CONTINUITY OF THE T SYSTEM WITH THE SARCOLEMMA IN RAT SKELETAL MUSCLE FIBERS

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INTRODUCTION

Interfibrillar spaces of skeletal muscle fibers contain two membranous systems, the T system and the sarcoplasmic reticulum. Most of the T system is oriented transversely between the fibrils. Occasionally longitudinal connections between the elements of the transversely oriented T system are seen (1). Electron microscope studies of fish skeletal muscle fibers have shown that the membranes of the T system are continuous with the sarcolemma at the Z band level, and that the tubular content of this system is continuous with the extracellular fluid (2–4). Similar continuities have also been reported for tadpole skeletal muscle fibers (4). Frog sartorius muscle fibers exposed, in the living state, to ferritin and subsequently examined with the electron microscope show ferritin molecules in the tubules of the T system, but do not show ferritin in any other part of the muscle fiber (5). These findings (5) indicate that the tubular content of the T system is continuous with the extracellular fluid in the adult frog. In reptiles, birds, and mammals the T system is located near the junction of the A and I bands. The sarcoplasmic reticulum is, in general, oriented longitudinally between the fibrils. However, the terminal segments of the sarcoplasmic reticulum face the T system and show transverse orientation at the level of the triads. Two terminal segments of sarcoplasmic reticulum and the tubular element of the T system between these segments have been called a triad (6). In numerous electron microscope studies on internal membranes and membrane-like structures of rat skeletal muscle fibers, we have occasionally observed connections of the T system with the sarcolemma. Although the majority of electron microscope studies have been made on fibers fixed in OsO_4, continuity between the tubular content of the T system and the extracellular fluid has never been seen when fixation with OsO_4 alone is employed. Such continuity has been seen in fibers fixed in glutaraldehyde and postfixed in OsO_4.

METHODS

Small bundles of fibers were removed from gastrocnemius muscle of rats anesthetized with Nembutal. The bundles of fibers were tied to Plexiglas stays and fixed in glutaraldehyde by the method of Sabatini *et al.* (7), slightly modified. The tissues were postfixed in OsO_4 by the Palade method as modified by Caulfield (8). The fibers were dehydrated for 10 minutes in 50 per cent alcohol and then placed overnight in 70 parts absolute alcohol and 30 parts saturated aqueous solution of uranyl acetate. After completion of dehydration with 95 and 100 per cent alcohol the fibers were embedded in Maraglas and sectioned with a Porter-Blum microtome. The sections were stained with $Pb(OH)_2$ solution according to the Lever method (9). Sections exhibiting gray interference colors and near 500 A in thickness were examined with a Siemens Elmiskop I.

RESULTS

The T system is readily identified in longitudinal and near-longitudinal sections of rat skeletal muscle fibers. Aside from its own characteristics, its close structural relationship with terminal segments of the sarcoplasmic reticulum makes identification virtually certain. Cross-sections of the T

system tubules usually show an elliptical shape in rat gastrocnemius muscle. The long axis of the ellipse is 800 to 1200 A and the short axis is 200 to 500 A. If it is assumed that the size and shape of the opening of the T system in the sarcolemma are similar to the size and shape of the T system tubule in cross-section, it should be possible to make 500-A thin sections whose thickness would fall within the long axis of the opening and exclude the boundary walls on either side of the opening. Sections, under these conditions, would show distinct continuity of the T system with the sarcolemma.

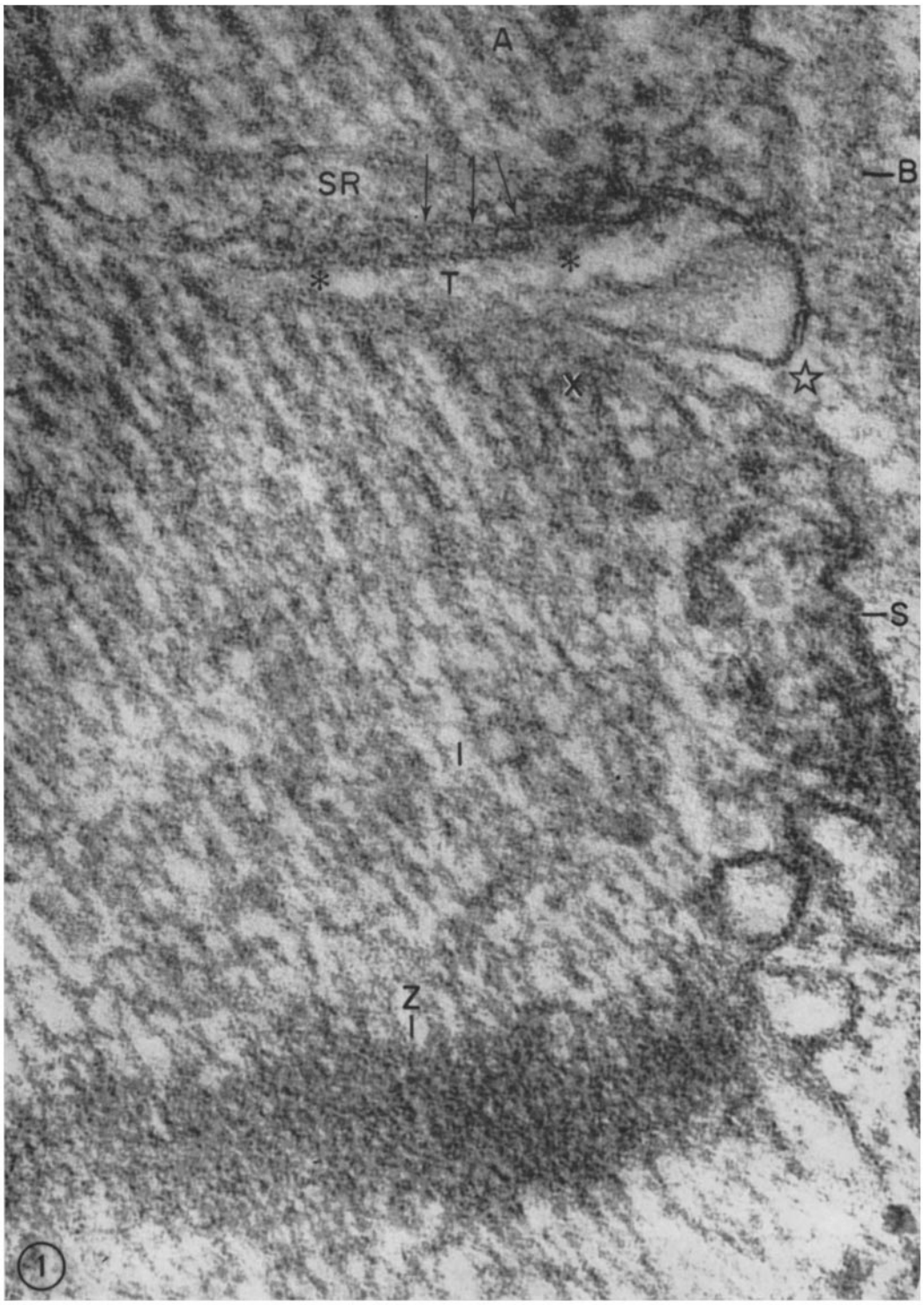
In fibers prepared by the methods described here, the tubules of the T system are somewhat convoluted and expanded at the subsarcolemmal level. Convolution and expansion of the tubules at this level are seen frequently in sections that do not show a continuity of the T system with the sarcolemma. In some cases a distinct invagination of the sarcolemma is observed at the level of the junction of the A and I bands, only to be obscured without showing continuity in the T system tubules passing toward the interior of the fiber. One of these cases is illustrated in Fig. 1. The invagination of the sarcolemma (star) is clearly shown in Fig. 1, and the width of the opening in the sarcolemma is about 175 A. Continuity of the invagination of the sarcolemma with the T system is not shown. This failure to demonstrate continuity is perhaps due to inclusion of the tubular wall in the section at the point indicated by *X*. Sparsity of electron-opaque material typical of ideal sections of the T system (T) is seen to the right of each asterisk. The section shown in Fig. 1 is about 15° oblique to the longitudinal axis of the fiber and does not show all of the components of the triad. A part of a terminal segment of sarcoplasmic reticulum (SR) is shown on the upper side of the T system. The apposed membranes of T and SR are about 100 A apart at points where both of the membranes are clearly defined. Dense zones of electron-opaque material (arrows) alternate with less dense zones at a few points in the space between the apposed membranes of T and SR.

Fig. 2 shows an invagination of the sarcolemma that appears to be continuous with the membranes that form the walls of the T system. The point above the *X* in Fig. 2 shows an electron opacity, traversing the T system tubule, which is apparently due to inclusion of a tangential section of the T system membrane at the level of a convolution in the tubule. Some points (asterisks) within the T system show very little electron-opaque material. The opening in the sarcolemma (star) which connects the extracellular fluid with the content of the T system is quite distinct although it is narrow. Through-focus studies on the section illustrated in Fig. 2 showed a distinct opening in the sarcolemma at all levels of focus observed. The plane of the section is about 15° oblique to the longitudinal axis of the fiber and it passes through only one terminal segment of the sarcoplasmic reticulum which can be seen at the upper side of the T system. The distance between the apposed membranes of T and SR is slightly less than 100 A at some points in Fig. 2. Dense zones (arrows) alternating with less dense zones of electron opacity can be seen by close inspection in the space between the apposed membranes of T and SR.

DISCUSSION

It follows from the observation of continuity between the T system and the sarcolemma in skeletal muscle fibers of fish (2–4) and the tadpole (4) that similar continuity of these structures in other vertebrates is the expected finding. In fact, Revel (1) has demonstrated close contact of the T system and the sarcolemma in a unique preparation of bat cricothyroid muscle fibers. Fibers fixed with OsO_4 in the presence of 0.01 M $CaCl_2$ and with no subsequent staining showed rather marked electron opacity in the T system and the sar-

FIGURE 1 Electron micrograph of rat gastrocnemius muscle fiber showing a section passing about 15° oblique to the longitudinal axis of the fiber. The star indicates an invagination of the sarcolemma (*S*) near the level of the T system (*T*). Presumably connection of the invaginated sarcolemma with *T* is obscured at a point above *X* by convolution of the T system. Dense zones in the space between apposed membranes of *T* and the terminal segment of sarcoplasmic reticulum (*SR*) are indicated by arrows. *A*, A band; *I*, I band, *Z*, Z band; *B*, basement membrane. × 230,000.


A
SR
B
T
S
I
Z
1

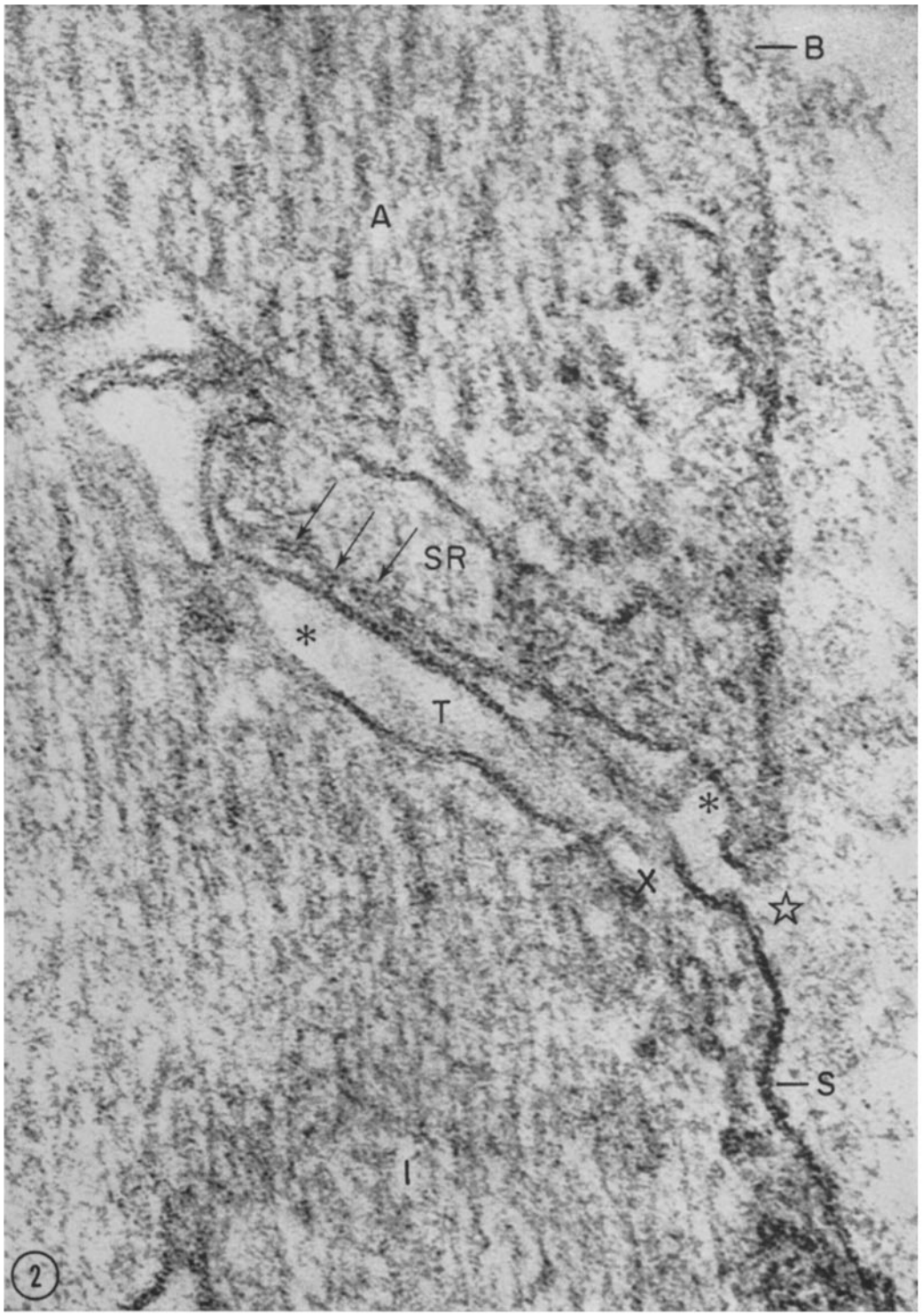
colemma, in sharp contrast with other structures of the fibers which showed virtually no staining at all. Since the terminal segments of the sarcoplasmic reticulum did not show electron opacity, it was possible for Revel (1) to conclude that the structure making contact with the sarcolemma at the level of the A–I junction is indeed the T system.

In electron microscope studies on rat gastrocnemius muscle fixed in OsO_4, contact between the T system and the sarcolemma has been seen several times. The use of glutaraldehyde as a fixative was suggested by the effectiveness of this fixative in studies (2–4) which revealed continuities between the T system and the sarcolemma in fish muscle fibers. The observations recorded here (star in Fig. 2) demonstrate the superiority of glutaraldehyde over OsO_4 as a fixative for study of structural relationships between the T system and sarcolemma in mammalian skeletal muscle fibers. The findings should be of general interest because the T system in reptiles, birds, and mammals is situated near the A-I junction in contradistinction to the T system located at the Z band in fish and amphibia.

The demonstrations of openings of the T system in the sarcolemma by electron microscope studies (2–5) on fish and frog muscle fibers, and the present study on mammalian muscle fibers, indicate that the T system might be the triadic structure that transmits excitatory effects from the surface to the interior of the fiber. The high sensitivity of the contractile mechanisms of the fibrils to local application of Ca^{++} (10) has increased interest in the possible relationship of excitatory effects to Ca^{++} release. Investigations on muscle homogenates treated with the relaxing factor have shown that the microsomal fraction (11–14) and, more specifically, the sarcotubular fraction (15) store Ca^{++}. Many investigations have suggested a relation of excitation to Ca^{++} release, and at least two (5, 10) have proposed that Ca^{++} is released from the sarcoplasmic reticulum when the stimulus for contraction is conducted to the fiber interior. Observations (16, 17) that frog skeletal muscle fibers treated with oxalate show precipitation of Ca^{++} mainly in the terminal segments of the sarcoplasmic reticulum (*SR* in Figs. 1 and 2) led to the simultaneous suggestions that Ca^{++} might be released specifically from *SR* as a result of membrane depolarization (18) or electrotonic spread of current (19, 20) in SR. It has been suggested (21) that continuity of the fluid contents of the T system with the extracellular fluid of the muscle fiber should be interpreted as evidence that Na^+ concentrations in these fluid compartments are equal and high. With this interpretation in mind it was further suggested (21) that a high Na^+ gradient across the membranous walls of the T system might exist, and that the presence of this gradient might facilitate conduction of self-propagating impulses along the tubules of the T system. Although a possible relationship between a Na^+ gradient and self-propagation in the T system is of interest, it should be pointed out that absence of all-or-none behavior during local stimulation of the fiber surface provides argument against self-propagation and has led to the suggestion that a passive electrical conduction along the T system tubules may be the mechanism of inward conduction (22).

The observations recorded here indicate that there is a space about 100 A wide between the apposed membranes of T and SR. Dense zones of electron opacity across this space (arrows in Figs. 1, 2, and 3) suggest the presence of connections between the apposed membranes of T and SR. Apparently the dense zones are comparable to the lines of density between apposed membranes of T and SR in bat cricothyroid muscle (1). It seems likely that the scalloped or beaded appearance observed in frog muscle fibers (5) at the junctional

FIGURE 2 Electron micrograph of a section passing about 15° oblique to the longitudinal axis of a fiber. The star indicates an invagination of the sarcolemma (*S*) which appears to be continuous with the membranes of the T system (*T*). Apparently continuity between the tubular contents of the T system and the extracellular space is partially obscured by a convolution at a point above *X*. Dense zones in the space between apposed membranes of *T* and the terminal segment of the sarcoplasmic reticulum (*SR*) are indicated by arrows. *A*, A band; *I*, I band; *B*, basement membrane. × 230,000.


B
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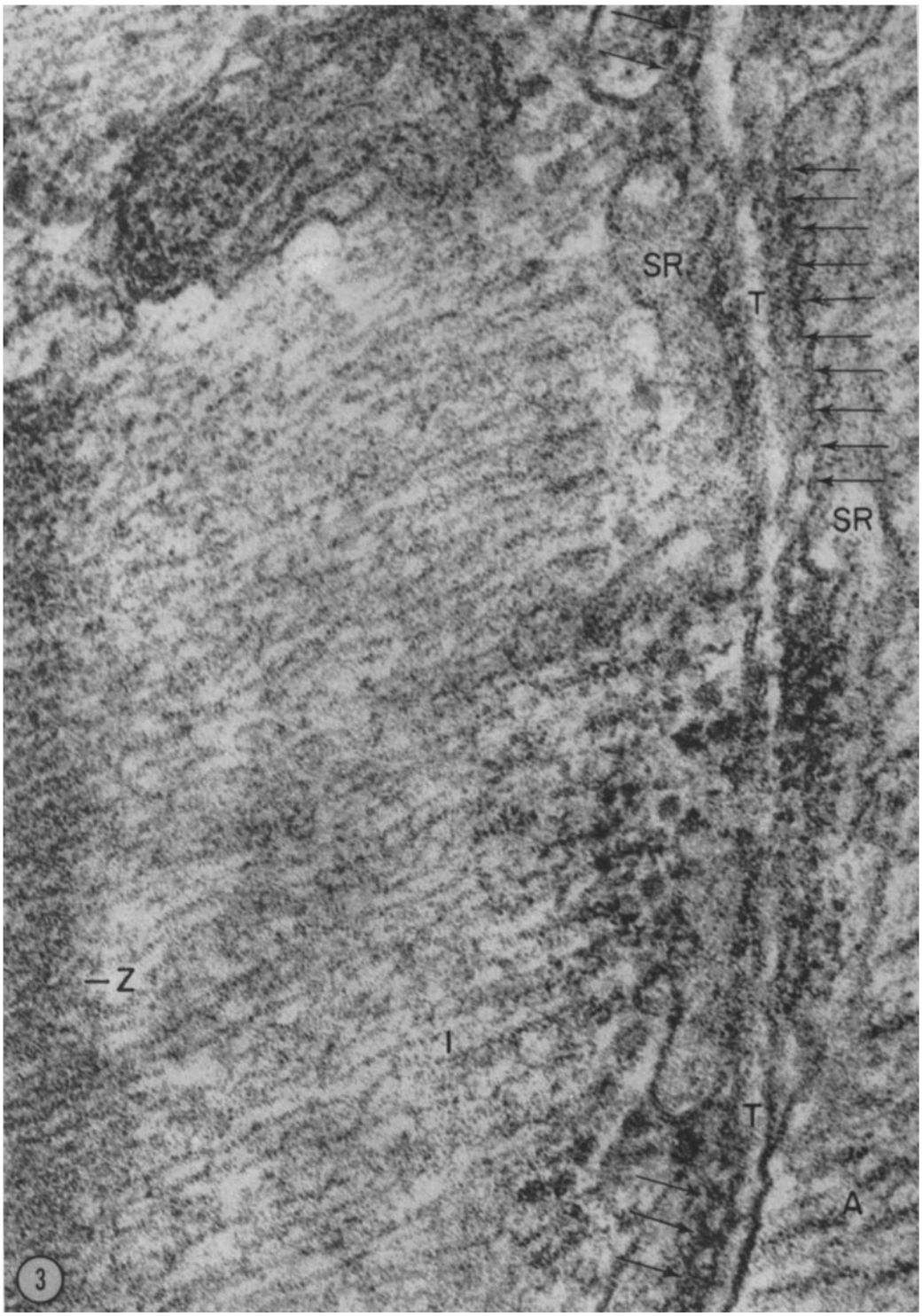

FIGURE 3 Electron micrograph of a section passing slightly oblique to the longitudinal axis of a fiber. Dense zones in the space between apposed membranes of the T system (*T*) and of the terminal segments of the sarcoplasmic reticulum (*SR*) are indicated by arrows. *A*, A band; *I*, I band; *Z*, Z band. × 215,000.

region between T and SR might be due to connections between the apposed membranes of T and SR. In electron microscope studies (3) on tail muscle fibers of the tadpole, the membranes of SR facing the T system are scalloped very regularly, giving the impression that there are small bridge-like structures joining the T system and SR membranes. In his report of a five-layered structure at the site of apposed membranes of T and SR in various striated muscles, Fahrenbach (18) suggested that a tight junction may be formed by these apposed membranes. The electron micrographs shown in the present study indicate that apposed membranes of T and SR do not fuse to form anatomical tight junctions like those reported for adjoining plasma membranes of smooth muscle cells (23), epithelial cells (24), and other cells (25). It is suggested that intermembranous connections (arrows in Figs. 1, 2, and 3), rather than anatomical tight junctions, form an integral part of apposition of T and SR membranes.

We thank Mr. C. D. Foreman for technical assistance. This work was aided by NSF Grant GB-2009 and NIH Grant NB 05444-01.

Received for publication, July 1, 1965.

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