ELECTRON MICROSCOPE STUDY OF THE SARCOPLASMIC RETICULUM AT THE Z LINE LEVEL IN SKELETAL MUSCLE FIBERS OF FETAL AND NEWBORN RATS

SHEPPARD M. WALKER, G. RANDOLPH SCHRODT, and MAXINE BINGHAM. From the Departments of Physiology and Pathology, University of Louisville School of Medicine, Louisville, Kentucky 40202

INTRODUCTION

In adult skeletal muscle fibers of reptiles, birds, and mammals each sarcomere contains two transversely oriented triads. The triads are located near the two A–I junctions in the sarcomere. The central element of the triad is a tubule formed by invagination of the sarcolemma and the transverse tubules thus formed are called the T system (T). The two lateral elements of the triad are terminal segments of the sarcoplasmic reticulum (SR). The terminal segments of SR are continuous with two networks of tubules, one traversing the M line level, the other the Z line level. Although a majority of the tubules in these networks are longitudinally oriented, many of the tubules make transverse connections in the networks (6). The interfibrillar SR and T of adult mammlain muscle fibers have been described in many electron microscope studies as a continuum of sleeves with each fibril surrounded by one of the sleeves. In a recent report (11) concerned primarily with triads in muscle fibers of the 19 day fetal rat, an interfibrillar network of tubules was also described. This network of tubules, which extends uninterrupted in successive sarcomeres, is continuous with lateral elements of triads. On the basis of this continuity the network was identified as SR (11). In further electron microscope studies on skeletal muscle fibers of fetal and newborn rats, close contact between the network of tubules and the Z line has been observed. A review of the literature shows that several investigations have called attention to the close association of interfibrillar tubules and the Z line. In early electron microscope studies on breast muscle fibers of the adult fowl, Bennett and Porter (2) observed strands of SR extending from Z line to Z line across the interfibrillar space. Bennett and Porter (2) described the SR as sleeves around each fibril and viewed the strands of interfibrillar SR at the Z line level as structures attached to SR encircling the Z line. In a recent study on developing muscle cells in embryonic and posthatched chicks, Allen and Pepe (1) found distinct Z lines in the 5 day embryo. The Z lines were occasionally associated with the interfibrillar tubular system. Tubules surrounding myofilaments at approximately 1.5 μ intervals were found in the 4 day embryo. Although no distinct Z lines were seen in the 4 day embryo, material resembling the Z line was found in place of the tubule in several cases. In fibers from breast muscle of the posthatched chick, small tubular connections were seen between interfibrillar vesicles and the tubules surrounding the fibrils at the Z line level (1). In a report on cultured breast muscle fibers from the chick embryo, Shimada, Fischman, and Moscona (9) showed elegant electron micrographs of the interfibrillar network of SR tubules at the Z line level of the sarcomere. However, the relationship of the Z line to the network of tubules was not considered in that report (9). At the same time, another report (5) on cultured breast muscle fibers from the chick embryo showed the interfibrillar network of tubules and called attention to the fact

that nothing is known of the events which order the Z line and/or the I band of the growing myofibril into their characteristic relationship with the SR or T system tubules. Ezerman and Ishikawa (5) suggested that one or both of these systems of tubules may play some role in the positioning of the sarcomeres of adjacent fibrils in register in mature muscle. Bergman (3) observed attachment of Z lines to large vesicles in electron microscope studies on myogenesis in fetal rats. The vesicles were interpreted as part of the SR (3).

The purpose of the present study is to examine the points of contact between networks of SR tubules and Z lines and to look for evidence of structural relationships at these points. The results of the study on skeletal muscle fibers of fetal and newborn rats suggest that connections exist between SR tubules and Z lines.

METHODS

The beginning of embryonic development of the rat fetuses was assumed to be about simultaneous with detection of sperm in the vaginal smear of females examined in the early morning. The gestation period is usually 21 days in the rat colony used. Gastrocnemius muscles were removed from rat fetuses taken from the uterus on the 18th to 21st days of pregnancy and fixed for 1–7 days in 3% glutaraldehyde by the method of Sabatini et al. (8) with 0.1 M phosphate buffer. Gastrocnemius muscles removed from rats 1 hr and 24 hr after parturition were fixed by the same method. Bundles of fibers from these muscles were postfixed in OsO_4 by the Palade method as modified by Caulfield (4). The fibers were dehydrated for 10 min in 50% 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% alcohol, the fibers were embedded in Maraglas and sectioned with an LKB microtome. Thick sections of the tissues were stained with toluidine blue and examined with a light microscope to obtain approximate orientation for longitudinal and cross-sections of fibers. The thin sections for electron microscope studies were triple-stained with lead, uranyl acetate, and lead. A modification of the Reynolds method (7) was used for lead staining. Sections exhibiting gray or light silver interference colors were examined with a Siemens Elmiskop IA electron microscope.

RESULTS

The interfibrillar space is somewhat wider in fetal than in adult rats muscle fibers. The prominent structure of the SR is a network of tubules (*SRN* in Fig. 1) that extends throughout each sarcomere and in successive sarcomeres. The SR tubules in this network form a labyrinth of diverging and converging pathways. Usually the tubules are oriented along the longitudinal axis of the fiber. However, transverse orientation of tubules can be seen at all levels of the sarcomere (stars in Fig. 1).

The SR tubules of the network are closely associated with the Z lines (arrows in Fig. 1.) In longitudinal sections of fibrils this association shows a wide variety of SR tubule images at the Z line level. The SR tubule image may appear to be a lateral extension from the Z line (asterisks in Figs. 1 and 2). It may be shown as an approximate tubule cross-section apposed at the Z line level (arrows in the left and right sides of Fig. 2). It can be seen as a longitudinal section apposed at the Z line level.

To obtain a more complete visualization of the structural relationship between SR tubules and the Z line, cross-sections of fibers were examined. In these cross-sections transversely oriented tubules are found more frequently at the Z line level than at other levels in the sarcomere (Fig. 4). Long segments of SR tubules are frequently seen at the Z line level in fibrils showing exact cross-sections of the entire fibril (arrows in the left side of Fig. 4). In approximate cross-sections passing through the Z line and the I band of a given fibril, SR tubules are seen more frequently at the border of that part of the fibril showing the Z line (arrow in the center of Fig. 4). At all points where the limiting membranes of SR tubules are clearly shown, there is a space between the tubules and the Z line. Usually this space is about 100 A wide.

Now, studies of cross-sections of fibrils provide two advantages over observations made on longitudinal sections. First, they show a more complete view of the extent of tubular encirclement of the fibril at the Z line level. Second, exact cross-sections can be viewed with the assurance that the plane of section passes perpendicular to the surface of the fibril. The relation of the plane of the section to the surface of the fibril is of particular importance to a possible explanation of the variety of SR tubule images found at the Z line level in longitudinal sections of fibrils. It is obvious that the plane of section may be either oblique or perpendicular to the surface of the fibril in longitudinal sections. If it is oblique, the space between the SR tubule and the Z line may be obscured (asterisks in Figs. 1 and 2). If it is perpendicular, the space between the SR tubule and the Z line may be visible. Fig. 3 illustrates a longitudinal section of a

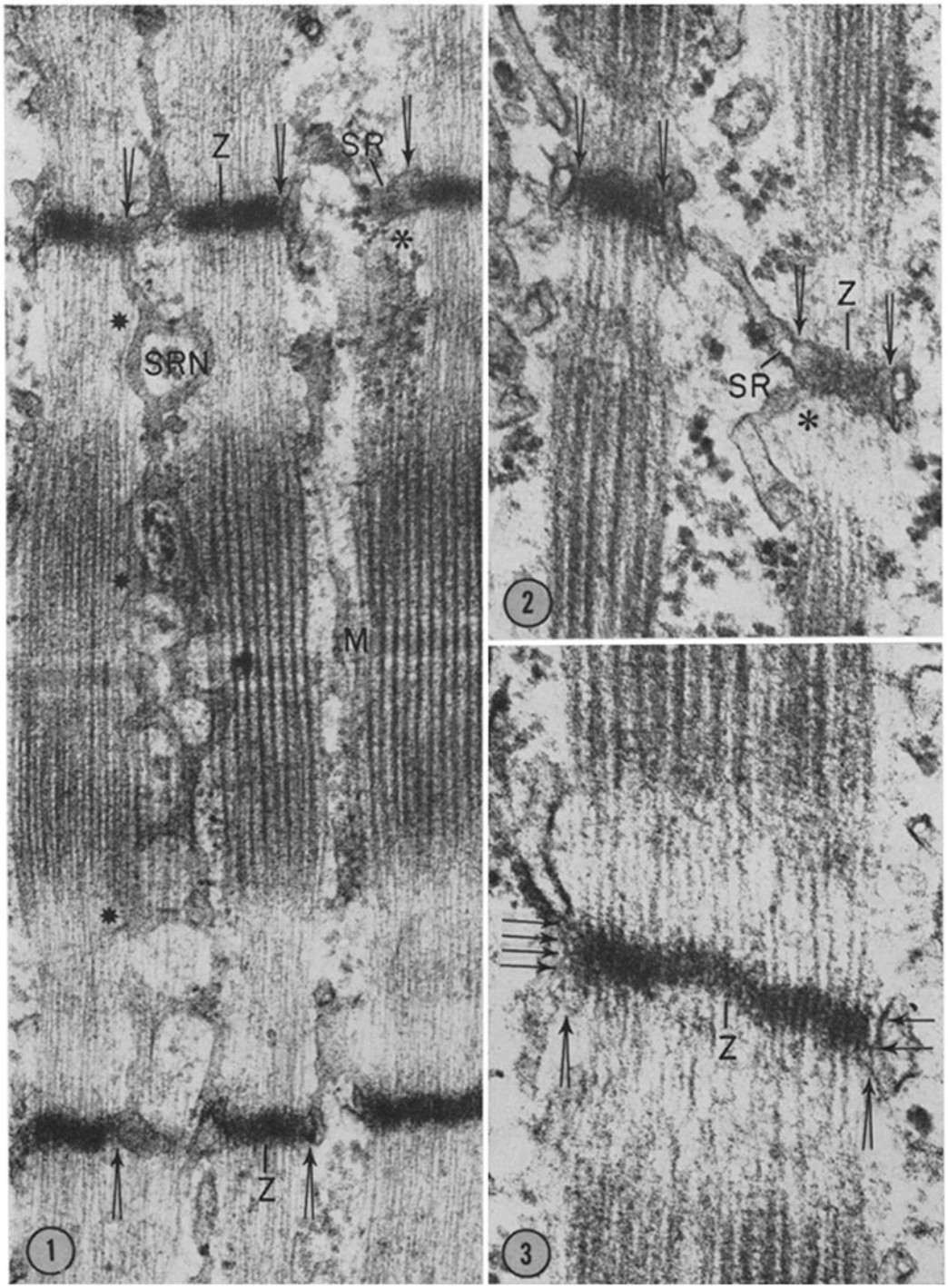
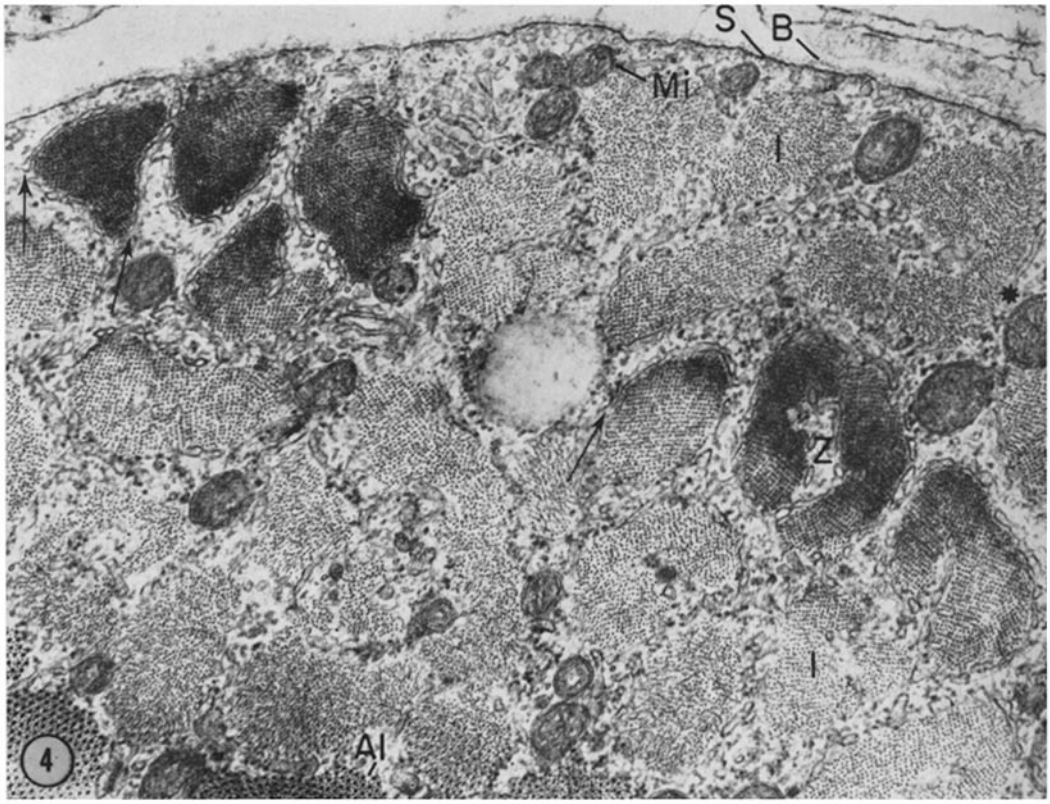


FIGURE 1 Electron micrograph showing a longitudinal section of a gastrocnemius muscle fiber removed from a newborn rat 1 hr after parturition. The arrows are directed toward points showing close association of SR tubules with Z lines. The continuity of the network of SR tubules (*SRN*) throughout the sarcomere is shown. Some of the transversely oriented tubules are indicated by stars. The asterisk is placed below a point where an SR tubule appears to be continuous with the Z line. *SR*, sarcoplasmic reticulum; *Z*, Z line; *M*, M line. × 50,000.

FIGURE 2 Longitudinal section of gastrocnemius muscle fiber from an 18 day rat fetus showing a close association (arrows) of SR tubules with Z lines (*Z*). The asterisk is placed below a point where an SR tubule appears to be continuous with the Z line. × 75,000.

FIGURE 3 Longitudinal section of gastrocnemius muscle fiber from an 18 day rat fetus showing electron-opaque strands (small arrows) traversing the space (double-stemmed arrows) between SR tubules and the Z line. × 87,500.



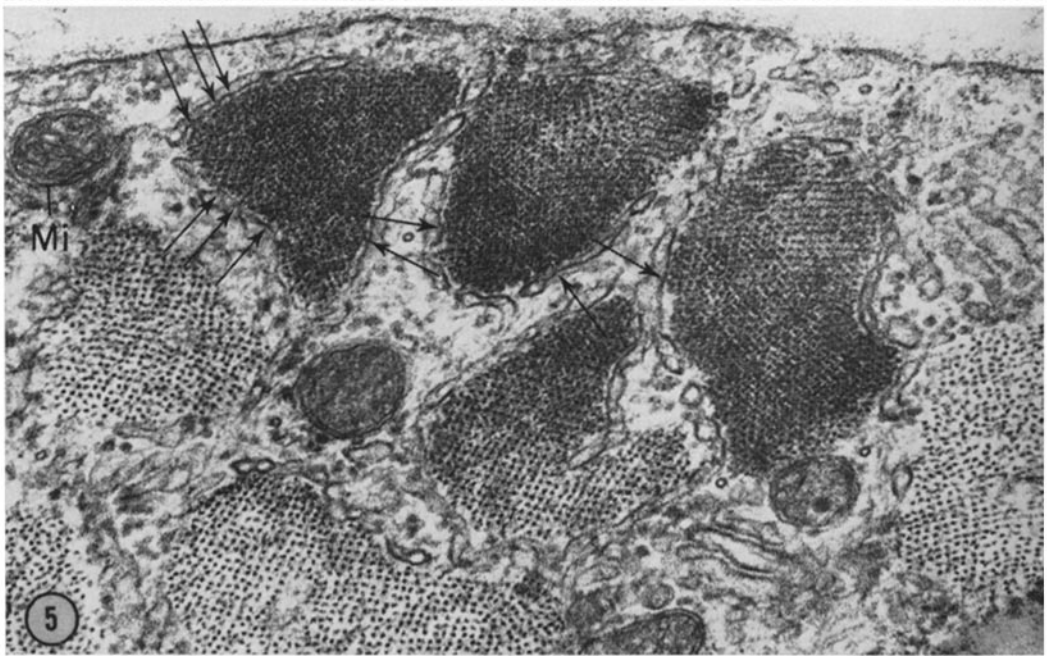


FIGURE 4 Cross-section of a gastrocnemius muscle fiber removed from a newborn rat 24 hr after parturition. The plane of the section passes through the Z line (*Z*) of several fibrils and through the I band (*I*) of others. Numerous SR tubules are seen at the borders of the Z lines (arrows). Star, transversely oriented SR tubule at the I band level; *Mi*, mitochondrion; *AI*, overlap of thick and thin filaments; *S*, sarcolemma; *B*, basement membrane. × 27,000.

FIGURE 5 A higher magnification of the upper left side of the micrograph shown in Fig. 4. The arrows are directed toward electron-opaque strands traversing the space between SR tubules and Z lines. × 55,000.

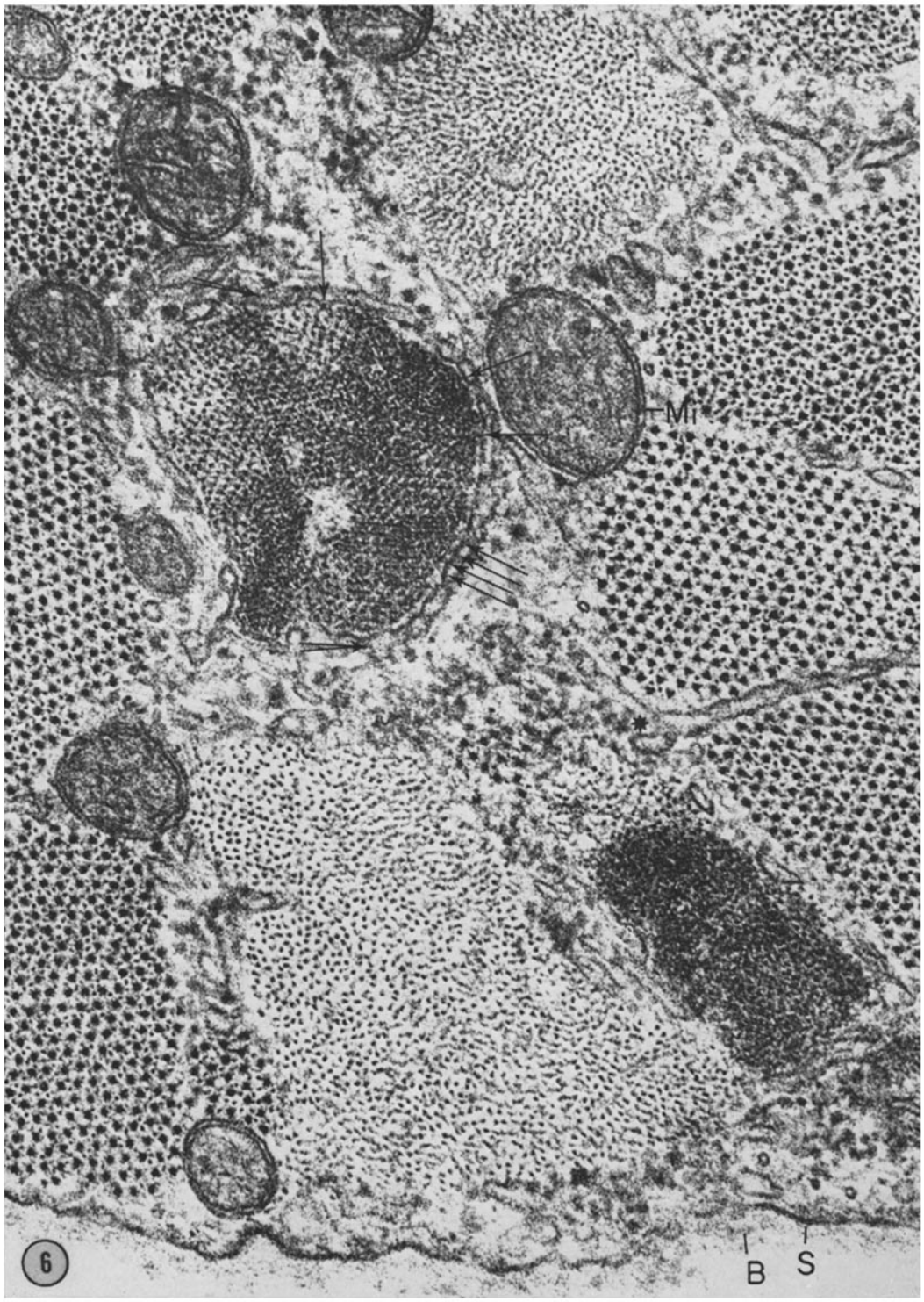


FIGURE 6 Cross-section of a gastrocnemius muscle fiber removed from a newborn rat 24 hr after parturition. The plane of the section passes almost exactly through the transverse axis of the fibril showing the Z line in the middle of the figure. This Z line is almost completely encircled by an apposed SR tubule (double-stemmed arrows). There are numerous electron-opaque strands traversing the space between the SR tubule and the Z line, some of which are indicated by small arrows. The SR tubules apposed at the Z line in the lower right side of the figure are shown intermittently. Star, transversely oriented SR tubule at the A band level; *Mi*, mitochondrion; *S*, sarcolemma; *B*, basement membrane. × 70,000.

fibril showing the space between the Z line and SR tubules on either side of it (double-stemmed arrows). The approximate cross-section of the tubule on the right side shows an image predictable from observation of longitudinal segments apposed at Z lines in cross-sections of fibrils (Fig. 4). The longitudinal section of the tubule on the left side of the Z line in Fig. 3 shows an image consistent with the observation that longitudinally oriented tubules of the SR network are frequently closely associated with the Z line (Fig. 1).

It is noteworthy that the space between SR tubules and the Z line in Fig. 3 is traversed by electron-opaque strands (arrows). Presumably these strands are identical with the strands observed more frequently in cross-sections of fibrils. The arrows in Fig. 5 are directed toward some of the electron-opaque strands that appear to be connections between SR tubules and Z lines. The Z line in the middle of Fig. 6 shows a virtually continuous SR tubule on the right side and some tubule segments of variable length on the left side. At numerous points the SR tubule appears to be connected with the Z line by electron-opaque strands. Some of the strands traversing the space between the SR tubule and the Z line are indicated by arrows. The lower side of Fig. 6 shows a Z line with the plane of section passing slightly oblique to the transverse axis of the fibril. Short segments of SR tubules are shown at the border of this Z line.

Transverse orientation of SR tubules is not infrequently found at the A band level (star in Fig. 6) and at the I band level (star in Fig. 4) of cross-sections. Similar transverse orientation of SR tubules is seen in longitudinal sections of fibers (stars in Fig. 1).

DISCUSSION

In the muscle fibers of 19-day fetal rats about 10% of the sections examined show triads (11). These triads are usually oriented along the longitudinal axis of the fibers near the level of the junctions of A and I bands. Triads are also sparsely distributed in muscle fibers taken from rats 1 hr after parturition. Therefore, it is not surprising to find that longitudinal sections of fibers from newborn rats show no triads (Fig. 1).

The observations of Allen and Pepe (1), showing SR tubules at the Z line level in longitudinal sections of muscle fibers from embryonic and post-hatched chicks, suggest a close association between SR tubules and Z lines. In longitudinal sections of ventricular and Purkinje fibers from hearts of guinea pig, rabbit, cat, dog, goat, and sheep, Sommer and Johnson (10) found a component of the SR that is closely associated with the Z line. However, studies on longitudinal sections of fibers show wide variations in the profiles of SR tubules associated with Z lines (Figs. 1–3). In cross-sections of fibrils a space with rather constant width is usually found (Figs. 4–6). The more consistent findings in studies on exact cross-sections of fibrils seem to warrant the suggestion that the electron-opaque strands across the space between SR tubules and Z lines are connections. Such connections might account for the observed fact that the fibril and the surrounding SR of a given fibril are in register. Furthermore, interfibrillar continuity of SR at the Z line level might contribute to transverse alignment of sarcomeres in adjacent fibrils across the fiber.

Studies on exact cross-sections of unstretched adult rat muscle fibers, fixed for prolonged periods in 3% glutaraldehyde, have not been made. It will be interesting to look for a structural relationship between SR and Z lines in adult fibers similar to that suggested for fibers of fetal and newborn rats.

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