SARCOPLASMIC RETICULUM AND T TUBULES IN DIFFERENTIATING RAT SKELETAL MUSCLE

A. M. KELLY

From the School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT

An electron microscope study has been made of the distribution of membrane couplings between the sarcoplasmic reticulum (SR) and either the plasmalemma or the T tubules in fetal and neonatal rat intercostal muscle. Within primitive muscle cells at 12 days of gestation, the SR forms both simple and specialized membrane junctions with the plasmalemma; caveolae are very few, and T tubules are not detected. Undifferentiated cells neighbor muscle cells. Occasionally these cells contain subsurface couplings between the endoplasmic reticulum and plasmalemmae. Possible relationships between these couplings and the peripheral couplings of muscle cells are discussed. By 15–18 days of gestation, caveolae and beaded T tubules, comparable to those of cultured muscle, develop; T tubules lie alongside myofibrils and are rarely transverse. SR couples both to T tubules and to plasmalemmae during this period. T tubules with lineal profiles appear after further development and their orientation transverse to A–I junctions becomes increasingly evident. Membrane couplings between SR and T tubules also increase in number, whereas the incidence of peripheral coupling declines rapidly Evidence suggests that peripheral couplings are swept into myotubes as caveolae proliferate and T tubules form. SR thus appears to initially couple with the plasmalemma and then to await T tubular growth. This contrasts with the developmental pattern described in cultured chick muscle in which peripheral couplings are not reported and T tubules with diads and triads occur at very primitive stages of muscle differentiation.

INTRODUCTION

The organization of the sarcoplasmic reticulum (SR) and the T system in muscle cells of newborn rats has recently been described by Schiaffino and Margreth (1969). This is the first detailed examination of these membrane systems in differentiating skeletal muscle in vivo since Veratti made his classic studies at the turn of the century (see reference 15). Investigation of the distribution of these membrane systems in the more primitive muscle of fetal animals is at present limited to isolated observations (16). By contrast, a great deal is known of the proliferation and arrangement of the sarcoplasmic reticulum and T system in cultured chick muscle as a result of the work of Ezerman and Ishikawa (1967) and Ishikawa (1968). These in vitro studies illustrate the presence of primitive T tubules at very early stages of muscle differentiation and provide a plausible explanation of the mode of T tubular growth. However, the authors are cautious in presuming that results obtained from their culture system apply to development in vivo. In particular, they stress the differences in speed of muscle development in vitro and in vivo and suggest that

the presence of the nervous system may influence T tubular growth.

For these reasons, a study of sarcoplasmic reticulum and T tubules in differentiating rat intercostal muscle cells at progressive stages of development is presented. The results have recently appeared in abstract form (5).

MATERIALS AND METHODS

Intercostal muscles were obtained from fetal rats after 12, 15, 18, and 20 days of gestation and from neonatal rats at birth. The materials were fixed according to the methods of Trelstad et al. (1966) (12 and 15 days), Kelly and Zacks (1969) (18 and 20 days), and Karnovsky (1965) (birth), and embedded in Araldite. Thin sections were stained in uranyl acetate and in lead citrate (9), using R. C. A., 3 F., Siemens Elmiskop, and A. E. I. electron microscopes.

OBSERVATIONS

12 day fetal rat intercostal muscle consists of cellular aggregates of both undifferentiated and primitive muscle cells surrounded by large, fluid-filled spaces. Within the cellular aggregates plasma membranes of neighboring cells approximate one another over considerable distances (Fig. 1). Apart from the presence of myofilaments loosely organized into myofibrils, the cytoplasm of undifferentiated cells and muscle cells is comparable; both cells contain prominent Golgi complexes, many ribosomes organized into polysomes, rough endoplasmic reticulum, and, at the periphery, profiles of smooth endoplasmic reticulum which make focal membrane couplings with the plasma membrane. Within undifferentiated cells, these intracellular membrane couplings are infrequent and consist of short, smooth-walled profiles of endoplasmic reticulum which run parallel to the plasma membrane and are separated from it by an intermembranous space of 100–150 A. Ill defined densities occasionally occupy this intermembranous space. Structures similar to these have been described in a wide variety of cells and are termed subsurface cisterns (7, 10, 13). In contrast to undifferentiated cells, coupling between sarcoplasmic reticulum and the plasma membranes within muscle cells is relatively common. Comparable structures, termed peripheral couplings (3), are a normal feature of some cardiac muscle. Many of the peripheral couplings in the present system are simple in structure, as in the undifferentiated cells (see Fig. 4), but a few are more specialized and contain periodic opacities in the intracellular space between the coupled membranes (Fig. 2). These more specialized junctions, which appear at very early stages of myofilament formation and compare with the sarcoplasmic reticulum–T tubular membrane junctions of mature skeletal muscle, are randomly distributed and without any association with myofibrils.

Caveolae (17) are sparse and rarely occur clustered in groups within either undifferentiated cells or muscle cells at 12 days of gestation. An apparent absence of T tubules within primitive muscle cells may correlate with this observation.

At 15 days of gestation the organization of the developing muscle compares with that at 12 days. Cells containing myofilaments, however, are more numerous and larger. Within these early muscle cells, both simple and specialized forms of SR–plasmalemmic couplings are frequent (Fig. 4). Commonly, the plasmalemma is depressed into the cell where it is coupled with the sarcoplasmic reticulum.

In addition to coupling with the plasmalemma, the sarcoplasmic reticulum also couples with the walls of vesicles lying near the borders of muscle cells (Fig. 4). The latter couplings include both simple and specialized forms; they are not as numerous as peripheral couplings, as Table I illustrates, but their presence indicates the development of a transverse tubular system. The vesicles have clearly defined walls and their electron-lucent lumina measure 2000–4000 A in diameter. Dual couplings formed by cisterns of SR with both the plasma membrane and T system vesicles are relatively common owing to the proximity of the vesicles to the cell wall (Fig. 3).

Caveolae are more numerous within 15-day than within 12-day fetal muscle cells, and occasional small clusters of them arranged as rosettes or short vesiculated tubules occur. Membrane continuity within these vesicular aggregates is common. The sarcoplasmic reticulum rarely attaches to the walls of the small caveolae, even though they may lie adjacent to profiles of SR or have membrane continuity with large vesicles to which SR is coupled (Figs. 3 and 8). Ezerman and Ishikawa (1967) and Ishikawa (1968) have previously suggested that proliferation of caveolae is associated with T tubular growth in cultured chick muscle cells; the present coincident appearance of clusters of interconnected caveolae and of T tubules indicates that this concept is also relevant to muscle development in vivo. In contrast,

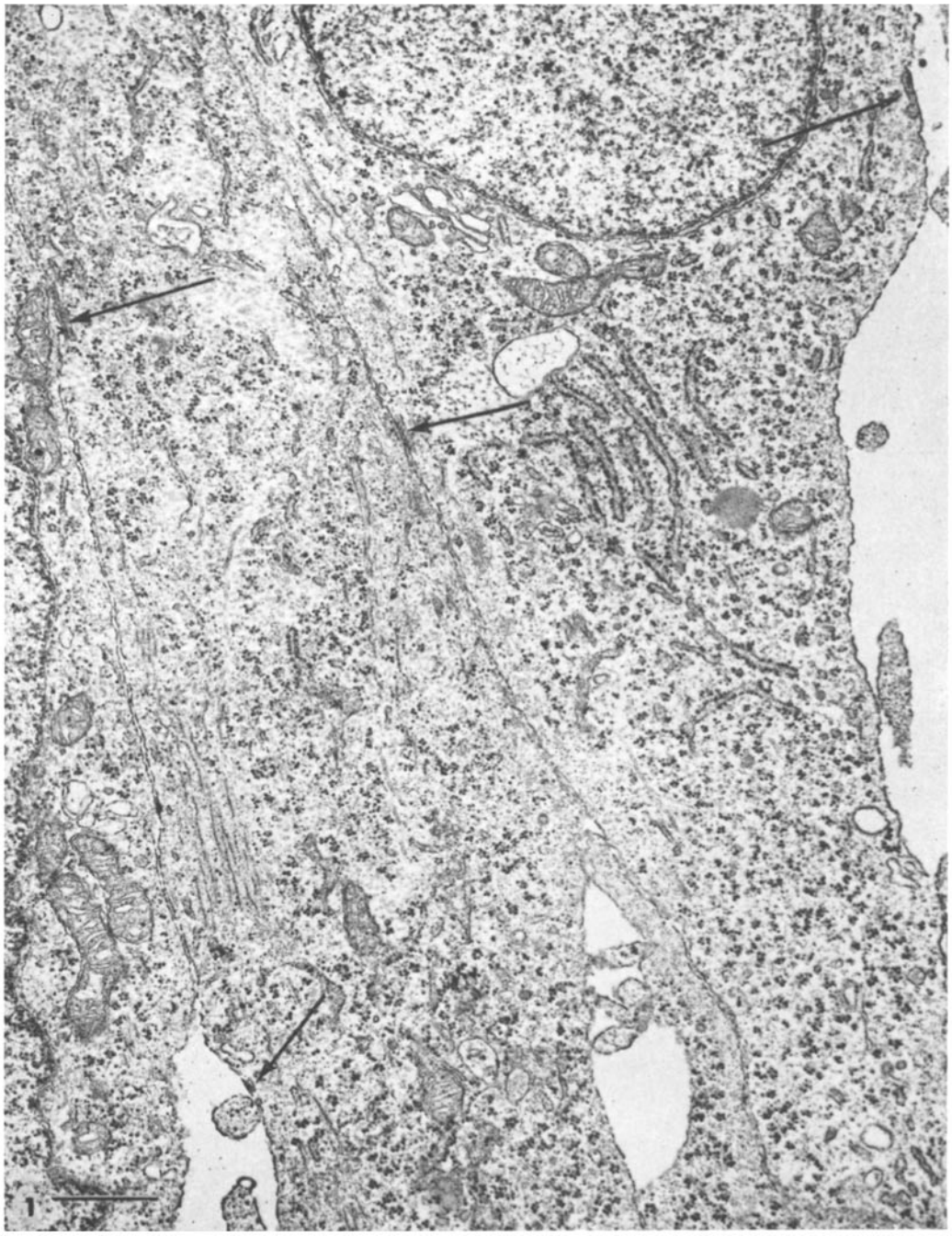


FIGURE 1 12 day rat intercostal muscle. Three muscle cells at very primitive stages of differentiation lie close to one another. Their cytoplasm contains many polysomes, profiles of rough endoplasmic reticulum, and a few loosely organized myofilaments. At the periphery of these cells are small opacities (arrows) caused by membrane coupling between SR and the plasma membrane. 1 μ marker. $\times$ 15,000.

TABLE I

The Relative Frequency of Coupling between the Sarcoplasmic Reticulum and either the Plasma Membrane or T Tubules at Successive Stages of Intercostal Muscle Development

	Peripheral coupling between the sarcoplasmic reticulum and the plasma membrane	Coupling between the sarcoplasmic reticulum and T tubules
12 days of gestation	100	0
15 days of gestation	84	16
18 days of gestation	57	43
20 days of gestation	18	82
Birth	3	97

These figures were obtained by surveying a number of muscle cells and recording the location of the first 100 couplings encountered at each of five stages of development.

however, caveolae do not appear to be as abundant in primitive rat fetal muscle cells as is described within comparable cultured chick cells, an observation which may relate to the greater rapidity with which muscle cells develop in vitro. In addition, the honeycomb, T tubular networks described by Ishikawa (1968) in cultured muscle are not found in fetal rat intercostal muscle cells.

At 18 and 20 days of gestation, the developing muscle consists of large myotubes measuring up to 20 μ in diameter, with smaller, less differentiated muscle cells and undifferentiated cells clustered around their walls (6). The numbers of peripheral couplings and the organization of the T system, however, differ between these two stages.

At 18 days of gestation, membrane couplings between the sarcoplasmic reticulum and either the plasmalemma or the walls of vesicles within myotubes are numerous (see Table I). Both simple and specialized forms of membrane junction are present and, at some of the more specialized junctions, cisterns of sarcoplasmic reticulum are distended and contain granular material comparable to that within lateral cisterns of triads in mature muscle (Figs. 5 and 6). Membrane couplings between sarcoplasmic reticulum and depressions of the plasmalemma are more numerous than at 15 days of gestation (Fig. 6), and cisterns of sarcoplasmic reticulum which couple both with plasmalemmal depressions and with the walls of vesicles in the subjacent sarcoplasm are relatively common (Figs. 5 and 7).

Caveolae and tortuous T tubules are more prevalent near the plasma membrane of 18-day fetal myotubes than previously; membrane continuity between the plasmalemma and T tubules is not infrequently encountered (Fig. 8). Comparable to those described in vitro (1), T tubules are tortuous and beaded and their diameter measures 300–800 A, characteristically increasing to as much as 3000 A where local coupling with sarcoplasmic reticulum occurs (Fig. 8).

In contrast to 18 days of gestation, sarcoplasmic reticulum is rarely coupled to plasma membranes at 20 days of gestation. Where couplings with the T system occur, not only are T tubules expanded, but also cisterns of sarcoplasmic reticulum are commonly distended and measure up to 0.5 μ in diameter. They contain a finely structured, threadlike material within their lumina (Fig. 10). Schutta and Armitage (1969) and Ovalle (1970) have recently described similar distentions of the lateral cisterns of SR in human thyrotoxic myopathy and normal rat intrafusal fibers, respectively, but the significance of these distentions with respect to muscle function is unclear.

Near the plasma membrane of 20-day myotubes, T tubules are tortuous, beaded structures comparable both to those of 18-day fetal myotubes and to those of cultured chick muscle (1). Between myofibrils in the deeper sarcoplasm, however, T tubules are frequently less curved and more lineal than at the cell periphery (Fig. 9). The diameter of these straighter T tubules measures 800–1000 A and increases to as much as 4000 A where couplings with the sarcoplasmic reticulum occur. Also present are parallel arrays of tubules which cannot be distinguished from Golgi complexes in the absence of coupling to the sarcoplasmic reticulum (Fig. 10).

Walker and Schrodt (1967) have reported that, in 19 day fetal rat gastrocnemius muscle, T tubules and triads are longitudinally oriented between myofibrils and are rarely disposed transverse to the A-I junctions of myofibrils. The present study correlates with these observations; at 20 days of gestation, almost all T tubules of intercostal muscle are disposed alongside myofibrils and transverse orientation is unusual.

Between 20 days of gestation and birth, considerable changes occur in rat intercostal muscle. Muscle cells mature to myofibers packed with myofibrils, and the cells segregate from one

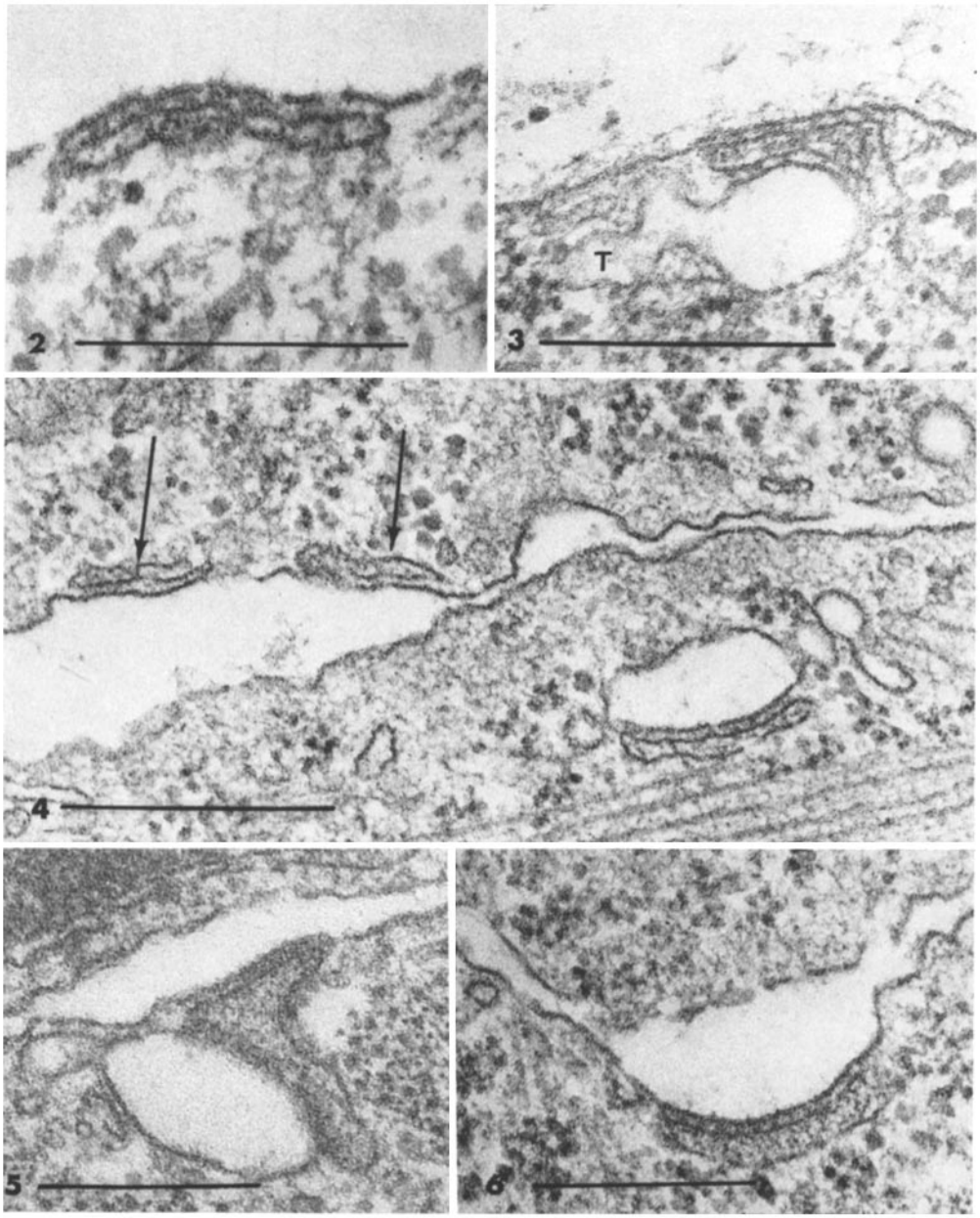


FIGURE 2 12 days of gestation. Membrane coupling between sarcoplasmic reticulum and the plasma membrane of a primitive muscle cell. Ill defined periodic opacities occur in the intracellular space between the coupled membranes. 0.5 μ marker. × 90,000.

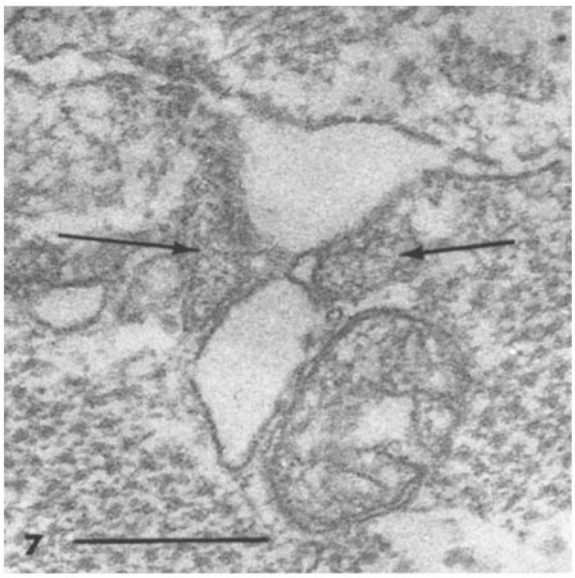
FIGURE 3 15 days of gestation. A profile of sarcoplasmic reticulum forms a dual coupling with both the plasma membrane and the walls of a primitive T tubule (*T*) within a myotube. This form of coupling is common at this stage of development, probably owing to the peripheral distribution of the primitive T system. 0.5 μ marker. × 80,000.

FIGURE 4 15 days of gestation. Sarcoplasmic reticulum coupled to the walls of a vesicle within a myotube indicates the development of a primitive T system. Note adjacent small vesicles and simple peripheral couplings in neighboring cell (arrows). 0.5 μ marker. × 74,000.

FIGURE 5 18 days of gestation. A cistern of SR which is distended and contains granular material is coupled both to the plasma membrane and to a T system vesicle in the adjacent sarcoplasm. 0.5 μ marker. × 60,000.

FIGURE 6 18 days of gestation. Sarcoplasmic reticulum is peripherally coupled with the plasma membrane. Where this occurs, the plasma membrane is depressed into the cell. 0.5 μ marker. × 60,000.

another. Within these myofibers the sarcoplasmic reticulum couples to T tubules and very rarely to the plasmalemma. T tubules follow a branching course alongside myofibrils. In the vicinity of I bands, distentions of these tubules and couplings with sarcoplasmic reticulum are invariably present; not all T tubules are swollen where they couple with SR as was the case earlier in develop-



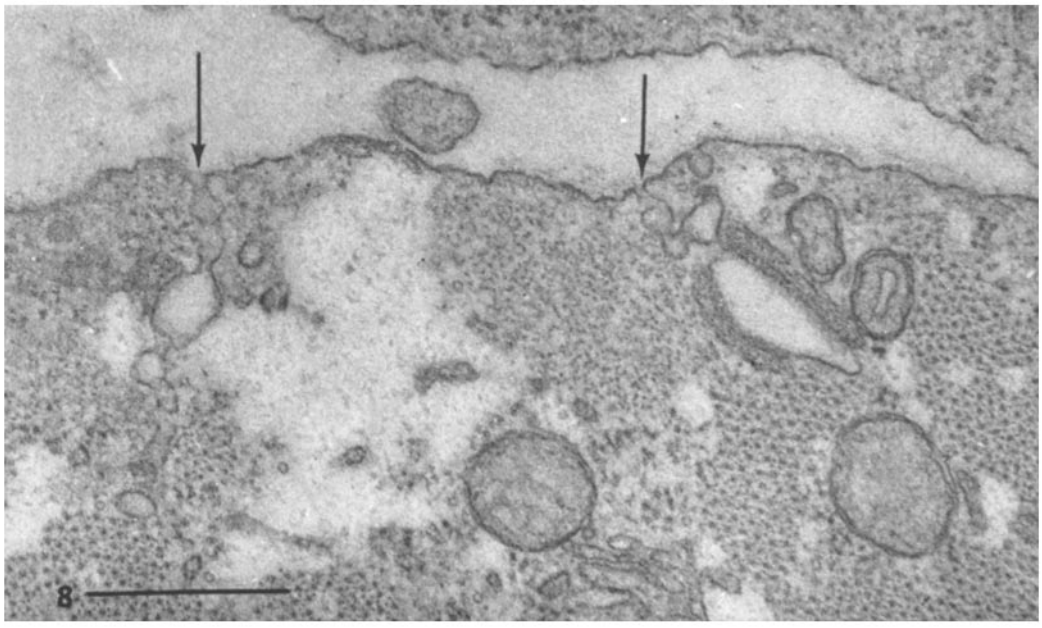
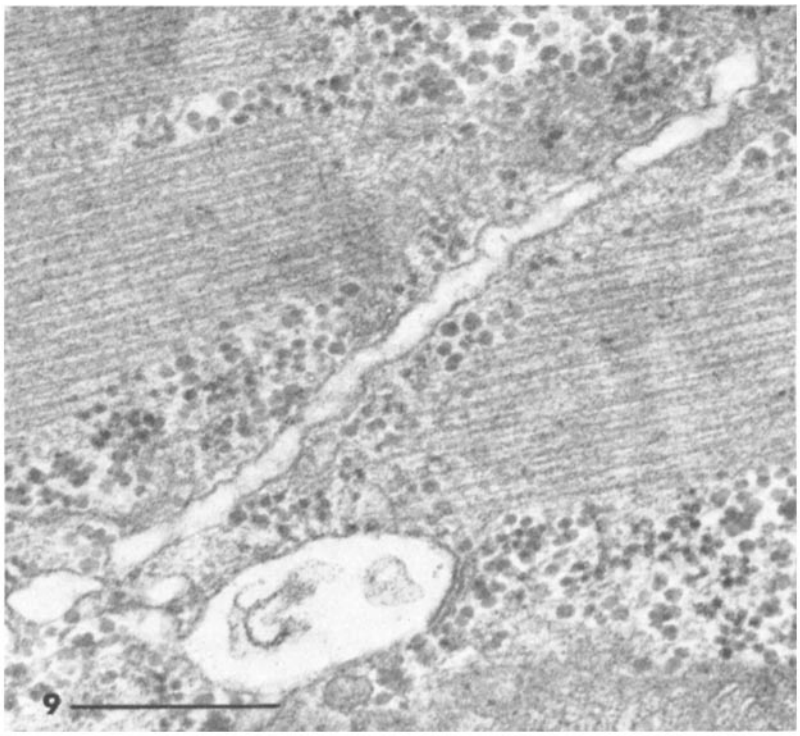


FIGURE 7 18 days of gestation. Cisterns of sarcoplasmic reticulum (arrows) peripherally coupled to vesicular-like depressions of the plasma membrane and to adjacent T system vesicles. 0.5 μ marker. $\times$ 54,000.

FIGURE 8 18 days of gestation. At two sites (arrows) membrane continuity between the plasma membrane and T tubules occurs. T tubules have a beaded contour and their lumina are characteristically distended where couplings with the sarcoplasmic reticulum occur. 1.0 μ marker. $\times$ 27,000.



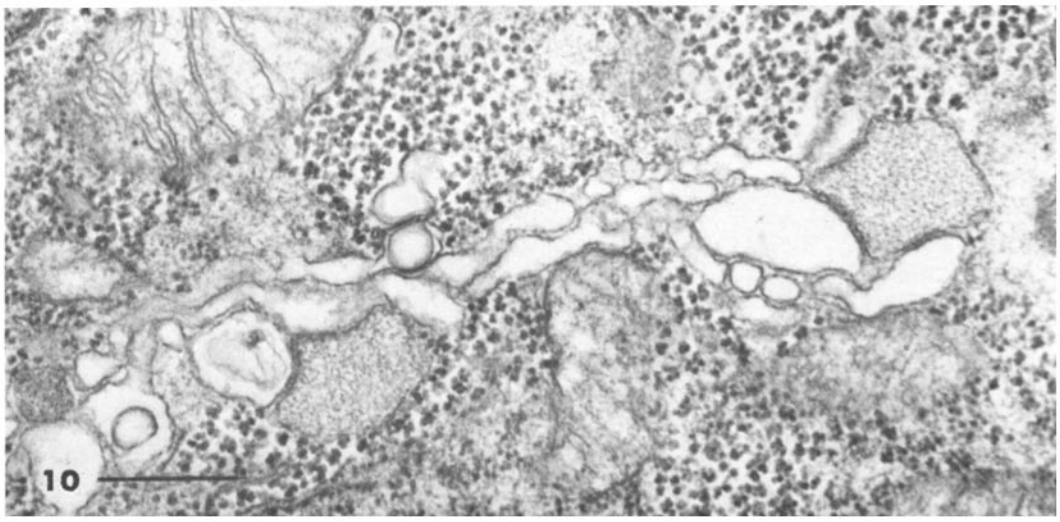


FIGURE 9 20 days of gestation. A long, relatively straight T tubule lying between myofibrils in a myotube. This tubule measures 600–1000 A in width and expands to 3000 A where couplings with sarcoplasmic reticulum occur. 0.5 μ marker. × 55,000.

FIGURE 10 20 days of gestation. Parallel arrays of T tubules surrounded by much glycogen occur within a myotube. Distended cisterns of sarcoplasmic reticulum couple to these tortuous tubules and facilitate their differentiation from the Golgi complex. 0.5 μ marker. × 40,000.

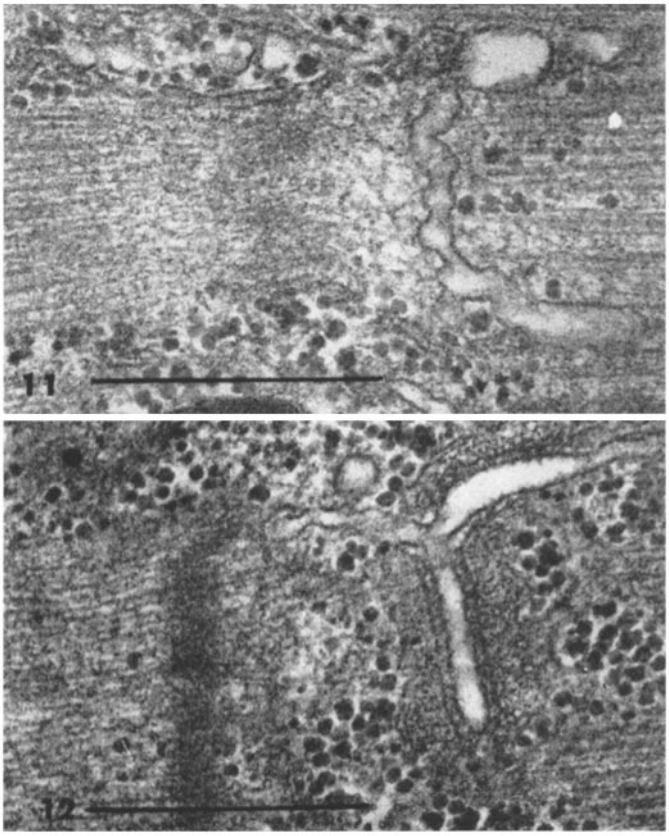


FIGURE 11 Myofiber at birth. A tortuous, beaded T tubule traverses the A-I junction of a myofibril unassociated with the sarcoplasmic reticulum. It measures 250–600 A in width. 0.5 μ marker. × 75,000.

FIGURE 12 Myofiber at birth. A T tubule coupled with lateral cisterns of SR follows a straight course across the A-I junction of a myofibril. The T tubule measures approximately 400 A in width. 0.5 μ marker. × 75,000.

ment. Nor do all T tubules which traverse the A-I junctions of myofibrils couple with sarcoplasmic reticulum. Many "naked" T tubules cross A-I junctions and follow a tortuous course as they do so (Fig. 11). By contrast, those that traverse A-I junctions and couple with sarcoplasmic reticulum are straight (Fig. 12). Such straight, transversely oriented T tubules measure 400 A in diameter, a dimension which is analogous to that of mature T tubules.

DISCUSSION

The present study, which describes the development of the T system and the formation of specialized connections between the sarcoplasmic reticulum and either the plasma membrane or T tubules in differentiating muscle cells in vivo, permits comparison with descriptions of the development of these same membrane systems within cultured chick muscle cells (1). The most obvious differences between the two systems are (*a*) the occurrence of peripheral couplings and (*b*) the stage of muscle cell differentiation at which T tubules commence formation. In cultured muscle cells, Ezerman and Ishikawa (1967) describe the presence of numerous small vesicles and of primitive T tubules coincident with the earliest stages at which myofilaments can be detected. In these primitive cells, T tubules are composed of chains of partially fused vesicles or caveolae. This configuration led Ishikawa (1968) to suggest that T tubules develop by repeated caveolation. Neither Ezerman and Ishikawa (1967) nor Ishikawa (1968) describe peripheral

couplings in the cultured material. By contrast, muscle cells at comparable early stages of differentiation in vivo contain very few caveolae (Fig 1); those present lie alone, not in groups, and so far no primitive T tubules have been detected in these cells. Furthermore, primitive muscle cells in vivo contain numerous peripheral couplings between the sarcoplasmic reticulum and the plasma membrane (Figs. 1–4). A few of these junctions possess periodic opacities in the interspace between the apposed membranes (Figs. 2 and 3), and morphological similarity of these to triadic junctions of mature muscle indicates that, despite the absence of T tubules, specific membrane specialization of the sarcoplasmic reticulum and plasmalemma takes place at about the same time as myofilaments begin to form.

Observations in the present study raise the possibility that there is a developmental progression from the subsurface cisterns of undifferentiated cells to the peripheral couplings of early muscle cells, since some of the undifferentiated cells which intermingle with myoblasts and myotubes early in gestation appear destined to enter into myofilament formation later in development (6). In support of this is the observation that, although specialized peripheral couplings are present within muscle cells at 12 and 15 days of gestation, they intermingle with many peripheral couplings which are simple in structure and similar to the subsurface cisterns of undifferentiated cells. Ezerman and Ishikawa (1967), who noted a similar intermingling of simple and specialized couplings between the sarcoplasmic reticulum and T tubules of cultured cells, also concluded that the simple forms of coupling are progenitors of the more specialized and characteristic membrane couplings of muscle triads.

What is the fate of the initially numerous peripheral couplings of differentiating rat intercostal muscle cells? Their numbers progressively decline with development, and they are rarely seen after muscle cells mature to myofibers (see Table I and also Schiaffino and Margreth, 1969). During the same period the number of sarcoplasmic reticulum–T tubular couplings increases, indicating that there is an inverse relationship between the distributions of these two types of membrane junction (see Table I). The following relevant observations suggest why this occurs. (*a*) Early in development sarcoplasmic reticulum–T tubular couplings are situated near the plasma membrane and there occur occasional junctions between the sarcoplasmic reticulum and both T tubules and the plasma membrane (Figs. 3 and 5). (*b*) At 15 and 18 days of gestation the sarcoplasmic reticulum is frequently coupled to vesicular depressions of the plasma membrane, the diameter of which is two to six times that of caveolae intracellulares (Figs. 6 and 7). (*c*) Within myotubes the sarcoplasmic reticulum couples to vesicular distentions of T tubules which are much larger than the interconnected chains of caveolae-like vesicles forming the rest of the primitive T tubules (Fig. 8). The interpretation is that with myotube development the initially formed peripheral couplings are swept into the cell by repeated caveolation and thus become part of the proliferating T system. The inverse relationship between the incidences of coupling of sarcoplasmic reticulum to plasma membranes and to T tubules can be readily explained by this interpretation, and the presence of sarcoplasmic reticulum–T tubular couplings close to the plasma membrane may be anticipated. One effect of membrane coupling is probably to increase locally the structural rigidity of the plasmalemma. Hence, large, vesicular depressions of the plasmalemma which are coupled with sarcoplasmic reticulum, and expansions of T tubules where coupled with sarcoplasmic reticulum, are probably related findings and represent different stages in the progressive submergence of membrane couplings into the cell.

Many SR–T tubular junctions undoubtedly form within myotubes and myofibers as the T system grows and is aligned at A-I junctions. The present data, however, indicate that during the initial stages of intercostal muscle development the sarcoplasmic reticulum attaches to plasma membranes and awaits T tubular formation.

This study was supported by United States Public Health Service Grants No. NB-08045 and NS 05572, and by a Faculty Development Award from the Merck Foundation.

Received for publication 13 July 1970, and in revised form 7 October 1970.

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