STUDIES OF THE TRIAD

IV. Structure of the Junction in Frog Slow Fibers

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ABSTRACT

The structure of the triadic junction in frog slow fibers has been studied and compared with that of twitch fibers. The junctional gap is wider (by approximately 13%) in slow fibers. The junctional feet have the same size and disposition as in twitch fibers, although the size and shape of the junctional areas are different. It is concluded that the role of triads in slow fibers is the same as in twitch fibers

INTRODUCTION

In frog slow fibers the development and maintenance of tension after depolarization in high potassium strongly depends on the concentration of calcium in the outside solution (Lüttgau, 1963 a). In this respect, slow fibers apparently differ from twitch fibers, where the calcium necessary for activation of the contractile material is all liberated from an internal store, the sarcoplasmic reticulum, or SR (see Lüttgau, 1963 b, and Sandow, 1965, for reviews).

On the other hand, it has been shown that slow fibers in the frog (Page, 1965), as well as in other vertebrates (Peachey, 1965; Hoyle et al, 1966, Hess, 1967; Page, 1969), possess structures equivalent to those thought to form the intermediary links between excitation and contraction in twitch fibers. Thus, slow fibers are pervaded by tubules, whose walls are continuous with the plasma membrane and which probably carry the surface depolarization to the interior of the fiber, similarly to the transverse (T) system tubules of twitch fibers (Page, 1965). The SR is present in frog slow fibers (Peachey and Huxley, 1962) and is fairly well developed (Page, 1965). Similarly to that of twitch fibers (Costantin et al., 1965), the SR of slow fibers contains a fairly high concentration of calcium, which can be visualized in the electron microscope after precipitation with oxalate (Costantin et al , 1967, Page, 1969). It is reasonably speculated that at least some of the calcium for excitation of slow fibers is released from this internal store (Page, 1969).

In twitch fibers it is thought that the signal to release calcium is transmitted from the T system to the SR at the triads, where specialized areas of junction exist between the two systems of membranes. In slow fibers, close proximity between SR and T system elements occurs at triads (and dyads) which differ in number and location from those of twitch fibers (Page, 1965) This paper shows that the fine structural details of the triadic junctions of slow fibers are not basically different from those of twitch fibers, thus providing further evidence that morphologically, at least, slow fibers have an excitation-contraction (e-c) coupling pathway analogous to that of twitch fibers.

MATERIALS AND METHODS

Lumbricalis muscles from the big toe of *Rana pipiens* hind legs were dissected and fixed either in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 (Figs. 1, 3–6, 11, 12), or in the same fixative with the addi-

tion of peroxide (Peracchia and Mittler, 1972) for 2 h at room temperature After a brief wash in buffer, small bundles of fibers were postfixed in 2% OsO₄ in the same buffer for 2 h at room temperature. Dehydration was in ethanol and propylene oxide and embedding was in Epon. Sections were stained in alcoholic uranyl acetate, followed by lead (Sato, 1968). Sections were cut on an A. F. Huxley pattern Cambridge microtome and examined in Siemens Elmiskop 101 and AEI 801 microscope. Where accurate measurements were required, the magnification of the AEI microscope was standardized by eliminating hysteresis of the lenses, as recommended by the manufacturer. This supposedly eliminates variations in magnification to within ±2%.

RESULTS

The majority of fibers in the lumbricalis muscle used are twitch type, with distinct fibrils (Fibrillenstruktur), a narrow Z line, and an evident M line. Slow fibers are few and they can be identified on the basis of their structure: large confluent fibrils (Felderstruktur), a wide Z line, lacking a regular structure, and lack of M line (Figs. 1 and 2) These are characteristics which have been described in slow fibers of other frog muscles (Peachey and Huxley, 1962; Page, 1965; Lännergren and Smith, 1966, Hess, 1967, Flitney, 1971)

The data in this paper were obtained from the examination of only two slow fibers. I consider such a limited sample sufficient to provide a reliable description of the triad in slow fibers, since adjacent twitch fibers in the same blocks, as well as others in many muscles fixed by the two techniques described in Materials and Methods, provide ample evidence that morphology and parameters of the triads are consistently uniform when fixed in glutaraldehyde The general appearance of SR and triads in the slow fibers described here is also very similar to that previously found in other frog slow fibers (Page, 1965, Flitney, 1971)

The sarcoplasmic reticulum (Fig. 1) is a loose network of intercommunicating tubules, running mostly longitudinally but also obliquely and transversely, without a precise pattern relative to the bands of the sarcomere and without interruptions at the Z line level (Page, 1965) Occasionally, mostly at the Z line, the SR forms a wider cisterna, which has a dense content analogous to that found in the lateral sacs of the triad of twitch fibers These enlarged SR sacs are usually interposed between the fibrils and a T system tubule, with which they form either triadic or dyadic junctions

(Fig. 1, arrow, Fig. 2). Dvads and triads are small and irregularly scattered between the fibrils.

Within the triads, T system elements are flat sacs, as indicated by the fact that their appearance is very similar in longitudinal and cross-sections of the fiber (Figs 1 and 2). Elsewhere, the T tubules are small cylinders one of these is transversely cut as it leaves the plane of section in Fig. 1 (double arrow). In most cases, due to similarity in size and general direction, the T tubules are not distinguishable from SR elements unless filled by an electron-opaque extracellular space tracer (Page, 1965, 1969) In the majority of triads, the T system runs approximately longitudinally, i.e., either parallel to or oblique relative to the fiber long axis (Figs 3–6) and the SR is interposed between T system and fibrils. In previous studies of slow fibers, triads were illustrated in which the axis of the T system was transversely oriented (Page, 1965, Flitney, 1971). Such triads are easily identifiable in the section because the SR lies under and above, rather than laterally to, the T system No transversely oriented triads have been noticed in this study, probably due to the limited number of observations. In all triads SR and T system are separated by a small junctional gap, which is crossed by evenly spaced densities, the SR feet.

In a previous description of the triad in frog twitch fibers (Franzini-Armstrong, 1970), three views were defined views a and b are obtained in longitudinal sections, when the plane of sectioning is either parallel or perpendicular to the T system long axis, respectively View c is obtained in cross-sections, when the section plane is parallel to and coincides with the junctional gap In those slow fiber triads where the axis of T system and triads are longitudinal rather than transverse, views a and c are obtained in longitudinal sections, whereas view b occurs in cross-sections of the fiber

In view a (Fig. 1, arrow; Figs. 3–6) the section cuts across T system and SR membranes and the intervening junctional gap. This view allows determination of the spacing between the feet—in a direction parallel to the T system long axis—and of the gap's width. The SR is slightly scalloped at regular intervals and in correspondence of each scallop a foot crosses the junctional gap. The center-to-center distance between the feet is 270 ± 15 Å (n = 22). In these preparations the SR scalloping is very shallow and the width of the junctional gap is not appreciably reduced by its occurrence: the gap is 140 ± 10 Å (n = 38) at its
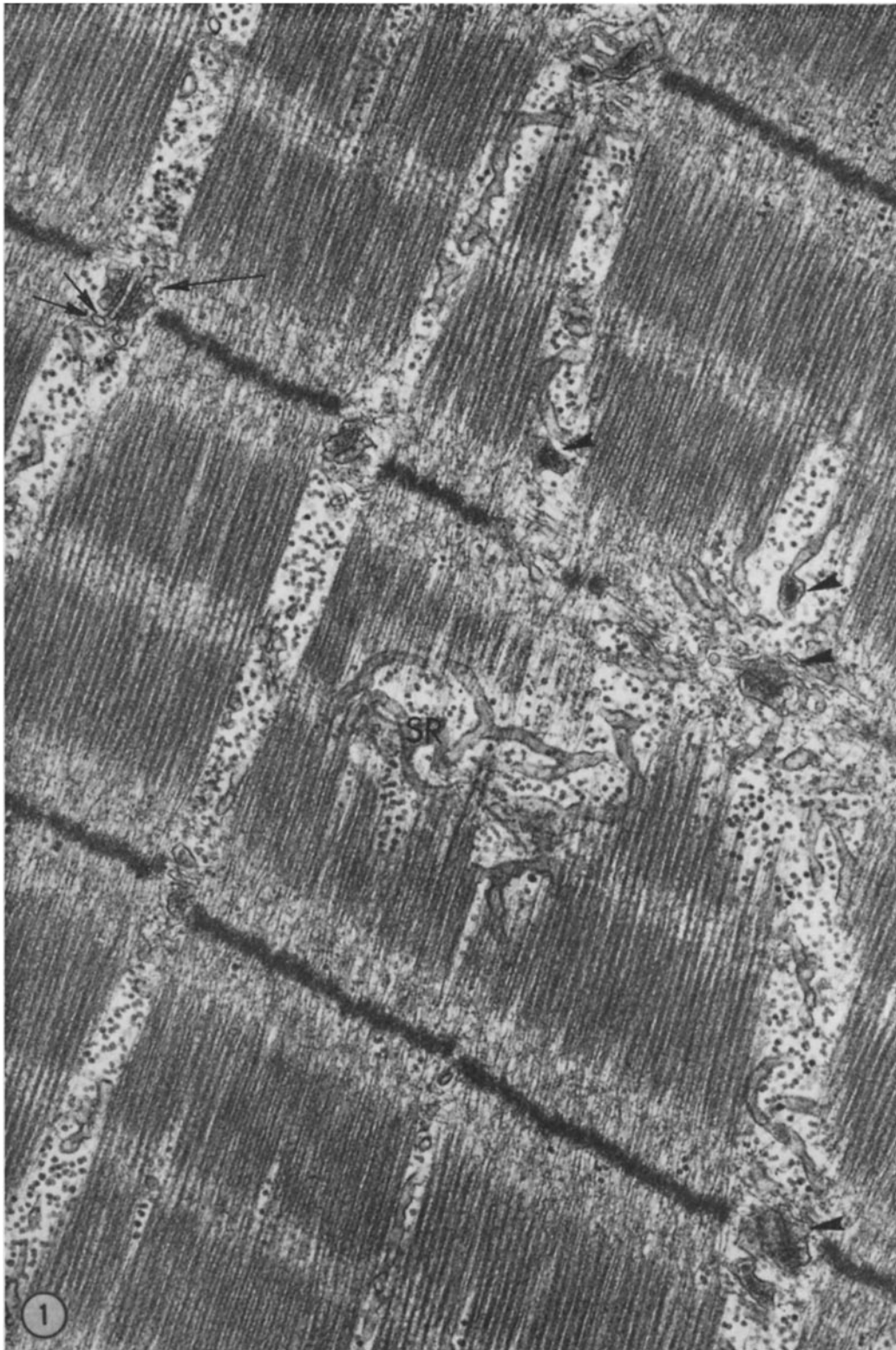
FIGURE 1   Longitudinal section through a slow fiber. Notice wide Z lines and lack of M lines. The sarcoplasmic reticulum (SR) forms a loose network. Many triads are present near Z lines. One (arrow) is cut crosswise, the others (arrowheads) tangentially. Double arrow indicates a T system profile × 46,000.
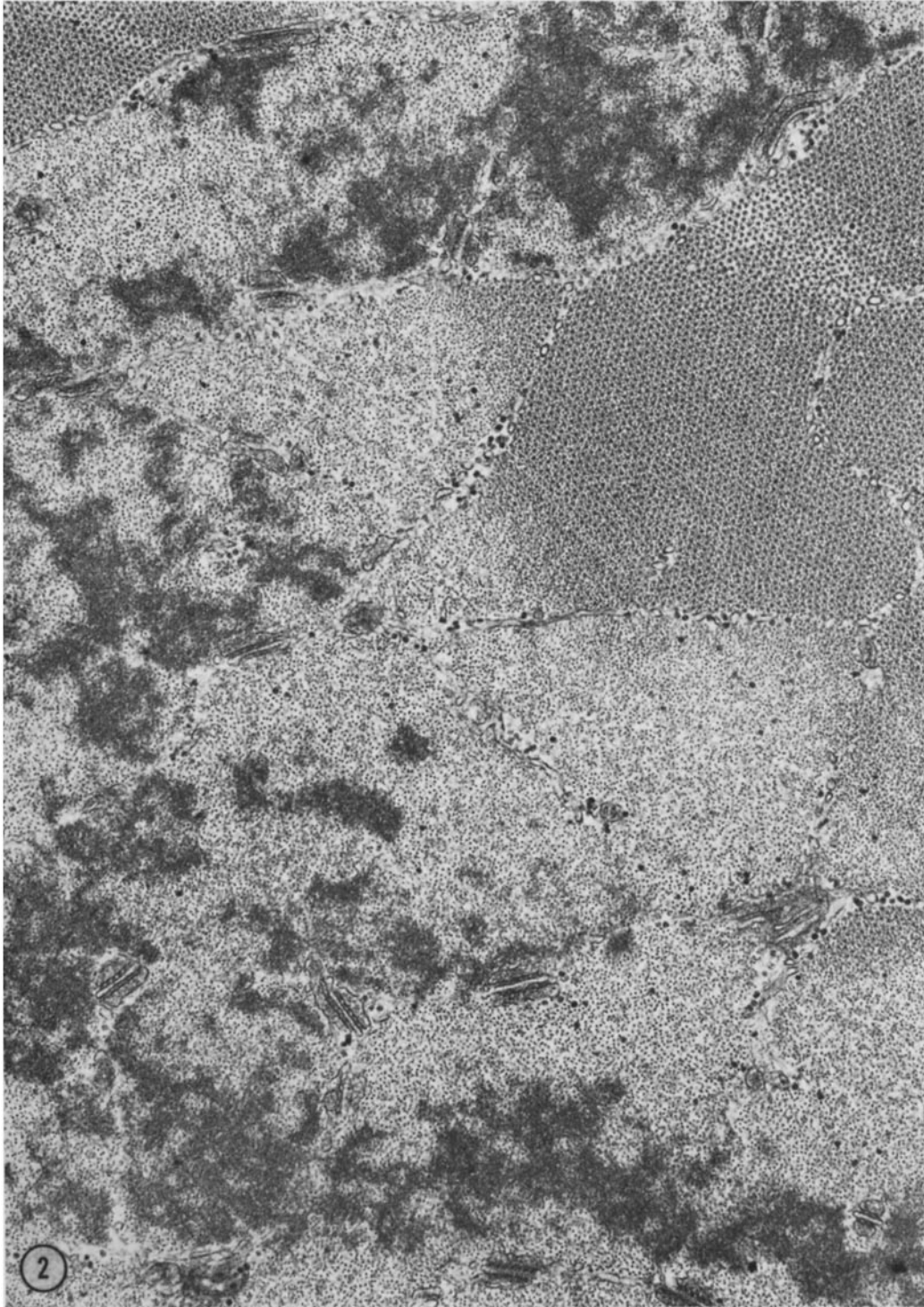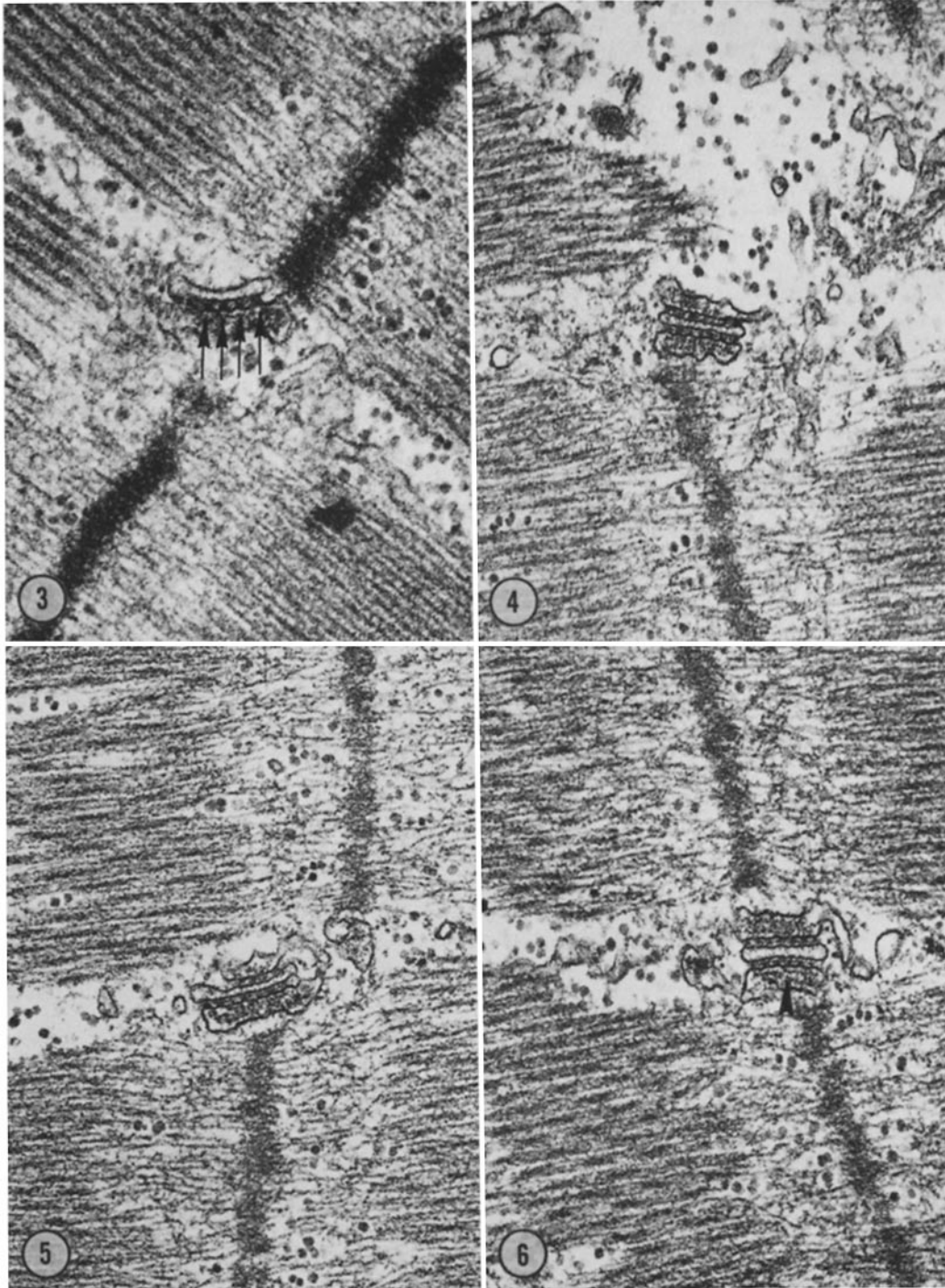
FIGURE 2 Cross-section through a slow fiber The fibrils are confluent, the Z line structure not discernible. Triads are scattered in the sarcoplasm. × 33,000.

FIGURES 3–6 Views *a* of the triad in longitudinal sections. Notice variable appearance of feet (arrowhead, see text) A slight SR scalloping and feet (arrows) are evident in Fig. 3. Fig. 3, × 72,000; Figs. 4–6, × 64,000.

widest and 120 ± 5 Å (n = 45) in correspondence of the tips of the scallops. This gap is slightly wider than that reported for twitch fibers in frog, which is approximately 120 Å at its widest (Franzini-Armstrong, 1970) To ascertain whether this difference is real, rather than due to variations in fixation, staining, and embedding between different experiments, the width of the gap was measured in two adjacent fibers, one twitch and one slow, from the same section Measurements were made on micrographs taken at the same magnification in the microscope and printed during a single session at a fixed enlargement Before exposing the plates, the microscope magnification had been standardized, as described in Materials and Methods The mean of 12 measurements on the two types of fibers confirm that the slow fibers' gap width is approximately 13% wider than that of twitch fibers

In view b (Figs. 7–10) SR and T system membranes, as well as the gap, are also cut crosswise, but the long axis of the T system is perpendicular to the plane of sectioning. The width of the junctional gap is the same as in view a, but the spacing between the feet is slightly larger: 325 ± 10 Å (n = 22) In view c, see below, the two different spacings can be measured in the same triad

In views a and b the triads have variable lengths. Some of them are so short that no more than 2 feet occupy the junctional gap, whereas others accommodate 7–10 feet (Figs 1–10) In this detail, slow fiber triads differ from those of twitch fibers, where in view b the feet are almost invariably two and in view a the number of feet may be very large and it is probably limited only by the fact that the junctions run out of the plane of the section (Franzini-Armstrong, 1970, 1972). The difference is due to the fact that whereas in twitch type fibers the junctional areas are narrow and elongated, accommodating two long parallel rows of feet, in slow fibers the junctions are small, round or oval patches of variable dimensions and the rows of feet are more numerous, but shorter. The rows run parallel to the T system long axis, and their number can be counted in view b, while view a shows how many feet are along a row In both twitch and slow fibers there are no parts of the junctional gap which are not occupied by regularly spaced feet.

The shape of the junction and the disposition of the feet are demonstrated to better advantage in view c (Fig. 1 arrowheads; Figs 11, 12), where the junctional gap of a whole triad (or most of it) is included in the plane of the section The feet are identifiable as small dense patches over a gray background representing grazing view of either SR or T system membrane (or both). In Fig 11 the axis of the junction forms a small angle with the longitudinal axis of the fiber and correspondingly the rows of feet are slightly oblique Three such rows are clearly visible (arrows), others are less evident (dashed arrows) because they are only partially included in the section The center-to-center distance between the feet along the rows is approximately 270 Å and the distance between adjacent rows is larger, approximately 320 Å. These values agree very well with the distances between the feet in views a and b, respectively The feet are in register in adjacent rows, forming a tetragonal pattern slightly elongated in the direction perpendicular to the T system long axis. In Fig 12 the junctional area is much smaller and it only accommodates two rows, which run almost transversely in the fiber. Each row is composed of four feet (arrows) If the rows were longer, this triad would be identical to a twitch fiber triad

In views a and b the appearance of the feet is variable, probably depending on section thickness and, more significantly, on the orientation of the section relative to the tetragonal pattern In some instances (Figs. 3 and 8, arrows) the feet are uniformly dense, discrete, and they touch both SR and T system membranes The space between the feet is mostly devoid of any structure More frequently, the portion of the feet lying midway between SR and T system is wider than their portions close to the two membranes and it appears as a thin dense line bisecting the junctional gap for short distances (Figs. 4 and 7). In some images, only this central portion of each foot is well visible and there apparently is a small gap (20–40 Å) separating the feet from the T system membrane The attachment to the SR is tenuous (Figs 5, 6, 9) The wider central portion of the feet may also be prolonged into a fine line, which crosses the space separating adjacent feet and apparently connect them to one another (Figs 6 and 9, arrowheads; Fig. 10). This intermediate line is located midway in the gap Where the intermediate line is more prominent, it fuses with the central portion of the feet to form an apparently continuous line, which occupies the center of the junctional gap (Figs. 6 and 9, arrows). Figs. 3, 5, 8, 9 (arrowhead), 6, 10, 9 (arrow) show successive steps in the transforma-
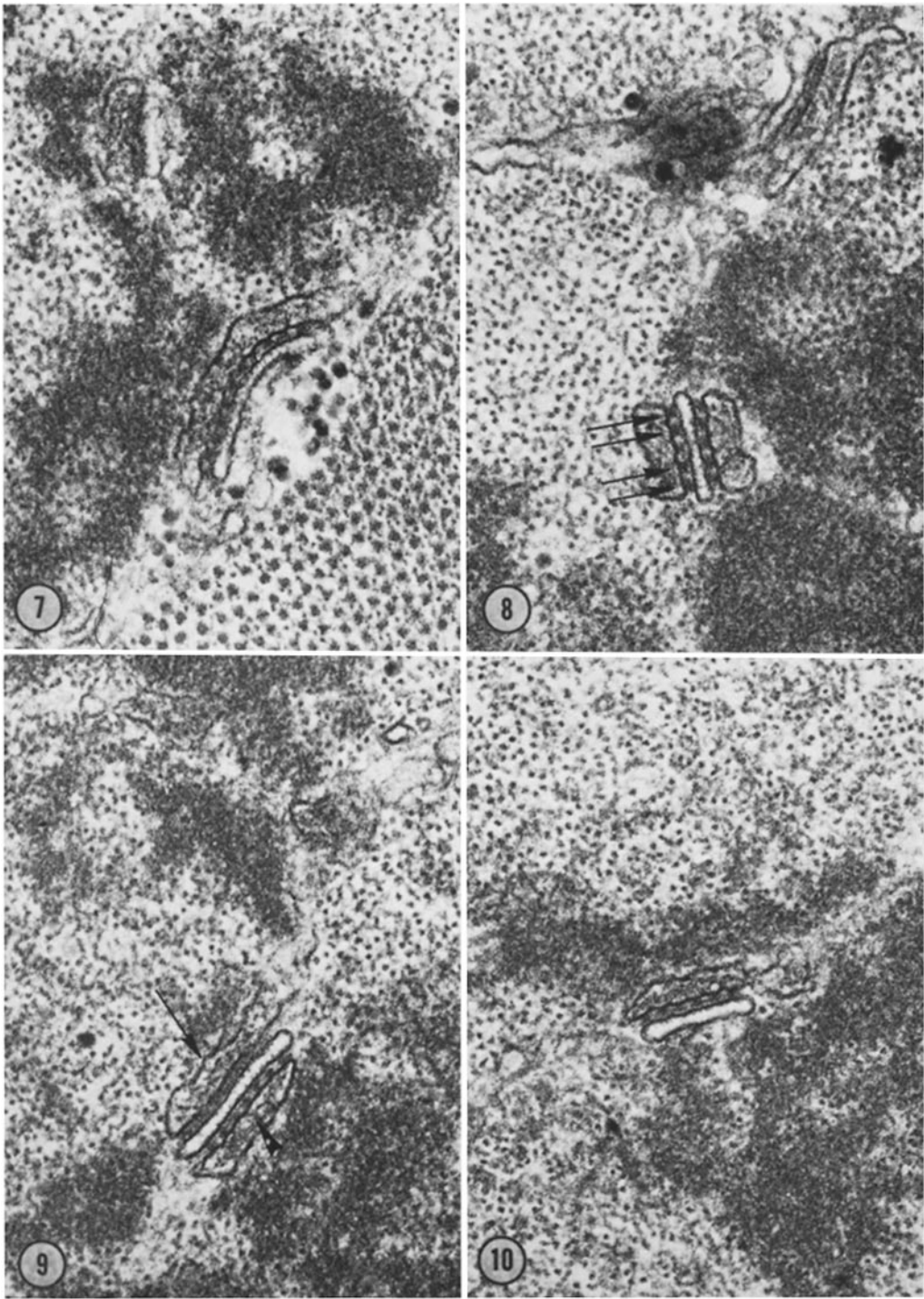
FIGURES 7–10 Views *b* of the triads in cross-sections. The rows of feet can be counted in this view. Here also appearance of feet is variable (arrows and arrowheads). × 87,000.
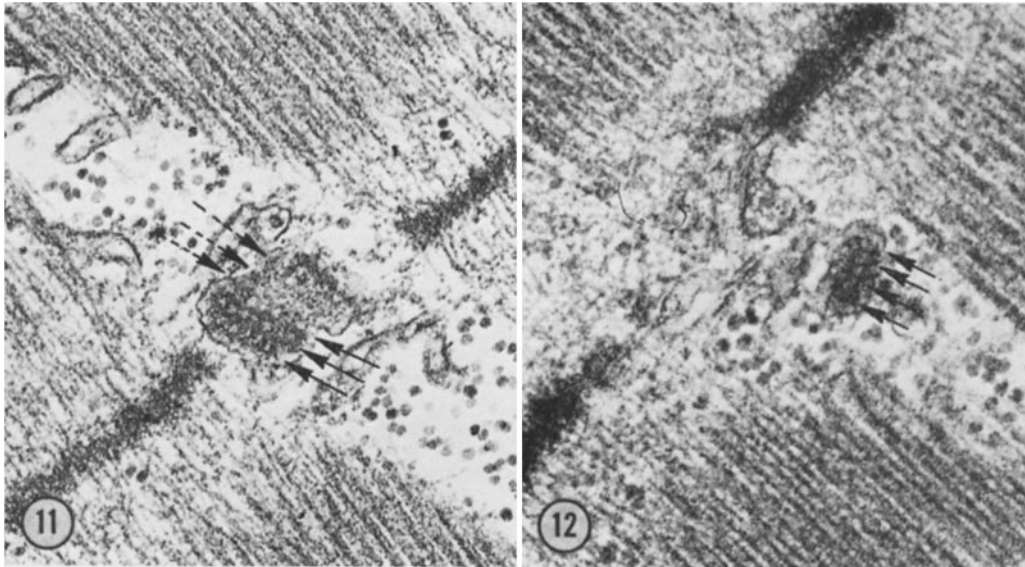
FIGURES 11 and 12   Views c of the triad in longitudinal section Arrows in Fig 11 indicate rows of feet, in Fig. 12 they point to individual feet along a row. In Fig. 12 triad axis is oblique. Fig. 11, × 64,000; Fig. 12, × 72,000.

tion of the image from that of discrete feet in the junctional gap to a continuous intermediate line.

In view c (Figs 11 and 12) the feet are not completely separated from one another, rather each foot has four projecting corners, which reach towards the two adjacent feet along the same row and the two in register in nearby rows. Due to the limitations imposed by the lack of contrast of the feet when viewed in superimposition with one or two membranes, these details of the feet are barely visible here Better images of view c have been obtained in twitch fibers (Franzini-Armstrong, 1972 b) and the model of the feet derived from those will be presented in a later report.

DISCUSSION

The junctional feet in frog slow fibers are disposed in register along multiple rows, so that the oval-shaped junctional areas are completely pervaded by a regular tetragonal pattern Number of rows and number of feet along the rows are dependent on the size and shape of each junctional area, which are very variable. The separation between the feet and their disposition are the same as in twitch fibers, the only differences being the length of the rows (which is shorter in slow fibers) and the number of parallel rows which is larger, since twitch fibers usually have two rows (Franzini-

Armstrong, 1970, 1972 a) and less frequently three or more rows (Kelly and Cahill, 1969).

In this study the feet display evidence of a sub-structure: they have a wider portion at a level equidistant from SR and T system membranes The feet are attached to the SR by a small neck On the other hand, a narrow gap is often present between feet and T system Only high resolution stereomicrographs will be able to demonstrate whether this is due to the fact that the feet attachment to the T system is tenuous or whether a real gap exists. This point is very important when considering the role of the triadic junction as the site of functional coupling between SR and T system Some of the structural details of the feet described in this paper were also visible in previously published micrographs of twitch fibers triads, but they were hardly commented upon in the text, because they were not very distinct (Franzini-Armstrong, 1970, 1972 a)

In some views of the triad the gap is apparently bisected by a continuous dense line, an image which would seem very difficult to reconcile with that of discrete junctional spots, more frequently described in the literature (Walker and Schrodt, 1966, Kelly, 1969; Franzini-Armstrong, 1970). A similar continuous line was also noticed in some triads of human twitch fibers after permanganate

staining (Fahrenbach, 1965) This paper brings evidence indicating that the discrete feet of some images and the continuous intermediate line of others are just different views of the same structure

The width of the junctional gap in slow fiber triads is slightly larger than in twitch fibers The possible significance of this difference should be considered in relation to the function of the triad. The mechanism of transmission of information between SR and T system at the triad has not yet been elucidated, but two major possibilities exist: one involves diffusion of a trigger substance across the junctional gap; the second requires ionic current to flow from T system to SR lumina, presumably along channels located in the feet (for discussion of the literature see Franzini-Armstrong, 1970). In either case, the extra e-c coupling delay caused by the additional 20–40 Å in gap's width would be several orders of magnitude smaller than the difference in total e-c coupling delay between slow and twitch fibers Thus events occurring at the triads are not responsible for the relatively slow development of tension in slow fibers.

On the basis of morphological similarity alone, it should be concluded that slow fiber triads have a role and a mechanism of action similar to those of twitch fibers, i.e., they are the site of calcium release during excitation. As shown by Page (1969), calcium for activation of chicken slow fibers comes demonstrably from two sources: an internal store (the SR) and the external solution The former causes a relatively rapid tension development, whereas the latter is responsible for maintenance of tension during the prolonged contracture of which these fibers are capable when depolarized in high potassium. In frog slow fibers, however, although an internal Ca store exists and it is functionally connected to the surface membrane via a T system and a triadic junction, measurable tension development is totally dependent on outside calcium (Lüttgau, 1963 a) Possibly, either calcium released from the SR is not sufficient to raise sarcoplasmic calcium concentration to threshold values for activation (Page, 1969), or else it does so, but only transiently, in close proximity of the triads. Since these are few and scattered in the fiber, the local areas of contraction thus

produced would not cause a detectable tension at the fibers ends

REFERENCES

COSTANTIN, L L., C. FRANZINI-ARMSTRONG, and R. J PODOLSKY. 1965. Science (Wash. D. C.) 147:158.
COSTANTIN, L. L , R. J. PODOLSKY, and L. W. TICE 1967. J. Physiol (Lond.). 188:261.
FAHRENBACH, W. H. 1965 Science (Wash. D C.). 147:1308.
FLITNEY, F. W. 1971. J Physiol. (Lond). 217:243.
FRANZINI-ARMSTRONG, C. 1970. J Cell Biol. 47:488
FRANZINI-ARMSTRONG, C. 1972 a Tissue and Cell. 4:471.
FRANZINI-ARMSTRONG, C. 1972 b. J. Cell Biol. 55(2, Pt 2):78 a (Abstr.)
HESS, A. 1967 Invest. Ophthalmol. 6:217.
HOYLE, G., P. A McNEILL, and B WALCOTT. 1966. J. Cell Biol. 30:197.
KELLY, D. E. 1969. J. Ultrastruct. Res. 29:37.
KELLY, D. E., and M. A. CAHILL 1969. J. Cell Biol 43(2, Pt. 2):66 a (Abstr.)
LÄNNERGREN, J , and R. S SMITH. 1966. Acta Physiol Scand. 68:263.
LUTTGAU, H. C. 1963 a. J Physiol. (Lond.). 168:679
LÜTTGAU, H. C. 1963 b. In Electrophysiology of the Heart. Pergamon Press, Inc., Elmsford, N. Y. 87.
PAGE, S. G. 1965 J. Cell Biol. 26:477.
PAGE, S. G. 1969. J. Physiol. (Lond). 205:131.
FEACHEY, L D. 1965. J. Cell Biol. 31(2):84A (Abstr).
FEACHEY, L. D , and A. F. HUXLEY. 1962. J. Cell Biol. 13:177.
PERACCHIA, C., and B. S. MITTLER. 1972 J. Cell Biol. 53:234.
SANDOW, A. 1965. Pharmacol. Rev. 17:265.
SATO, T. 1968. J Electron Microsc. 17:158
WALKER, S. M., and G. R. SCHRODT. 1966. Anat. Rec 155:1.