 60 nm in diameter and are sandwiched between the contractile material. The tubules form fenestrated networks around the center of the sarcomere (M rete) and around the Z line (Z rete) (*21–23*). The retes are connected to the junctional SR (terminal cisternae) through membranous and luminal continuity of a few rather long tubules running parallel to the fiber axis. The junctional SR is morphologically different from the remainder of the SR, called the free SR (*11*). In skeletal muscle the junctional SR always appears distended with electron-dense granular material, the junctional granules (*21*), which probably represent calsequestrin (*24*). In cardiac muscle the junctional SR is not distended, and the granular material it contains often forms a continuous line bisecting the lumen. Junctional SR has junctional processes which look like caterpillar feet (*25*). In cardiac muscle they are commonly seen on both sides of the flat junctional SR while in skeletal muscle they are exclusively on one side, namely that which is closest to the plasmalemma. The processes usually make contact with the cytoplasmic face of the plasmalemma either at the cell surface or at transverse tubules. A SR specialization, the so-called extended junctional SR, which shows the characteristic morphologic features of junctional SR but has no contact with plasmalemma, is an invariant feature of the ultrastructure of bird cardiac muscle at Z lines.

Ca^{2+} is sequestered in the SR by an ATP-driven Ca^{2+} pump residing in the SR membranes, presumably in order to ensure relaxation (*26, 27*). Calcium and ATPase activity have been demonstrated in the SR using cytochemical techniques (*28–30*). Morphologically the pump proteins are presumably represented by a dense population of particles visible on the P faces of freeze-fractured SR both *in situ*

and *in vitro* (*31, 32*).* There is a reason to believe that the junctional SR may be a storage sink within a sink for Ca^{2+}. For example, cytochemical techniques have demonstrated that the junctional granules within the junctional SR are highly negatively charged (*33*). This is consistent with the observed presence of large amounts of calsequestrin, an acidic protein, in the heavy junctional SR fraction of isolated SR vesicles. In contrast, the light free SR fractions have few junctional granules and contain little calsequestrin (*24*). The structural complexes of junctional SR, junctional processes and plasmalemma in both cardiac and skeletal muscle are called "couplings" (*10*) and their consistent occurrence in most muscles has led to the tempting assumption that they are the anatomical sites at which the action potential is translated into calcium release for contraction. Indeed, calcium movements toward the Z line (where most couplings are located during relaxation), and toward the A band during contraction have been intimated by several kinds of experiments (*34, 35*). Nevertheless, the direct demonstration of Ca^{2+} release from junctional SR has not been accomplished as yet, and although the free and junctional SR are clearly Ca^{2+} sinks, it does not necessarily follow that Ca^{2+} is released for contraction from either or both. Moreover, in birds the extended junctional SR which makes up almost half of the junctional SR of cardiac cells has no contact with plasmalemma.

There are other locations in a cell in which Ca^{2+} may be stored for subsequent release. One such location is the cytoplasmic surface of the plasmalemma. This location seems attractive, if only because of its proximity to the electrical activity which, after all, elicits contraction. However, this plasmalemmal location for Ca^{2+} release becomes less attractive when one considers the fact that the numerous SR tubules occupying the subplasmalemmal space would interfere with the free passage of released Ca^{2+} to its target, the contractile material. Indeed, from this point of view junctional SR is much more propitiously situated, since it is always located within about 1 μm of the I band, a distance within a favorable range for intracellular diffusion of Ca^{2+} in muscle (*36*). Moreover, since the junctional SR is intercalated into the tubules of the free SR network, there is little chance that the free SR tubules are accidentally interposed between the junctional SR and the contractile material, which would impede the diffusion of Ca^{2+} to its locus of action. For this topographic argument to be valid, however, one must assume permanent topographical relationships between the integral structures. This topographic relationship between the various organelles in muscle cells may be maintained by an extensive cytoskeleton to which the organelles are anchored. Bundles of 10 nm fibrils can be seen extending transversely from the cytoplasmic surface of the plasmalemma into the interior of the cells connecting Z lines with Z lines (*37*). Transverse tubules when present with their interior couplings provide additional support. Thus the invariant topographic position at the Z lines of the so-called Z tubules (*38*), the junctional and extended junctional SR may find its explanation in this way.

The Transverse Tubules

The transverse tubules are tubular invaginations of the plasmalemma. Their diameter in skeletal muscle is about 20–30 nm. In cardiac muscle, the diameters vary greatly from 20 to 200 nm, thin-tubular segments alternating with large bizarre outpouchings. This is especially well demonstrated in electron photomicrographs of thick sections (1 μm) taken with the high voltage electron microscope (*32*). The observed polymorphous geometry of the transverse tubules does not exclude the possibility that this geometry represents only the static expression of dynamic processes such as, for example, peristaltic waves. The lumens of the transverse tubules are open to the extracellular space whence they can be filled with electron-dense tracers, such as lanthanum, exogenous peroxidase, or india ink. In fact, the filling of tubules with india ink was the means by which the continuity of the transverse tubules with the extracellular space was first proven in cardiac muscle. In contrast to skeletal muscle, transverse tubules in cardiac muscle carry a laminar coat continuous with that covering the cell surface. In skeletal muscle the laminar coat of the cell surface appears to cover the openings of the transverse tubules as well as of the caveolae from which transverse tubules often arise. The transverse tubules often communicate longitudinally across several sarcomeres in both skeletal and cardiac muscle (*32*). The result is an elaborate tridimensional tubular network of extracellular space that is quasi-interiorized into each cell. Such an arrangement must profoundly influence at least two parameters of physiologic importance. One has to do with the diffusion of metabolites into and from cells. Indeed, the discoverer of the transverse tubules in cardiac muscle cells called them "Saftkanälchen," little juice channels. The other has to do with the electrical activity at the plasmamembrane which,

* Freeze-fracture splits the lipid bilayer of unit membranes in half, exposing two faces: one, the P face, shows the fracture surface of the hydrophobic layer toward the inner lamella of the unit membrane; the other, the E face, is the complementary face toward the outer lamella of the unit membrane.

moreover, could be further influenced by momentary ion accumulations occurring in unstirred compartments generated by the intricate geometry of the tubular system. The occurrence of transverse tubules in heart muscle cells is apparently associated with cell diameters beyond about 8 μm. Immature mammals have no transverse tubules until several weeks after birth (*39*), at a time when the cells approach diameters larger than 8 μm (*40*). On the other hand, conduction fibers in adult mammals may reach diameters well in excess of 8 μm, yet they do not have transverse tubules. It is very interesting to note that the volume fraction of transverse tubules keeps pace with hypertrophy, suggesting that the transverse tubules are capable of expansion (*41*). It is not at all known whether the presence of transverse tubules is dependent strictly on cell size, or due to a membrane property unrelated to cell size. For example, bird cardiac muscle cells have an average diameter of about 8 μm, and they do not have transverse tubules. It would be interesting to enquire whether the cardiac cells in birds would develop transverse tubules if one succeeded in hypertrophying them.

Other Structural Features

The remaining organelles which are found in cardiac cells, mitochondria, lysosomes, etc., show no apparent peculiarities in comparison with similar organelles found in other eucaryotes. Many atrial cells, however, contain a large number of so-called specific granules (*42*) the nature of which is still obscure. They are membrane-bound vesicles that contain electron-dense material. Similar granules are seen also in ventricular cells of several lower vertebrates.

Within the myocardium, many nonmyelinated nerve fibers can be found, especially in the nodal regions of the right atrium. It is not possible with the electron microscope to distinguish, unequivocally, between adrenergic and cholinergic components.

In the preceding paragraphs an attempt was made to provide a cursory introduction into the ultrastructure of cardiac muscle. For greater details the pertinent literature must be consulted (*43, 44*). We have also pointed to some physiologic parameters which are likely to be profoundly influenced by the anatomy of the individual cells as well as by the geometry of bundles of cells. Cardiac muscle does not offer itself as an easy object for the study of ultrastructural alterations caused by all manner of pharmacologic intervention for the simple reason that isolated preparations of viable individual cells are difficult to obtain. Single skeletal muscle fibers, which are more readily obtained and are much larger, offer better opportunities for exacting studies. Nevertheless, several suitable preparations can be used to great advantage (*45, 46*) for the study of cardiac muscle although the influence of factors residing in the geometry of cell bundles often make interpretations of experimental results equivocal, and for some studies the small dimensions of isolated single heart muscle cells are prohibitive. Finally, it should be pointed out that all ultrastructural studies depend on preparative procedures prior to electron microscopy that introduce morphological alterations of their own which make informative structure-function investigations exceedingly difficult, especially on whole heart preparations.

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