
THE SARCOPLASMIC RETICULUM
AND ITS ASSOCIATION WITH THE
T SYSTEM IN AN INSECT

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ABSTRACT

The fine structure of the sarcoplasmic reticulum and the transverse tubular system of the femoral muscle of the cockroach, *Leucophaea maderae*, was studied after prefixation in glutaraldehyde, postfixation in osmium tetroxide, and embedding in Epon. The sarcoplasmic reticulum in this muscle reveals features not previously reported. The sarcoplasmic reticulum is abundant, consisting mainly of a fenestrated envelope which surrounds each myofibril at all levels in the sarcomere. This sarcoplasmic reticulum envelope is continuous transversally as well as longitudinally along the myofibrils. Dyadic junctions are formed by a single T system element which contacts the unfenestrated sarcoplasmic reticulum of adjacent myofibrils in an alternating manner at the ends of the A band. At the dyads, regularly spaced thickenings of the sarcoplasmic reticulum membranes bordering the dyadic spaces are noted. These thickenings, however, do not contact the T tubule membrane. Typical dyadic contacts also are seen between the cell surface membrane and sarcoplasmic reticulum. Z line-like material is seen in contact with the membranes of the cell surface and longitudinal branches of the T systems.

INTRODUCTION

There is abundant morphologic and physiologic evidence that the transverse tubular system is distinct and separate from the sarcoplasmic reticulum in a variety of muscles that have been studied (1, 2, 7, 12, 26, 27). Smith (23) has described these systems in great detail in insect flight muscles. The insect transverse tubular system (*T*) has an interrelationship with the sarcoplasmic reticulum (*SR*) at specialized sites to form dyads. This association is termed a dyad since it is composed of two components, a portion of the T system and only one

segment of the *SR*. On the other hand, triads, which are found in vertebrates and in many invertebrate muscles, are composed of a portion of the T system in association with two segments of the *SR*. Information of the *SR* associated with a dyad in insects is scarce, and certain aspects of the *SR* and T system require clarification.

The general cytological features of the femoral muscle of the cockroach, *Leucophaea maderae*, have been described in an earlier paper (8). The present article describes additional features concerning

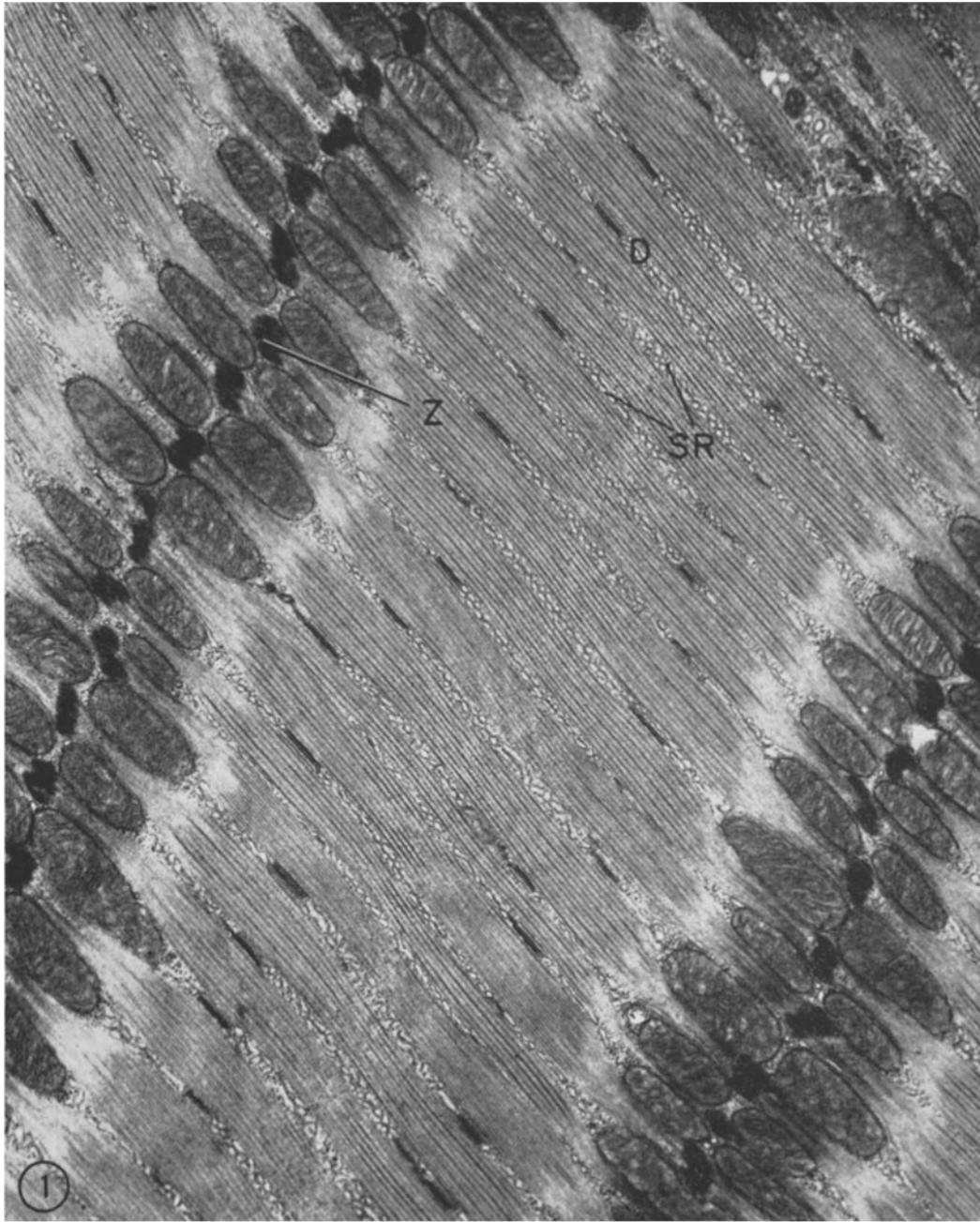


FIGURE 1 Survey electron micrograph of a longitudinal section of a cockroach femoral muscle. Note the long sarcomeres (Z disc (*Z*) to Z disc) which are about 6.4μ long, the abundant profiles of the sarcoplasmic reticulum (*SR*) which are on each side of the myofibrils, and the many dyads (*D*) which are in the intermyofibrillar spaces. $\times 18,000$.

the disposition of the *SR* and its association with the T system in an insect leg muscle.

MATERIALS AND METHODS

The metathoracic leg (hind leg) of the cockroach, *Leucophaea maderae*, was removed from the thorax and perfused with phosphate-buffered 5% glutaraldehyde at pH 7.4 for 4 hr (4°C) (22). To complete prefixation, the femoral muscle without the exoskeleton was placed into 0.2 M sucrose solution for 18 hr at 4°C, and then immersed at the same temperature into a 1% solution of phosphate-buffered osmium tetroxide plus sucrose at pH 7.4 (15). Dehydration in various grades of ethanols and embedding in Epon 812 (13) followed. Thin sections were stained with a combination of uranyl acetate (30) and lead citrate (20) and studied in a Siemens Elmiskop I at 80 kv.

RESULTS

The femoral muscle of *Leucophaea* illustrates the continuous and fenestrated *SR* which is typical for synchronous skeletal fibers of insects. This system of *SR* membranes forms a fenestrated envelope surrounding each straplike or polygonally shaped myofibril (Figs. 1, 2). Transverse sections of muscle reveal that the fenestrations or pores are formed by the fusion of the membranes of the sarcoplasmic reticulum (Fig. 6). The interior of the *SR*, therefore, does not communicate with the sarcoplasm. The pores themselves are in continuity with the sarcoplasm, and both have similar electron-opacities (Figs. 2, 3).

The irregularly disposed fenestrations are present over the entire sarcomere, I band as well as A band. However, at the lateral margins of the A band nonfenestrated portions of *SR* (Figs. 2, 3), which are continuous with the fenestrated portions, are seen. It is the nonfenestrated component of the *SR* which contacts the T system at this level of the sarcomere. The *SR* appears continuous longitudinally at all levels of the sarcomeres and myofibrils. Lateral continuity of the *SR* between the adjacent myofibrils is also present. The pores have an average diameter of 300 Å and occupy about 15–20% of the *SR* surface (excluding the nonfenestrated *SR* portions associated with the T system) (Fig. 4).

The T system originates at regular intervals from the invagination of the sarcolemmal plasma membrane (Fig. 9) at the lateral margins of the A band. Thus, the T system of the femoral muscle is seen in direct contact with the hemolymph although the basement membrane of the sarco-

lemma generally remains at the surface of the fiber. The resulting convoluted T system consists of flattened tubules whose larger diameters are parallel to the myofilaments. These transverse tubules traverse the surface of the myofibrils at the ends of the A band. In general, the membranes of the T system are of greater density than those of the *SR*. Upon occasion the transverse tubule forms branches which are parallel to the myofibrils (Fig. 10). At the Z line levels, these longitudinally oriented tubule branches (*T*) are dilated and have thickened walls (Figs. 11, 12). The increase in wall thickness is due to the presence of electron-opaque material resembling that of Z lines on the sarcoplasmic aspect of the *T* tubules. Similar contiguity between the cell surface membrane and Z line also is seen (Fig. 13).

As described above, each myofibril is surrounded by its own envelope of *SR*. A single tubule of the transversely oriented portion of the T system courses between the two *SR* envelopes. This single tubule in well-oriented sections alternates in forming dyads, first with the *SR* of one myofibril and then with that of the adjacent myofibril. This alternation may occur after each dyad or after pairs of dyads (Figs. 7, 8). The pairs of dyads actually may be a single dyad since the plane of sectioning might pass through and re-enter the same dyad. Nevertheless, a single T system is shared by the *SR* of two adjacent myofibrils. The dyadic junctions are recognized easily because at the junction the *SR* membrane adjacent to the T system has an increased electron-opacity and, in this regard, resembles the membranes of the T system. Both pairs of membranes exhibit a unit membrane structure (Fig. 15) and are separated by a space about 100 Å in thickness (Figs. 14, 15). The dense *SR* membrane bordering the dyadic space also shows, in certain planes of section, areas of regularly spaced thickenings extending toward the T system membrane. No actual contact or communication, however, between the *SR* and T system is present (Fig. 14). The dyadic portion of the *SR* also contains granular material of moderate electron opacity which is not present in either the nondyadic portion of the *SR* or T system tubules (Figs. 5, 7, 8, 14, 15). The longitudinally oriented branches of the T system also form multiple dyadic junctions with the *SR*, in a single sarcomere, at the edges of the A bands. The structure of these dyads is similar to that formed by the transversely oriented component of the T systems.

The tubules of the T system and *SR* are often in very close apposition to the myofilaments. In such regions the circlet of thin myofilaments surrounding the thick myofilament is interrupted by the presence of either tubular components (Figs. 7, 8, 15). Occasional *SR* vesicles make contact with the cell surface membrane in a manner analogous to that seen at true dyadic junctions.

DISCUSSION

The femoral muscle of the cockroach is probably of the synchronous type. The presence of an extensively developed *SR* in this muscle is in accord with previously published studies of other insect synchronous muscle. The insect muscles which are asynchronous, however, are distinguished by relatively poorly developed *SR*.

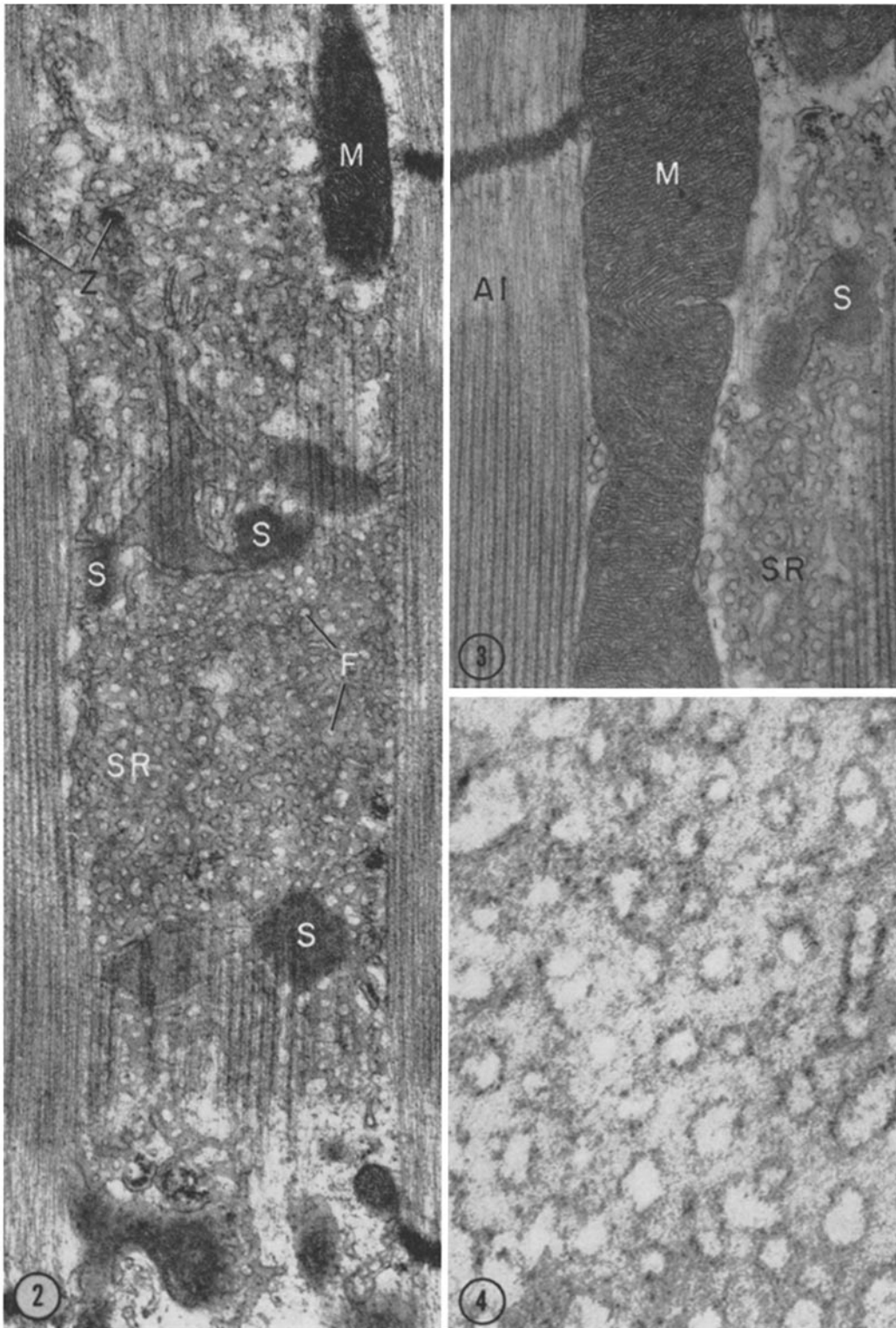
Significant differences in the organization of the *SR* and associated T systems in the femoral muscle of the cockroach, on the one hand, and vertebrate skeletal muscles, on the other hand, are also apparent. In this insect muscle, the *SR* is abundant and consists of a fenestrated envelope surrounding all levels of the sarcomere. This envelope forms a longitudinally continuous structure along the entire length of the myofibrils. Moreover, lateral continuity in a plane transverse to the long axis of the muscle fiber also can be demonstrated. The straplike profiles of the majority of the myofibrils in this muscle, however, make it difficult to evaluate the extent of transverse continuity which is revealed best by

grazing sections through the *SR*. In vertebrate muscle, the *SR* is not so well developed, and the fenestrated envelope is limited to the A band region. Furthermore, in vertebrate muscle the longitudinal connections of the *SR* in a direction parallel to the myofibrils are relatively rare. For the most part, the *SR* of vertebrate muscle is discontinuous longitudinally and consists of isolated segments confined to adjacent sarcomeres and portions of adjacent sarcomeres. In vertebrates, therefore, the T system traverses the fiber in direct contact with the myofibrils and makes triadic contacts with two terminal cisternae of the discontinuous *SR* in a plane at right angles to the muscle fiber. In this insect, however, the T tubules pass external to the more or less continuous layer of *SR* and form dyadic junctions in a plane parallel to the long axis of the myofibrils. The formation of triads in vertebrates may be the result of the longitudinal discontinuities of the *SR* where the T system forms complexes with two lateral cisternae. Other investigators have demonstrated fenestrations in the *SR* envelope which are limited to the A band region (2, 5, 17, 24). In contrast to these previous observations dealing with the crayfish, dragonfly, and frog, the present study indicates that the fenestrations are not limited to the A band but are present over the entire sarcomere in the femoral muscle of the cockroach. The elucidation of this complex structure in three dimensions requires numerous planes of sections since serial sections are generally too thick for this purpose.

FIGURE 2 An extensive view of a surface section of a longitudinally oriented femoral muscle fiber. It depicts the fenestrated sarcoplasmic reticulum (*SR*) and the unfenestrated sarcoplasmic reticulum (*S*) which associates with the transverse system. The fenestrations in the *SR* have the same electron density as the general sarcoplasm. Longitudinal continuity of the *SR* across the Z line is evident. *F*, fenestrations; *M*, mitochondrion; *S*, unfenestrated sarcoplasmic reticulum; *SR*, fenestrated sarcoplasmic reticulum; *Z*, Z line. $\times 33,000$.

FIGURE 3 A portion of a grazing section of the sarcoplasmic reticulum (*SR*). The position of the unfenestrated sarcoplasmic reticulum (*S*) is at the lateral ends of the A band. The end of the A band is seen in the stretched myofibril to the left of the elongated mitochondrion (*M*) at the A band and I band junction (*AI*). Longitudinal continuity of the *SR* is again seen. The unfenestrated portion of the *SR* is continuous with the fenestrated portions which overlie the A band and I band, respectively. $\times 33,000$.

FIGURE 4 A high power view of the surface of the fenestrated sarcoplasmic reticulum. The fenestrations are spaced irregularly and about 300 Å in diameter. $\times 160,000$.



The femoral muscle of the cockroach, unlike vertebrate skeletal muscle, is characterized by longer myofilaments and sarcomeres as well as by more numerous (10–12) thin myofilaments surrounding each thick myofilament. Moreover, the thick myofilaments in this insect muscle are greater in diameter (180–200 μ) than those of vertebrate muscle. These structural features imply more numerous reactive sites between actin and myosin in femoral cockroach muscle. This probably necessitates an extensively developed *SR* which forms a more or less complete investment of the sarcomere in order to ensure adequate Ca binding and relaxation commensurate with the speed of the contraction. The fenestrations in this otherwise continuous layer probably permit diffusion of ATP and possibly other essential substances from the sarcoplasm into the myofibrils.

Other investigators also have described an increase in the electron opacity of both the *SR* contents and the apposing *SR* membrane where this system forms a junction with the T system (1, 11, 12, 17, 18, 19, 24, 29). Two other structural specializations in this region of the *SR* have been observed. The *SR* in the regions of dyadic junctions, unlike that of remaining *SR*, lacks fenestrations. Moreover, regularly spaced thickenings of the dyadic membrane of the *SR* are noted here

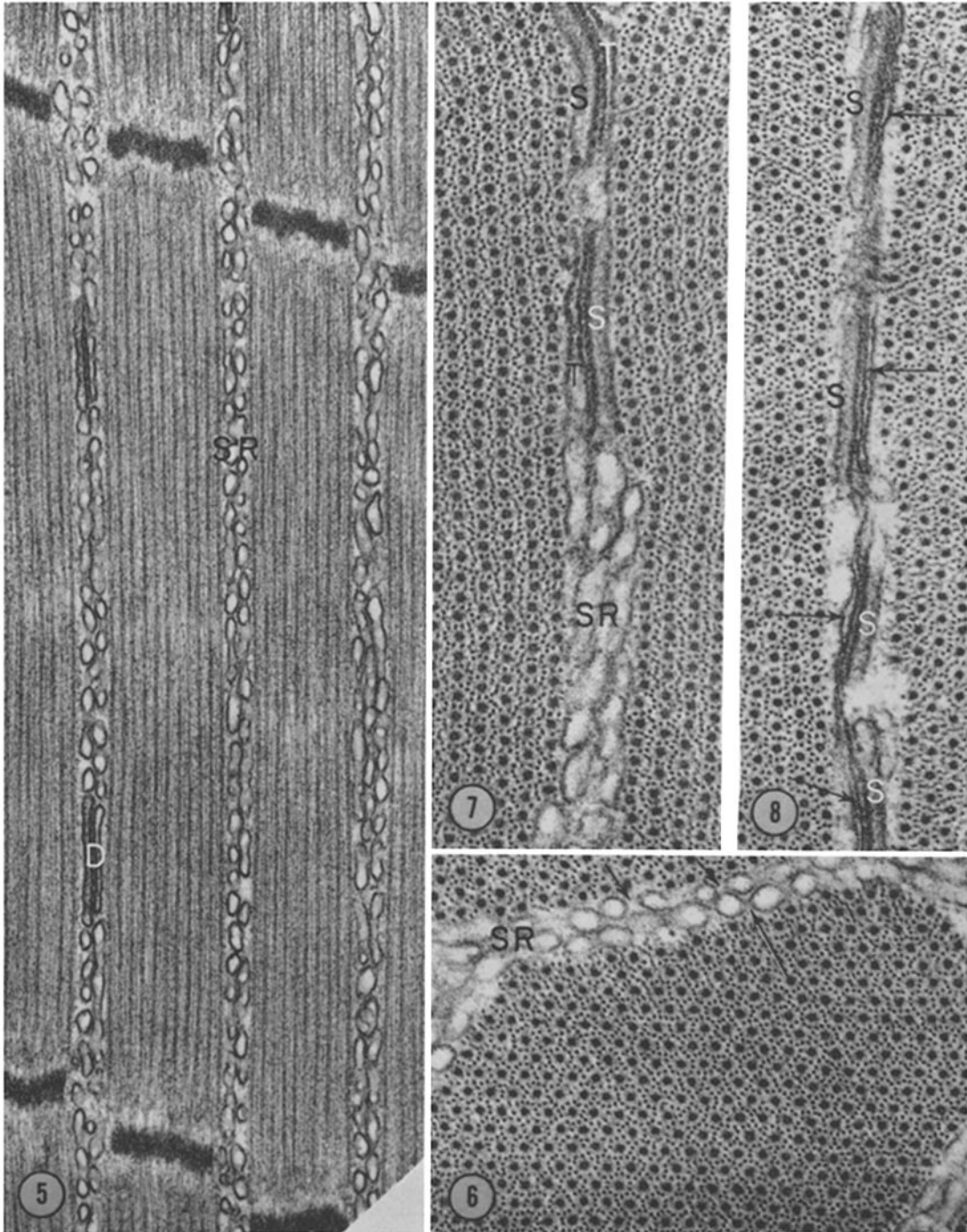
although no structural continuity between the unit membrane of the *SR* and unit membrane of the T system can be demonstrated. The role of these focal area thickenings of the membrane, in terms of functional interaction between *SR* and T system, is at present not clear. Fahrenbach (3) recently speculated, after a study of triads in muscle from humans, copepods, ostracods, and barnacles, that the triads show a five-layered construction similar to that of a tight junction (4). He further suggested that this structural organization at the triads facilitated the coupling of membrane depolarization and calcium release. Hoyle (11) described a row of regularly spaced granules connecting the two elements (T system and *SR*) in the dyads of invertebrate muscle. Peachey (17), on the other hand, suggested that the fine structure of triadic junctions in frog muscle was similar to that of the septate junctions formed between some epithelial cells (16, 31, 32). This study of the femoral muscle dyad is at variance with that of the preceding investigators. The cockroach femoral muscle dyad is neither a tight junction nor a septate junction, and no continuous structures linking the T system and *SR* can be demonstrated in well-resolved micrographs, as stated above. Hoyle (11) examined the corresponding junctions in striated muscles of some insects, crabs, crayfish, lobster, and barnacle and

FIGURE 5 For the most part two layers of *SR* are seen between adjacent longitudinally oriented myofibrils. *D*, dyad; *SR*, sarcoplasmic reticulum. $\times 38,000$.

FIGURE 6 Transverse section to show the disposition of *SR* in this plane. The arrowheads are directed between vesicles in order to indicate the pores of the fenestrated envelope. The upper layer of vesicles (two arrows) is the *SR* of the upper myofibril, while the layer of lower vesicles (one arrow) belongs to the *SR* of the lower myofibril. $\times 54,000$.

FIGURE 7 A micrograph of a cross-section of the muscle fiber showing how a single transverse system tubule (*T*) associated first with the *SR* of one myofibril and subsequently with the *SR* of an adjacent myofibril to form dyads. At the top of the picture the transverse element (*T*) of the dyadic contact alternates to make a similar contact with the unfenestrated sarcoplasmic reticulum (*S*) of the neighboring myofibril. The sarcoplasmic reticulum component of a dyad is always unfenestrated and always contains an amorphous substance, while the transverse system (*T*) has none. Lateral continuity between *SR* elements is seen. *S*, unfenestrated sarcoplasmic reticulum; *SR*, fenestrated sarcoplasmic reticulum; and *T*, transverse, system. $\times 50,000$.

FIGURE 8 Another end-on view of the myofilaments, showing a more extensive area of the transverse system (arrows) alternating with the unfenestrated sarcoplasmic reticulum (*S*) of one myofibril to the unfenestrated sarcoplasmic reticulum (*S*) of the adjacent myofibril to form dyads. $\times 56,000$.



observed that the majority of the invertebrate contacts are dyadic; that is, the transverse element (which he calls the E element for excitation) is shared with a single cisternal element of the SR. The characteristic relationship between the T system and the SR in the femoral muscle of the cockroach hitherto has not been observed. In this muscle, a single T element forms a dyadic junction with the SR of one myofibril, and then at a different level forms another junction with the adjacent myofibril. This alternation of T system associated with the SR of adjacent myofibrils has not been reported previously in either invertebrate or vertebrate muscle. The alternation of dyadic junctions may be related to the rather extensive and continuous SR surrounding individual myofibrils in this muscle. The SR in vertebrates, on the other hand, is discontinuous, not so abundant, and appears to be shared by adjacent myofibrils to a greater extent so that the T tubule can contact the SR of both myofibrils simultaneously in these muscles. It is interesting that typical dyadic junctions also are formed between surface SR and cell surface membrane, in view of the extensive SR in the femoral muscle.

Previous investigators generally have found such surface dyads only in muscles where the SR is relatively sparse (6, 9, 10, 14, 21). Rosenbluth (21) resolved the unit membrane structure as well as tight junctions at the dyadic contacts of SR and sarcolemma in *Ascaris*. The dense material subjacent to the sarcolemma and adjacent to the longitudinal branches of the T system borders at the Z line levels merits comment. Such attachments could maintain the structural relationships between sarcotubules and myofibrils. These dense zones contiguous with the membrane of the T system are reminiscent of desmosomes. This is of interest in view of recent work (28) which suggests that Z lines may be derived from desmosomes either at the surface of the muscle fiber or adjacent to the primitive T system in developing chick heart muscle.

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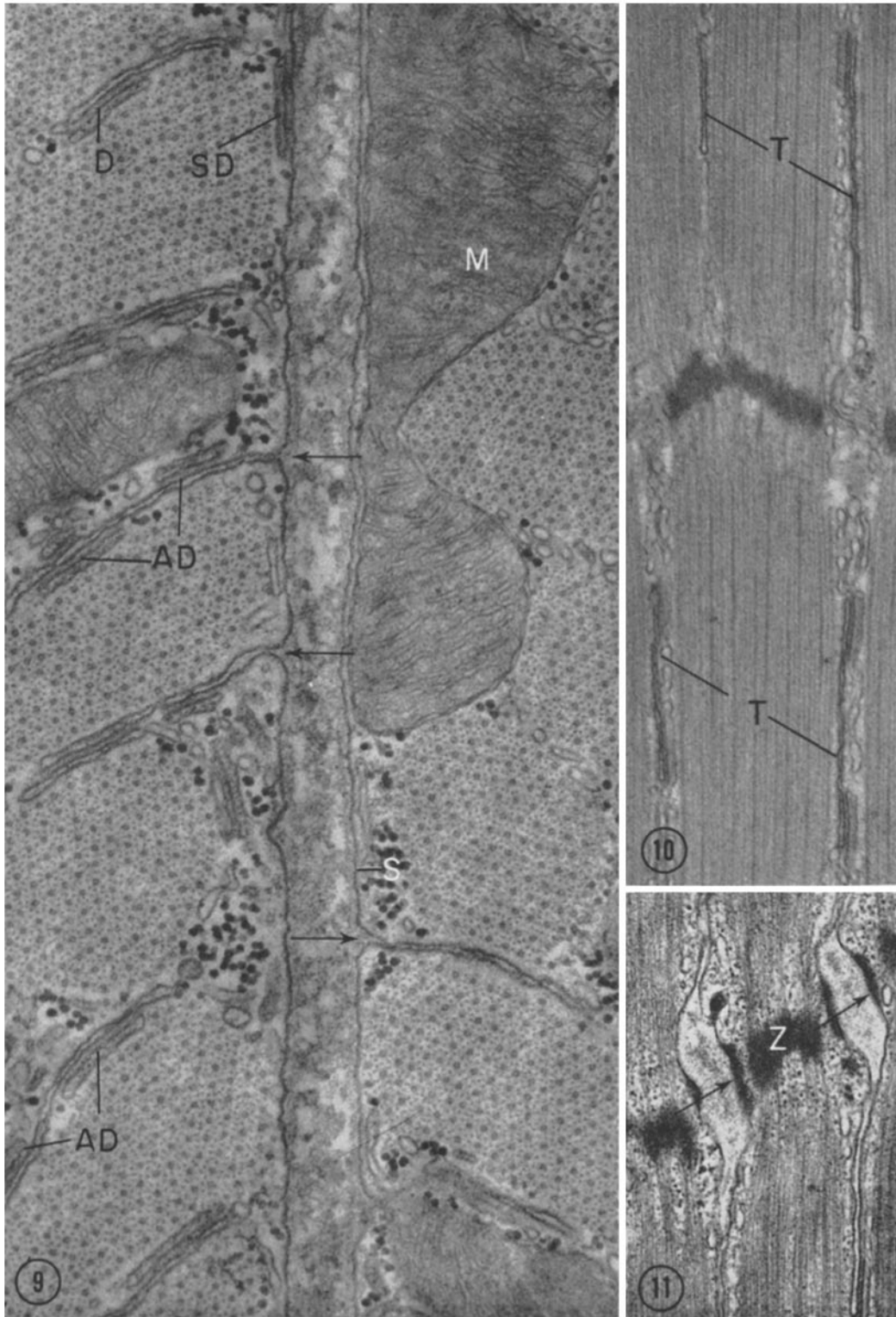
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FIGURE 9 A transverse section of two adjoining muscle fibers, revealing the invaginations of their sarcolemmae (arrows) to form the transverse elements. The transverse system originates, at regular intervals, from the sarcolemma at the lateral margins of the A bands. Also note the alternating dyads (*AD*) and a sarcolemmal dyad (*SD*). *AD*, alternating dyad; *D*, dyad; *M*, mitochondrion; *S*, sarcolemma; *SD*, sarcolemmal dyad. $\times 65,000$.

FIGURE 10 A portion of a longitudinal section of the femoral muscle fiber, depicting some longitudinal branches (*T*) which are derived from the transverse system. The T system elements are readily differentiated from SR elements by the greater density of their membranes. $\times 36,000$.

FIGURE 11 A portion of two tubular branches stemming from the transverse system crossing the Z line. Note the increase in thickness of their membranes and the dilation of the tubules in the region of the Z line (arrows). These thickened areas are perhaps attachment points between the branch of the T system and the Z line. $\times 45,000$.



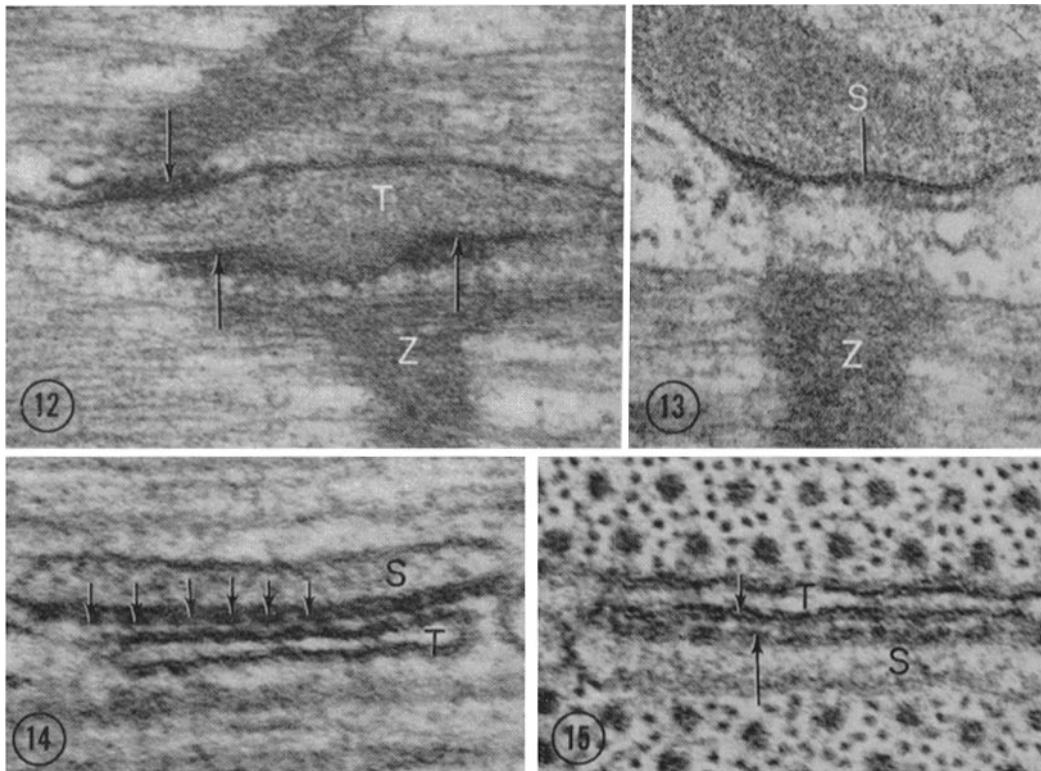


FIGURE 12 A high power micrograph of a branch of the transverse system in the intermyofibrillar space bordering the Z line. The three arrows indicate the unit membrane structure of the branch (T). Z line-like material is seen adjacent to these branches. $\times 108,000$.

FIGURE 13 A high power micrograph showing the Z line-like material adjacent to the sarcolemma (S) in the region of the Z line (Z). The sarcolemma demonstrates a unit membrane structure. $\times 102,000$.

FIGURE 14 A high power micrograph of a longitudinally oriented muscle fiber in the region of a dyad. It illustrates quite clearly the regularly spaced thickenings (arrows) of the unfenestrated sarcoplasmic reticulum (S) membrane apposing the dyadic space. These areas of membrane thickenings, however, do not contact the T element (T) of the dyad. $\times 192,000$.

FIGURE 15 A high power micrograph of a transverse section of the muscle fiber in the region of a dyad. The unit membrane is resolvable in the transverse element's membrane (short arrow) apposing the dyadic space and in the unfenestrated sarcoplasmic reticulum membrane (long arrow). The dyadic space in both longitudinal and transverse sections is about 100 A. Also note that the circles of actin filaments (small dots) around the myosin filaments (large dots) are incomplete, adjacent to both the transverse system (T) and the unfenestrated sarcoplasmic reticulum (S). $\times 190,000$.

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