BODY MUSCLES OF THE LAMPREY

Some Structural Features of the T System and Sarcolemma

DONALD JASPER. From the Department of Zoology, Columbia University, New York. The author's present address is the Department of Biology, University of York, England

INTRODUCTION

In the skeletal muscle fibers of a number of vertebrates, the transverse tubular or T system (TTS) has been found to be in apparent continuity with extracellular space (11, 15, 16), and its membranes have been shown to be invaginations of the plasma membrane (4, 5, 17–19, 30). Although in vertebrates the TTS is generally oriented in a plane which is transverse or perpendicular to the fiber's long axis, it sometimes gives rise to longitudinal branches and extensions (11, 16–18, 24).

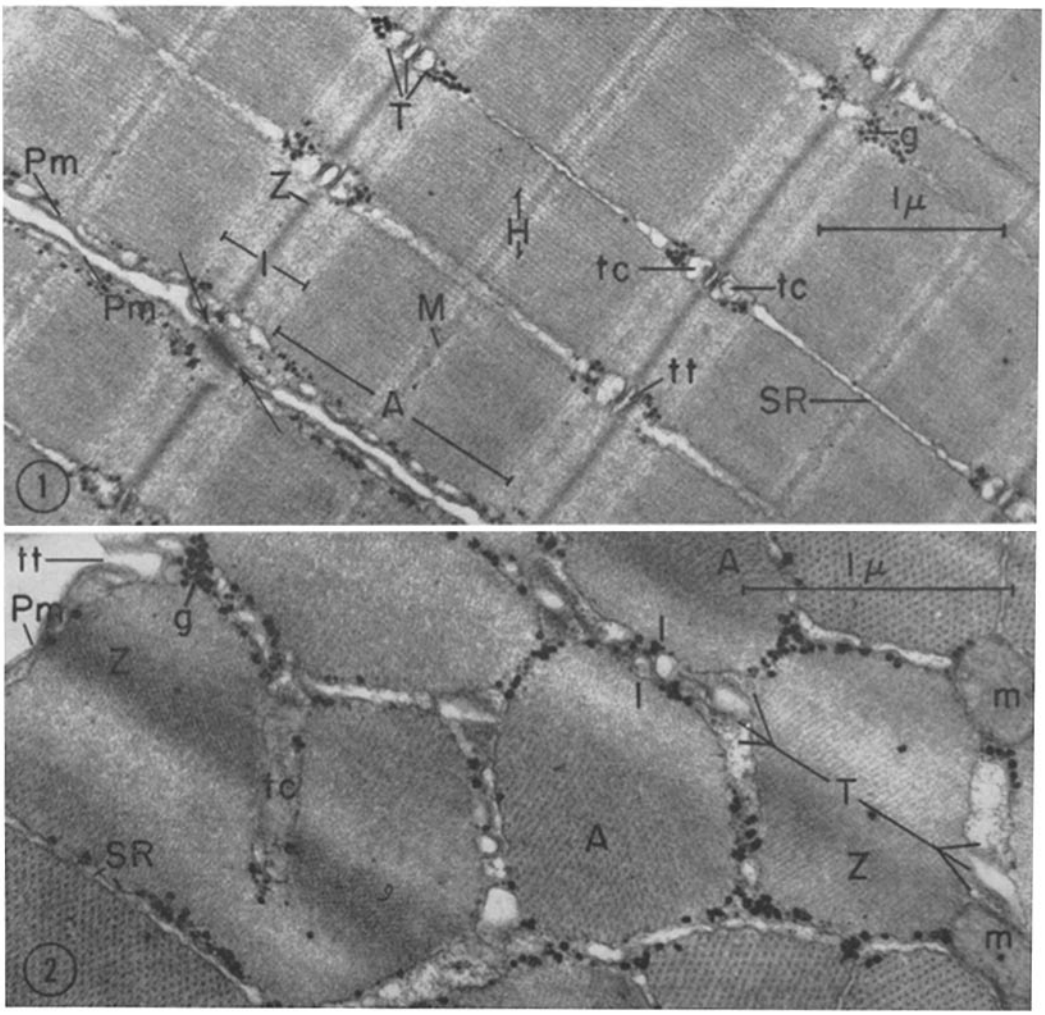

FIGURE 1 Electron micrograph of a sagittal section of two central fibers in which the TTS (*tt*) as central elements of triads (*T*) extends transversely at levels of Z lines (*Z*). Other sarcomere line and band divisions are as labeled. Note interfiber junction (arrows). *Pm*, plasma membrane. *g*, glycogen. *tc*, terminal cisternae of sarcoplasmic reticulum (*SR*). *P. marinus*. × 25,000.

FIGURE 2 Transverse (somewhat oblique) view of part of a central fiber displaying a number of triads (*T*) in cross-section. A transverse tubule (*tt*) is shown opening into extracellular space. Myofibrillar sarcomere divisions are as labeled. *tc*, terminal cisternae of SR. *Pm*, plasma membrane. *g*, glycogen. *m*, mitochondria. *P. marinus*. × 35,000.

Also, in vertebrates, as it extends inward and across the fiber, the TTS typically is found as the middle component of a three-part structure, the triad (23). The two outer components of the triadic structure are the terminal cisternae (5, 17, 18, 23) of the sarcoplasmic reticulum (SR), a separate system of membrane-bounded sacs and tubules (5, 17, 18). Depolarization of small regions of the surfaces of several kinds of vertebrate muscle fibers leads to an inward spread of localized contraction only when the depolarizing pulse is applied at the sarcomere levels of the triads (8–10). Thus, the membranes of the TTS and SR are implicated in the mediation and intracellular

transmission of electrical activity leading to muscular contraction (5, 8–10, 17, 19, 20, 22, 23, 27, 31).

Recent work on the lamprey has revealed some features on the fine structure of its myotomal fibers and also an interesting arrangement of the nerves which innervate them (21). Nothing, however, has been reported on the fibers' TTS or SR. This paper includes some of the fine structural aspects of triads of central fibers after glutaraldehyde fixation. Because of unexplained variability in appearance of intracellular components of parietal muscle fibers, these fibers will not be reported or discussed in as much detail. Some structural manifestations of the surfaces of both central and parietal muscle fibers will be presented.

MATERIALS AND METHODS

Myotomes of young adult brook lampreys (*Entosphenus appendix*), 20–25 cm in body lengths, and larval forms as well as recently metamorphosed forms of marine lampreys (*Petromyzon marinus*), 10–20 cm in body lengths, were doubly fixed. For the initial fixation, caudal and middle regions of the bodies were cut into 2–10-mm thick slices and placed for ca. 2 hr into 2 or 6% glutaraldehyde (28) buffered with 0.15 M sodium phosphate + 2 mM of calcium chloride at pH 7.0–7.2. Subsequently, the slices were rinsed twice (1–2 hr/rinse) in 11% sucrose with 0.15 M sodium phosphate + 2 mM calcium chloride, and

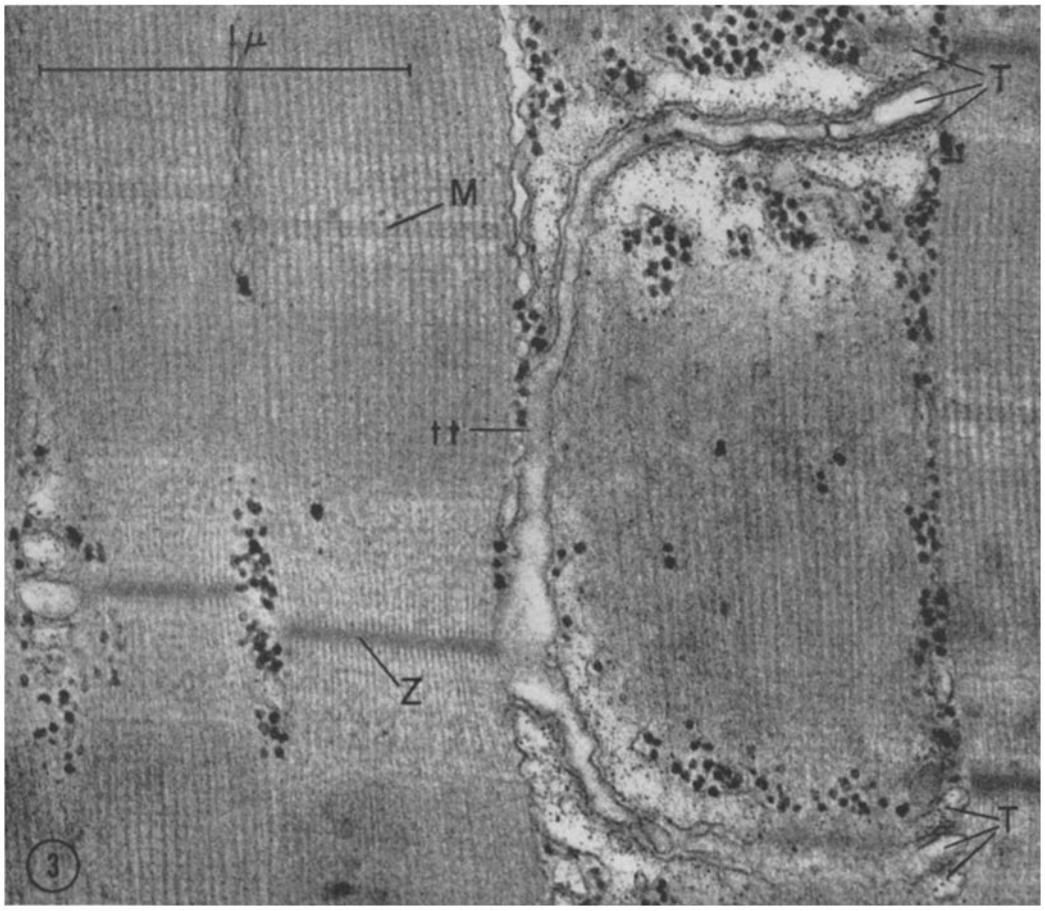

FIGURE 3 Longitudinal view of part of a central fiber in which a single transverse tubule (*tt*) forms the central element of two longitudinally adjacent triads (*T*). Myofibrils show a "vernier" shift. Compare with Fig. 4. *Z*, Z line. *M*, M line. *P. marinus*. × 50,000.

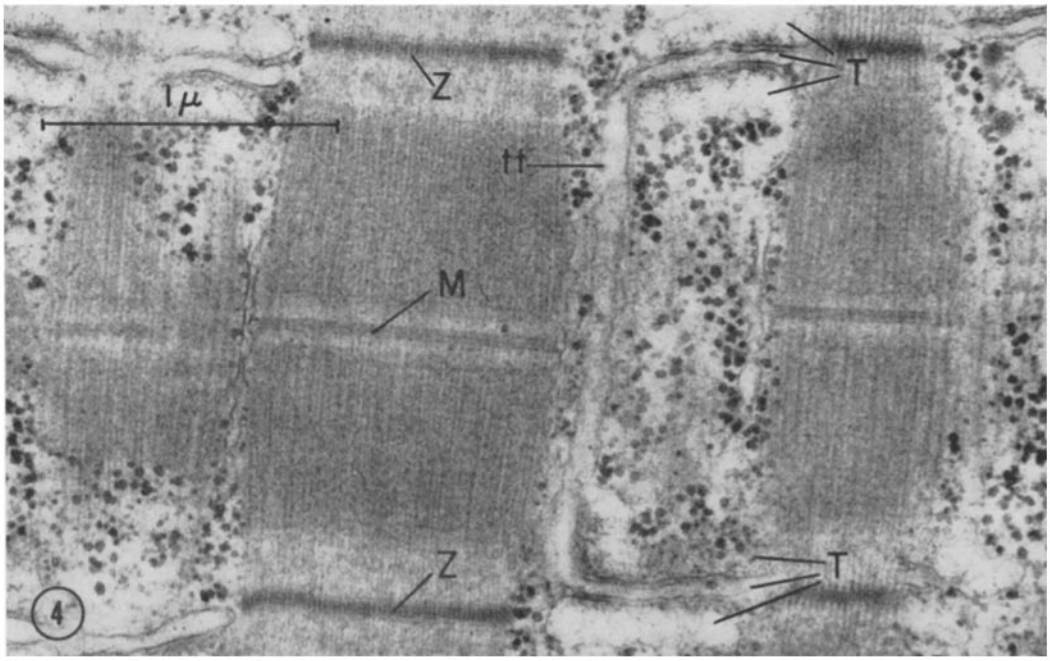

FIGURE 4 A section of a central fiber with a longitudinal profile of the T system (*tt*) connecting longitudinally adjacent triads (*T*) as in Fig. 3, except that here adjacent fibrils are more in register and do not show a vernier formation. *Z*, Z line. *M*, M line. *E. appendix*. × 40,000.

some were cut into 1–2-mm cubes. These smaller pieces of tissue were then fixed for 1 hr in 1% osmium tetroxide buffered with 0.15 M sodium phosphate at pH 7.0–7.2, and dehydrated in ethanol-water mixtures. Finally, the cubes of tissue were soaked overnight in a 1:1 mixture of propylene oxide and Epon 812 resin, and afterwards embedded in fresh resin (14). Fixations were done routinely at 0–5° and also at room temperature (ca. 22°C).

Sections were cut with glass and diamond knives on Porter-Blum MT-1 or MT-2 microtomes (Ivan Sorvall, Inc., Norwalk, Connecticut). Thick sections (1–2 μ) were mounted on glass slides and studied in the light microscope. Thin sections (~300–800 A) were mounted on bare 200-mesh copper grids. After staining with lead citrate (25) or a saturated solution of uranyl acetate followed by lead citrate, the thin sections were viewed with either a modified Philips EM 100A or an RCA EMU 3F at 60 kv.

RESULTS

The body muscles of the lamprey consist of a number of overlapping myotomes, with the medial surface of any one always situated anterior to its lateral surface. All myotomes are subdivided into layered units (lamellae), each containing a group of central muscle fiber "plates" or "sheets," which seemingly extend the medial to lateral myotomal width. Each central fiber group (usually seen as four "plates" per group) is horizontally flattened and surrounded dorsally, laterally, and ventrally by parietal muscle fibers. All fibers are attached anteriorly and posteriorly at intermuscular septa. Sizes of fibers, as well as over-all myotomal sizes, vary with location in the animal's body. In this study, myotomal measurements from medial to lateral surfaces in caudal and midbody regions were 1–4 mm and distances between intermuscular septa were of the order of 0.3–3 mm. Fibers' thicknesses (both parietals and centrals) varied from ca. 1 μ (larval parietals) to 50 μ (young adult centrals). This brief light microscope description of myotomes of *P. marinus* and *E. appendix* agrees generally with that of Peters and Mackay (21) for *Lampetra fluviatilis* and *L. planeri*, and also that of Grenacher (6) for *P. marinus*. Evidently, the architecture of lamprey body musculature, as pointed out (21), is constant.

The fine structure of central muscle fibers of the lamprey in many aspects is similar to that

observed in skeletal muscle fibers of the frog (17, 23). Myofibrils, about 1 μ or so in diameter, have sarcomeres between 1.8 and 2 μ long in these preparations and show the usual pattern of A and I bands with Z and M lines. These myofibrils also contain the customary array of contractile elements (in the A band, six thin filaments around each thick one). Surrounding the contractile elements are other sarcoplasmic contents which include sparsely concentrated mitochondria, densely staining glyocogen granules, and components of the SR and the TTS. The terminal cisternae of the SR are continuous with the nuclear envelope. The TTS is located as a separate system between these terminal sacs of the SR at levels of Z lines, where together the two systems are arranged as triads (Figs. 1 and 2).

This arrangement of triads at the Z line in lamprey is like that in skeletal muscles of amphibians (8–11, 15–19, 23) and body muscles of the fish (4, 5, 18, 19), but unlike that in skeletal muscles of reptiles (7, 9, 26) and mammals (23, 24, 31), or the swimbladder muscles of the Atlantic toadfish (3). In the latter instances, triads are found two per sarcomere and are disposed near ends of the A bands or near the A–I junctions. Physiological as well as taxonomic factors may determine arrangement of the triads in different muscle fibers (18).

By noting the accumulation of ferritin molecules in the TTS, H. E. Huxley (11) and Page (16; see also Peachey, 18) determined that portions of this central element of the triads could extend for considerable distances in a longitudinal direction in frog muscle fibers. Similar extensions of the TTS were directly observed in the central muscle fibers of lamprey. Furthermore, in places in which the plane of section was favorably oriented with respect to the tubular direction, a profile of a single transverse tubule could clearly

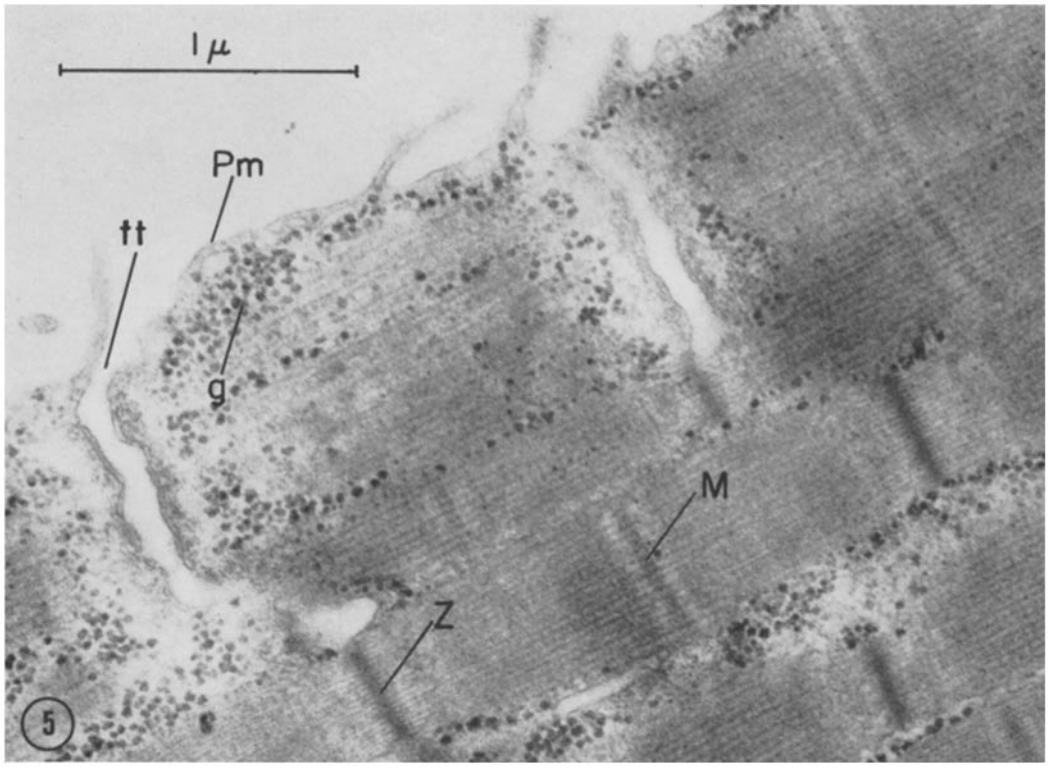

FIGURE 5 A longitudinal section of lamprey central fiber showing an opening of the TTS (*tt*) in continuity with extracellular space, and its membranes in continuity with the plasma membrane (*Pm*). Note that the surface of the fiber is modified into thin outward extending projections, one of which overlies the surface opening of *tt*. *Z*, Z line. *M*, M line. *E. appendix*. × 40,000.

FIGURE 6 A cellular junction (arrows) is shown between a parietal muscle fiber (left) and a central fiber. The parietal fiber here is only one myofibril thick. *Pm*, plasma membrane. *tt*, transverse tubule. *M*, M line. *Z*, Z line. *g*, glycogen. *P. marinus*. × 50,000.

FIGURE 7 A desmosome (arrows) forming the intercellular contact between two central fibers cut in cross-section (oblique). *Z*, Z line. *Pm*, plasma membrane. *P. marinus*. × 50,000.

be followed extending "in the longitudinal direction" from one triad and entering the next triad (Figs. 3 and 4). This phenomenon was observed more than a dozen times in separate experiments on both marine and brook lampreys. In some cases, it was observed where there was a "vernier" shift in the striations of adjacent fibrils (Fig. 3), but it was also observed where no such vernier formation was seen (Fig. 4). This direct coupling of triads of two adjacent Z lines is apparently a new observation and will be discussed later.

In central muscle fibers the TTS is found often to open into the extracellular space, with its membranes continuous with the plasma membrane (Figs. 2 and 5). This finding in lamprey myotomes is another instance in which central triadic elements are invaginations of a muscle fiber's surface membrane. The functional importance of such structural continuities to muscle physiology has been well related in studies by others (e.g., 5, 11, 19).

Adjacent fibers of a myotomal lamella are frequently closely stacked and their surfaces are often modified by various forms of protrusions. In *P. marinus* the protrusions are seen many times as interfiber adhesions or linkages, and they have been found between parietal and central fibers (Fig. 6) and between adjacent centrals (Figs. 1, 7,

and 8). Such units were more frequently observed in sections which were cut in a sagittal plane and seemed to be more prevalent in those lamellae in which fibers are stacked less than 0.5 μ apart. But close fiber proximity does not seem to ensure the presence of these junctions, as there were some lamallae in which they appeared to be absent. This was especially so in young adults of *E. appendix*, in which the surface areas sometimes displayed outward extending projections as shown in Fig. 5, rather than interfiber adhesions. The characteristics of the linkage unit are those of a kind of desmosome with a 200-A adhesion area within which is a somewhat homogenous material (Figs. 1, 6–8). Although cell junctions or desmosomes with variations in substructure are present in many vertebrate tissues, including smooth (1, 2) and cardiac (12) muscle, there seem not to be other known instances of their presence in any form in a vertebrate's voluntary muscle.

DISCUSSION

The cell junctions observed in this study are evidently a new finding for the voluntary muscles of a vertebrate. The only other known study in which a similar kind of structure has been found was included in a recent report on the visceral muscles of an insect (29). The exact nature of the interfiber junctions found in lamprey body muscles is not understood, but cell junctions or desmosomes are structures which seem, in some degree, to function in relation to the types of tissues in which they are found.

The filamentous framework of newt epidermal desmosomes (13) suggests that those attachment sites may participate in mechanical support. In lamprey, the major portions of central fibers are apparently suspended within a fluid matrix of the lamellar units. An obvious role here of the button-like fiber junctions, seemingly, would be attachment areas of support, but this does not preclude other possible functions. Because the junctions are adhesion sites they also, perhaps in a passive manner, may be involved in the coordination of muscle fiber activity, thus enabling each lamella to function more as a unit during certain active phases.

The apparent lack of the adhesions in certain lamellae may be attributed to an unfavorable orientation of the tissue block. On the other hand, since surface protrusions were not evident in every fiber, and if this is indicative of their rarity or absence in some lamprey myotomes, it is not impossible that certain surface areas of a fiber

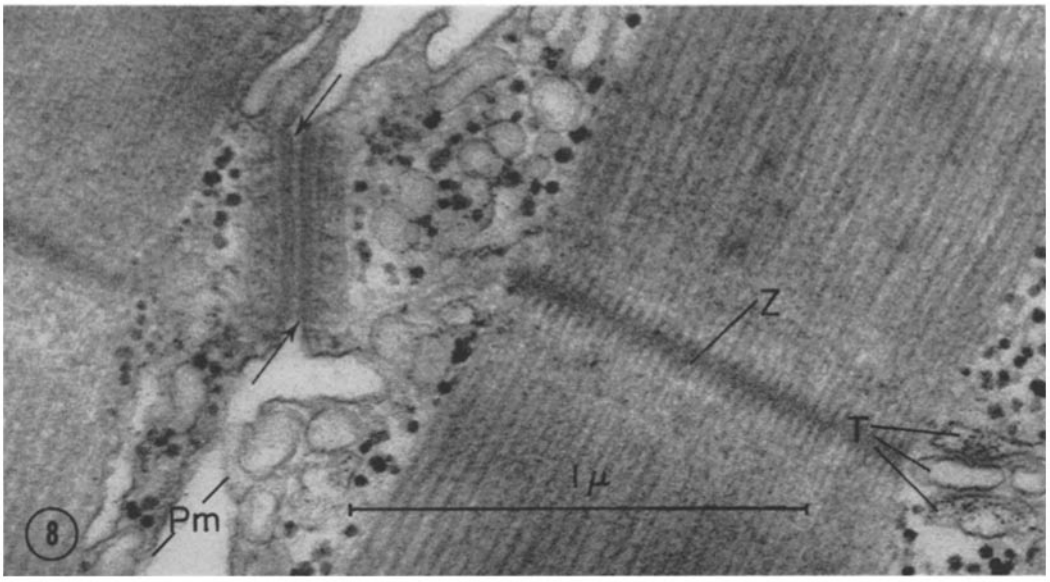

FIGURE 8 Electron micrograph illustrating the several layers of a desmosome (arrows) of two central muscle fibers in longitudinal section. *T*, triad. *Z*, Z line. *Pm*, plasma membrane, *P. marinus*. × 60,000.

undergo a transition or transformation. It is hoped that further studies will clarify this problem.

Another new and an interesting result of the present study was the observation of a single transverse tubule forming the central element of each of two longitudinally adjacent triads. If such structures were to be activated in a local stimulation experiment, such as done by A. F. Huxley and collaborators (8–10), one would expect to observe contraction of two longitudinally adjacent I bands: a phenomenon, in fact, observed once in frog muscle by Huxley and Taylor (10). This single observation was made in a region of the fiber in which the myofibrils were arranged in a vernier formation. In the present study, morphologically linked triads were found both in such regions and also in regions in which no vernier formation was present. It is possible that such links in absence of a vernier formation are peculiar to lamprey muscle, or that they are only infrequently found in frog muscle, at least near the cell surface, and thus escaped detection in Huxley and Taylor's experiments.

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