

THE FINE STRUCTURE OF SHEEP MYOCARDIAL CELLS; SARCOLEMMA INVAGINATIONS AND THE TRANSVERSE TUBULAR SYSTEM

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ABSTRACT

An electron microscope study of sheep myocardial cells has demonstrated the presence of a transverse tubular system, apparently forming a network across the cell at each Z band level. The walls of these tubules resemble the sarcolemma in consisting of two dense layers—plasma membrane and basement membrane; continuity of the tubule walls with the sarcolemma can be seen when longitudinal sections of a cell are obtained between two subsarcolemmal myofibrils and at the same time perpendicular to the cell surface. The demonstration of communication between the lumen of the transverse tubular system and the extracellular space appears to be more definite in this study than in any work hitherto published. It provides anatomical evidence of a possible direct pathway for transmission of the activating impulse from the sarcolemma to the myofibril Z bands.

The hypothesis that in striated muscle the tubules of the sarcoplasmic reticulum are responsible for the intracellular transmission of the activating impulse has recently been reviewed (1, 2, 28), and it appears to be well founded. A. F. Huxley (3) considers that the simplest form of the hypothesis would be exemplified if the lumen of the tubules were continuous with the extracellular space, but, as he remarks, such a continuity has hitherto not been demonstrated by electron microscopy.

The present paper describes some features of the fine structure of sheep myocardial cells (already briefly reported (4)), and we believe that the findings provide definite evidence of a continuity in these cells between the lumen of certain intracellular tubules and the extracellular space.

MATERIAL AND METHODS

Small portions of sheep ventricle were removed under general anesthesia, fixed in buffered 1 per

cent osmium tetroxide (5) at about 4°C for 2 hours, dehydrated in alcohol, and embedded in methacrylate in the standard way. Sections were examined with the aid of a Siemens Elmiskop I electron microscope.

OBSERVATIONS

The material shows some fixation and embedding artifact, but, while this may have obscured valuable points which would otherwise have been cleared, it is not thought to invalidate the present findings.

The *sarcolemma* (or surface membrane complex (6)) is about 250 to 300 Å thick (Figs. 1, 3 to 6) and at this level of resolution it consists of a narrow (60 to 80 Å) inner dense layer or plasma membrane, a middle almost unstained layer, and an outer moderately dense layer (140 to 170 Å)

or "basement membrane."¹ In longitudinal sections of cells, the sarcolemma is seen to be indented opposite each Z band of the subsarcolemmal myofibril. At some of these points there is a small aggregation or "plaque" of dense material (Fig. 4, *Pl*).

Within the cell, appearances suggest that there

The thicker walled tubules are much more readily seen, lying almost always opposite Z bands (Figs. 1 to 5). Their shape on section depends on whether they have been cut straight across or down their length. When cut down their length, the tubules are usually seen to be orientated across the cell (Figs. 3, 4, and 5). The tubules are present

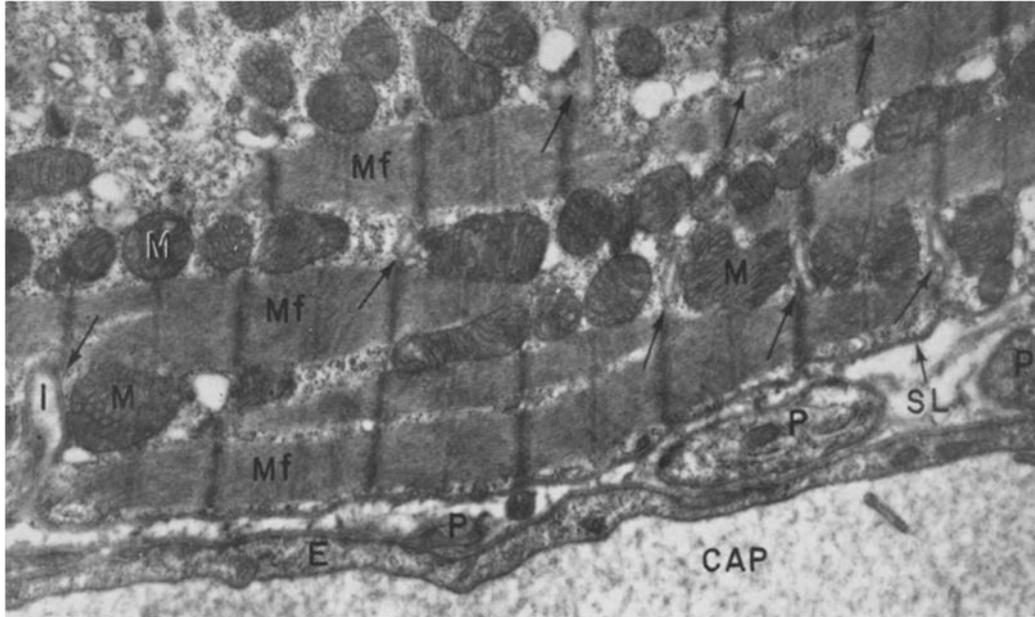


FIGURE 1

Slightly oblique, longitudinal section near the edge of a cell, showing sarcolemma (*SL*), myofibrils (*Mf*), mitochondria (*M*), and sections of the thick walled, transverse tubules (arrows) usually lying at Z band levels. The appearance of one tubule (*I*) suggests that it is in communication with the extracellular space. Part of a capillary (*CAP*) is also seen, with a thin endothelial covering (*E*) and parts of pericapillary cells (*P*). $\times 15,500$.

are two distinct and different *sarcotubular systems*, one of which has quite thick walls, identical with the sarcolemma, while the other is very much finer and more delicate. The latter elements are not always well seen in these specimens, probably because of artifact, although they can be discerned in some places.

¹ The term "sarcolemma" has been used by some authors (28) to designate the plasma membrane alone. In the present paper it has been used to designate the "surface membrane complex" (6); *i.e.* the plasma membrane + basement membrane + intervening translucent layer. The term "basement membrane" is used because in spite of criticism it seems a convenient and reasonable one and because there is no unanimity about an alternative term.

in the granular, mitochondrion-filled spaces at the ends of the nuclei, but only at the edges of these spaces next to myofibril Z bands (Fig. 2). At times a small cluster of two or three thick walled tubules have been sectioned close together, possibly representing the branching of a tubule (Fig. 2, *B*).

As already mentioned, the walls of these tubules closely resemble the sarcolemma, and evidence has been found which appears to put their continuity and identity with the sarcolemma beyond doubt. In some longitudinal sections, passing between two subsarcolemmal myofibrils, deep invaginations of the sarcolemma have been noted at Z band levels (Figs. 3 to 5). These invaginations appear as longitudinally sectioned tubules running

transversely into the cell. In some cases identical tubules are seen which do not communicate with the extracellular space in the plane of the section but which are clearly in series with the sarcolemmal invaginations (Figs. 3 to 5). Serial sections (Figs. 4 and 5) demonstrate the continuity of the tubular lumen and the extracellular space. Somewhat similar appearances, with longitudinally cut tubules lying transversely at Z band levels, have been noted in the interior of the cells where

space, but it is not possible to be certain whether such communication with the extracellular space occurs at every Z band level and between all subsarcolemmal myofibrils. The latter at least is unlikely, because the ramifications of the transverse tubular networks within the cell do not extend between *all* the myofibrils. The question of communication between adjacent transverse networks is also uncertain.

Along the cytoplasmic aspect of the walls of

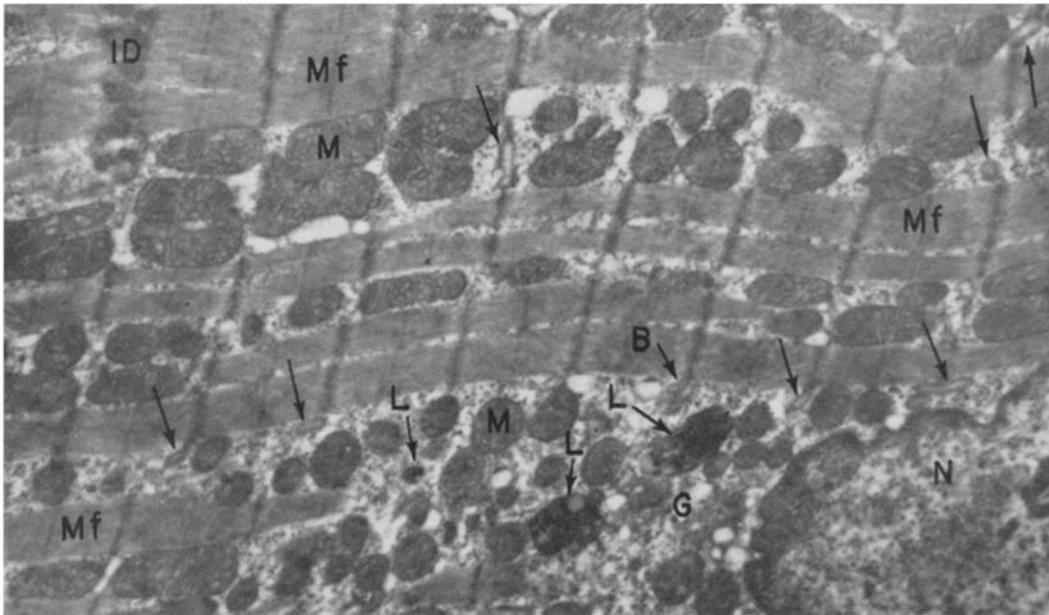


FIGURE 2

Longitudinal section, showing nucleus (*N*), myofibrils (*Mf*), intercalated disc (*ID*), mitochondria (*M*), lysosomes (*L*), Golgi zone (*G*), and cross-sections of thick walled, transverse tubules (arrows). The latter are present only near Z bands, but they are not invariably present between adjacent Z bands; they occasionally occur in clusters, as if having branched (*B*). $\times 12,000$.

the plane of section has passed between two myofibrils. Several examples of the sarcolemmal invaginations have been seen, so the appearances are not merely a chance finding. It seems likely that such appearances depend on the plane of a longitudinal section passing between two subsarcolemmal myofibrils and at the same time perpendicular to the circumference of the cell.

It seems to be safe to conclude that the elements of thick walled tubules which are seen on longitudinal section of a cell do in fact represent parts of transverse tubular networks lying at each Z band level. It is also evident that these networks communicate with the extracellular

the transverse tubules, elements of the thin walled tubules may be seen (Figs. 4 to 6, arrows). Some small loops of these appear to be continuous with the plasma membrane of the transverse tubules (*e.g.* Fig. 4, tubule *P*; Fig. 5, tubule *S*). Other thin walled tubular elements appear to terminate against the transverse tubules without being continuous with them. Structures which appear to be cross-sections of flat, thin walled tubules can be seen at fairly regular intervals along the transverse tubules (Figs. 4 to 6); their nature and connections cannot be entirely determined from these electron micrographs.

Mitochondria are numerous. They tend to lie

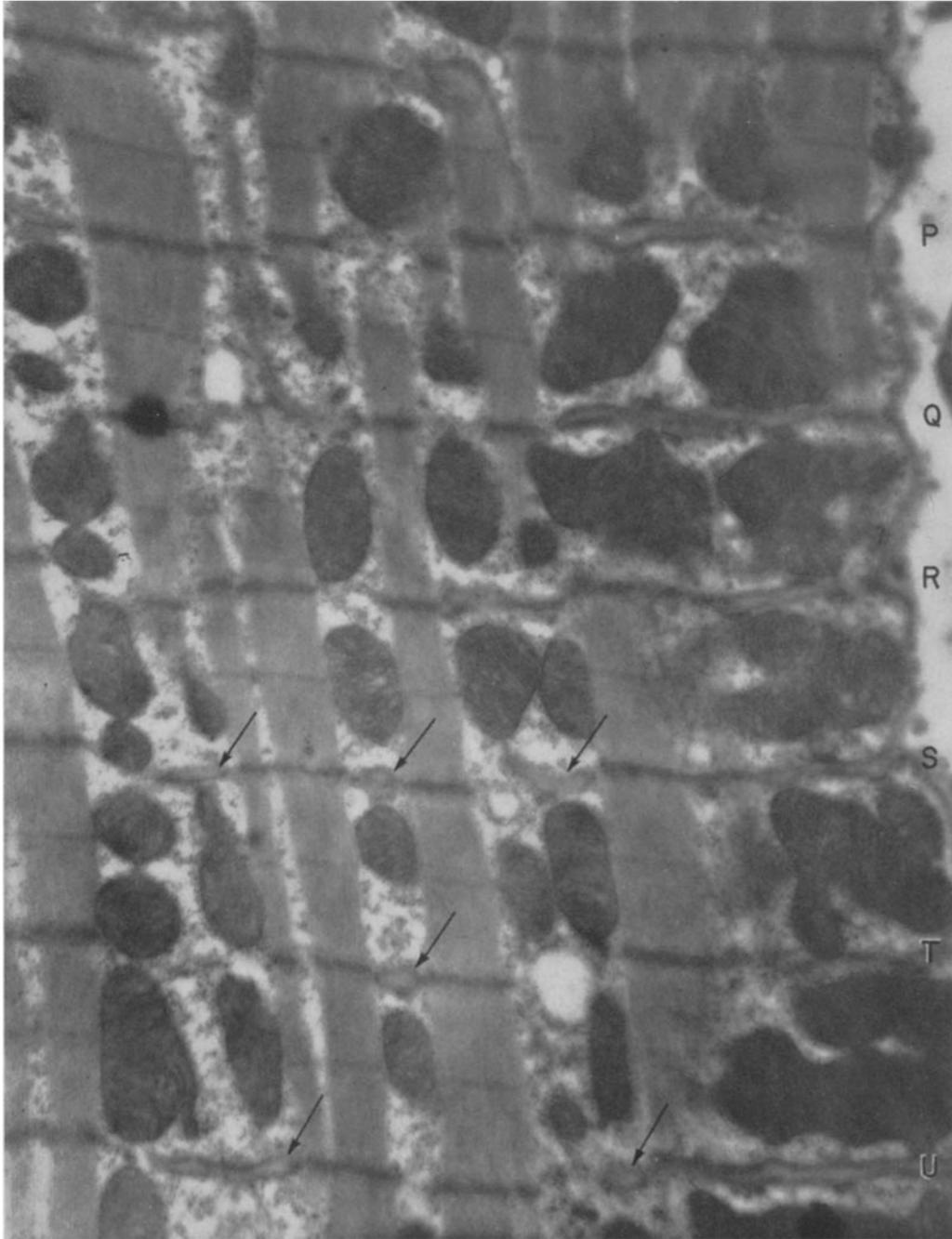


FIGURE 3

Longitudinal section, showing cell edge at right; the plane of section at the upper part has passed *through* a subsarcolemmal myofibril, and at the lower part *between* two subsarcolemmal myofibrils. The Z band levels have been labeled *P* to *U*; a sarcolemmal invagination is seen at *S*, and a smaller one at *T*. The tubules seen at *P*, *Q*, *R*, and *U* are clearly in series with the invaginations. Cross-sections of other parts of the transverse tubular network are seen within the cell (arrows). $\times 19,000$.

singly or in pairs between myofibril segments. They are usually oval and less than $1\ \mu$ long, but occasionally long ones are seen, extending over two to three myofibril segments. In the longitudinal sections passing between two subsarcolemmal myofibrils (Figs. 3 to 5), it can be seen that the subsarcolemmal mitochondria are large and of bizarre shapes. It is evident that in other planes of section they would appear as several small mitochondria. Mitochondria in other parts of the cell also sometimes appear to be larger than usual and of irregular or "horseshoe" shape.

DISCUSSION

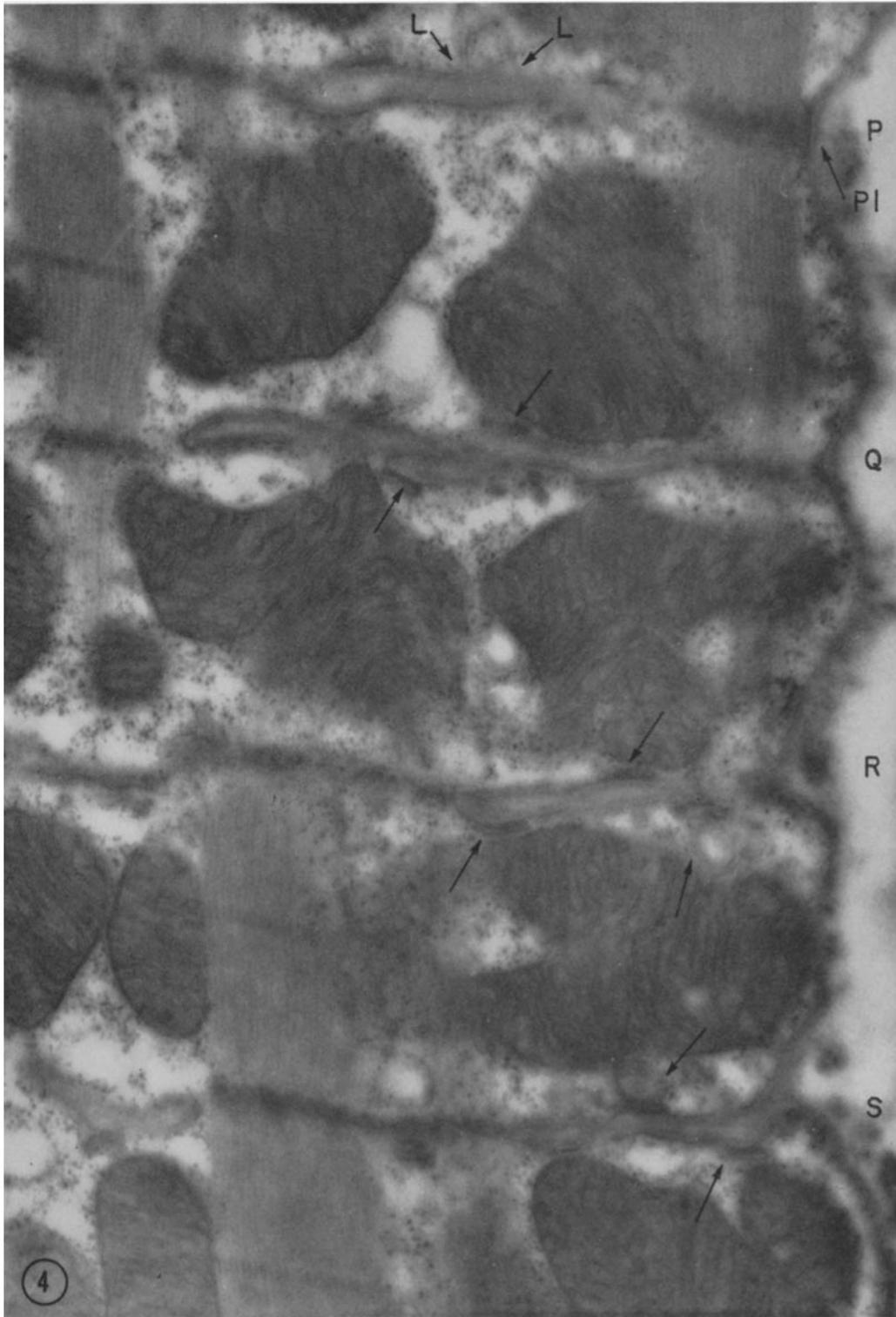
The most interesting feature of these observations lies in the light which they throw on the relationship of the transverse tubular system to the sarcolemma and hence on the problem of intracellular conduction of the activating impulse. The latter has stimulated much speculation even as early as 1881 (7) but more especially since the advent of electron microscopy (8-16) and the experiments of Huxley and Taylor (17), who demonstrated the likelihood of some form of individual stimulation for each myofibril segment. The main theory has been that the membrane of the sarcoplasmic reticulum may separate two phases of the cytoplasm, so that a chemical and electrical gradient is possible across the membrane; the membrane may then be depolarized in the same way as the sarcolemma. This type of theory is propounded in detail by Peachey and Porter (2) on the basis of Porter and Palade's work (15) on the sarcoplasmic reticulum, and by Ruska *et al.* (1), and is also discussed by Andersson-Cedergren (16) in her extensive study of mouse skeletal muscle, in which she also distinguishes clearly between two types of sarcotubular system: the transverse system, which she considers may carry the impulse from the sarcolemma to the neighborhood of the myofibril (A-I junction); and the A and I system, which may act as the final link in the chain of events. The role of the transverse sarcotubular system as a conductor of impulses seems a very likely one; this is borne out by the agreement between the experiments of Huxley and Taylor (18) and Huxley (19) showing the positions of maximum sensitivity to microstimulation of skeletal muscle fibers in the frog and the lizard, and the electron microscopical localization of the transverse sarcotubular systems in the lat-

er species (11) and in amphibian muscle (15). Of recent writers, Bergman (20) appears to be alone in postulating conduction via the Z membranes and "interfibrillar transverse membranes which link adjacent Z membranes to each other."

The relationship of the sarcotubular systems (sarcoplasmic reticulum) to plasma membrane in striated muscle has been investigated by means of electron microscopy by various authors. The possibility that the lumen of the tubules may communicate with the extracellular space was considered by Sjöstrand (12), Andersson (13), and Moore and Ruska (14) as well as by Huxley (19), but the Swedish group have not confirmed this theory in later work (16), and Ruska *et al.* (1) later depict and discuss only a closed intracellular system of tubules. Bergman (20) attributes theories of a system of tubules filled with extracellular fluid to various authors (1, 2, 8, 14-16), but apart from Moore and Ruska (14) it seems doubtful whether the papers cited in fact suggested this. Lindner (21) recognizes the similarity between the sarcolemma and the walls of some intracellular tubules in the dog heart, and postulates a connection between the tubular lumen and the extracellular space via sarcolemmal invaginations which however are not very clearly shown in his illustrations. Smith (22) has recently suggested that in the flight muscle of a beetle the invaginations of plasma membrane which accompany the tracheoles may afford a pathway for intracellular conduction.

A. F. Huxley (3) has maintained that a transverse tubular network, with its lumen communicating with the extracellular space, is the simplest way of explaining the physiological phenomena of the intracellular spread of activation in skeletal muscle. According to this theory, the wave of depolarization would simply spread down such extensions of the sarcolemma to each individual myofibril segment or half segment.

In the present observations, there is probably too much artifact to permit any definite conclusions about the thin walled sarcotubular system. It is also difficult to be certain of the relationship between the thick walled tubules and the tubular elements described by other authors in various types of striated muscle, but there seems at least to be no doubt that there exists in sheep myocardial cells a system of transverse tubules with walls similar to, and continuous with, the sarcolemma, thus lending support to Huxley's theory.



There are two possibilities: either that the transverse tubules in sheep myocardial cells are an *extra* structure, additional to any structures corresponding to those described in other species by other authors, or else that they are analogous to (for instance) the intermediary vesicles and transverse tubules described in rat cardiac muscle by Porter and Palade (15) and to the transverse sarcotubular system of skeletal muscle described by Andersson-Cedergren (16). The latter possibility seems to be the more likely, but it raises the question whether these transverse tubules described by other authors may not also communicate with the extracellular space.

The fact that the transverse tubules in sheep myocardium communicate with the extracellular space carries other implications apart from the probability that they act as impulse conductors. For instance, the total area of sarcolemma must be greater than the mere external surface of the cell (as Siekevitz (23) has pointed out for the membrane of certain other cells), and this may help to explain the rapid ionic fluxes which take place. The extent to which extracellular fluid actually perfuses the system of tubules is, of course, a matter for speculation, but it is not impossible that the lumen of the tubules during life is greater than it appears after fixation. It is also possible that the contraction and relaxation of the cell alternately empties the tubules and permits them to refill by a sort of pumping action.

The nature and function of the little plaques of dense material which are present at the point of contact of sarcolemma and Z band are uncertain. They have been noted also in turtle atrium (24) and toad myocardium (25), and may be analogous to "desmosomes," which occur in other cells at points of contact, and they may have a purely mechanical, strengthening function. On the other hand, they may in some way facilitate passage of the impulse to the Z band.

In the light of our present findings, it seems possible that in some types of muscle each Z band has an attachment or other close functional relationship to the sarcolemma or to its extension in the transverse tubular system. In certain muscle tissues, the myofibrils may nearly all have contact with the external sarcolemma, because each cell is either very flat (*e.g.* amphioxus (2)) or very narrow (toad myocardium (25, 26)). In such cases, it is evident that no elaborate tubular system for intracellular propagation of the impulse will be needed, as Peachey and Porter (2) point out, and this may apply also to the atrium of the turtle, where the paucity of sarcoplasmic reticulum has been noted (24).

The significance of the variety in shape and size of mitochondria is uncertain. No definite difference in internal structure has been seen, but it is not impossible that the variously shaped mitochondria may have different functions. It is, however, also possible that mere mechanical factors may influence their size and shape, and it has been suggested that their shape is constantly altering (27). It is at least evident that mitochondria may be larger and of more complicated shape than they appear to be in single sections, as Andersson-Cedergren has also pointed out (16).

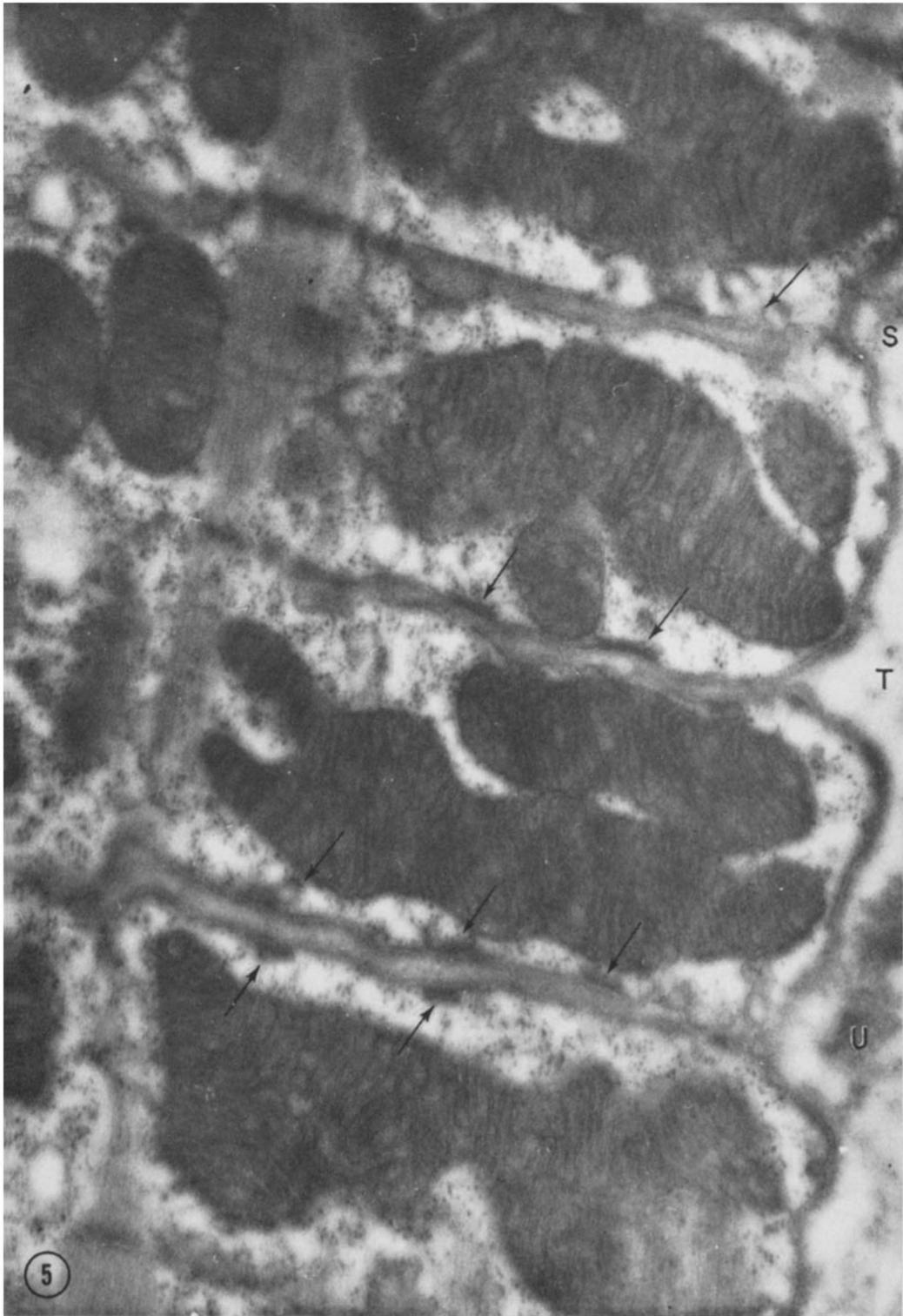
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FIGURE 4

Higher power view of part of Fig. 3, showing the invagination at *S*, and the tubules at *P*, *Q*, and *R*. The tubule at *Q* appears to be branching. Elements of the thin walled tubules can be seen in several places (arrows), often lying close to the transverse tubules, and in one case (*L*) possibly continuous with the plasma membrane of a transverse tubule. A sarcolemma-Z band "attachment plaque" (*Pl*) is seen. $\times 35,000$.



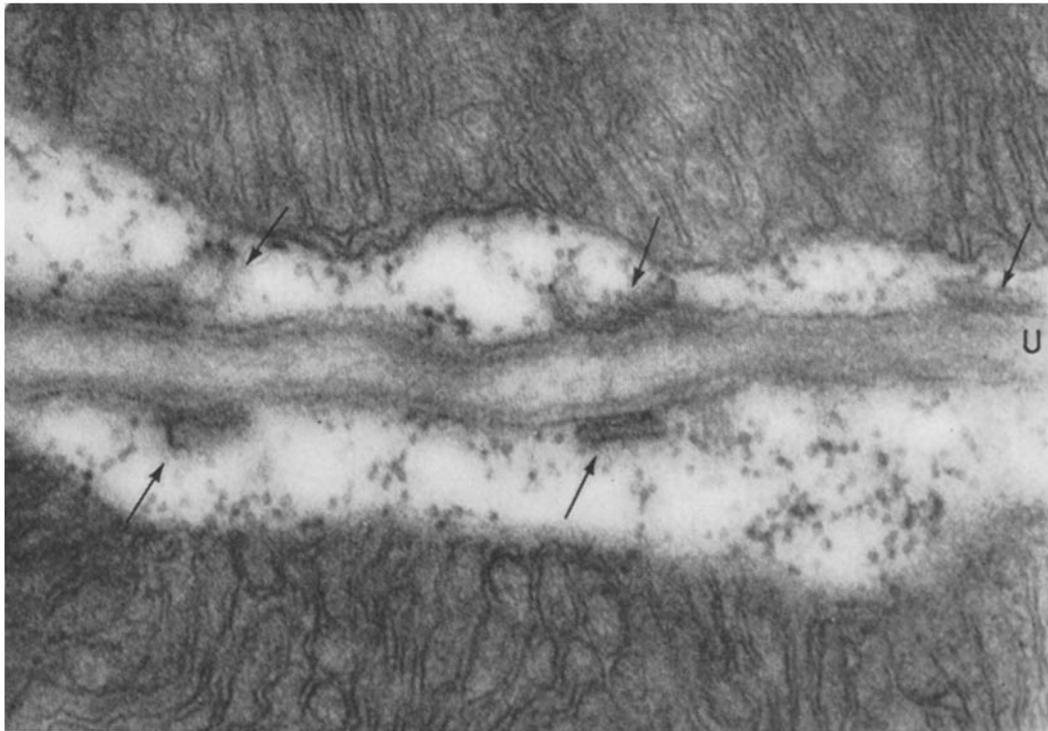


FIGURE 6

High power view of part of tubule *U*, as seen in the section shown in Fig. 5. The wall of the tubule at this level of resolution consists of a plasma membrane and a basement membrane with paler layer between. The basement membrane forms the lining of the lumen of the tubule. Elements of thin walled tubules can be distinguished lying against the transverse tubules (arrows); there seems to be a regular pattern in their distribution (see also Figs. 4 and 5), but the resolution is not sufficient to permit definite conclusions. $\times 82,000$.

FIGURE 5

Another section covering part of the same area as that shown in Fig. 4 and at the lower right hand corner of Fig. 3. The two sections are not necessarily directly consecutive; there may have been one section between them. The lettering corresponds to that in Figs. 3 and 4. In this section the tubule at *S* does not open to the exterior but there is now at *T* a deep tubular invagination (compare with Fig. 3). The inner end of tubule *U* suggests a change of direction or a bifurcation. Elements of thin walled tubules lying against the transverse tubules (arrows) can be only barely distinguished owing to poor definition. $\times 35,000$.

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