HELICOIDS IN THE T SYSTEM AND STRIATIONS OF FROG SKELETAL MUSCLE FIBERS SEEN BY HIGH VOLTAGE ELECTRON MICROSCOPY

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ABSTRACT Reconstruction from thick serial transverse slices of frog skeletal muscle fibers stained with peroxidase and examined by high-voltage electron microscopy has revealed that the T system networks at successive sarcomeres are connected together in a helicoidal fashion. From zero to eight helicoids have been found in each of a group of 21 fibers reconstructed in cross section. Helicoids can have either right- or left-handed screw senses, and both senses can be found in one fiber cross section. Because the T system maintains a relatively precise alignment with the myofibrillar striations, it follows that the striations must also have a helicoidal arrangement. This has been found before, but has not been widely accepted in recent times. The presence of helicoids in the bands and membrane networks is not thought per se to alter very much our thinking about excitation and contraction mechanisms in skeletal muscle fibers.

INTRODUCTION

The T system (or transverse tubular system) of a striated muscle fiber is generally understood to consist of networks of branched tubules that invaginate into the fiber from its surface. These networks are believed to play an important role in the activation of contraction by conducting action potentials from the surface of the fiber throughout its interior. These tubular action potentials then induce the sarcoplasmic reticulum to release calcium, which activates the contraction of the myofibrils (for review, see Costantin, 1975). In amphibian skeletal twitch muscle fibers, T tubules are almost completely localized in triads adjacent to the Z lines of the myofibrillar striations (Porter and Palade, 1957; Page, 1965; Peachey, 1965; Franzini-Armstrong, 1973). T tubules have been detected running in the longitudinal direction alongside the myofibrils (Jasper, 1967; Peachey and Schild, 1968), but these represent a minor proportion of the total tubules present (Eisenberg, 1972). Therefore, it generally has been believed that T system networks, like the striations, lie largely in planes perpendicular to the fiber axis, there being one such T system network in the plane of each Z disk. Although the T system planes and the Z disk might be irregular or undulate, each one would remain separated from longitudinally adjacent ones by a distance equal to the length of one sarcomere.

This model for T system structure has been deduced from study of thin sections by electron microscopy, where only very small portions of a T system network appear in any one section. In a high-voltage electron microscope (HVEM) study of the T system of frog skeletal muscle designed to gather further structural information from views of larger areas of T system network seen in relatively thick slices of tissue, we have found that the model described above is incorrect in a rather surprising and curious way. The T system networks, and the striations of the fiber as well, are not independent planes, but adjacent networks and striations connected together in a helicoidal manner. In fact, this helicoidal form of muscle fiber striations has been described earlier, for example, by Tiegs (1924), but generally has been ignored. A preliminary account of our work has been presented (Peachey and Eisenberg, 1975).

METHODS

Sartorius muscles were dissected from frogs (*Rana pipiens*), tied at body length, and submerged in Ringer's fluid containing 0.1% horseradish peroxidase (type II or VI, Sigma Chemical Co., St. Louis, Mo.) for 30 min. Some muscles were stimulated once every 3 s during this time and the others were agitated to stir the solution. Only one muscle from the stimulated group and two muscles from the other group were well infiltrated with peroxidase, and these three muscles were used for all the results reported here. Fixation, incubation with H_2O_2 and 3, 3'diaminobenzidene tetrahydrochloride, postfixation, and tissue processing were as described in Eisenberg and Eisenberg (1968). Serial transverse slices of approximately 0.7 μm thickness were cut with LKB-Huxley (LKB Instruments, Inc., Rockville, Md.) or Sorvall MT2B microtomes (DuPont Instruments, Sorvall Operations, Newtown, Conn.). This was near the maximum thickness that gave good visibility of the T system with this staining method. Serial slices were encouraged to stick into a ribbon by coating the top and bottom of the block with printer's paste-up wax. The ribbons were picked up on single-hole specimen grids previously covered with a film cast from 0.4% Formvar (Monsanto Company, St. Louis, Mo.), and then coated with carbon by vacuum evaporation. Care was taken to keep track of the order of slices in each series.

The finished grids were checked in the light microscope by using phase optics. Photographs were taken to check for peroxidase staining and to choose fibers with good cross-section orientation and without tissue folds or other imperfections throughout the series. These were used as maps to aid location of fibers and to assure correct slice identification in the HVEM.

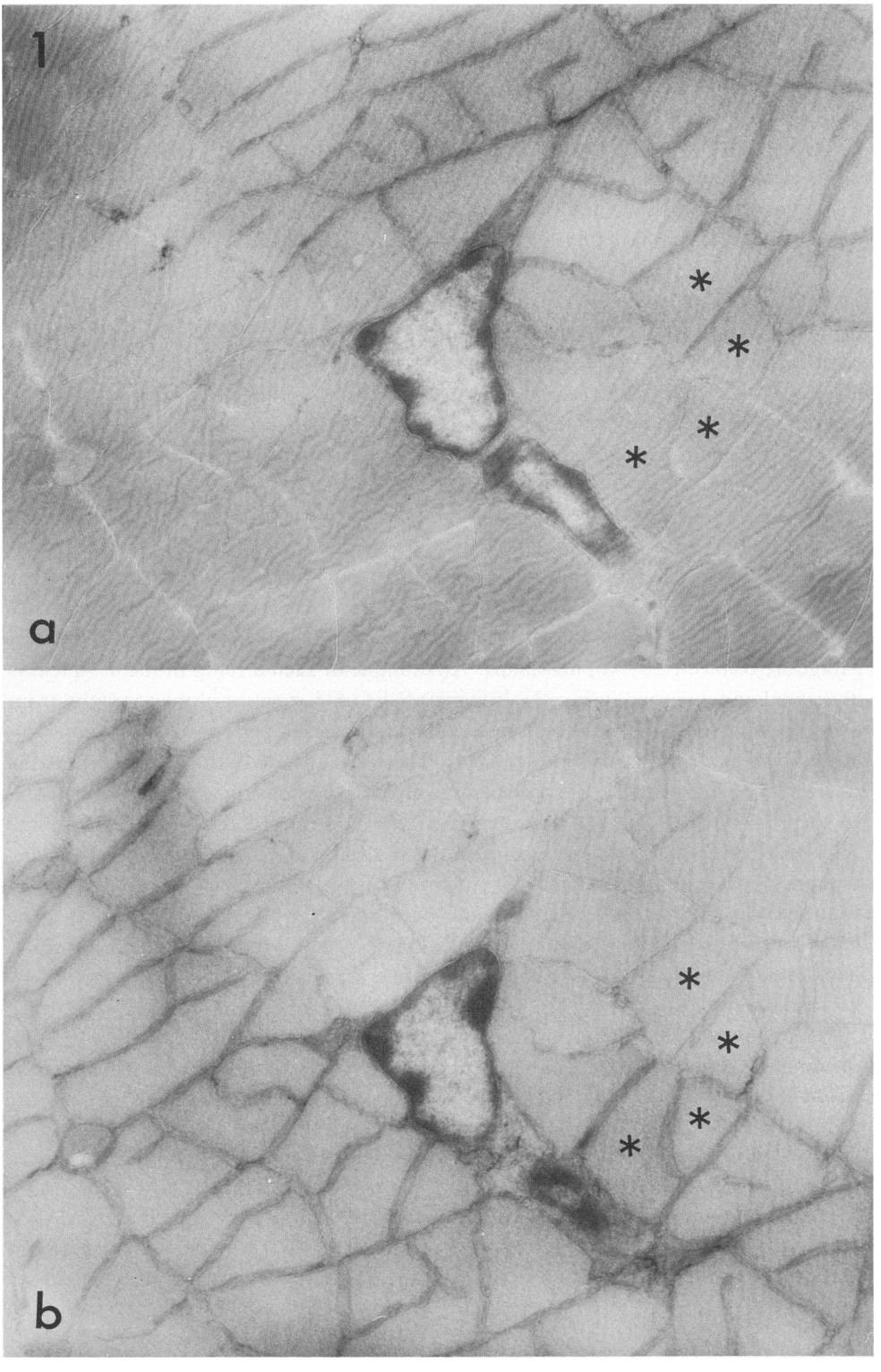
Electron micrographs were made at 1,000 kV on the JEM-1,000 high-voltage transmission electron microscope at Boulder, Colorado. Initially, magnifications of from 1,500 to 3,000× were used. The entire transverse area of most fibers could be covered in four to eight micrographs. In prints at about 6,000×, the T system tubules could be seen and the T system network followed through a series of successive micrographs from consecutive serial slices. Later, lower magnifications, down to about 400×, were used in the HVEM, with enlargement to about 2,000× in the prints. It was more difficult to visualize individual tubules in these lower magnification prints, but reconstruction of the T system in three dimensions as reported here could be done. Reconstruction was made by tracing on a plastic sheet laid over successive micrographs in a series. In one fiber, each tubule in the entire tubular network was traced completely across the fiber cross section. In other cases, only the outlines of areas occupied by the T system network in each micrograph were traced, forming a sort of topographic map. These maps were extended through enough consecutive slices to cover at least two or three sarcomeres in the longitudinal direction. In one fiber, this form of reconstruction was done in two regions separated longitudinally by eight sarcomeres.

RESULTS

In prints at 6,000×, it usually was possible to trace T tubules through each micrograph and into the following micrograph in the series (Fig. 1), using the peroxidase stain to identify T tubules. For one fiber, we made a tracing completely across the fiber in this way (Fig. 2), which produced a two-dimensional projection of the T system network in a transverse plane. This network is not very different from what we had imagined a T system network would look like from examination of thin sections. Dead-end tubules are more common than we might have expected. These appear to be real ends of tubules and not regions of poor peroxidase infiltration, because, at high magnification, a membrane can be seen around the end of these tubules. There also are two regions where the tubules have a preferred orientation toward the central region of the fiber. Except for these, the T system appears as an isotropic, irregular network extending across the whole cross section of the fiber. This complete network and partial areas of networks from other fibers have been used for quantitative estimation of network parameters for the T system, as reported elsewhere (Eisenberg and Peachey, 1975).

Analysis of the T system network through a series of slices in three dimensions gave a result that was more surprising. This analysis was more difficult than the two-dimensional projection, and could be understood only when it was appreciated that successive layers of T system in the longitudinal direction could be connected together by sloping portions of the network, much the way successive floors of a multilevel garage are connected by sloping ramps. An example of such a ramp in the T system is illustrated and analyzed in Fig. 3. In panels S-1 to S-5, a series of micrographs from five successive slices through the same region of a muscle fiber shows the regions occupied by T system network in each slice. These regions are outlined in the drawing of Fig. 3. In Fig. 4 they are represented in three dimensions, reassembled in a stack. In the real preparation, the T system network is arranged obliquely through the thickness of each slice (we have confirmed this in several places by stereoscopic viewing of pairs of micrographs taken at different specimen tilts). Fig. 5 is a representation of what the plane of the network might have been like before it was cut into slices. The main conclusion from this reconstruction is that T system networks that earlier would have been thought of as being entirely separate are, in fact, connected together by helicoidal ramps.

Fig. 6 shows a complete reconstruction of another fiber. As in the drawing of Fig. 3, the numbers and lines indicate areas in the micrographs where T system network was found in the slice of that number. This particular muscle had a sarcomere length close to four times the slice thickness, and therefore similar areas of the fiber cross section were occupied by a T system every fourth slice. Dotted lines are boundaries where a dislocation was found in the T system, that is, where continuity could not be traced across this boundary from one slice to the next. As shown by the circular arrows, helicoids are found eight places in this cross section of this particular fiber. Four of these helicoids are well separated from each other, and four others are grouped closely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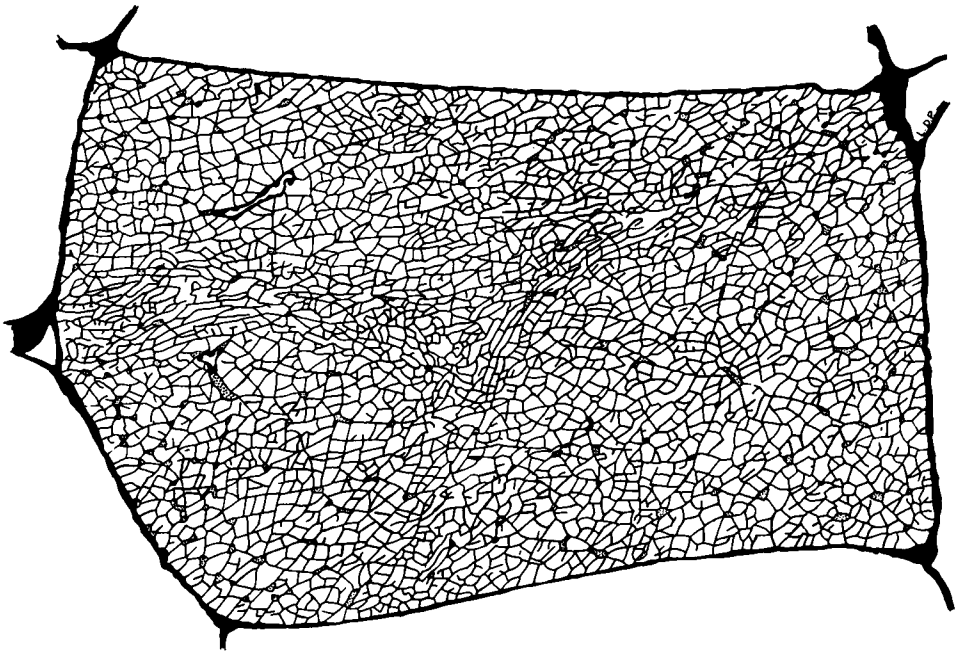

FIGURE 2 Reconstruction of the T system network over the whole cross section of a muscle fiber made by tracing successive portions of the network from micrographs of serial transverse slices about 0.7 μm in thickness. The fiber border and interfiber space are black. Two nuclei are indicated, at the left, as areas with an irregularly thickened border. Small stippled areas are mitochondria. The fine lines represent T tubules. Over most of the area of the fiber the network is irregular, but not obviously anisotropic. Along a line extending toward the center of the fiber from the left, and along another line from the upper right toward the fiber center, there are regions where the tubules have a preferred orientation toward the center of the fiber. Many tubules extend into the region occupied by myofibrils and end without completely crossing the myofibrils or connecting to the T system network at the other side. Dislocations (tears) in the T system are not indicated in this reconstruction $\times$ 1400

together, near the upper left in the figure. There are ambiguities in the order in which the slices and micrographs are viewed or imagined to be stacked, and therefore it is not possible to say whether any particular helicoid we observed has a right-handed or a left-handed sense in the fiber. It is clear, however, that both handednesses exist because three of the helicoids in this fiber have one sense and the other five have the opposite sense. It would be possible to work out the true sense of any given helicoid by keeping track of all possible image and slice order reversals, but we have not done this.

FIGURE 1 Identical areas from two successive 0.7-μm slices in a series, showing how T tubules can be followed from one slice to the next. A nucleus is at the centers of the figures. T tubules appear darker than sarcoplasmic reticulum or myofibrils, but are somewhat low in contrast because of the thickness of the slice. The same myofibrils can be identified in the two micrographs, as indicated by the stars. Where T tubules leave one slice, they can be found entering the other slice, making it possible to trace the T system network completely across the fiber cross section. $\times$12,000.

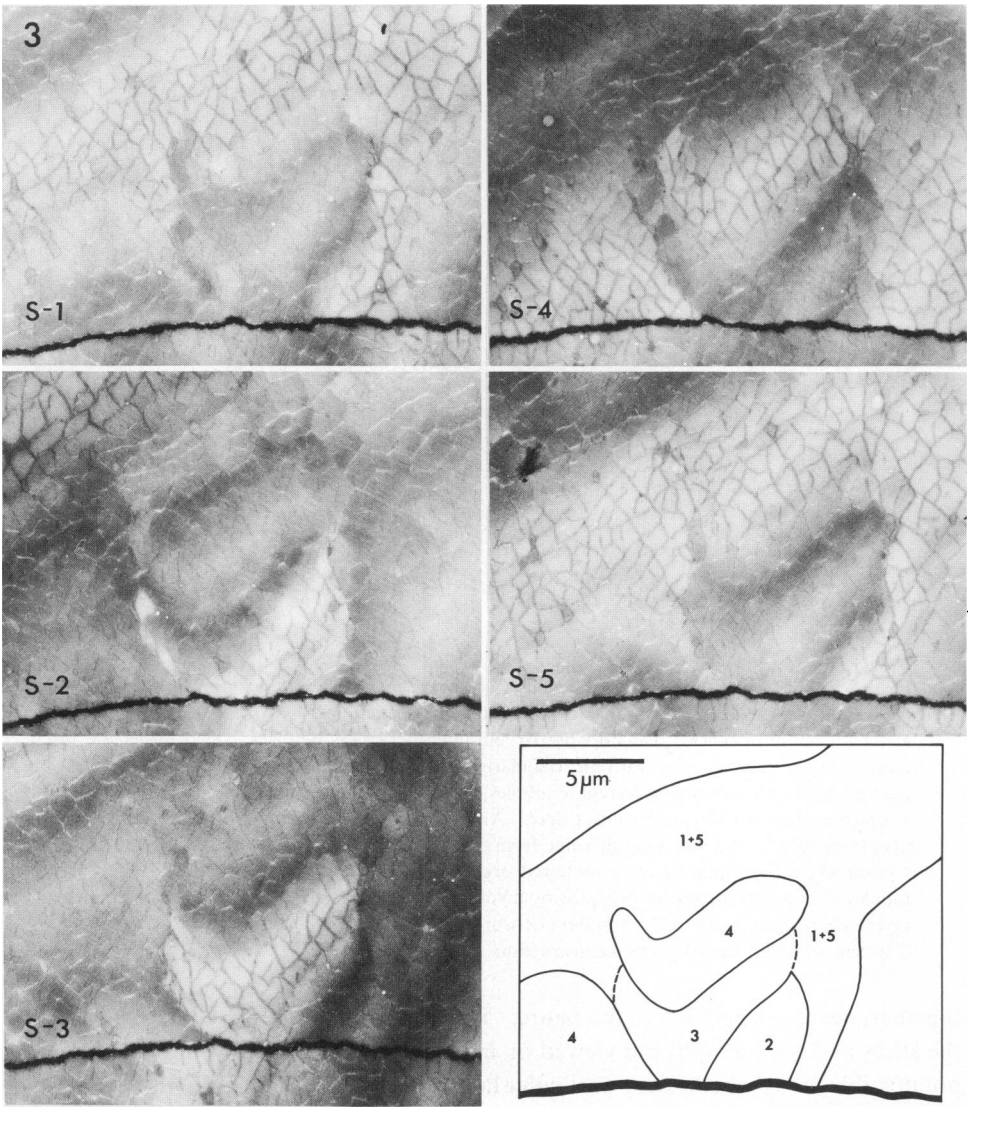

FIGURE 3 Illustrations of part of another fiber, showing how the analysis of T system helicoids was done and how the results are interpreted. Panels S-1 to S-5 show a series of micrographs from five serial slices through one region near one edge of the fiber. The helicoid is located against the fiber surface, inside a C shaped region of T system that appears in slices 1 and 5. The ramp of the helicoid appears in segments in the micrographs of slices 2-4. At the bottom right there is a tracing of the region occupied by T system network in slices 1-5. Dashed lines indicate regions where the T system does not connect directly into the next slice, which are equivalent to dislocations in the plane of the T system.

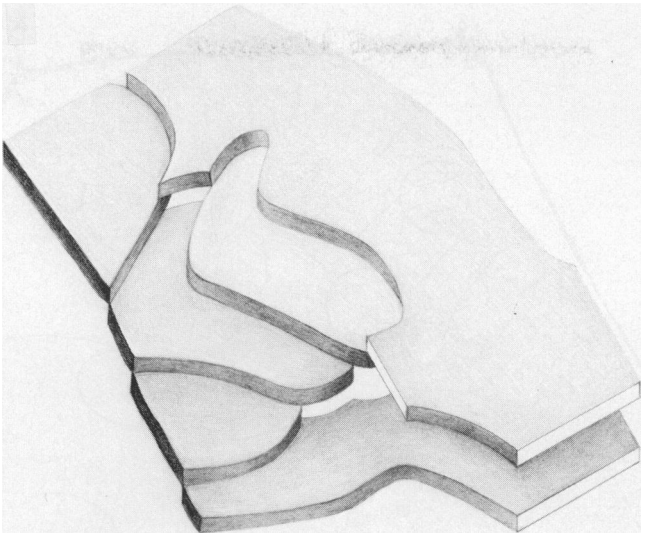

FIGURE 4 Drawing of the area reconstructed in Fig. 3 as a pile of slabs, each slab representing the area occupied by T system in one slice. Dislocations appear as holes in the stack.

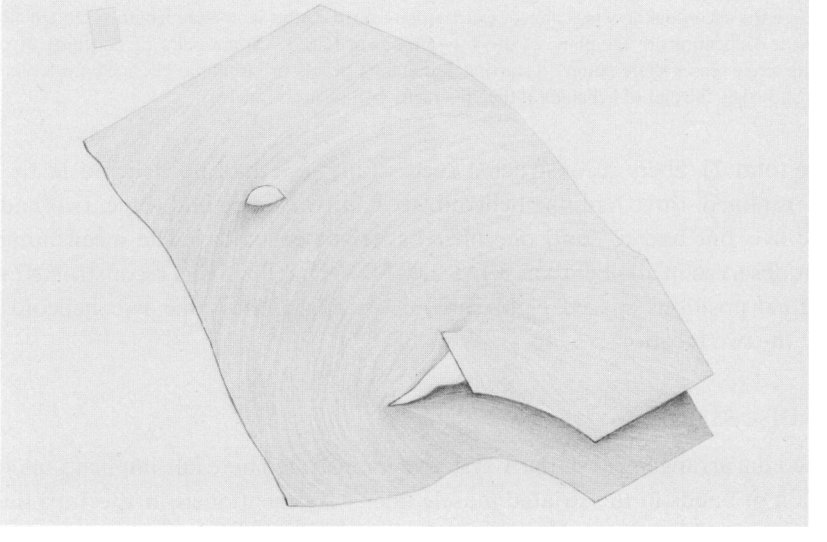

FIGURE 5 A smoothed version of the previous drawing, showing how the plane of the T system might have been arranged before it was cut into slices and projected into two-dimensional micrographs. Dislocations appear as holes in the plane made by tearing and shearing the plane in the longitudinal direction of the fiber.

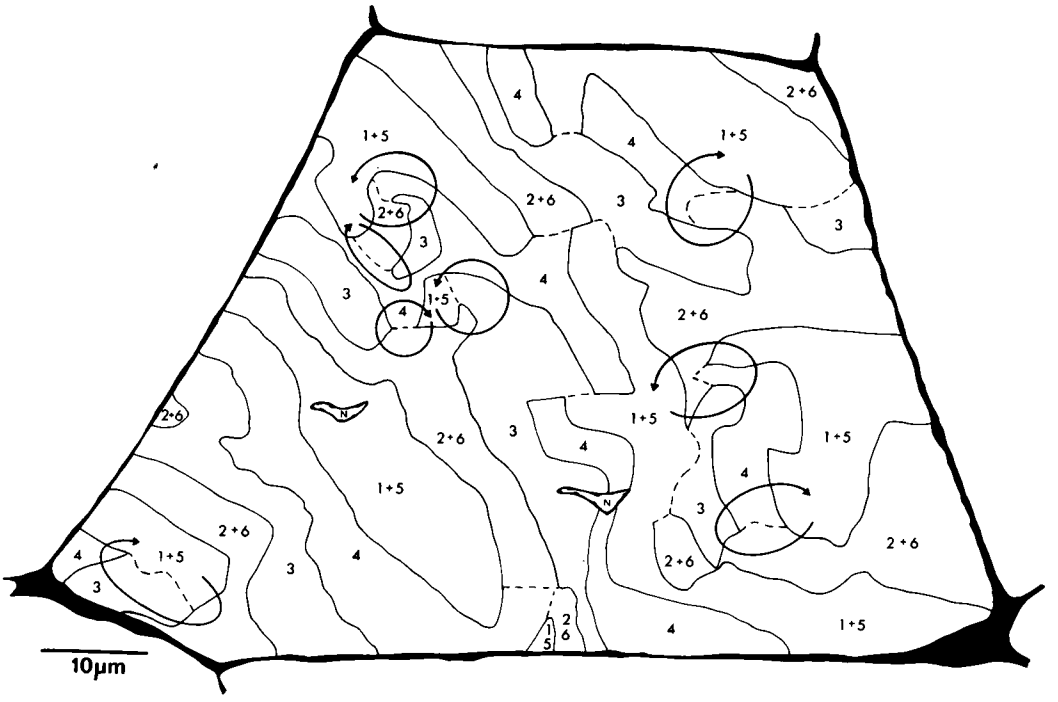

FIGURE 6 A complete reconstruction of another fiber. This fiber has eight helicoids in the cross section shown, as indicated by arrows. Each arrow runs from slice 1 through slices 2, 3, and 4 to slice 5: in one case the helicoid first passes "up" to slice 6 and "back" to slice 5 to complete the turn. The area occupied by T system in slices 1 and 5 and slices 2 and 6 are almost identical, because the slice thickness was almost exactly one-fourth of the sarcomere length. Dotted lines indicate dislocations in the plane of the T system, as in Fig. 3. Some pairs of helicoids of opposite screw senses share common starting and ending points or common intermediate levels as best shown by the pair of helicoids at the upper left. N indicates a nucleus.

Of the total 21 fibers reconstructed successfully, one had no helicoid in the cross section examined, three had one helicoid, six had two, three had three, two had four, four had five, one had six, and one fiber had eight helicoids. The mean number of helicoids observed in all fibers was 3.1 $\pm$ 2.0 (SD). One fiber was reconstructed in two longitudinal positions spaced eight sarcomeres apart: the same five helicoids were found at the two locations.

DISCUSSION

The helicoidal arrangement of the T system demonstrated here has implications for the disposition of bands in the striated muscle fiber. As mentioned in the Introduction, observations on thin sections and freeze-fracture replicas of skeletal muscle fibers of amphibians show that the T systems are largely confined to the regions of the interfibrillar space adjacent to the Z disks of the myofibrils. It then follows that the striations of the myofibrils must also have a helicoidal disposition. Descriptions of heli-

coidal striations in striated muscle fibers are not absent from the literature, though they have not often been quoted in recent years. Tiegs described helicoids in the striations of both skeletal and cardiac muscle fibers more than 50 yr ago (Tiegs, 1922, 1923, 1924), and reviewed his observations in 1955 (Tiegs, 1955). In his review, he mentions several 19th-century proponents of helicoidal striations, and provides a quotation to the effect that Leeuwenhoek knew of them in 1718, only 6 yr after he reported his first observation of striations as circular rings or girths around the striated muscle fibers of a whale (Leeuwenhoek, 1712): we have not seen the 1718 reference cited by Tiegs (Tiegs, 1955).

Tiegs interpreted his light microscope observations as showing a double helicoid. If this were correct, our reconstructions would have required a number and thickness of slices equivalent to two sarcomere lengths before a full turn of the helicoid was completed. In no case did we see this. All of the 66 helicoids we observed were completed in the length of one sarcomere, and we must disagree with Tiegs on this point, at least for frog sartorius muscle fibers.

It is not at all clear what physiological implications this helicoidal arrangement of bands and membrane networks might have. If the fibrils are independent force generators acting in parallel, then the fact that their striations are not in perfect planar alignment laterally should have no mechanical implications. There is nothing about the helicoidal arrangement of bands as we have observed it that is inconsistent with a constant sarcomere spacing among all fibrils.

The T system and sarcoplasmic reticulum networks must have dislocations associated with the helicoids. We have observed these, and have also found other dislocations not associated with helicoids. Recently, Peachey (1975) reported that longitudinally oriented T tubules were common in regions of band dislocations. This may function to maintain a certain degree of continuity of the T system across the fiber in spite of the dislocation. The small quantity of these longitudinal T tubules, the small amount of T system network involved in ramps as part of helicoids, the low slope of these ramps, and the presence of irregularities and undulations in the plane of the T system anyway, all suggest that the effect of the helicoidal arrangement per se on the cable properties of the T system may be small. Mathias[1] has analyzed a helicoidal T system theoretically, and has shown that the helicoid makes only an unimportant contribution to the passive electrical properties of the T system.

We are left with the conclusion that the helicoidal arrangement of bands and membrane networks in muscle fibers is an interesting anatomical arrangement whose physiological importance, if any, is not obvious at present.

This paper is dedicated to the memory of our colleague and friend L. L. Constantin, who died on 7 November, 1974, in the midst of a remarkable research career in muscle cell physiology.

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[1]Mathias, R. T. An analysis of the electrical properties of a skeletal muscle fiber containing a helicoidal T system. Submitted for publication to Biophys. J.

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