ON THE STRUCTURAL CONTINUITIES OF THE TRANSVERSE TUBULAR SYSTEM OF RABBIT AND HUMAN MYOCARDIAL CELLS

DOUGLAS A. NELSON, M.D., and ELLIS S. BENSON, M.D.

From the Department of Laboratory Medicine, University of Minnesota Medical School, Minneapolis

ABSTRACT

An electron microscopic study of rabbit and human myocardium provides further evidence of the existence of two distinct components of the sarcoplasmic reticulum. A thin-walled tubular system (termed longitudinal system) is arranged in anastomosing channels sursurrounding each sarcomere and has transverse and possibly also longitudinal connections with the tubules of adjacent sarcomeres. A thick-walled tubular system traverses the myofiber transversely at the level of the Z lines of the myofibrils. The structure of these tubules very closely resembles that of deep sarcolemmal invaginations. Indeed, the membranes of the tubules appear to be continuous with the sarcolemma in favorable sections so that there seems to be an extension of the cell membrane and extracellular fluid to all depths of the myocardial fiber. Certain physiologic data which support this concept are discussed. The calculations of A. V. Hill comparing the kinetics of diffusion and the time-distance relationships between excitation and activation in frog sartorius muscle are reconsidered for cardiac muscle.

After Bennett and Porter's first electron microscopic observations of the sarcoplasmic reticulum in the breast muscle of the domestic fowl (1), Porter and Palade presented detailed descriptions of this subcellular structure in different types of striated muscle (2). These findings provided a structural basis for consideration of the mechanisms of excitation-contraction coupling in muscle.

Using a micropipette electrode to achieve local depolarization of the cell membrane, Huxley and Taylor (3) demonstrated transverse, two-dimensional conduction of excitation toward the central axis of an isolated intact fiber of frog striated muscle. The geometry of excitation and spread indicated very strongly that conduction inward took place along some structure in the plane of the Z lines of the myofibrils lying in register with one another. This conclusion conformed with an earlier prediction by A. V. Hill (4), based on consideration of the kinetics of diffusion and the time-distance relationships between excitation and activation in frog sartorius muscle. Hill proposed that, while excitation undoubtedly occurred at the cell surface, diffusion was far too slow a means of bearing the impulse into the interior of the fiber. He concluded that a *process*, not a *substance*, must carry the excitation inward.

The electron microscopic observations of Porter and Palade (2) suggested the possible role of transversely oriented elements of the sarcoplasmic reticulum in excitation-contraction coupling.

These authors described membranous structures, termed "triads," which lie in the interfibrillar space and are arranged at the Z line level in *Amblystoma* skeletal muscle and at the A-I junction in rat sartorius muscle. The term "triad" refers to the appearance in longitudinal section of three round or oval membranous elements in close proximity. The outer two profiles correspond to cisternae which are continuous with longitudinally oriented tubules surrounding the myofibril but not with the central elements of the triad, the intermediary vesicles, which are closely associated

Legends for Figures

The length of the solid line on each picture represents 1 micron. Figs. 1 to 10 are of rabbit myocardium; Figs. 11 and 12 are of human myocardium. All tissue was embedded in Vestopal W. The following letter designations apply to all figures.

C, capillary endothelial cytoplasm
CB, cell boundary within the myofiber
DB, dense body, granular
F, collagenous fibrils
FD, homogeneous dense body, presumed to be lipid (fat droplet)
ID, intercalated disc
L, longitudinal system of the sarcoplasmic reticulum
MI, mitochondrion
MF, myofibril
N, nucleus
R, rough-surfaced elements of sarcoplasmic reticulum
S, sarcolemma
SI, sarcolemmal invagination
T, transverse system of the sarcoplasmic reticulum
Z, Z line

FIGURE 1

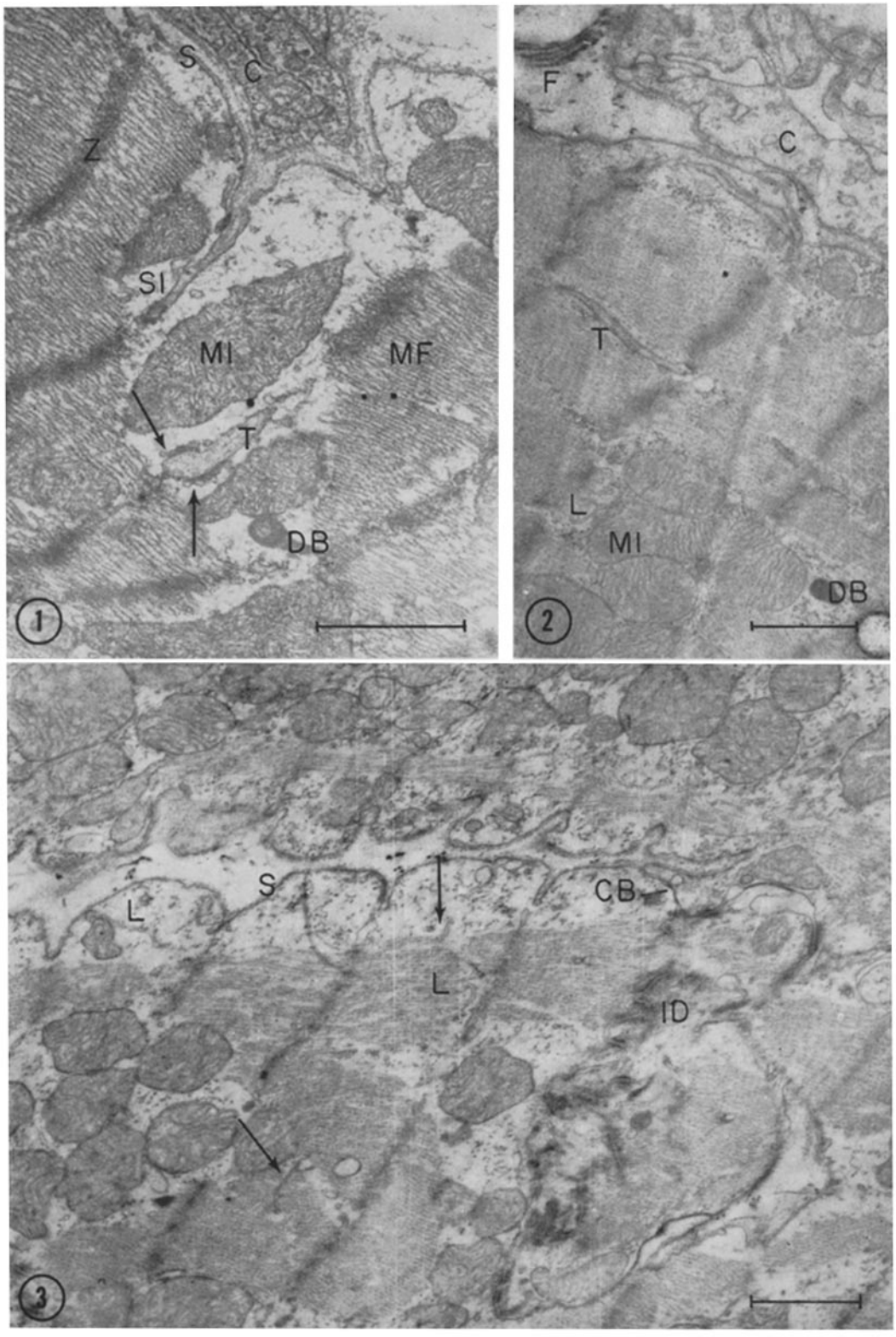
The perimembrane (the outer membrane of the sarcolemma, *S*) is structurally similar to the basement membrane of the capillary (*C*). The contracted myofibril (*MF*) lacks the I band and has heavy contraction bands at the Z lines (*Z*). Where the myofibril is out of the plane of the section, deep sarcolemmal invaginations (*SI*) become closely associated with the Z line at a deeper level. A profile (*T*) of the transverse tubular system is located also at the Z line level and has the same structure as the invaginated sarcolemma; indeed, it may be just a section of the same. Closely associated with *T* at the arrows are somewhat irregular elongated profiles and vesicles lined by a single membrane which could be interpreted as the lateral elements of a triad. The mitochondria (*MI*) have closely packed but here somewhat irregularly arranged cristae. Close to one mitochondrion is a small dense body (*DB*). Rabbit, phosphotungstic acid. × 24,500.

FIGURE 2

Collagenous fibrils (*F*) are seen in the extracellular space. Between the obliquely cut myofibrils is a longitudinally oriented tubular profile (*T*) which is limited by membranes similar to those of the sarcolemma and has an expansion at the Z line level. Small, thin branching tubular profiles (*L*) are probably components of the longitudinal system of the sarcoplasmic reticulum. Numerous granules of varying density, 150 to 250 A in diameter, are present in the subsarcolemmal and interfibrillar sarcoplasm; most of these are probably ribosomes and glycogen particles. The mitochondria here have parallel cristae. Rabbit, uranyl acetate. × 17,500.

FIGURE 3

The sarcolemma (*S*) is scalloped with indentations at the Z line levels. Profiles of thin tubules (*L*) lie along the surface of the myofibril and in the subsarcolemmal sarcoplasm where one such element becomes closely associated with the plasma membrane. At the arrows, elements belonging to this system may provide some transverse continuity in the middle of the sarcomere. At the surface of the myocardial fiber only the plasma membrane turns inward to form the intercellular boundary within the fiber (*CB*); the perimembrane continues over the surface of the fiber. This boundary becomes convoluted and associated with adjacent electron-opaque material where it crosses the myofibrils forming an intercalated disc (*ID*). Rabbit, uranyl acetate. × 17,000.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with the sarcolemma. In a detailed study of mouse skeletal muscle in which serial sections were examined and a three-dimensional model assembled, Andersson-Cedergren (5) concluded that the intermediary vesicles represented sections of a convoluted tubule continuous for long distances transversely within the fiber. This she called the transverse system. Although close proximity with the plasma membrane was demonstrated, she found no direct continuity of the membranes of this transverse tubular system with the plasma membrane.

If the membranes of these elements were electrically continuous with the cell membrane and formed a two-dimensional network extending throughout the interior of the fiber, the transverse type of conduction seen by Huxley and Taylor could be expected to follow local membrane depolarization. A simple mechanism might be provided by physical continuity of the lumen of this transverse tubular system with the extracellular space, a relationship not yet demonstrated in vertebrate skeletal muscle (2, 6, 7).

On the other hand, Smith has recently presented evidence for deep infoldings of the plasma membrane extending throughout the flight muscle of the adult beetle (8) and also for regular tubular invaginations of the plasma membrane traversing the fibers of the flight muscle of the dragonfly (9). Peterson and Pepe (10) illustrated deep infoldings of the sarcolemma which appeared to be continuous with the sarcoplasmic reticulum in crayfish stretch receptor muscle fibers opposite nerve endings.

Lindner (11) in 1957 described invaginations of the cell membrane (sarcolemma) of canine cardiac muscle which appeared to be continuous with transverse elements of the sarcoplasmic reticulum. Similar observations were described by Edwards and Challice (12) in a study of the cockroach heart. Recently Simpson and Oertelis (13, 14) have illustrated convincingly such a continuity in electron micrographs of sheep myocardium. We, too, have noted deep invaginations of the cell membrane which appeared to be directly continuous with the transverse tubules in rabbit cardiac muscle (15).

The present communication extends these observations (15) and supports those of Simpson and Oertelis (13, 14) and others (11, 12). Additional features of the sarcoplasmic reticulum of rabbit and human cardiac muscle are described.

MATERIALS AND METHODS

Small portions of ventricular muscle were obtained from anesthetized adult white rabbits and from human subjects at open heart surgery. Small blocks were cut, fixed in 1 per cent osmium tetroxide buffered with Veronal acetate at pH 7.4–7.6 (16), dehydrated in acetone, and embedded in Vestopal W following the procedure of Ryter and Kellenberger (17). Sections were cut with glass knives using a Porter-Blum microtome. In some instances, phosphotungstic acid was incorporated as a 1 per cent solution in the anhydrous acetone used in dehydration. When this was omitted, sections were stained by floating them on a saturated aqueous solution of uranyl acetate for 30 to 120 minutes. Electron micrographs were made with RCA microscopes, EMU 2 or EMU 3F.

OBSERVATIONS

Rabbit Myocardium

At the level of resolution of our micrographs the cell membrane or sarcolemma is composed of an inner, dense plasma membrane (protomembrane, Lindner, 11) approximately 75 A thick; a middle layer of lower electron opacity 150 A thick; and an outer, irregular membrane (perimembrane, Lindner) 75 to 200 A thick (Figs. 1 to 5). The perimembrane is similar in appearance to the capillary basement membrane (Figs. 1, 2, 5). Characteristically the sarcolemma is scalloped with indentations occurring in register with the Z lines of the underlying myofibril (Figs. 3, 4). This scalloping appears to be the result of contraction of the myofibrils which presumably occurs in obtaining the muscle sample or in the initial stages of fixation.

In favorable sections long invaginations of the sarcolemma are seen at the Z line level (Figs. 1, 4, 5). Where the superficial myofibril leaves the plane of the section, these invaginations extend across the width of the myofibril and become closely associated with the Z line at a deeper level (Figs. 1, 4).

Between myofibrils, elliptical profiles are transversely oriented at the Z line levels (Figs. 1, 4 to 9). These transverse profiles are bounded by an outer dense membrane about 75 A thick, an intermediate non-stained layer about 150 A thick, and an irregular, flocculent inner layer. These membranes bear a striking resemblance to those lining the sarcolemmal invaginations, as has been noted by Lindner (11) and by Simpson and Oertelis (14). The lumen of these profiles

FIGURE 4

There are extremely dense contraction bands in the obliquely cut myofibril (*MF*) which leaves the plane of the section on the right side of the figure. Cross-bridges are seen between myofilaments and have a periodicity of about 320 A at this angle of sectioning. Note again the deep invaginations of the sarcolemma (*SI*) which are distinguished from transverse tubular profiles (*T*) only by the lack of continuity between the two in the plane of the section. Both become closely associated with Z line substance. Rabbit, phosphotungstic acid. × 32,000.

often contains material which is denser than the sarcoplasm and resembles the amorphous dark material often associated with the perimembrane. This is true especially where the inner membrane of the tubular profile is least well defined (Figs. 1, 6, 9). It is recognized that images such as *T* and *SI* in Fig. 4 could represent relatively shallow infoldings of the sarcolemma resulting from a tangential-longitudinal section of the muscle fiber. In addition to the usual transversely elongated profiles in the sarcoplasm at the Z line level, a widening of the tubular profile into that of a poorly defined vesicle extending the length of one sarcomere along the myofibril is occasionally seen (Fig. 6). Elongated tubular profiles with membranes comparable to those of the sarcolemma (or the transverse system) are occasionally found oriented longitudinally from one Z line to the next (Fig. 2). These structures may represent either longitudinal connections between the transversely disposed tubules or perhaps deep longitudinal infoldings of sarcolemma.

In Fig. 9, transverse tubular profiles lie close to the nucleus at the Z line levels, and in Fig. 8, a tubular profile whose structure resembles that of the transverse type appears to make contact with the nuclear envelope. The exact relationship of the membranes cannot be determined.

Evidence of another type of membrane-lined tubular system, the longitudinal system of the sarcoplasmic reticulum, is seen within the cells. This system has been well described by others (2, 18) and will be considered only briefly here. Its components in section are profiles of vesicles and thin, anastomosing tubules (Figs. 2, 3, 6, 7, 10) which are limited by a single membrane. These elements are found in the perinuclear, subsarcolemmal, and interfibrillar sarcoplasm. The tubular nature of these profiles is most often apparent immediately adjacent to areas where a myofibril just leaves the plane of the section (Figs. 3, 7, 10), indicating the close apposition of this tubular network to the surface of the myofibrils. In addition to longitudinal continuity of this network within the individual sarcomere, in some places there appears to be transverse orientation of profiles at mid-sarcomere level between fibrils and just under the sarcolemma (Fig. 3). Narrow tubular profiles limited by a single membrane are occasionally in close contact with a transverse tubular profile near the Z line (arrows, Figs. 1, 5, 6, 7), but no direct communication between the two systems has been seen.

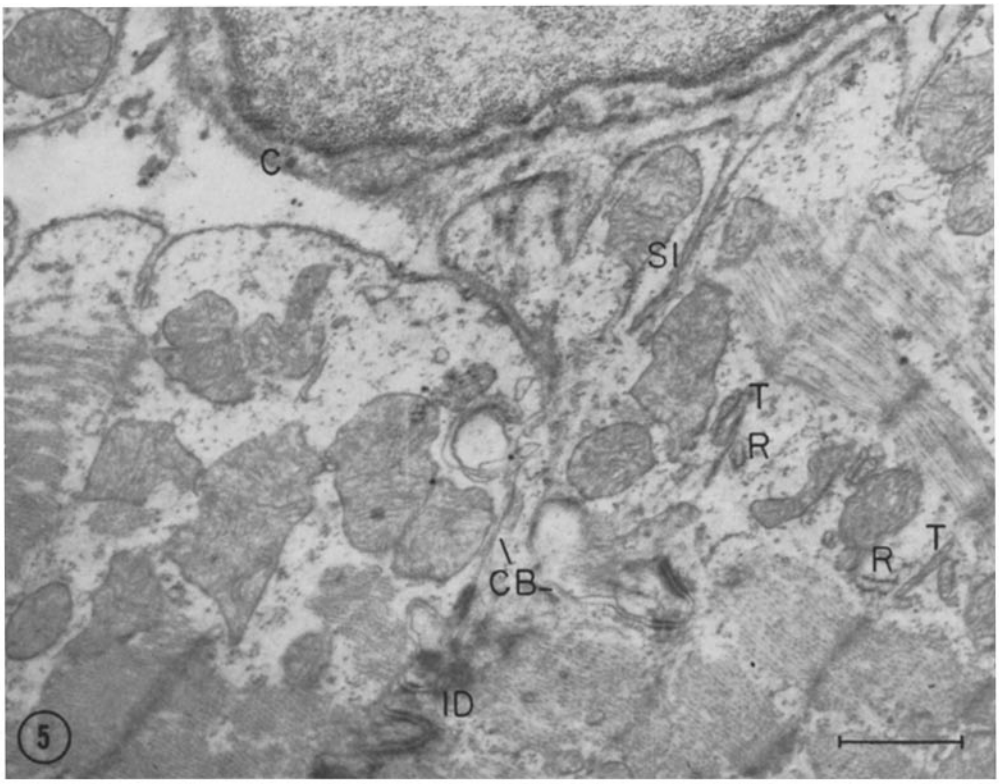
Elliptical profiles with 150 A dense particles attached to the external surface of the single limiting membrane are sometimes encountered in the perinuclear or interfibrillar sarcoplasm (Figs. 5, 7). These rough-surfaced elements of the endoplasmic reticulum on occasion appear to be continuous with characteristically smooth-surfaced components of the longitudinal system of the sarcoplasmic reticulum (Fig. 7).

FIGURE 5

The nucleus at the top belongs to a capillary endothelial cell. In the myofiber note the complex convolutions of the cell boundary (*CB*) separating the sarcoplasm of the two adjacent cells and the intercalated disc (*ID*) at the Z line level. The sarcolemma is indented at the levels of the Z lines and where the cell boundary originates. Structures with the characteristics of transverse tubular profiles (*T*) are occasionally seen side by side at the same Z level. Elongated profiles with externally attached 150 A particles (*R*) represent rough-surfaced elements of the sarcoplasmic reticulum. Rabbit, uranyl acetate. × 17,000.

FIGURE 6

The profiles of the transverse system (*T*) of the sarcoplasmic reticulum here are somewhat irregular. At T_1 and T_2 they dilate into large expansions. At T_2 such a dilated element of the system, although cut tangentially, appears to extend for the length of one sarcomere. Arrows indicate sites of close approximation of narrow profiles (probably continuous with or belonging to the longitudinal system of the sarcoplasmic reticulum) with the transverse tubular elements to form dyads or triads. The homogeneous dense bodies (*FD*) probably represent fat droplets and are closely associated with mitochondria. In the interfibrillar sarcoplasm note the numerous mitochondria with abundant, closely packed cristae. Rabbit, uranyl acetate. × 25,000.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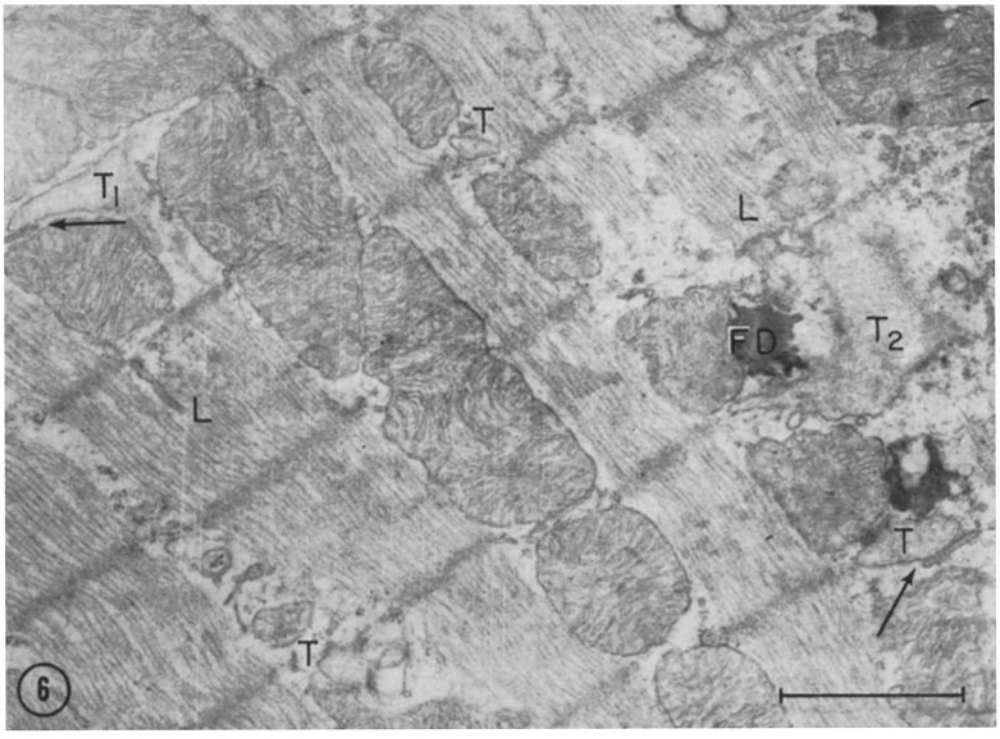
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Short invaginations of the sarcolemma may be seen at the point where the membranes continuous with an intercalated disc meet the cell surface (Fig. 5). Only the plasma membrane of the sarcolemma turns inward to become a component of the intercellular boundary and the intercalated disc within the fiber. The perimembrane, with its rough surface, bridges the intercellular gap at the surface of the fiber (Fig. 3). There appears thus to be no direct communication between the intercellular space of the intercalated disc and the extracellular space.

Human Myocardium

Invaginations of the sarcolemma and transversely oriented tubular profiles at the Z line level (Figs. 11, 12) are entirely comparable to those described above in the rabbit. Although the connection of the two is not so convincingly illustrated, the invaginations appear to extend deeply into the cell. Budding from the plasma membrane of these deep invaginations are stubby branches or small vesicular pouches about 500 to 600 A in diameter (Fig. 11). Small vesicles of similar size are sometimes closely associated with transverse tubular profiles (Fig. 12). The sarcolemmal invaginations appear to pass over the surface of the superficial myofibril (Fig. 11). Both the sarcolemmal invaginations and the transverse tubular profiles are limited by membranes identical in appearance to those of the sarcolemma, contain within their lumens heterogeneous material of like density, and are located precisely at the Z line levels of the myofibrils.

DISCUSSION

Our observations on rabbit myocardium added to those of Simpson and Oertelis on sheep cardiac muscle (13, 14) and of others (11, 12) support the suggestion that the transverse tubular system of the sarcoplasmic reticulum in cardiac muscle is a direct extension of the extracellular fluid space. This suggestion is based both on what appears to be direct communication between invaginated cell membrane and transverse tubules and on the striking similarity in membrane structure between sarcolemma and transverse tubular membrane.

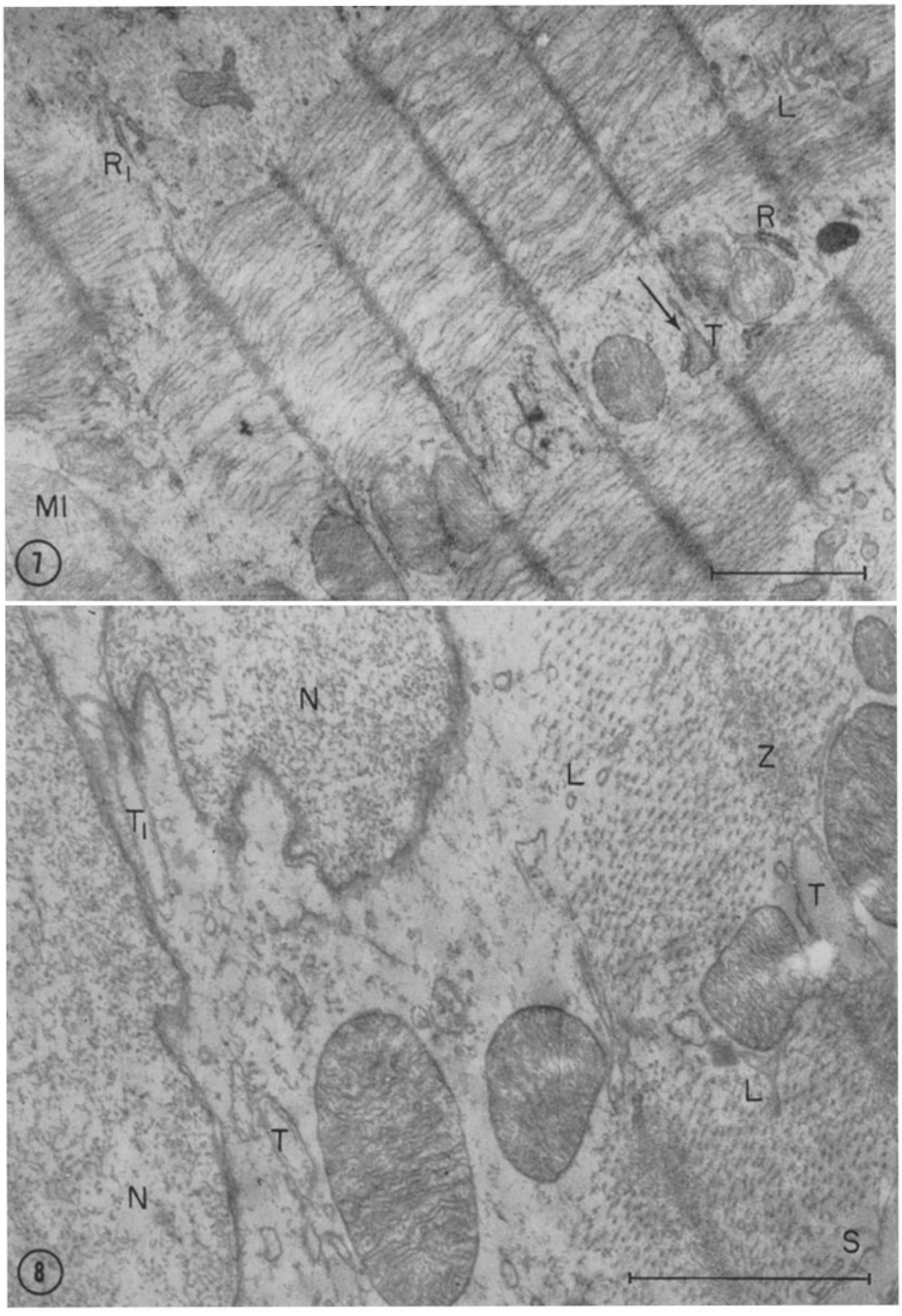
Porter and Palade (2) demonstrated structural variations in the triads in different muscles. In rat cardiac muscle, the sarcoplasmic reticulum was less highly developed than in skeletal muscle, the intermediary element of the triad was large relative to the lateral profiles, and occasionally the intermediary element was associated with only one lateral profile to form a "dyad." We assume that the transverse tubular system in rabbit and human myocardium corresponds to the series of vesicles at the Z line level in rat cardiac muscle (2). In most of our material, however, the transverse tubular profiles have the structure of sarcolemmal invaginations. Structures which could be interpreted as triads are seen at the arrows in Figs. 1 and 6, and as dyads at the arrows in Figs. 6 and 7. The lateral elements of these structures

FIGURE 7

A transverse tubular profile (*T*) is closely associated with a small elongated element lined by a single membrane (arrow). Profiles of small, intercommunicating tubules (*L*) of the longitudinal system are seen where the myofibril is cut tangentially to its surface. Some elements of the sarcoplasmic reticulum have attached 150 A particles (*R*). At *R* and R_1 these rough-surfaced elements appear to be directly continuous with smooth-surfaced profiles which probably belong to the longitudinal system. Note the abundant 150 to 300 A diameter granules in the interfibrillar sarcoplasm. Rabbit, uranyl acetate. × 24,500.

FIGURE 8

The transverse tubular profiles (*T*) at the Z line level of the obliquely cut myofibrils are limited by membranes similar to those of the sarcolemma (*S*). One profile (T_1) which may represent a transverse tubule bends toward and makes contact with the membranes surrounding the nucleus; whether or not continuity exists cannot be determined. Some of the smaller vesicles lined by a single membrane are probably elements of the longitudinal system of the sarcoplasmic reticulum. Rabbit, phosphotungstic acid. × 38,000.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probably correspond to cisternal endings of the longitudinal system of the sarcoplasmic reticulum, as indicated by Porter and Palade. The relative infrequency of triads and dyads and of the *en face* views of the longitudinal network compared with skeletal muscle indicates that this component of the sarcoplasmic reticulum is less well developed in cardiac than in skeletal muscle (2).

Fig. 13 represents our conception of the sarcotubular systems in cardiac muscle. The *longitudinal system* is a rather sparse network of single membrane-lined tubules closely applied to the surface of the myofibrils affording longitudinal continuity within the length of one sarcomere but rarely if ever with adjacent sarcomeres of the same fibril. Some transverse continuity is present at the mid-sarcomere level, as previously observed (2), and tubules of this system may closely approach the plasma membrane and the nucleus, but direct continuity with these structures is not observed. The longitudinal tubules may terminate in narrow cisternae closely applied to the outer membrane of the transverse tubules. Sporadically, some tubules of this system have externally attached 150 A particles, probably ribonucleoprotein.

We interpret the *transverse system* of the sarcoplasmic reticulum as continuous with and identical with the deep invaginations of the sarcolemma. This system thus is made up of channels filled with extracellular fluid extending throughout the fiber in close association with the Z lines of the myofibrils. Transverse tubules at successive Z line levels may occasionally be connected by tubules or cisternae of like structure lying parallel to the myofibril. Similar connections between adjacent levels of transverse tubular elements of the triads have been clearly shown in bat cricothyroid muscle by Revel (19).

In the intercalated disc, the cell boundary is formed by apposed plasma membranes of adjacent cells without an intervening perimembrane; the latter is continuous over the surface of the fiber. If the perimembrane (basement membrane) is a restricting ion barrier as suggested by Ruska, Edwards, and Caesar (20), it would appear to aid in the maintenance of a potential difference between the interior of the fiber and the extracellular fluid (despite the multicellular composition of the myocardial fiber) and thus to facilitate the propagation of the action potential along the surface of the fiber. If the membranes of the transverse tubular system are indeed continuous with the sarcolemma, the action potential may spread directly in a transverse plane across the fiber at each Z line.

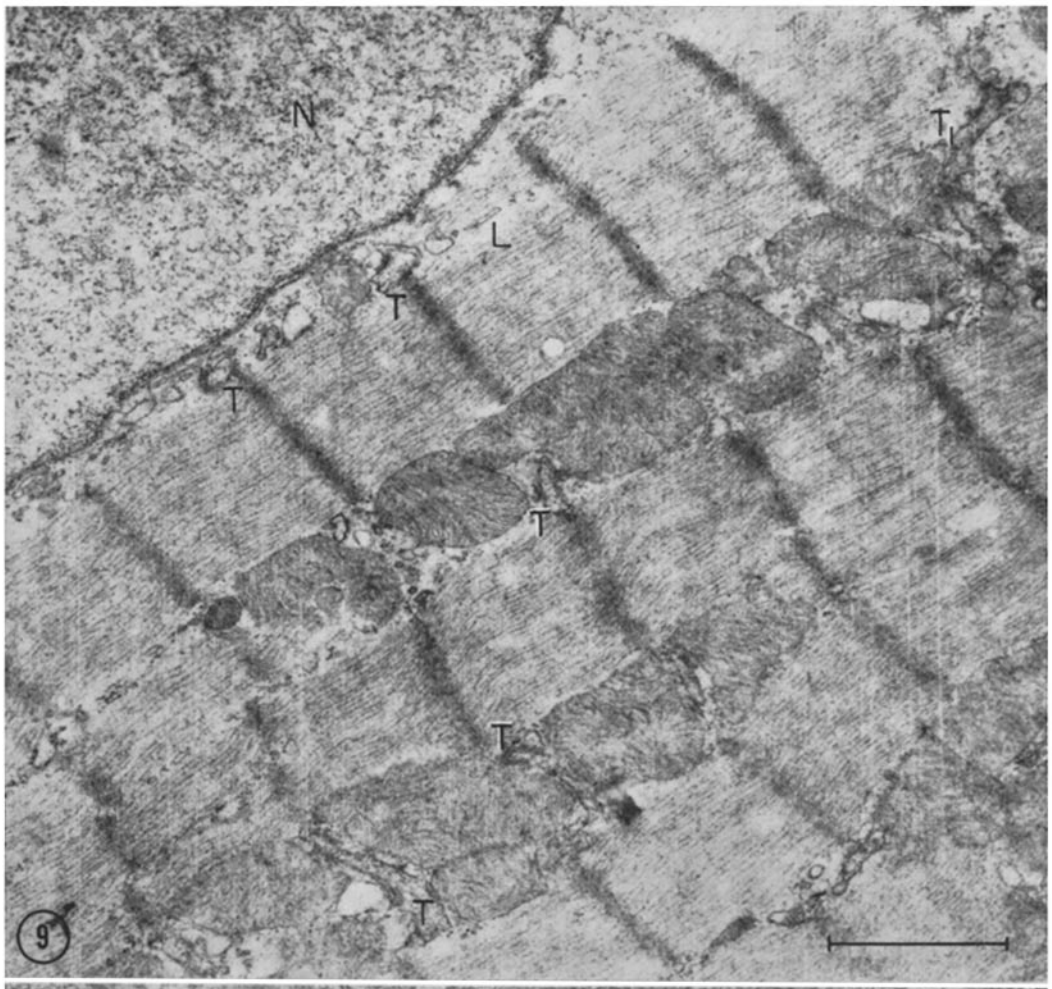
Assuredly, proof of the proposed continuity between the transverse tubular system and the extracellular space must await completion of the arduous task of reconstructing cells from electron photomicrographs of serial sections. In the meantime, the proposed concept of continuity is in harmony with another type of observation, the comparison of estimates of the extracellular volume of rabbit heart muscle made by a histologic method and those made by measurement of the

FIGURE 9

In the interfibrillar and perinuclear sarcoplasm at the Z line levels are profiles (T) which are lined by membranes comparable to the sarcolemma. Where the inner membrane is not clear, the heterogeneous contents are similar in density to the perimembrane material of the sarcolemma. At T_1 such a profile is curved, but appears at both ends to be oriented in the same transverse plane. Some of the smaller vesicles or oval profiles (L) probably belong to the longitudinal system of the sarcoplasmic reticulum. Note also the numerous mitochondria with tightly packed cristae in the interfibrillar areas. Rabbit, uranyl acetate. × 24,500.

FIGURE 10

Where the obliquely cut, contracted myofibril drops from the plane of the section, numerous mitochondria and profiles of interconnecting tubules (L) are seen in close apposition to the myofibrillar surface. They appear to be limited by a single membrane 50 to 75 A thick, have a homogeneous substance in the lumen slightly denser than the sarcoplasm, and are probably components of the longitudinal system of the sarcoplasmic reticulum. Rabbit, phosphotungstic acid. × 53,000.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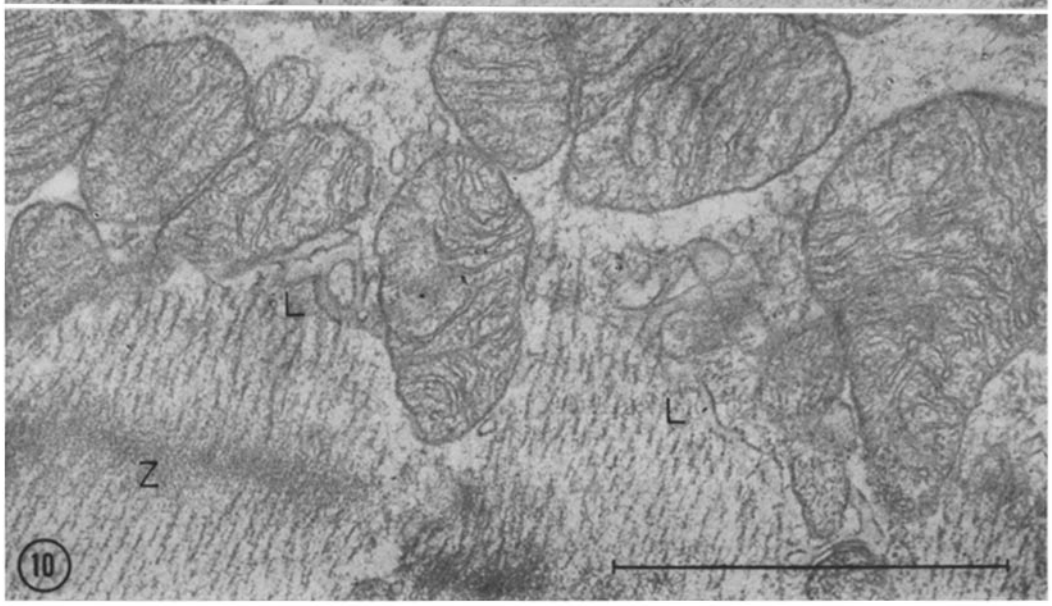
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volume of distribution of sucrose, which resulted in considerably higher values for the latter (21). There is good reason to believe that sucrose does not cross the cell membrane and is, therefore, confined to a preponderantly extracellular distribution (22). In the study by Johnson and Simonds (21), the histologic method of determining cellular and extracellular spaces proposed by Chalkley (23) was used and compared in the same hearts with sucrose space. The tissue was fixed and embedded by freeze-substitution. A random distribution of points in each of two components of the tissue, cellular and extracellular space, was counted and the ratio of points lying outside the cell boundaries to total counts was determined. By the sucrose method the extracellular space occupied 40 per cent of the total tissue space while the histological method defined only 26 per cent of the total space as extracellular. Obviously, a membrane-limited tubular space lying within the area bounded by the cell membrane but continuous with the extracellular space would be penetrated by sucrose molecules yet be assigned to the intracellular space in the histological method of space estimation.

In Huxley and Taylor's experiments (3) contraction occurred only when the microelectrode was placed on the cell membrane of frog muscle in the neighborhood of a Z line of the underlying myofibril. The induced contraction involved only the I bands of the half-sarcomeres on either side and adjacent to the Z line specified. When the contraction spread, it did so either transversely or circumferentially for a distance as much as 10 μ recruiting only I bands adjacent to Z lines in register with the microelectrode at the cell membrane. No way of explaining these observations on the basis of a diffusion process from the cell membrane seems possible unless the diffusion is sharply restricted by barriers to a transverse plane. The electron microscopic observation of a transverse membrane-bounded system at the Z line level makes very plausible the implication of this system in the transmission phenomenon observed by Huxley and Taylor in frog skeletal muscle.

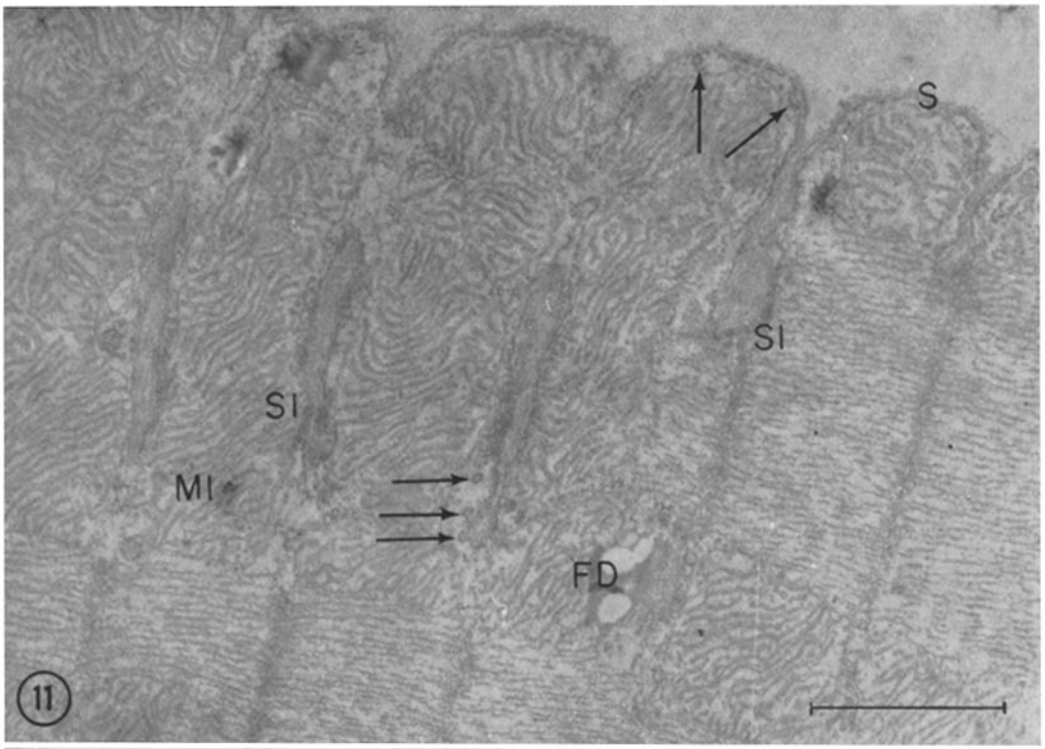
In postulating that a pure diffusion process was inadequate to explain the time relationships between excitation and activation of the contractile elements, A. V. Hill noted that at 0°, the active state is fully developed within 40 milliseconds after a shock in frog sartorius muscle (4), representing about one-tenth of the time required to reach maximum tension. Assuming that the entire cross-section of the fiber is activated in a twitch, and that the diffusing substance is not used up

FIGURE 11

Note the scalloped sarcolemma and sarcolemmal invaginations (*SI*). At the left three Z line levels the continuity of the profiles labelled *SI* with the sarcolemma is not apparent in the plane of the section. Indeed, whether one labels them *SI* or *T* seems arbitrary, since all such profiles in these illustrations might well be continuous. Here they extend below the depth of the most superficial myofibril. Note the vesicular buds extending into the cell from the plasma membrane component of the sarcolemmal invagination and adjacent vesicles 500 to 600 A in diameter (arrows) and compare them with similar structures associated with the sarcolemma itself; these may be a structural representation of pinocytotic activity. The mitochondrial cristae are abundant in this section; note the loss or fragmentation of the outer mitochondrial membranes with relative preservation of the cristae which is true in much of our human material. Human, uranyl acetate. × 26,500.

FIGURE 12

Profiles (*T*) of the transverse tubular system which are oval or quadrangular appear in the sarcoplasm at the Z line levels and are more definitely lined by an inner membrane similar to the perimembrane. Small vesicles (arrows) are occasionally numerous adjacent to these elements; whether these 500 to 600 A round structures represent cross-sections of narrow tubules of the longitudinal system or sections of pinocytotic vesicles is difficult to say. Other features are similar to those in Fig. 11. Human, uranyl acetate. × 26,500.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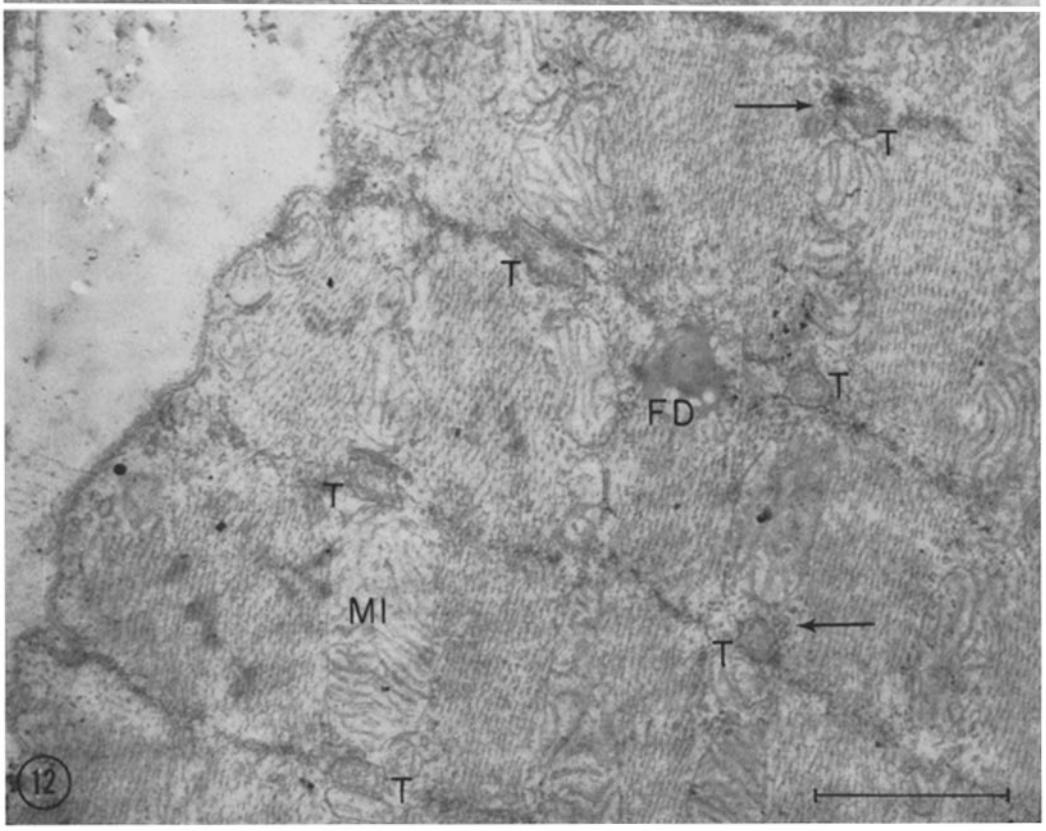
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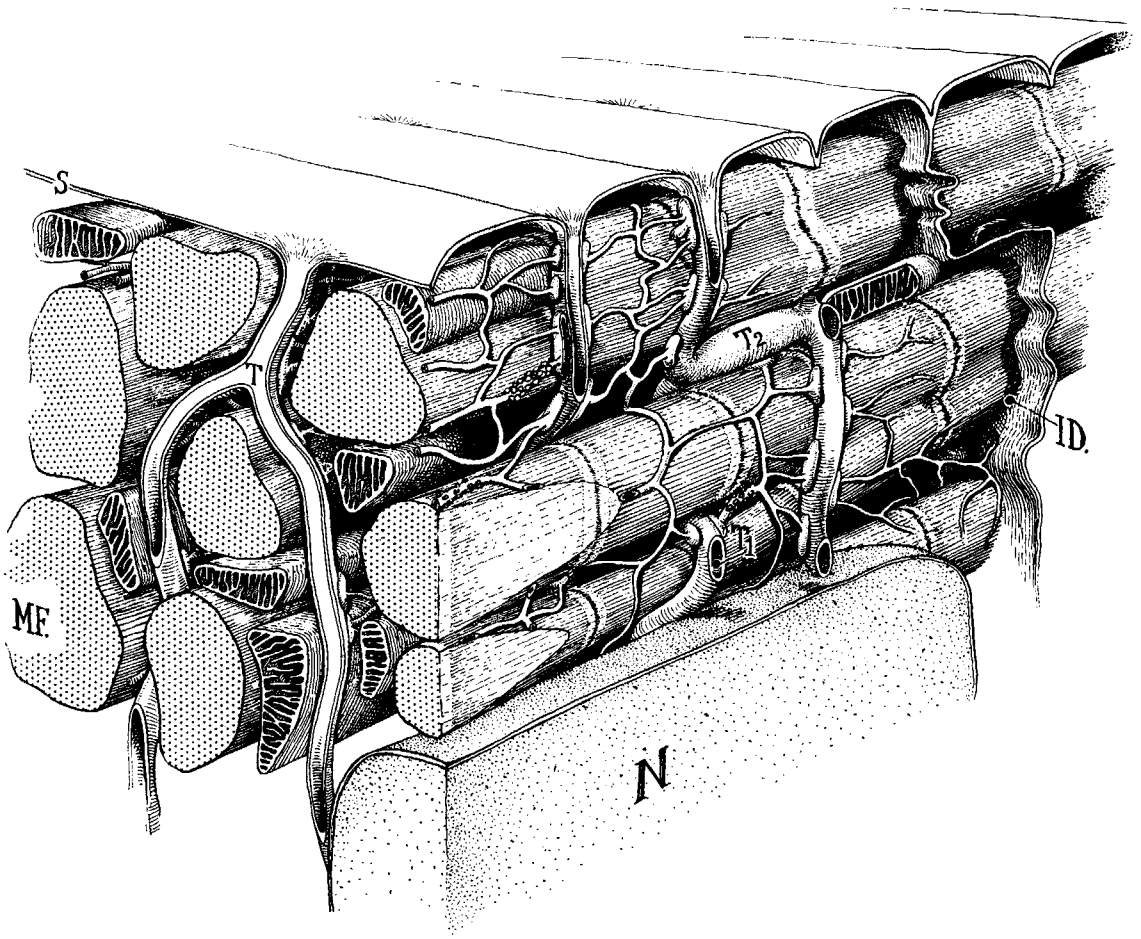

FIGURE 13

Model of a portion of a myocardial fiber demonstrates our idea of the relationships of the two components of the sarcoplasmic reticulum. A discussion is found in the text. Oval profiles (T_1) designate somewhat tangential sections through parts of the transverse system to show the structure of two membranes; this is also shown in sections through the sarcolemmal invaginations which seem to be part of this system. Longitudinal connections between adjacent transverse tubules are occasionally seen (T_2).

during its passage inward, he applied a formulation from an earlier paper (24) and considered the diffusion of a substance such as calcium ion into a cylinder from its surface. At the time at which activation is complete, the relative concentration of the diffusing substance at the axis of the cylinder he found to be zero (to five decimal places) (4). Thus, diffusion appeared to be too slow to account for the abrupt transition from rest to full activity.

To our knowledge there is no evidence bearing on the time required for full activation of heart muscle after stimulation. Assuming, however, that the time of activation bears the same relationship to that of development of peak tension as in frog sartorius, one might try to apply Hill's line of reasoning to rabbit heart muscle. By the histological method of estimating cell space, single rabbit myocardial fibers were found to average 20 μ in diameter (25). At 30°, the time required to reach peak tension is approximately 150 to 200 milliseconds. Applying the symbols and relationships used by Hill (24), an estimate is made of y, the concentration of the diffusing substance, at time t and distance r from the axis; y_∞ is the concentration when diffusion is complete. This estimate is made using the relationship kt/a^2,[1] where k, the diffusion coefficient of calcium ion at 30° is 1.5×10^{-5}, t, the time available for diffusion, is taken as 15 milliseconds (the estimated time for full activation to develop) and a, the radius of the fiber, is 10 μ. Solving kt/a^2, one obtains a value of 0.225, and y/y_∞ at $r = 0$ is 0.9. Since this is a reasonable value, diffusion is not ruled out by this line of reasoning.

On the other hand, the diffusion coefficient used is that of calcium in free solution. One might reasonably expect it to be somewhat lower in the muscle fiber. There may be internal barriers to diffusion and, more importantly, the diffusing substance would very likely be used up in the activation process. Since these factors tend to reduce y/y_∞, the likelihood that free diffusion from the cell membrane to the axis is the mechanism of spread of excitation into the fiber is diminished. Furthermore, the time of activation is assumed in our calculation; it could be shorter, though heart muscle is a relatively slow acting muscle.

If the transverse tubular system is a direct extension of the cell membrane, as seems to be the case, estimates of the surface area of the cell based upon a cylindrical model are likely to be quite inadequate. Notably, estimates of ionic flux per unit area of cell membrane may have to be modified. Humphrey and Johnson (25), for example, in measuring potassium flux using K_{42} in an isolated rabbit heart, found large discrepancies between estimates of the difference in flux between heart rates of 100 and 200 beats per minute and estimates derived from the theory of Hodgkin (26) based on the electrical activity of muscle cells.

Current concepts of the structure and function of the sarcoplasmic reticulum have been recently reviewed by Porter (7) and seem to indicate that two distinct components exist. We have presented further evidence that the tubules of the transverse system have the structure of the sarcolemma, probably are continuous with it, and may be responsible for conduction of excitation into the fiber.

The distribution and the role of the longitudinal system, which is probably analogous to the endoplasmic reticulum of other cell types, are not clear. That it is in part rough-surfaced suggests that protein synthesis is one function; others may include storage and transport of materials. Muscatello *et al.* (27, 28) recently have reported findings on frog striated muscle which suggest the identity of the sarcoplasmic reticulum and the "granular" relaxing factor of Marsh (29). The significance of this observation with respect to heart muscle must await further study. The relative sparseness of the thin-walled sarcoplasmic reticulum with respect to that of skeletal muscle may have some relevance in regard to observations made by Finkel and Gergely (30). These workers found that cardiac myofibrils of rabbit and dog heart are relatively insensitive to the granular relaxing factor system of skeletal

[1] Hill (24) used the equation

$$\frac{y}{y_\infty} = 1 + \sum_{\alpha_1, \alpha_2 \cdots} \frac{J_0(\alpha r/a)}{J_0(\alpha)} e^{-\alpha^2 kt/a^2}$$

where α_1, α_2, $\alpha_3 \cdots$ are positive roots of $J_1(\alpha) = 0$. (J_0 and J_1 are functions used in differential equations involving cylindrical coordinates and are termed Bessel functions.) A graphic relationship between y/y_∞ and kt/a^2 can be set up by reference to which values of y/y_∞ can be determined for different values of r when a and t are known in rough approximation.

muscle. They also were unsuccessful in obtaining a relaxing factor from heart muscle by methods effectively used for skeletal muscle.

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