FORMATION OF ELABORATE NETWORKS OF T-SYSTEM TUBULES IN CULTURED SKELETAL MUSCLE WITH SPECIAL REFERENCE TO THE T-SYSTEM FORMATION

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ABSTRACT

Muscle cells, cultured for 1–28 days from 11-day chick embryo breast muscles, often show elaborate, three-dimensional networks of a membranous system. The network consists of tubular units which are quite regularly arranged. The tubular units composing the network are accessible to ferritin particles suspended in the culture medium; this suggests continuity with the extracellular fluid. These networks can be regarded as a special morphological elaboration of the T-system tubules. Such network formations can be seen much more often in well-developed myotubes. The networks usually exhibit a hexagonal pattern, which is formed of tubular units of a constant diameter. However, some early myotubes contain tetragonal networks, which are composed of spherical pockets with channels of lesser diameter connecting the pockets. Networks are also observed which probably represent a transitional form between these two patterns. Myotubes show many inpocketings of the sarcolemma similar to what are commonly referred to as caveolae or micropinocytotic vesicles. The similarity in configuration and dimension of the tubular units of the network to the caveolae leads to the plausible suggestion that repeated caveola-formation from the sarcolemma or T-system tubule may result in formation of these networks. In this connection, a possible mechanism of the T-system tubule formation is discussed.

INTRODUCTION

With glutaraldehyde fixation, the T system has been shown to be continuous with the sarcolemma in the vertebrate skeletal muscle fiber (Franzini-Armstrong and Porter, 1964; Jasper, 1967). Continuity of the T-system tubule with the extracellular space has also been demonstrated by a ferritin-diffusion method (Huxley, 1964; Page, 1964). Thus, the T-system tubule should be considered to be part of the plasma membrane of the muscle fiber, and not part of the sarcoplasmic reticulum (SR) (Peachey, 1965).

Our previous observations on the differentiation of the SR and T system in developing skeletal muscles in vitro have shown that the T-system tubule can be clearly distinguished from the SR and is formed by invagination of the sarcolemma (Ezerman and Ishikawa, 1967).

In cultured skeletal muscle cells, elaborate three-dimensional networks of a tubular form have often been observed. Similar networks have been reported in denervated rat skeletal muscle (Pellegrino and Franzini, 1963). The purpose of this paper is to describe these networks with special reference to the T-system tubule formation.

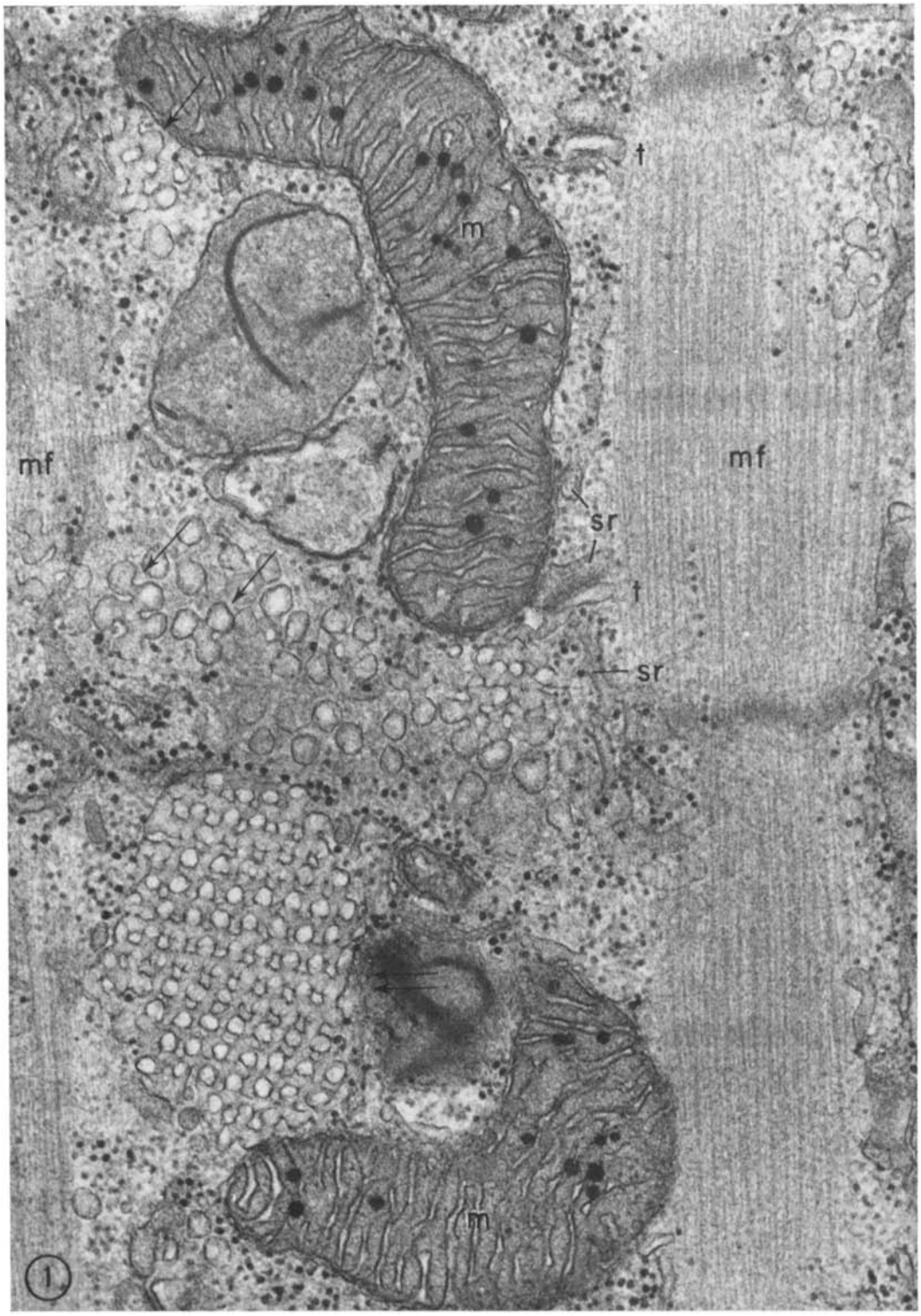

FIGURE 1 Longitudinal section of a well-developed myotube. An elaborate membranous network (double arrow) can be seen between the myofibrils (*mf*). The network is so well organized that it appears crystalline. Simpler, and presumably early configurations of the network also can be found (single arrows). *t*, triadic connections between the T system and SR (*sr*); *m*, mitochondria. 16 day culture. × 54,000.

MATERIALS AND METHODS

Breast muscle tissues from 11-day chick embryos were dissected and trypsinized aseptically (Holtzer et al., 1958). The cultures were grown on fibrin clots 0.1–0.3 mm thick at a concentration of 1 or 2 × 10^6 cells per milliliter at 37°C in a 5% CO_2 and water-saturated atmosphere. The culture medium consisted of Eagle's medium, horse serum, and embryo extract (8:1:1), to which was added 1% by volume of a penicillin-streptomycin solution (penicillin, 1000 U/ml; streptomycin, 1000 μg/ml) and 1% of a 200 mM solution of L-glutamine.

Cultures of various ages (1–28 days) were fixed for approximately 15 hr in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 2 mM $CaCl_2$ at pH 7.2 and room temperature (22 ± 2°C), rinsed in the same cacodylate buffer with 10% sucrose for 15 min, and then postfixed for 1 hr in cold 1% OsO_4 in the same buffer. All specimens were dehydrated in graded concentrations of ethanol (75, 95, 100%) and embedded in Epon 812 (Luft, 1961).

Specimens grown on the fibrin clots could easily be removed from the plastic culture dishes in propylene oxide after dehydration. The clots were easy to handle during subsequent stages of preparation and orientation. Sections were cut on a Sorvall Porter-Blum MT-2 microtome, stained sequentially with a saturated solution of uranyl acetate in 50% ethanol and lead citrate (Reynolds, 1963), and observed with an AEI EM6B electron microscope. Measurements of dimensions presented were made on the printed micrographs enlarged photographically 260,000 times.

Ferritin Experiments

Crystallized ferritin from horse spleen (Nutritional Biochemicals Corporation, Cleveland, O.) was suspended in 1 mM EDTA and centrifuged at 40,000 rpm (average 170,000 *g*) for 120 min at 6°C in a

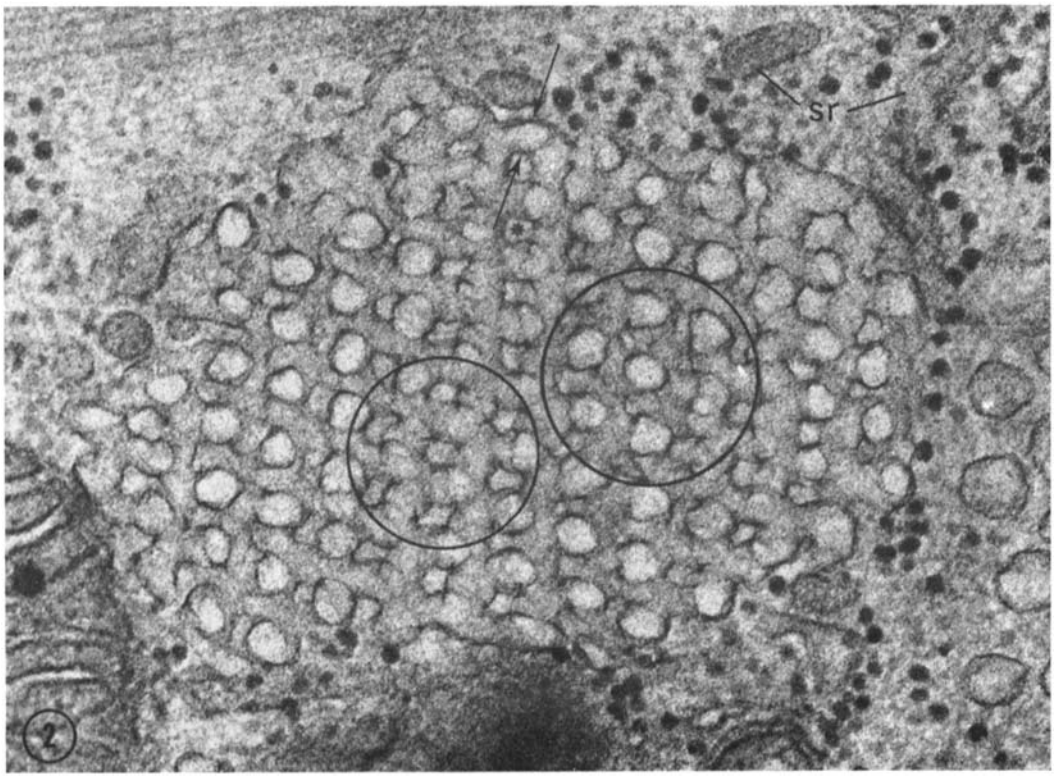

FIGURE 2 High magnification shows that the network consists of tubular units (arrows), which are regularly arranged in an hexagonal pattern. The bigger circle indicates the pattern of the tubular units cut in cross-section. Variation in the pattern is due to a different plane of section (small circle). The tubular units show a sharp limiting membrane and a clear lumen. Note the extratubular space (asterisk) of the network. *sr*, SR membrane. 16 day culture. × 98,000.

Beckman model L-2 ultracentrifuge. The process was repeated two additional times, after which the final pellet was diluted to about five times its volume with culture medium. The ferritin solutions were warmed to 37°C and substituted for culture medium of living muscle cultures (6, 8, 14, 21 days). After 30 min, the cultures were rinsed quickly with the culture medium and fixed according to the techniques described above. Thin sections of ferritin preparations were stained weakly with lead citrate or not stained.

OBSERVATIONS

Formation of T-System Network

Elaborate networks of a membranous system quite often can be found in developing muscle cells in vitro. These networks of varying sizes are seen in the interfibrillar spaces inside the myotubes and more often in the subsarcolemmal regions. Most of the networks appear ovoid in contour, and some of them are as large as 2.0 × 1.2 μ in section. The networks are found much more often in well-developed myotubes. However, even early myotubes in 1 day cultures can contain such networks.

Most of the networks are so well organized that they appear crystalline at lower magnification (Fig. 1). Higher magnification shows that the networks consist of tubular units which have a diameter of about 550 A and are regularly arranged in an approximately hexagonal pattern (Fig. 2). The shapes of the profiles of the individual tubules depend on the angle of the plane of section to the planes of the three-dimensional array (Fig. 3 *a* and *b*). If a section is made exactly parallel to one of the planes of the network, one can see an hexagonal pattern (Fig. 3 *a*). If a section is made obliquely to the plane, alternating bands of tubular profiles sectioned at different levels of the network can be observed (Fig. 3 *b* and also Fig. 2). Very thin serial sections indicate that the network is arranged in successive planes in which the tubular units form an hexagonal pattern in two dimensions. The hexagonal networks of tubules in successive planes are connected

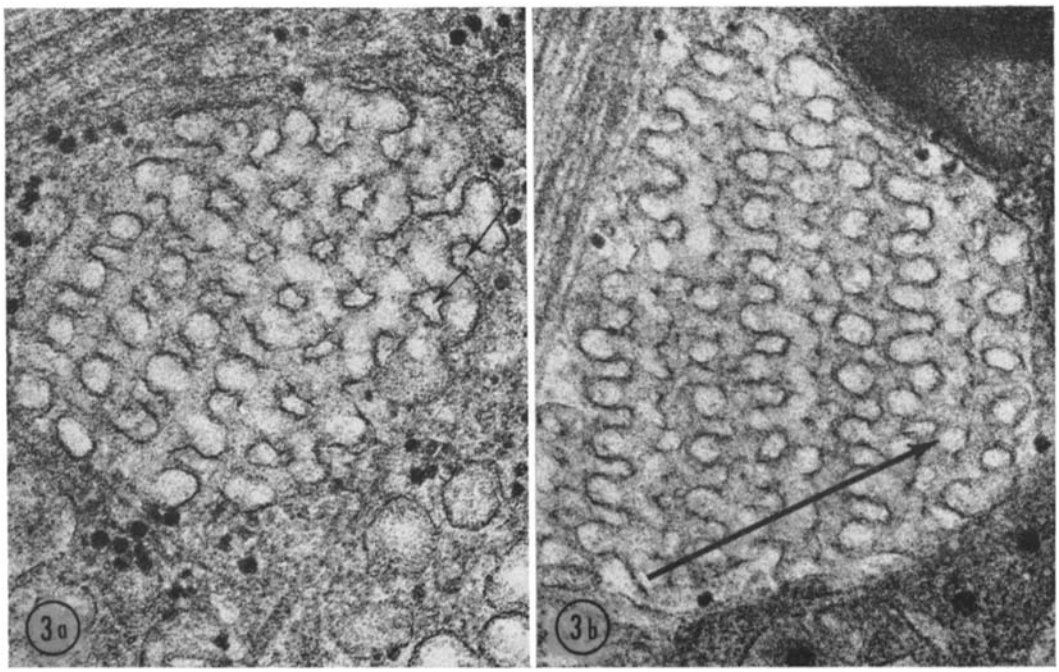

FIGURE 3 *a* and *b* Hexagonal networks in well-developed myotubes. 16-day culture. Fig. 3 *a*, a regular hexagonal pattern of the tubular lattice can be seen on the right, where section is made in a plane of the network. Note extratubular spaces (arrows). Fig. 3 *b*, alternating profiles of tubular units (direction of repeat, see arrow) sectioned at different levels are seen when section is made obliquely to the successive planes. × 100,000.

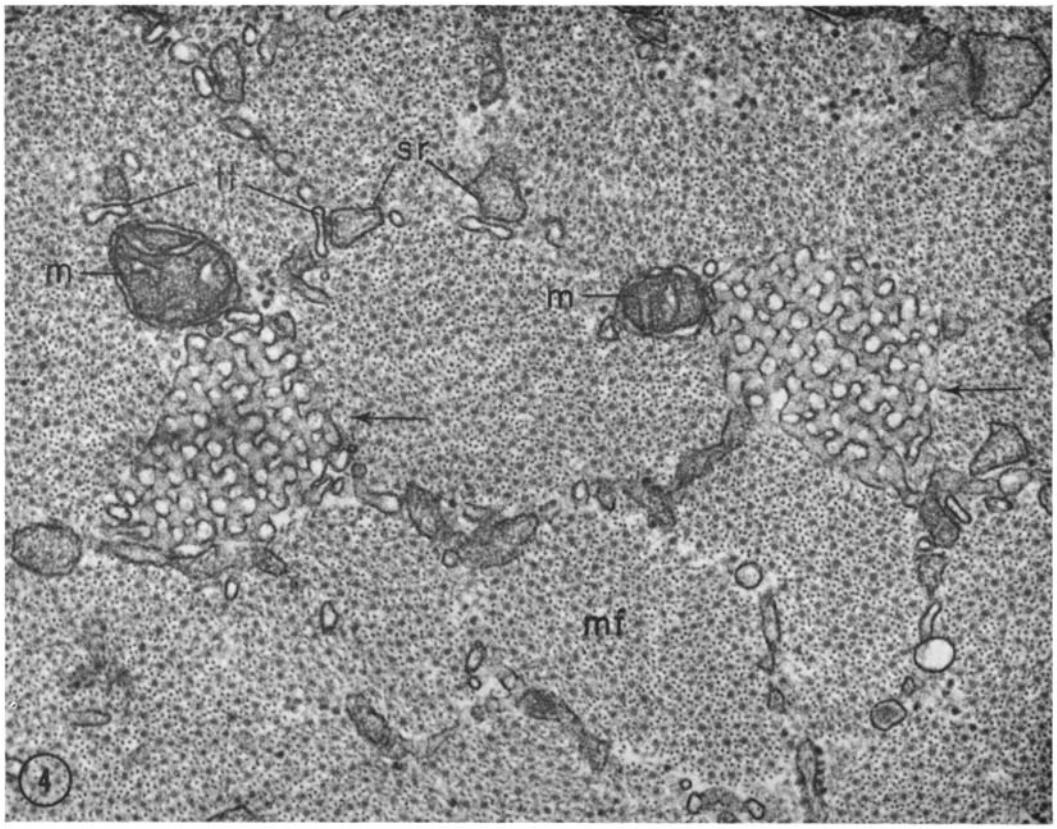

FIGURE 4 Transverse section of a well-developed myotube. The networks (arrows) are seen in the interfibrillar spaces deep inside the myotube. The membrane of the network tubules is similar in appearance to that of the T-system tubules (*tt*), while the lumen of the SR has a diffuse, dense content (*sr*). Since this section is oblique to the plane of the networks, the hexagonal pattern is not clearly seen. *mf*, cross-sectioned myofibrils; *m*, mitochondria. 14 day culture. × 52,000.

to each other by tubular units running roughly perpendicular to the planes.

The units show a clear limiting membrane and clear lumen (Fig. 4) similar to those of the differentiating T-system tubule as described in a previous paper (Ezerman and Ishikawa, 1967). The lumen of the tubular units composing the network is accessible to ferritin particles suspended in the culture medium; this suggests continuity with the extracellular fluid (Figs. 5, 6, and 9). Ferritin particles are never found in the sarcoplasmic reticulum (SR) seen near the network (Fig. 5 and 9). The network is in this way divided into two phases: one is the membrane-limited intratubular phase, the other is the sarcoplasmic matrix lying between the tubular units (see arrows, Fig. 6 *a* and *b*). In Fig. 6 *b*, the grayish areas in the middle part (*M*) of the network represent tubular membranes included in the thickness of the section. Careful observations on the distribution of ferritin particles in the lateral areas (*L*), where no obliquely cut membranes are expected, indicate that the particles are found only inside the tubular units composing the network and never in the sarcoplasmic matrix, i.e. extratubular space (see arrows, Fig. 6), of the network.

T-system tubules forming triadic connections with the SR are often seen to be continuous with the network tubules (Fig. 7). Thus, the network can be regarded as a special morphological elaboration of the T-system tubules and is distinguishable from the perforated (or fenestrated) collar of the SR (Franzini-Armstrong, 1963; Peachey, 1965). Bristle-coated pits (Roth and Porter, 1962) are

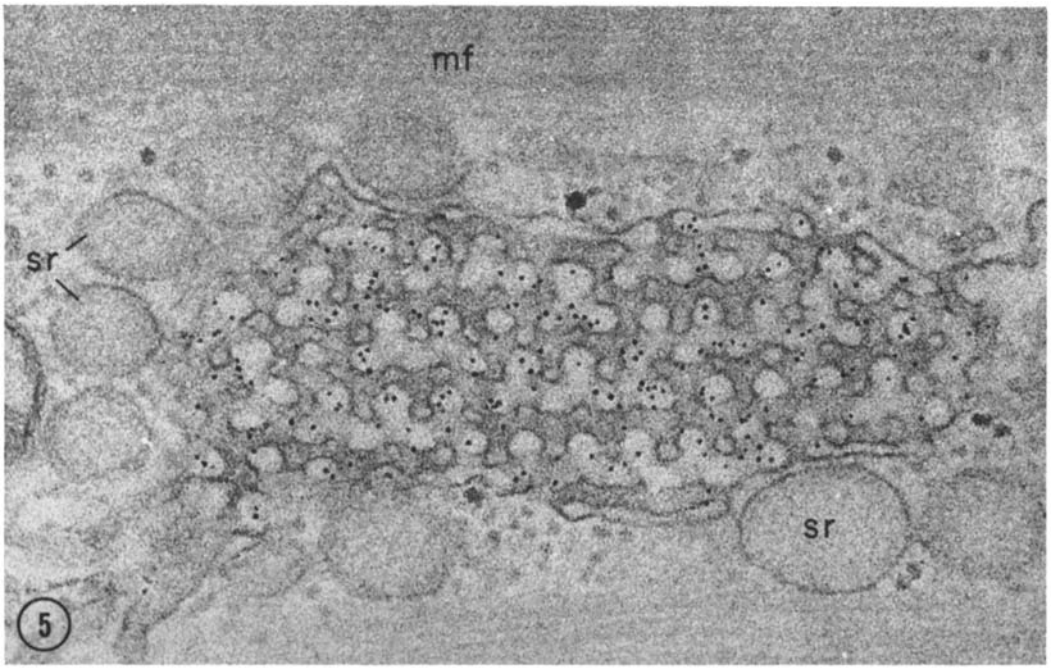

FIGURE 5 Ferritin diffusion. The network is accessible to ferritin particles about 110 A in diameter suspended in the culture medium. The SR vesicles (*sr*) seen near the network do not contain any ferritin particles. *mf*, myofibril. Weakly stained with lead citrate. 21 day culture. × 90,000.

occasionally seen connected to the peripheral tubules of the T-system network (Fig. 8) and to the sarcolemma. There is no finding to suggest that these bristle-coated pits and inpocketings of the sarcolemma are the sites of Z-disc precursor formation (Heuson-Stiennon, 1965). The SR shows a close relationship to the T-system network. There are many images which show branched and tortuous T-system tubules intermingled with the SR tubules. In some cases, narrow tubules are seen occupying the hexagonal interstices of the network (Fig. 9). Such narrow tubules (see arrows, Fig. 9) are never found to contain ferritin particles. These observations strongly suggest that the narrow, inner tubules within the T-system network are SR tubules.

In early myotubes, the networks show a tetragonal (or rectangular) pattern more often than a hexagonal one. The tetragonal networks are usually seen in the subsarcolemmal regions and consist of subunits which are spherical pockets about 830 A in diameter, connected by channels about 470 A in diameter (Fig. 10 *a* and *b*). These subunits are similar in size and shape to the single flasklike inpocketings of the sarcolemma (see Fig. 12 *a*, and also Fig. 10 *c*), and are arranged in a three-dimensional tetragonal array, approximately 1140 A apart center to center. The walls of the channels are continuous with the sarcolemma at many points spaced quite regularly along the network (Fig. 10 *a* and Fig. 12 *f*). At its periphery within the cell, the network often terminates in blind outpocketings (Fig. 10 *b*).

Occasionally, one sees loosely packed networks. Structures are also seen the subunits of which are arranged in a rather tetragonal pattern, but do not consist of the typical spherical pockets characteristic of the regular tetragonal networks (Fig. 11). These structures may possibly be regarded as transitional forms. However, it is uncertain at this time whether the tetragonal network converts to the hexagonal one during culture. Indeed, the simplest, and presumably earliest configurations of the network formation can show a somewhat hexagonal pattern (see Fig. 1).

Invagination of the Sarcolemma and T-System Tubule Formation

Differentiating T-system tubules in skeletal muscle cells in vitro have been described in a

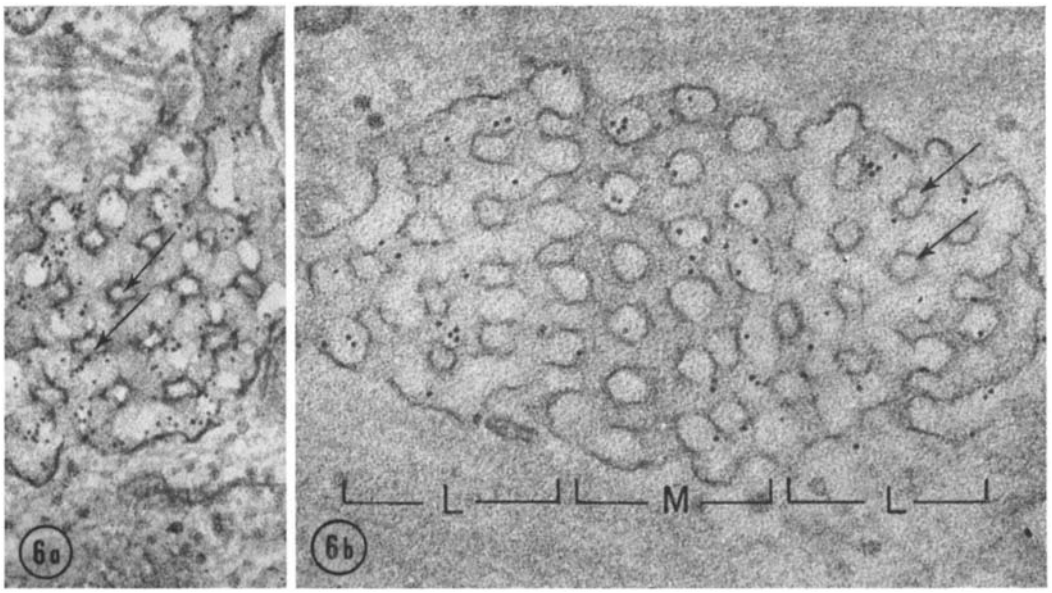

FIGURE 6 *a* and *b* Hexagonal networks showing ferritin entry into the tubular units. No ferritin particle is found in extratubular spaces (arrows). Fig. 6 *a*, a thicker section. 14 day culture. Fig. 6 *b*, a thinner section. Ferritin particles are found only inside the tubular units, as seen in the lateral parts (*L*) of the network. The middle part (*M*) of the network includes horizontally running tubules (gray areas) as well as vertically running tubules (clear areas). Thus, ferritin particles are present in both areas. 21 day culture. Fig. 6 *a*, × 75,000; Fig. 6 *b*, × 98,000.

previous paper (Ezerman and Ishikawa, 1967), in which particular attention was paid to the distinction between the T system and the SR. The T-system tubules in developing skeletal muscle cells appear first as inpocketings and invaginations of the sarcolemma. Then, the T system develops as an inward extension of these invaginations. The developing T-system tubules are highly curved, with a radius of curvature of approximately 380 A (380 A ± 40).

In developing myotubes in vitro, one can see many inpocketings of the sarcolemma which are similar to *caveolae intracellulares* (Yamada, 1955) or micropinocytotic vesicles. An important characteristic of these sarcolemmal inpocketings is that they usually form elongated, branched channels (see Figs. 10 *c* and 13). A single inpocketing, i.e. caveola, is a spherical pocket, about 810 A in diameter, connected to the surface by a tunnel-like channel 300-500 A in diameter (Fig. 12 *a*). More commonly, multiple (1–5) spherical pockets, extending by way of channels from the presumably original caveola, which is open to the outside, show a multilobed or rosette-like configuration (Fig. 12 *b–e*, and Fig. 13). Such a configuration appears so uniquely in cells showing some other characteristic of myogenesis that it seems to be yet another clue in identifying differentiating skeletal muscle cells in these cultures. The caveolae and multilobed inpocketings often communicate with each other beneath the sarcolemma (Fig. 12 *f*).

All these vesicular or tubular forms appear to be accessible to ferritin particles (Figs. 9 and 13). One cannot tell, by using the ferritin diffusion technique, whether these vesicles beneath the sarcolemma remain open to the outside space or pinch off as do the pinocytotic vesicles seen in endothelial cells (Palade, 1961). But serial sections indicate that many vesicles are continuous with the sarcolemma; other vesicles can not be traced with certainty to the sarcolemma. The concentration of ferritin does not seem to be higher in these vesicles than in the outside space (Rosenbluth and Wissig, 1964). Soaking the muscle cells in the ferritin solutions does not appear to stimulate caveola formation significantly.

The possibility that some of the vesicles are pinched-off, micropinocytotic vesicles has been examined in developing muscles by using the lanthanum-staining technique described by Revel

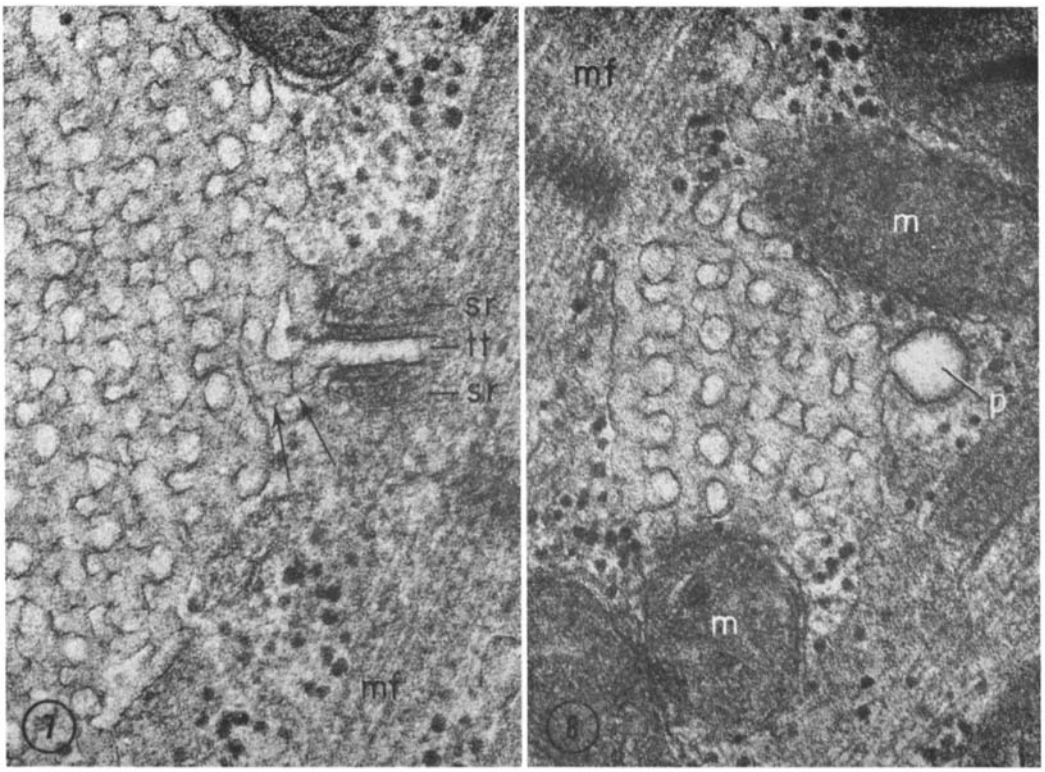

FIGURE 7 A T-system tubule (*tt*) forming a triadic connection with the SR (*sr*) is seen to be continuous with the network tubule (arrows). *mf*, myofibril. 16 day culture. × 84,000.

FIGURE 8 A bristle-coated pit (*p*) connected to the peripheral tubule of the network. *mf*, myofibril. *m*, mitochondria. 16 day culture. × 84,000.

and Karnovsky (1967). Lanthanum in the fixatives does not penetrate the plasma membrane and could be used to demonstrate a lack of continuity of an extracellular space. However, although most but not all of the structures showing vesicular profiles in sections are stained with the lanthanum, the interpretation is still limited by the fact that lanthanum is easily washed out.

Details of the structures described above are summarized in the diagram shown in Fig. 16.

DISCUSSION

These observations show that, in vitro, elaborate three-dimensional networks of tubules are formed in conjunction with the development of the T system. These networks are continuous with tubules forming triad connections with the SR, and thus can be considered to be part of the T system. Furthermore, the networks seem to arise in a manner analogous to T-system formation. This prompts us to discuss these results in terms of possible mechanisms of formation of the T system.

The T-system tubule appears first as an inpocketing of the sarcolemma. This structure reminds us of what is referred to as micropinocytotic vesicles (Odor, 1956) or caveolae (cavelike inpocketings, *caveolae intracellulares*, see Yamada, 1955). Micropinocytosis is considered to be a widespread phenomenon in animal cells involved in the intake or transcellular transport of substances. Caveolae have been described in skeletal muscle fibers by many electron microscopists, e.g., Bennett (1960). Although some generalized functions of caveolae and vesicles associated with the plasma membrane have been suggested for those seen in skeletal muscle fibers (Bennett, 1960), no one has demonstrated yet that caveola-formation is actually a morphological manifestation of any form of transport in skeletal muscle.

The situation becomes more complex when one

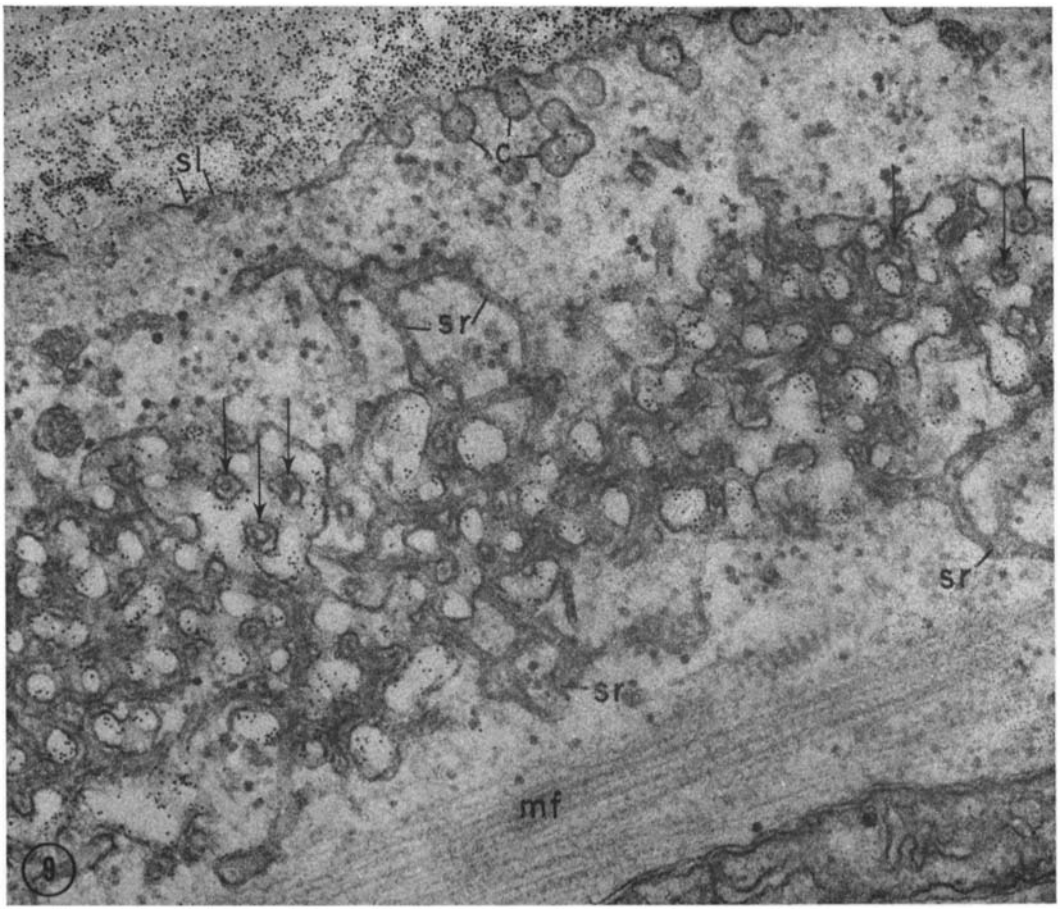

FIGURE 9 A close relationship between the network and SR membrane. Narrow tubules of the SR (*sr*) are seen reaching the network. Note narrow tubules occupying the extratubular spaces inside the network (arrows). Ferritin particles are found only inside the network tubules and inpocketings (*c*) of the sarcolemma (*sl*), and not inside the narrow, inner tubules. *mf*, myofibril. 14 day culture. × 59,000.

considers, in addition to caveolae, the formation of T-system tubules in developing muscle cells. Besides the single caveolae, elongated and branched invaginations are seen continuous with sarcolemma. Some of them are multilobed, or in a rosette configuration, which has been described in detail by Williamson (1964) in lipid-depleted fat cells. Such multilobed forms are also found in other cells such as endothelial cells (Jennings et al., 1962; Williamson, 1964). Williamson considered it unlikely that pinocytotic vesicles originating from the plasma membrane could form this rosette in the fat cell. However, in our ferritin experiments on developing skeletal muscle cells, the multilobed forms beneath the sarcolemma always contain ferritin particles; this indicates that these vesicles, at least sometime during the soak in ferritin, were connected to the sarcolemma. We have no evidence that caveolae are pinched off to form free vesicles in the skeletal muscle cell. It seems very likely, from the findings obtained, that the multilobed and rosette-like configurations are formed by repeated outpocketing from the original caveolae ("caveolation"), similar, at least morphologically, to caveola formation seen in other types of cells. When a simple caveola is formed from the sarcolemma ("primary" caveola), one or more "secondary" caveolae may be extended from the primary caveola. These secondary caveolae are similar in size and shape to the primary caveola. Considering the possibility of repeated caveolation without pinching off, we

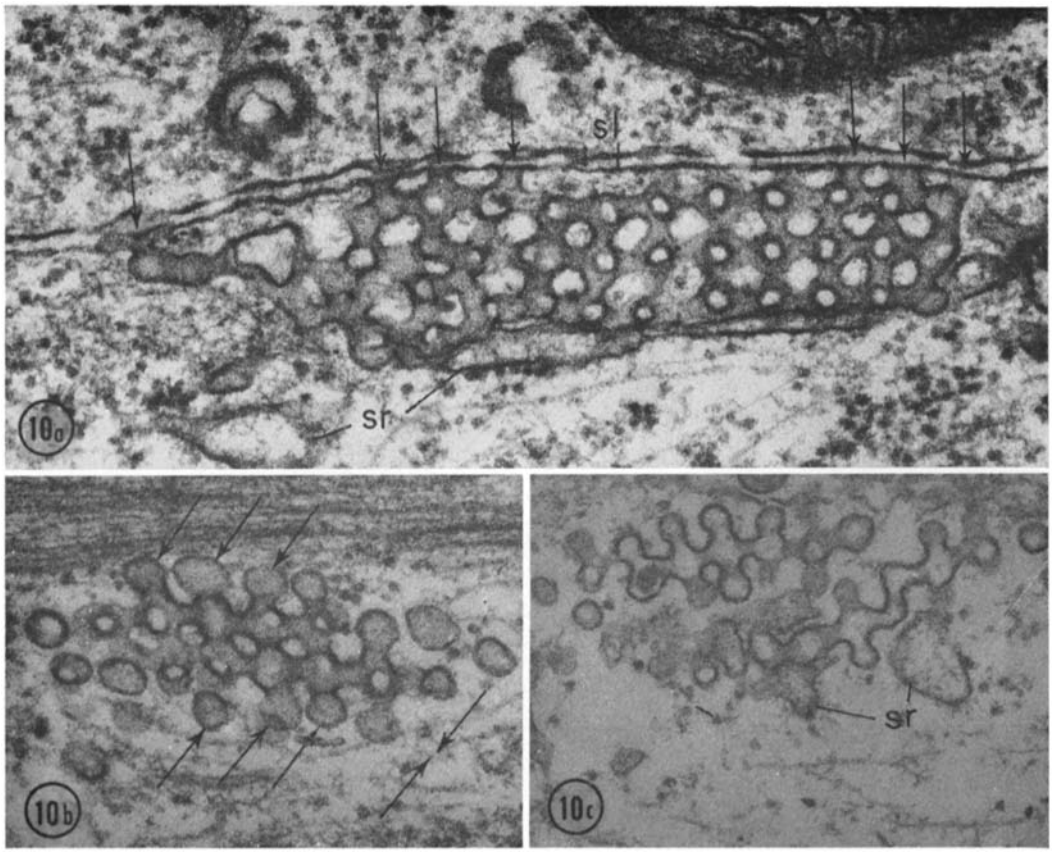

FIGURE 10 *a–c* Tetragonal networks seen in early myotubes. The network consists of caveolar units which are spherical pockets with channels of lesser diameter connecting the pockets. 1 day culture. × 62,000.

Fig. 10 *a*, a typical tetragonal network seen beneath the sarcolemma (*sl*). The network is continuous with the sarcolemma by way of the walls of channels at many points (arrows) spaced quite regularly along the network. Fig. 10 *b*, a somewhat distorted feature of the tetragonal network. This is presumably due to compression of this section (direction shown by large arrows), as judged on the direction of knifemark. The network consists of caveolar units. The units seem to terminate peripherally in blind out-pocketings (small arrows). Fig. 10 *c*, extended tubules with tetragonal arrays of caveolar units. *sr*, SR membrane.

recall that the developing T-system tubules are always highly curved, with a radius of curvature approximately the same as that of the caveolae. It is interesting to speculate that the T-system tubule is formed by repeated caveolation. It seems possible that the process of caveolation is somehow altered in a cell which is forming T-system tubules so that very little, if any, pinching off can occur. A continuous tubule could result from the repeated caveolation. In addition, the developed T system in vivo is much more curved and branched than had generally been believed (see Peachey and Schild, 1968). It seems that the T-system tubules are straight only where they are connected with the SR membranes, i.e. terminal cisternae, in the triads.

It is difficult to distinguish the initiating T-system tubule from pinocytotic vesicles or channels, if any exist in skeletal muscle. The T system is only recognized in its subsequent development by its association in triad structures with the SR. If a tubule, however long, does not connect with the SR to form a triad, this tubule might be regarded as useless in terms of excitation-contraction coupling. However, it may retain the ability later to form connections with the SR.

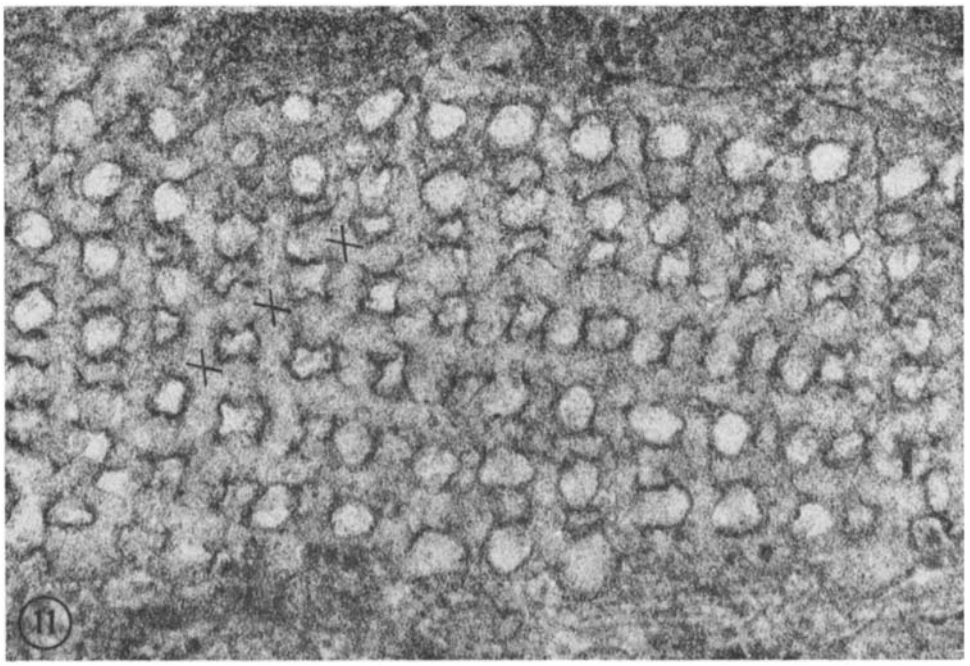

FIGURE 11 A network showing a tetragonal pattern (see *X*). The network, however, does not consist of typical caveolar units characteristic of the regular tetragonal pattern. This presumably represents a transitional form between a typical tetragonal pattern and an hexagonal one. Compare with Figs. 3 *a* and 10 *a*. 16-day culture. × 117,000.

The observation that three-dimensional tetragonal networks are found mostly in early myotubes and that hexagonal ones are encountered with increasing frequency in older cultures suggests that the hexagonal pattern is a more stable form and possibly is formed from the tetragonal one. A three-dimensional reconstruction of the hexagonal network was made as interpreted from serial sections (Fig. 14). In this connection, Fig. 15 shows a schematic illustration of the relationship between the level of sectioning and planes of the hexagonal network.

Structurally, the two patterns described here are not very different from each other. As shown schematically in Fig. 16, the transformation from the tetragonal to the hexagonal network observed could be explained by a deformation of the tetragonal network in certain directions; this would result in tubular units of a constant diameter (see *a*, *b*, and *c*, Fig. 16). The fact that the subunits of the tetragonal network are similar in dimensions to the caveolae beneath the sarcolemma strongly suggests that multiple and repeated caveola formation, and the fusion of the caveolae with one another, result in the elaborate three-dimensional networkds observed. A similar but simpler process may be postulated for the formation of the T system, since it can be formed by a linear array of caveolae.

Elaborate membrane networks somewhat similar to the T-system networks seen here have been reported in the chloride cells of the fish gill (Copeland and Dalton, 1959; Doyle and Gorecki, 1961), in the "clear" cells of the dendritic organs in some marine catfishes (Van Lennep and Lanzing, 1967), in mitochondria (Pappas and Brandt, 1959) and in the plastids of some plants (Gunning, 1965; Newcomb, 1967). A study of extracted proteolipids in vitro (Revel and Ito, 1967) strongly suggests a basic role for proteins in producing and/or stabilizing such membranous networks.

In the denervated rat skeletal muscle, Pellegrino and Franzini (1963) have described an overproduction of the T-system tubules with the formation of networks similar to those seen in cultured

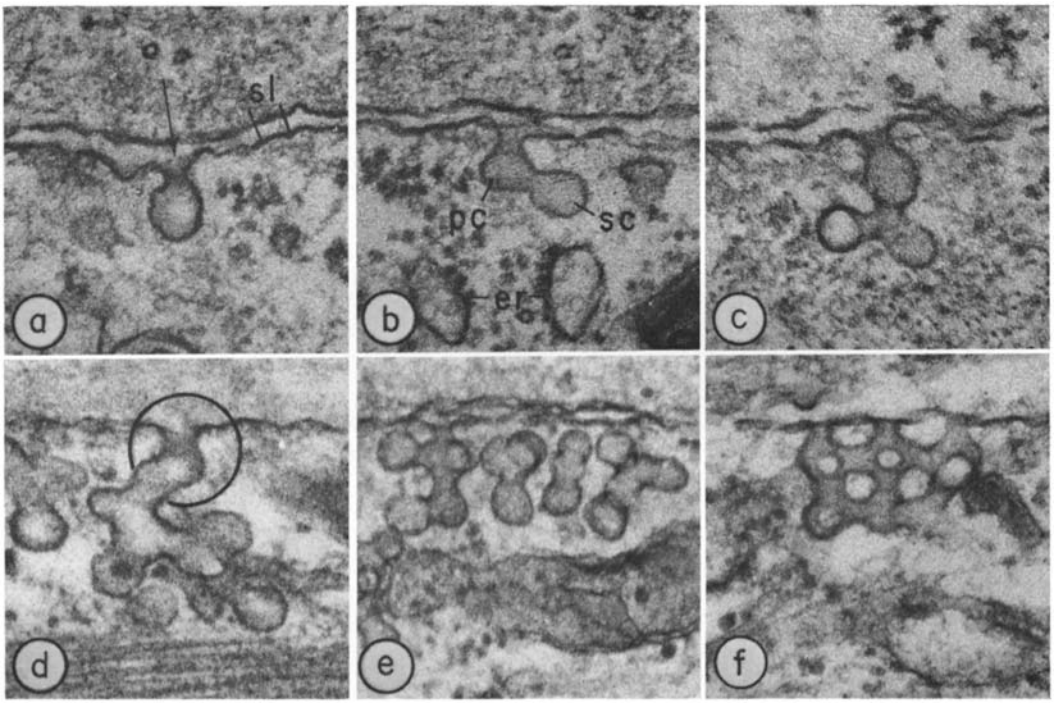

FIGURE 12 *a–f* Various features of the inpocketings of the sarcolemma (*sl*). × 76,000. Fig. 12 *a*, a single inpocketing (caveola). The spherical pocket is connected to the surface by a tunnel-like channel (arrow). 2 day culture. Fig. 12 *b*, a secondary caveola (*sc*) extending from a primary caveola (*pc*). *er*, rough ER. 2 day culture. Fig. 12 *c*, a multilobed inpocketing. 2 day culture. Fig. 12 *d*, an inward extension of the invagination of the sarcolemma presumably from a pimary caveola (circle). 6 day culture. Fig. 12 *e*, multilobed inpocketings lining up beneath the sarcolemma. 6 day culture. Fig. 12 *f*, multilobed inpocketings communicating with each other beneath the sarcolemma, resulting in a tetragonal network. 1 day culture.

muscle cells. These same authors have claimed to have seen this T-system network in one case of atrophy of the bulbocavernosus muscle following castration (Gori et al., 1967). It is interesting to note some factors existing both in cultured and in atrophied muscles. An important point is that, in the muscle fibers showing the overdevelopment of the T system, such as the networks and pentad formations, we see no degenerative alteration, at least not in the same areas, although there may be some loss of contractile material. In our cultured materials, we did not find any evidence suggesting that the T-system network formation is related to degenerative changes, although degeneration with pyknosis is seen in occasional muscle cells. The functional significance of the T-system network remains to be understood.

The formation of the T-system tubule is clearly one of the important events in muscle differentiation. It is known that the muscle cells cultured from 11 day chick embryo skeletal muscle differentiate rapidly (Okazaki and Holtzer, 1965). Excessive and unusual formations of the T system such as the network observed might be associated with high-speed or unbalanced growth in vitro. In this connection, the abnormal T-system proliferation in both cultured and atrophied muscle might be somehow associated with the absence of innervation. It is possible that proteins or other substances in our culture medium stimulate multiple caveolation in the muscle cells much as they have been shown to stimulate pinocytosis in amebae (see Holter, 1959 and 1961) and in some mammalian cells (Barrnett and Ball, 1960). The overproduction of the T-system tubules in denervated muscle fibers (and in rather later stages after denervation, Pellegrino and Franzini, 1963) might be explained by assuming that some mate-

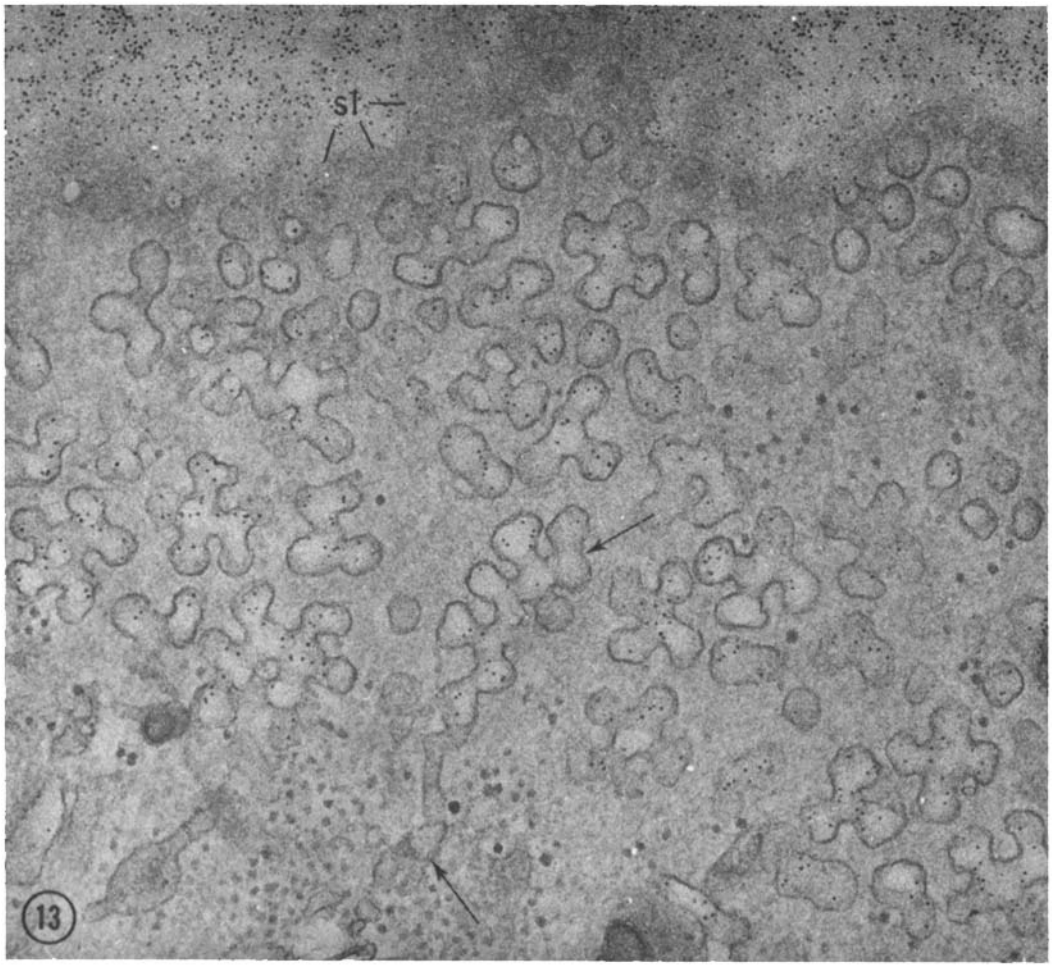

FIGURE 13 Ferritin experiment. The sarcolemma (*sl*, gray zone) is cut obliquely in longitudinal section of a myotube. Numerous multilobed, rosette-like inpocketings are seen containing ferritin particles. Beneath the sarcolemma, some of them appear as elongated, tubular forms (arrows). 21 day culture. × 65,000.

rials such as proteins are released to the extracellular space from the disrupted areas in the atrophying fibers and stimulate the caveola formation on the T-system tubules in nondegenerated areas. It may be possible to inhibit or accelerate selectively the formation of the T system by changing components of the outside medium. A breakdown in the coordination of the events, such as T-system formation, proliferation of SR, and myofibril formation, in differentiating muscle cells could be helpful for an understanding of cell differentiation.

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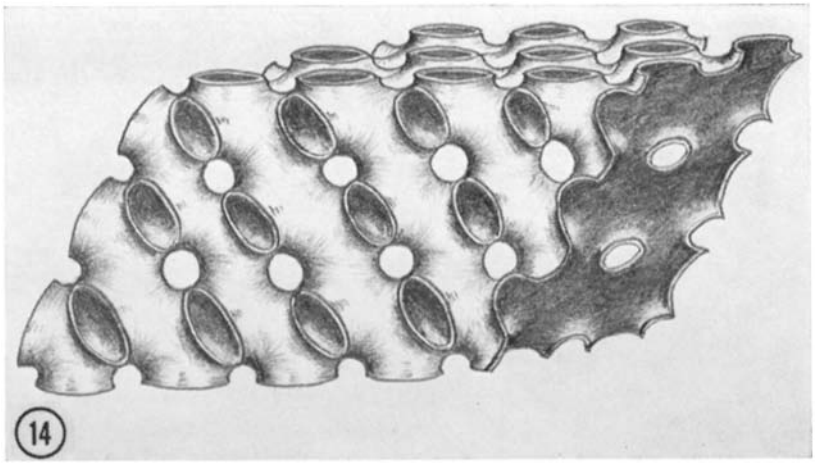

FIGURE 14 Three-dimensional reconstruction of the hexagonal T-system network. Dark areas on the right represent the interior surface of the network tubules. The network is arranged in successive planes in which the tubular units form an hexagonal pattern. The "vertical" tubular units connecting the successive "horizontal" planes themselves form a plane, which is inclined about 60° from the horizontal planes. Thus, any plane with the typical hexagonal pattern intersects other such planes at 60°. × 170,000.

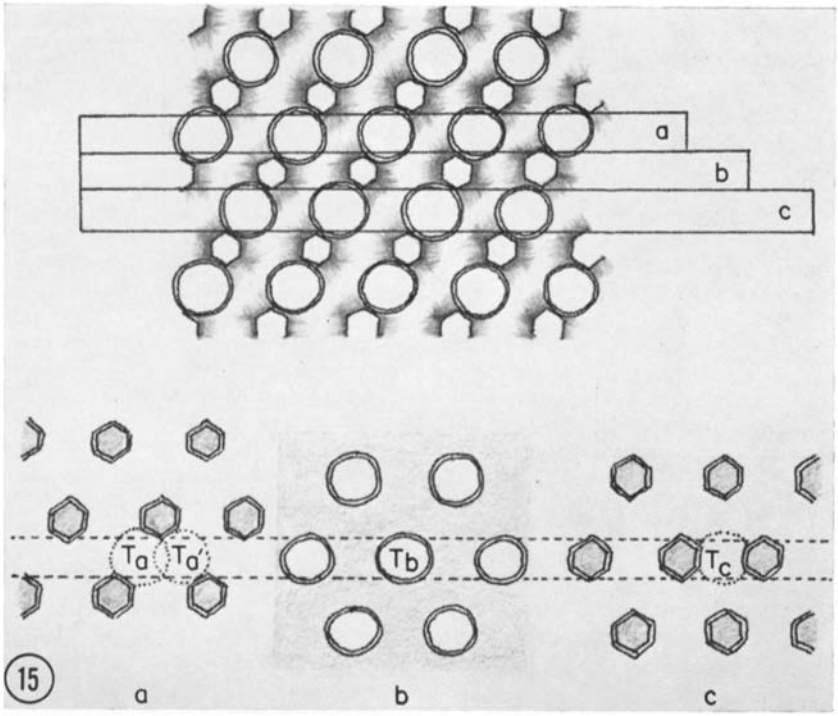

FIGURE 15 Schematic illustration of the relationship between the level of sectioning and planes of the network. An hexagonal pattern of the tubular units is expected when a section is made in the plane of the network (section *a* and *c*), and a rhombic or equilateral array of transversely sectioned tubules when a section goes between the two planes (section *b*). Grayish areas in illustrated hexagonal patterns (below) represent extratubular matrix. The broken circles show the relative location of a cross-sectioned tubule (T_b) in sections *a* and *c*. The tubule (T_b) originating at location (T_c) in the section *c* terminates at location T_a or T_a' in the section *a*. Compare with Fig. 2 (circles).

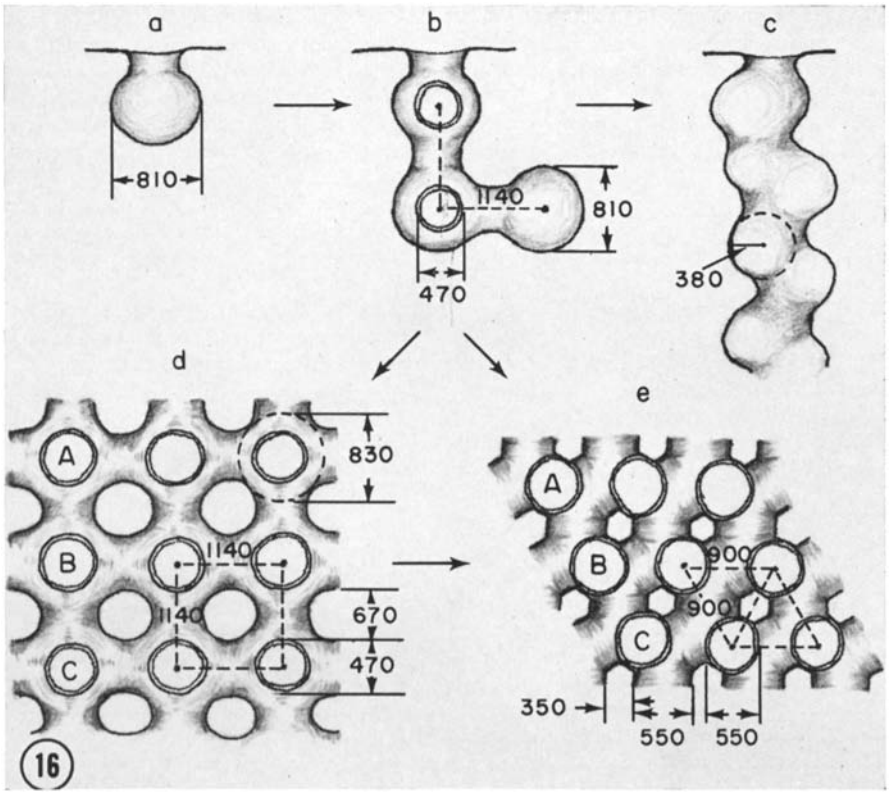

FIGURE 16 Diagram summarizing the details of the dimensions (mean values; given in angstroms) of the structures described. The dimensions given are the values in embedded tissues. This also shows possible mechanisms of the formation of the T-system tubule and networks (see direction of arrows). *A*, *B*, and *C* in *d* and *e* are corresponding tubules. *a*, single inpocketing or caveola. *b*, multilobed inpocketing. *c*, T-system tubule. *d*, tetragonal network. *e*, hexagonal network. All are drawn at approximately the same magnification, 170,000. Mean values of the dimensions are given below with the standard deviations (± SD) (*n*, number of determinations). Caveola: diameter of the spherical pocket, 810 ± 60 (n = 22). T-system tubule: radius of curvature, 380 ± 40 (n = 60). Tetragonal network: diameter of the pocket, 830 ± 60 (n = 50); diameter of the channel connecting the pockets, 470 ± 40 (n = 50); center-to-center distance of the pockets, 1140 ± 110 (n = 30). Hexagonal network: diameter of tubular units, 550 ± 30 (n = 50); center-to-center distance of tubular units (see *e*), 900 ± 70 (n = 50).

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