

THE HISTOGENESIS OF RAT INTERCOSTAL MUSCLE

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ABSTRACT

Intercostal muscle from fetal and newborn rats was examined with the electron microscope. At 16 days' gestation, the developing muscle was composed of primary generations of myotubes, many of which were clustered together in groups. Within these groups, the membranes of neighboring myotubes were interconnected by specialized junctions, including tight junctions. Morphologically undifferentiated cells surrounded the muscle groups, frequently extended pseudopodia along the interspace between adjacent myotubes, and appeared to separate neighboring myotubes from one another. At 18 and 20 days' gestation, the muscle was also composed of groups of cells but the structure of the groups differed from that of the groups observed at 16 days. Single, well differentiated myotubes containing much central glycogen and peripheral myofibrils dominated each group. These large cells were interpreted as primary myotubes. Small, less differentiated muscle cells and undifferentiated cells clustered around their walls. Each cluster was ensheathed by a basal lamina. The small cells were interpreted as primordia of new generations of muscle cells which differentiated by appositional growth along the walls of the large primary myotubes. All generations of rat intercostal muscle cells matured to myofibers between 20 days' gestation and birth. Coincidentally, large and small myofibers diverged from each other, leading to disintegration of the groups of muscle cells. Undifferentiated cells frequently occurred in the interspaces between neighboring muscle cells at the time of separation. Myofibers arising at different stages of muscle histogenesis intermingled in a checkerboard fashion as a result of this asynchronous mode of development. The possibility of fusion between neighboring muscle cells in this developing system is discussed.

INTRODUCTION

It has been recognized for many years that the number of fibers in a muscle progressively increases during fetal life (7). Accounts of this developmental process depict primary generations of muscle cells differentiating in close proximity to each other and the subsequent appearance of secondary and tertiary generations around their walls (11, 12, 49). The manner in which these new generations form, however, has been the subject of debate for most of this century. Many investigators (12, 16, 19, 30, 49, 54) have de-

scribed secondary and tertiary muscle cells originating as buds from the walls of primary cells from which they subsequently separated by longitudinal fission. The nuclei of these new generations of muscle cells were thought to come from central nuclei of myotubes which constantly replicated by amitosis. Light microscope investigations of apparent longitudinal fission amongst muscle cells differentiating in vitro (38) have supported this theory which is perpetuated in some current literature (2). A conflicting view, that

secondary and tertiary generations of muscle cells develop from mononucleated cells in close association with primary generations of myotubes, was held by Morpurgo (1898), Bardeen (1900), Meves (1909), and Couteaux (1941). Theoretically, this hypothesis is the most likely to be correct since many recent studies (4, 5, 9, 10, 17, 22, 27, 28, 46, 48) have demonstrated that neither mitotic nor amitotic nuclear division occurs in striated muscle cells. However, the problem has remained unresolved to the present time, mainly due to the limited resolving power of the light microscope.

The purpose of the present study is to investigate with the electron microscope the cellular relationships between the successive generations of muscle primordia which contribute to the formation of rat intercostal muscle.

MATERIALS AND METHODS

Skeletal muscle was obtained from the proximal third of the rib cage of individual animals from two separate litters of Sprague-Dawley rats at each of the following intervals: 16, 18, and 20 days *in utero*, birth (after 22 days' gestation), and 5 days postpartum. No one fixative proved satisfactory for the series of developmental stages, and best results were obtained by use of the following:

16-day fetus: 2.5% glutaraldehyde, 4% paraformaldehyde in phosphate buffer (pH 7.2) (Trelstad et al., 1967);

18-day fetus: 6% glutaraldehyde in phosphate buffer (pH 7.2);

20-day fetus; 2.5% glutaraldehyde, 1% paraformaldehyde in phosphate buffer (pH 7.2);

Birth and 5 days postpartum: 5% glutaraldehyde, 4% paraformaldehyde in phosphate buffer (pH 7.2) (Karnovsky, 1965).

After fixation, tissues were postfixed with Dalton's solution (13), flat embedded in Araldite (Ciba), orientated, cut into small blocks, and affixed to plastic rods. 0.2-1 μ sections cut from these blocks were mounted on glass slides and stained with a heated solution of 0.1% crystal violet in 1% sodium borate (S. Guyer, personal communication). Selected areas were thin sectioned, stained with uranyl acetate and lead citrate, and examined in an RCA EMU III F electron microscope. Measurements of the size of muscle cells were made with a planimeter placed over electron micrographs of transversely sectioned material.

Definitions

1. Small cells with prominent nuclei and cytoplasm which contained many ribosomes but few profiles of

rough endoplasmic reticulum are termed morphologically undifferentiated cells.

2. Myotubes are elongated cells with multiple, centrally located nuclei (7).

3. Myofibers have multiple, peripherally located nuclei and cytoplasm packed with myofilaments (37).

OBSERVATIONS

Differentiating intercostal muscle in the 16-day fetus contained groups of muscle cells at various stages of development which were separated from similar muscle groups by a large extracellular space (Figs 1, 2). The most differentiated muscle cells were small (2-10 μ) and had large central nuclei, numerous myofibrils, and focal accumulations of glycogen. They are interpreted as the primary generation of myotubes. The groups of muscle cells were irregular in composition, but frequently included several adjacent myotubes of comparable differentiation (Fig. 1). Plasma membranes of such neighboring myotubes lay close to each other for considerable distances, and were separated by an intercellular space measuring up to 400 A. These apposed membranes were more intimately connected in some areas by close junctions (51) characterized by parallel electron-opaque membranes separated by an intercellular space measuring 20-100 A (Fig. 3), and by pentalaminar structures with an over-all width of 130 A which are interpreted as tight junctions (Fig. 4). Similar membrane specializations also occurred in areas of contact between pseudopodial processes of myotubes and the plasma membranes of neighboring muscle cells. There was no basal lamina.

Cells which were morphologically undifferentiated closely surrounded the aggregates of muscle cells and were focally connected to the myotube plasma membranes by close junctions (Fig. 2). Frequently, undifferentiated cells or their processes were insinuated to varying depths into the interspace between adjacent muscle cells (Fig. 5). These undifferentiated cells appeared to separate the adjacent myotubes from one another. Undifferentiated cells and occasional fibroblasts also lay free in the tissue space between groups of muscle cells.

The intercostal space was increased at 18 days. In the central area of the developing tissue, muscle cells were again distributed in clusters but the clusters were smaller and more regularly organized than at 16 days. Individual, differenti-

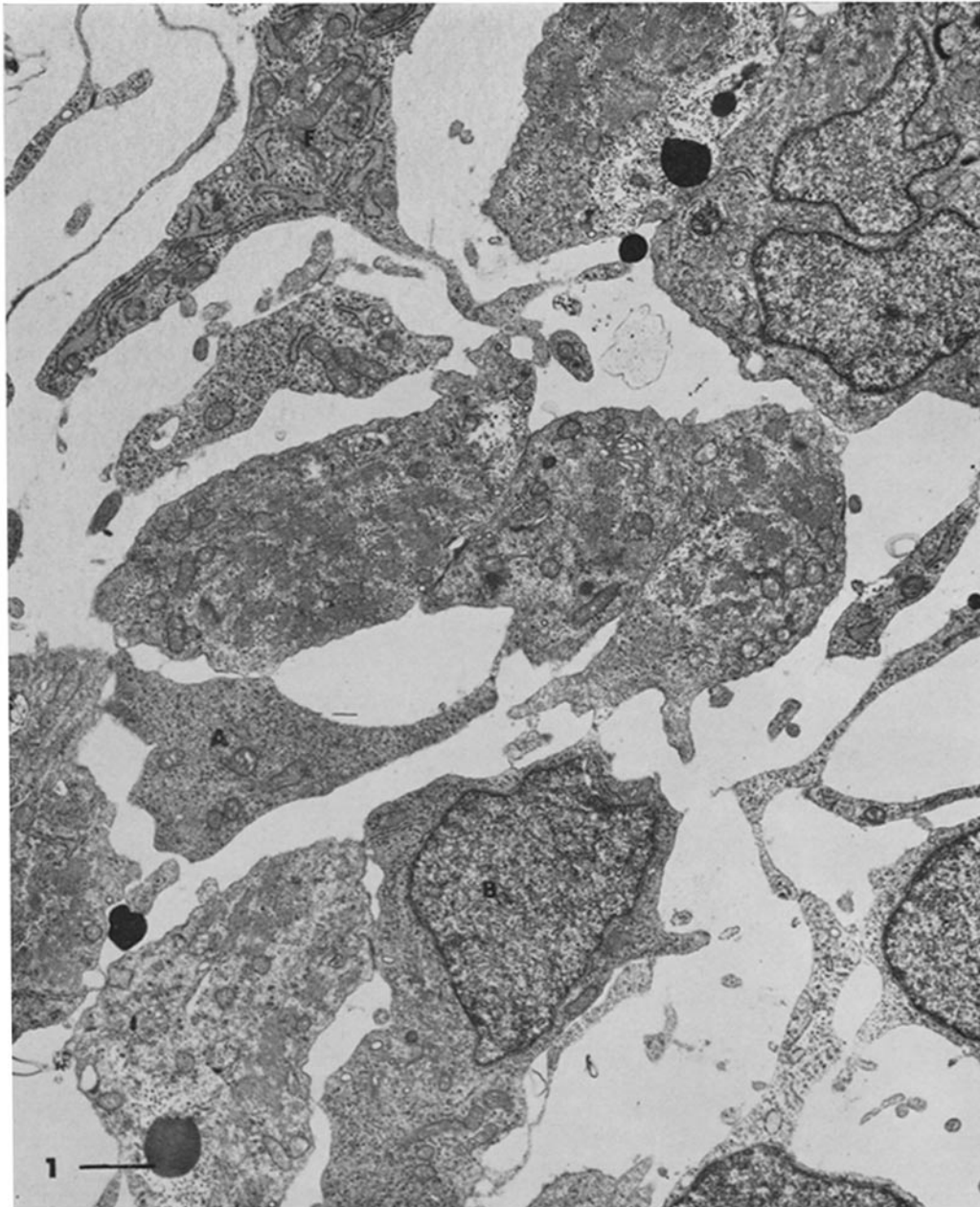


FIGURE 1 16 days' gestation. Cells are irregularly distributed and several contain myofilaments. There is a large extracellular space. Three cells interpreted as small myotubes are aggregated together in a group. Undifferentiated cells (*A* and *B*) lie adjacent to this muscle cell group. A cell which contains much rough endoplasmic reticulum (*F*) is interpreted as a fibroblast. $\times 10,000$. Marker = 1μ .

ated myotubes which contained considerable accumulations of glycogen and peripheral myofibrils dominated the groups (Fig. 6). These myotubes are interpreted as primary myotubes which

had developed from the groups of cells seen at 16 days. They were individually surrounded by undifferentiated cells and by small cells which contained myofilaments and focal deposits of glyco-

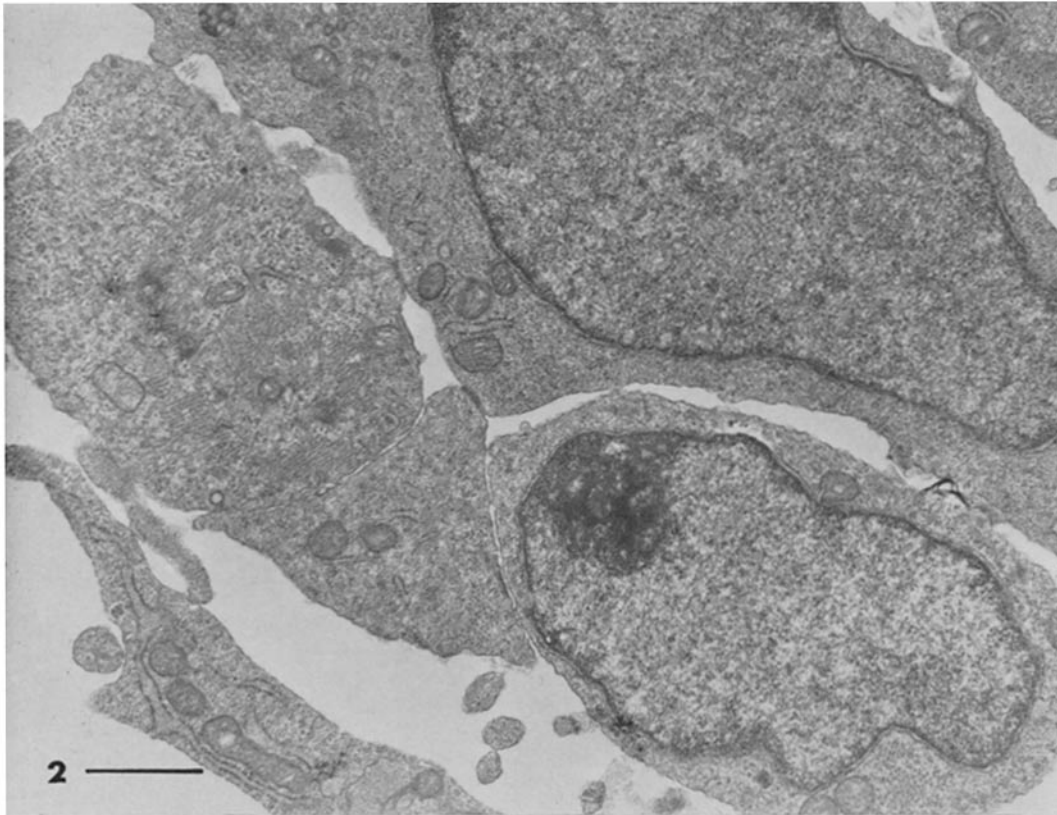


FIGURE 2 16 days' gestation. Three muscle cells lie adjacent to each other. An electron-opaque membrane contact is formed between the central muscle cell and an undifferentiated cell to the right. A cell process in the lower left contains much rough endoplasmic reticulum and is interpreted as part of a fibroblast. $\times 15,000$. Marker = 1μ .

gen (Figs. 6, 7). The small cells containing myofilaments are interpreted as new generations of muscle cells. They were found immediately adjacent to the walls of large myotubes at 18 days and rarely occurred elsewhere. A rudimentary basal lamina peripherally ensheathed each group of muscle cells and did not penetrate between apposed cell membranes within the groups (Fig. 7). Fibroblasts associated with scant collagen loosely subdivided the clusters of muscle cells into rudimentary muscle bundles. Surface membranes of large myotubes and of the neighboring new generations of small muscle cells were usually closely apposed and in some areas were interconnected by close and tight junctions. These apposed membranes followed a tortuous course and included pseudopodia which penetrated into invaginations of the walls of large myotubes (Figs. 7

and 8). Where such interdigitations occurred, membrane separation between the two muscle cells was frequently indistinct (Fig. 8). Whether this indistinct appearance was due to focal fusion between the cells or to variations in the plane of section, however, could not be ascertained. Many of the small cells with myofilaments and some undifferentiated cells occupied depressions in the walls of large myotubes which were occasionally of such depth as to completely engulf the small cell and cause the contours of the two cells to appear continuous (Fig. 9).

Undifferentiated cells or their processes insinuated between neighboring large and small muscle cells were constantly observed at this stage of development. This distribution of undifferentiated cells resembled the arrangement present at 16 days. Mitotic figures were occasionally seen

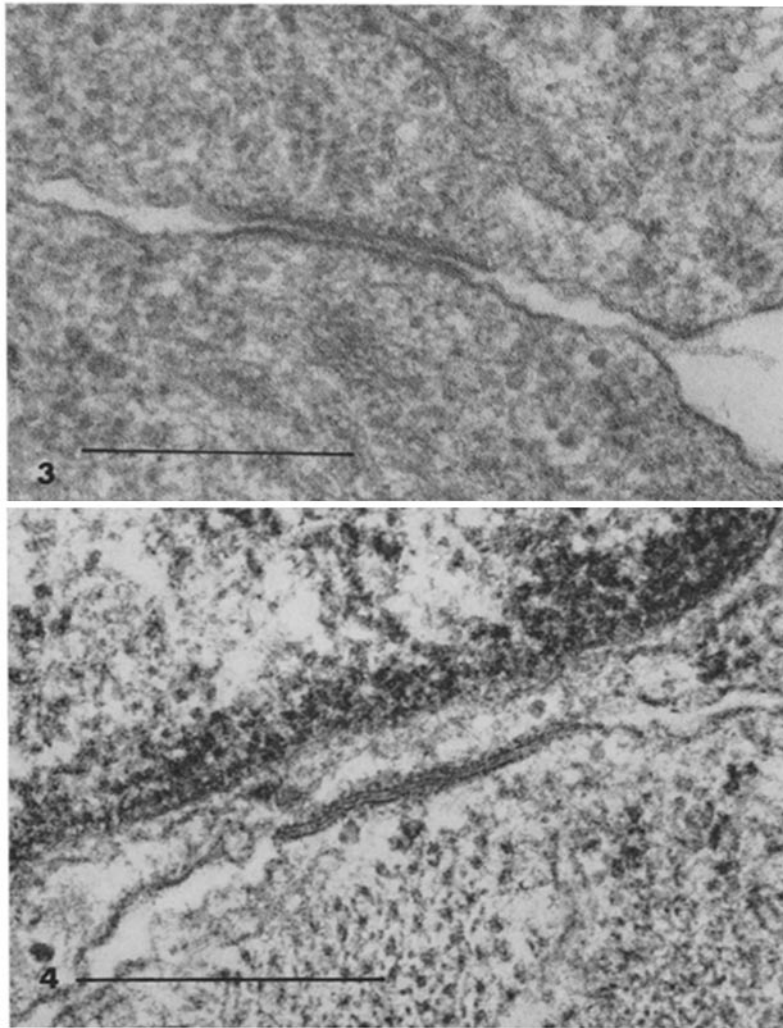


FIGURE 3 16 days' gestation. A close junction characterized by parallel electron-opaque membranes which are separated by an intercellular space measuring 100 Å is formed between neighboring muscle cells. $\times 77,000$. Marker = 0.5μ .

FIGURE 4 16 days' gestation. A pentalaminar tight junction with an overall width of 130 Å connects the membranes of neighboring muscle cells. $\times 80,000$. Marker = 0.5μ .

amongst the undifferentiated cells including those enclosed within the basal lamina surrounding large myotubes.

At the costal extremities of the developing muscle were aggregates of large myotubes; the surface membranes of these myotubes were interconnected by membrane specializations. These aggregates of myotubes resembled those seen at 16 days. This arrangement indicated that at 18 days many myotubes were separated from each

other in the midpart of the muscle but were still interconnected at the costal extremities.

For a fuller investigation of the cellular composition of the muscle aggregates in the middle of this developing muscle, the size of myotubes dominating each group was measured with a planimeter placed over micrographs of transversely sectioned material. The values were plotted in histogram form. The incidence of new generations of small muscle cells and of undifferentiated cells

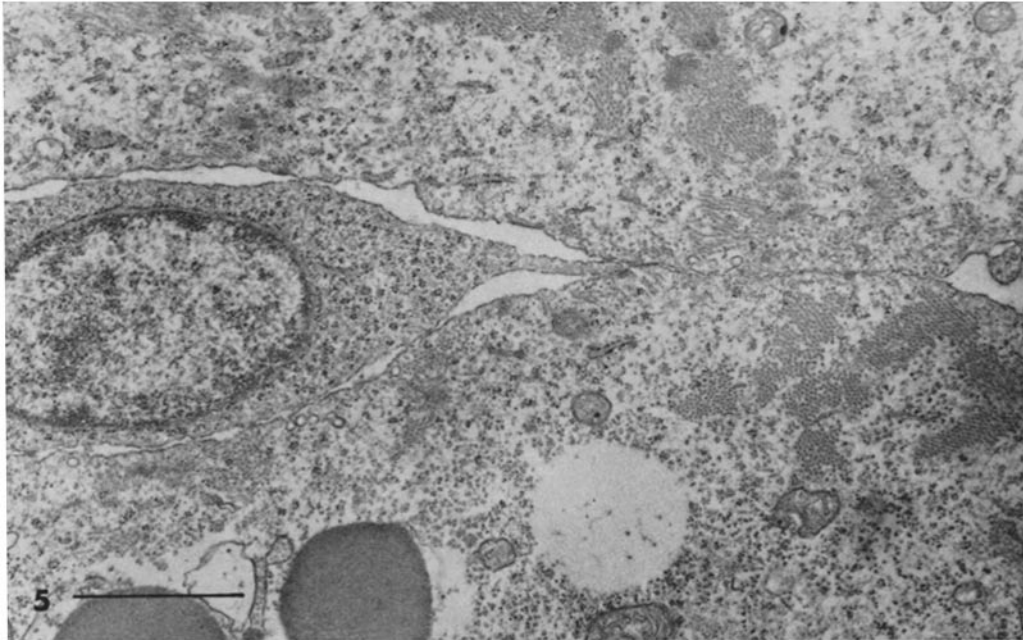


FIGURE 5 16 days' gestation. An undifferentiated cell is situated between two neighboring muscle cells. It extends a process towards an area where the muscle cell plasma membranes lie close to each other. $\times 22,000$. Marker = 1μ .

which lay closer than 300 A to walls of each myotube was also recorded. Neighboring large and small myotubes which were separated from each other by distances of over 300 A appeared as individual units of differentiation and were measured and recorded separately.

At 18 days' gestation (Histogram I, Fig. 14), myotubes dominating the groups of muscle cells had a bell-shaped population distribution. Whereas undifferentiated cells were associated with myotubes of all sizes, they were the only type of cell attached to myotubes measuring less than $10 \mu^2$. Of these small myotubes, 64% had undifferentiated cells applied to their walls. A second population of small myofilament-containing cells were attached to the walls of myotubes measuring over $10 \mu^2$.

At 20 days' gestation, the morphology of the developing intercostal muscle was similar to that at 18 days. Muscle cells at various stages of differentiation were clustered in groups, each of which centered upon a well differentiated myotube. Histogram II (Fig. 14) which was constructed in the same way as Histogram I, illustrates that the size of myotubes dominating each group is in-

creased compared to the size at 18 days. This increase was largely due to further accumulation of glycogen by the myotubes. The distribution of myotubes dominating each group and the arrangement of undifferentiated cells and of new generations of small myofilament-containing cells clustered about their walls were comparable to those at 18 days.

At birth, the midzone of the developing muscle differed considerably from that at previous stages. Almost all muscle cells, irrespective of size, were myofibers with peripheral nuclei and cytoplasm packed with myofibrils (Fig. 10). Most myofibers were individually surrounded by a basal lamina and lay independent of each other (Fig. 12). They were rarely seen clustered together in groups. Processes of fibroblasts loosely subdivided the muscle into bundles within which large and small myofibers intermingled in a checkerboard fashion. Histogram III (Fig. 14) illustrates that the myofiber distribution was skewed in favor of the small myofibers and was not bell-shaped as at previous stages. This suggests that there was an increase in the incidence of small muscle cells compared to the incidence at 20 days. Correlated with this was

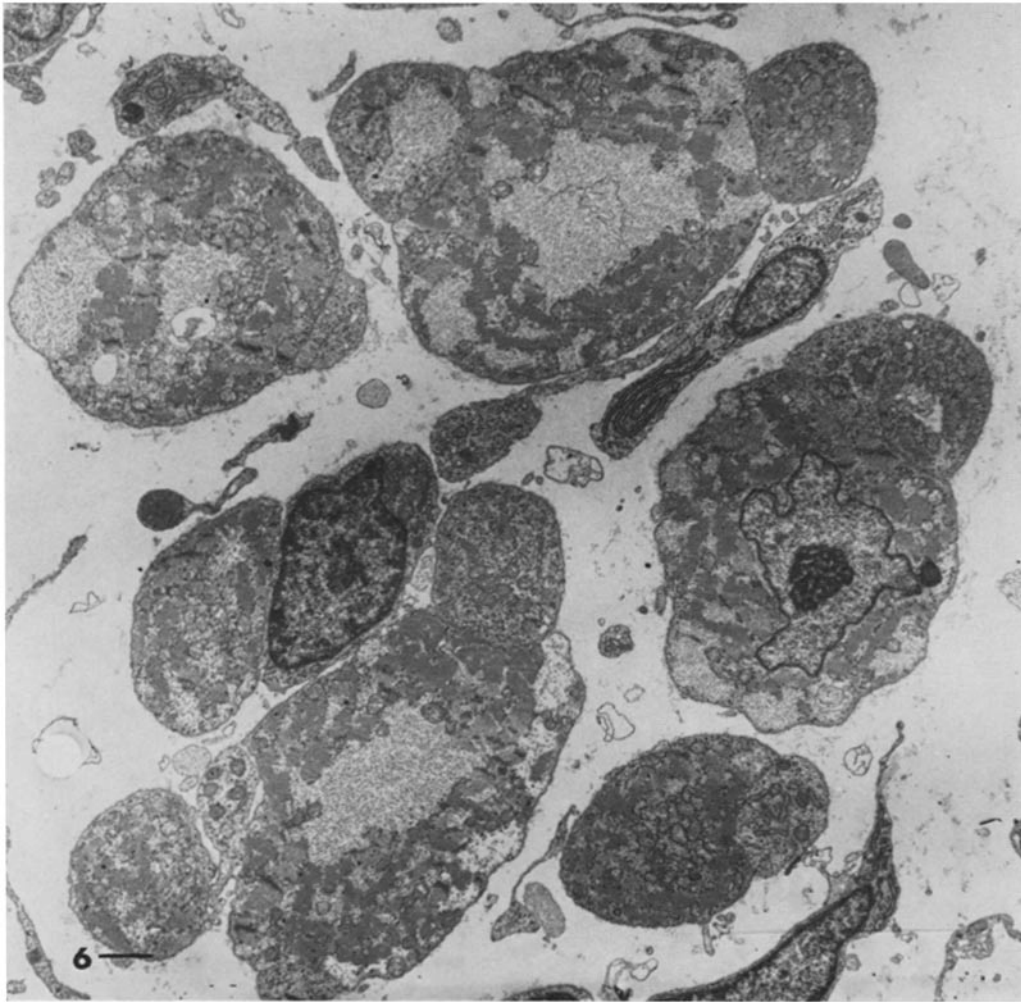


FIGURE 6 18 days' gestation. Large myotubes which contain much glycogen and peripherally dispersed myofibrils dominate cell groups. They are surrounded by undifferentiated cells and small cells which contain myofilaments. The small cells are interpreted as new generations of muscle cells. At lower left, two small myotubes are separated from the walls of a neighboring large myotube by the interposing processes of undifferentiated cells. $\times 7,000$. Marker = 1μ .

a low incidence of small muscle cells associated with the walls of large myofibers. The few small muscle cells which were observed clustered around large myofibers were packed with myofilaments and appeared to be small myofibers (Fig. 11). There were infrequent close junctions between adjacent large and small muscle cells.

Undifferentiated cells were rarely seen clustered to the walls of large myofibers. By contrast, numerous undifferentiated cells clustered around the walls of small myofibers (Fig. 12). The frequency

with which undifferentiated cells were encountered neighboring small myofibers was comparable to the incidence of undifferentiated cells associated with small myotubes at 18 and 20 days' gestation. Occasionally, such undifferentiated cells protruded pseudopodia into plasmalemmal invaginations of small myofibers (Fig. 12). As the plasmalemma invaginations occurred in register with I bands of adjacent myofibrils, they possibly represent expanded T tubules. Undifferentiated cells insinuated between large and small myofibers were

common. Mitoses occurred in undifferentiated cells included within the basal lamina surrounding myofibers (Fig. 13). Comparable mitoses have been previously reported (31).

At 5 days postpartum, the architecture of the midzone of the developing muscle resembled that at birth (Histogram IV, Fig. 14). Undifferentiated cells lay adjacent to the walls of 60–70% of small myofibers, but rarely occurred in similar relationship to large myofibers. No small myofilament-containing cells were found in association with

myofibers used for this histogram. However, in some more obliquely sectioned material these cells were occasionally seen.

DISCUSSION

It is now well recognized that during development striated muscle cells increase their numbers of nuclei by fusion with specific neighboring cells. From the present study, we anticipate that fusion occurs between some of the intercostal muscle cells clustered together in groups at 16, 18, and 20



FIGURE 7 18 days' gestation. A small muscle cell occupies a shallow depression of the wall of a large myotube. Pseudopodial processes protrude from the small cell into invaginations of the wall of the large myotube. The intercellular space between these two cells measures 80–100 Å. Basal lamina forms a common sheath for these two cells but does not penetrate between them. $\times 33,000$. Marker = 1μ .

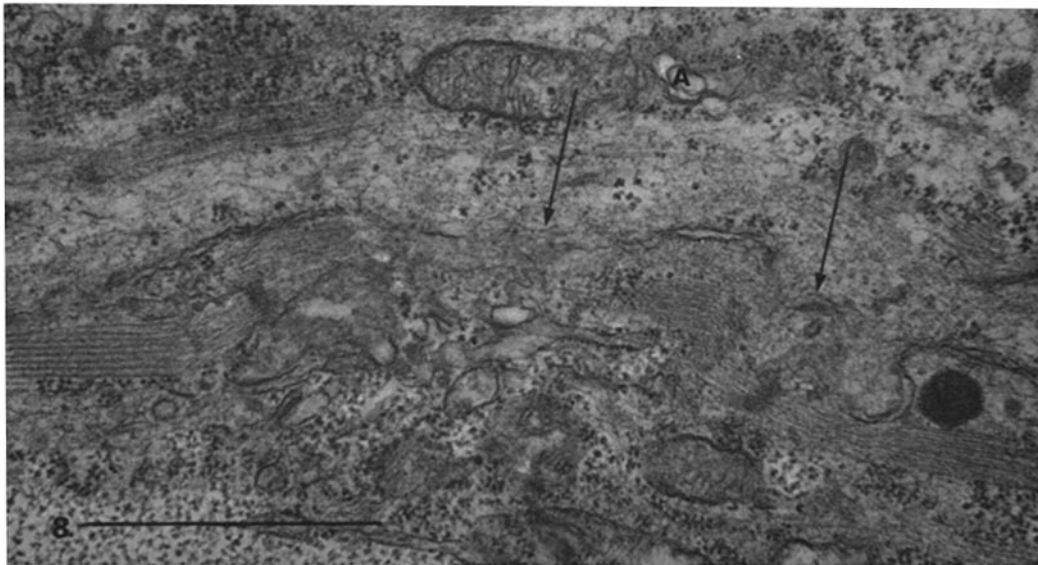


FIGURE 8 18 days' gestation. A primitive muscle cell (A) lies adjacent to the wall of a large myotube. The apposed plasma membranes of these two cells (arrowed) follow a tortuous course. In some areas, the definition of these membranes is indistinct. It cannot be determined whether this indistinct appearance results from local cell fusion or is due to a tangential plane of section through the membranes. $\times 39,000$. Marker = 1μ .

days' gestation. The large myotubes which dominate the muscle groups at 18 days' gestation, for example, are probably derived, through fusion, from several of the muscle cells which occurred together within groups at 16 days' gestation. Possibly, the specialized junctions found between neighboring muscle cells at these stages of development (Figs. 3, 4) potentiate the process of fusion, for, as sites of specialized membrane attachment, they must maintain plasma membranes of neighboring muscle cells in close proximity to one another. The morphological events of fusion, however, have not been unequivocally recorded either in the present or in previous studies, largely owing to the problems of sampling and interpretation of membranes in various sectional planes in electron micrographs. It cannot be determined, for instance, whether the two adjacent muscle cells illustrated in Fig. 8 have undergone local fusion or whether the discontinuities in definition of the apposed, tortuous plasma membranes are the result of an oblique plane of section. Hay (1963) and Shafiq (1963) have suggested that numerous vesicles bordering the juncture between myogenic cells represent membrane breakdown before fusion. The vesicles they describe are morphologically

similar to pinocytic vesicles, structures which are common in many types of cell, but which in developing muscle are associated with T tubule differentiation (15, 25). In discussing the process of fusion of mononuclear cells to the lateral walls of myotubes in vitro, Betz et al. (1966) describe small fusing cells gradually sinking into the myotube cytoplasm so that the contours of the two cells merged. Fig. 9 illustrates a cell with ribosome-studded cytoplasm which lies completely within a depression in the walls of a large myotube such that the contours of the two cells blend with each other. That this cell is analogous to the fusing cell described by Betz correlates with the hypothesis that some of the small cells lying along the walls of large myotubes at 18 and 20 days' gestation fuse laterally into the substance of the myotube and contribute to myotube multinucleation.

Fischman (1967) has described groups of muscle cells in the chick which are morphologically similar to those of the 18- and 20-day fetal rat intercostal muscle and has suggested that all cells within each group are destined to fuse with each other and produce one large multinucleated myofiber. This hypothesis is neither supported by the present study nor by a number of previous studies. In-

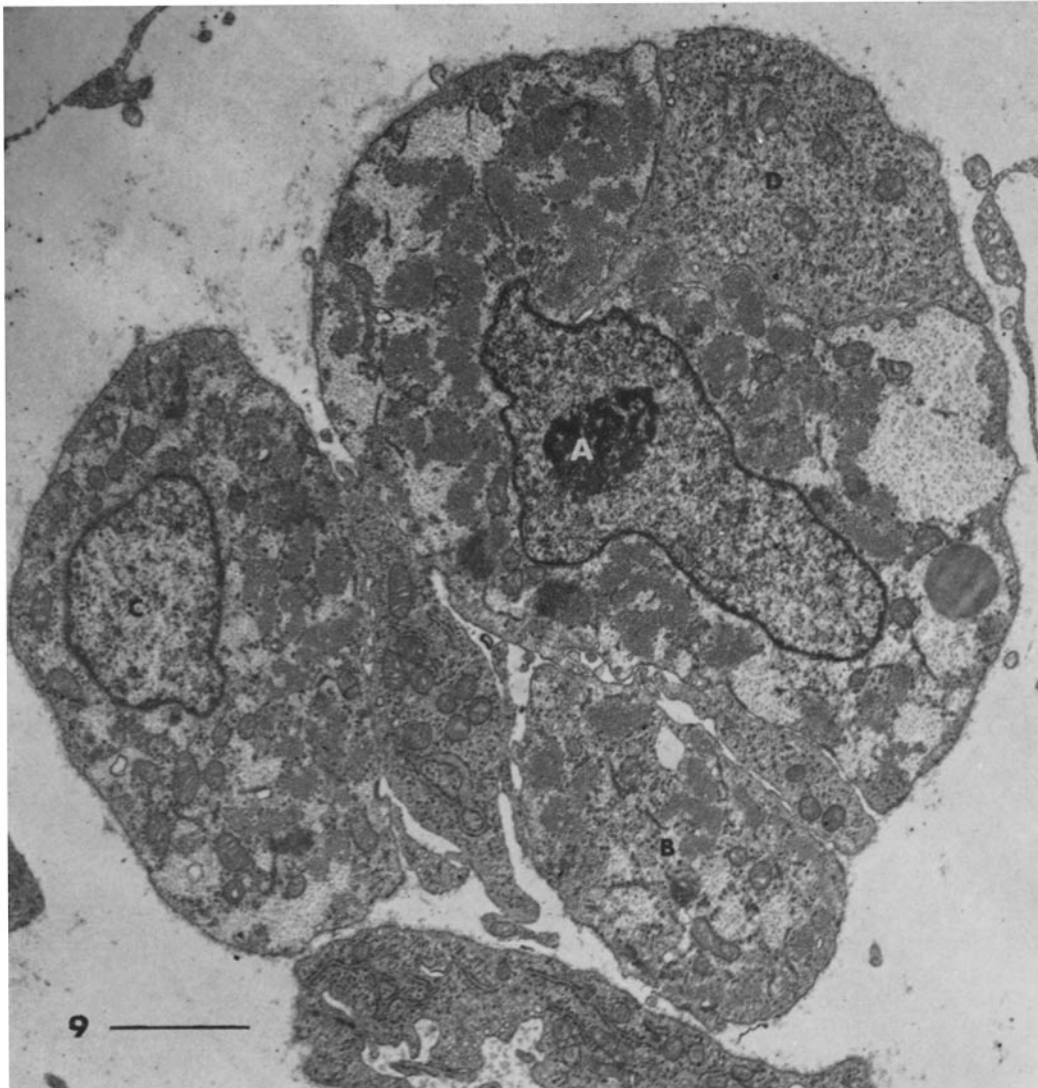


FIGURE 9 18 days' gestation. Cell marked *A* is interpreted as a primary myotube. Two smaller myotubes (*B* and *C*) lie adjacent to this large cell, but are separated from it by processes of undifferentiated cells. A small undifferentiated cell (*D*) is entirely accommodated within a depression of the wall of the large myotube, and the contours of the two cells merge. $\times 17,400$. Marker = 1μ .

vestigations of striated muscle histogenesis by Meves (1909), Tello (1922), Couteaux (1941), and Cuajunco (1942) all describe groups of muscle cells from which primary, secondary, and tertiary generations of cells separate as the muscle is progressively built up. Comparison between histograms I, II, and III (Fig. 14) in the present study also illustrates that successive generations of muscle cells separate from each other as intercostal muscle

progressively matures. At 20 days' gestation, numerous small muscle cells cluster to the walls of myotubes measuring over $20 \mu^2$ in diameter (histogram II). By contrast, at birth (histogram III) the incidence of these small muscle cells clustered to the walls of myofibers measuring over $20 \mu^2$ has declined precipitously. A coincident rise in the incidence of separate small myofibers is indicated by a skewing of the population distribution of

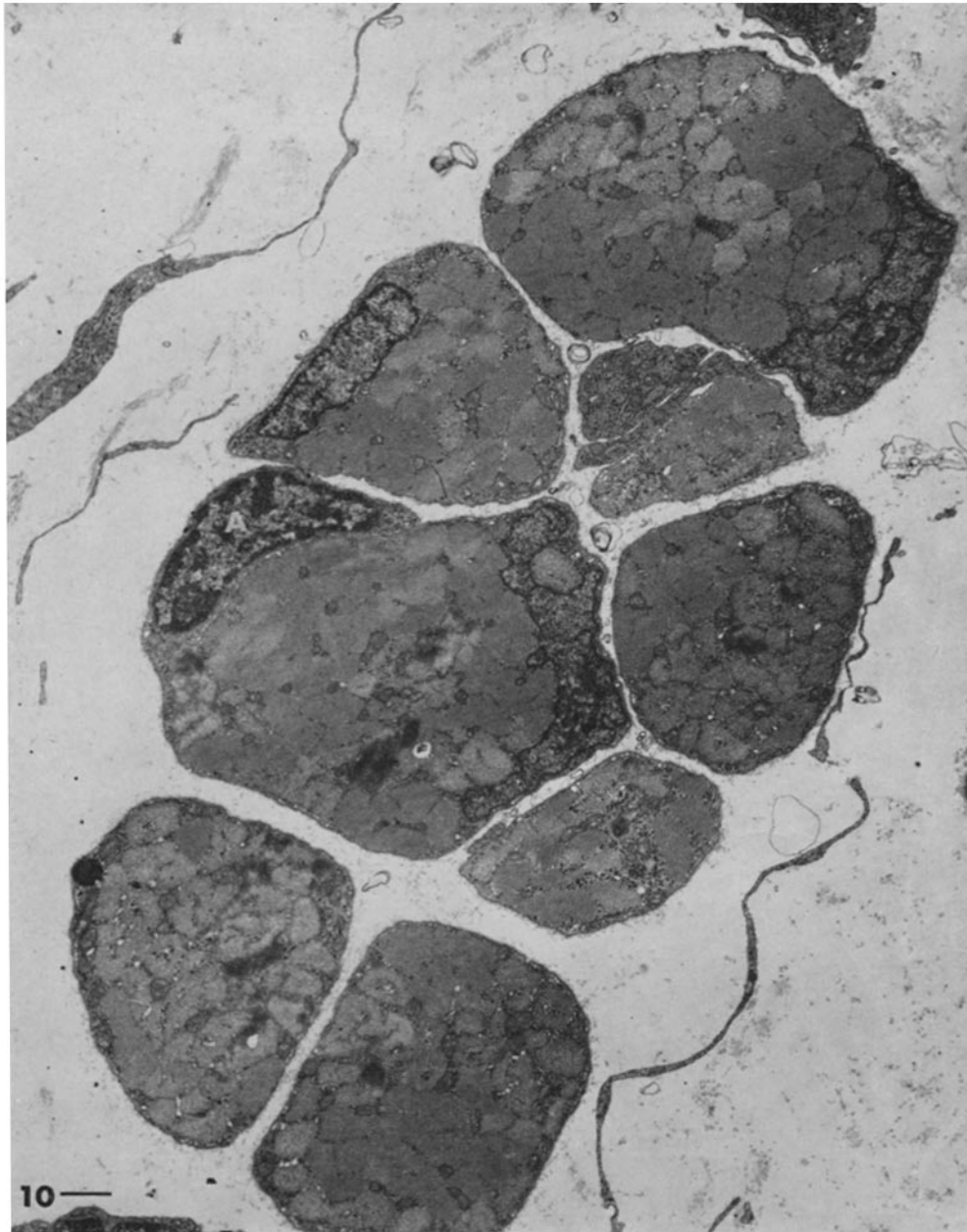


FIGURE 10 Birth. Myofibers packed with myofibrils compose a muscle bundle. The myofibers vary greatly in size and intermingle in a checkerboard pattern. The small myofibers are interpreted as new generations of muscle cells which have initially developed along the walls of the large myofibers. An undifferentiated cell (A) lies closely adjacent to the walls of a large myofiber. $\times 7,000$. Marker = 1μ .

separate myofibers to the left of histogram III. These developmental events undoubtedly are correlated and indicate the initial development of new generations of small muscle cells around the walls

of the large myotubes and then their separation. Possibly, the new generations of cells differentiate along the walls of large myotubes in order to obtain support for growth. By developing in this

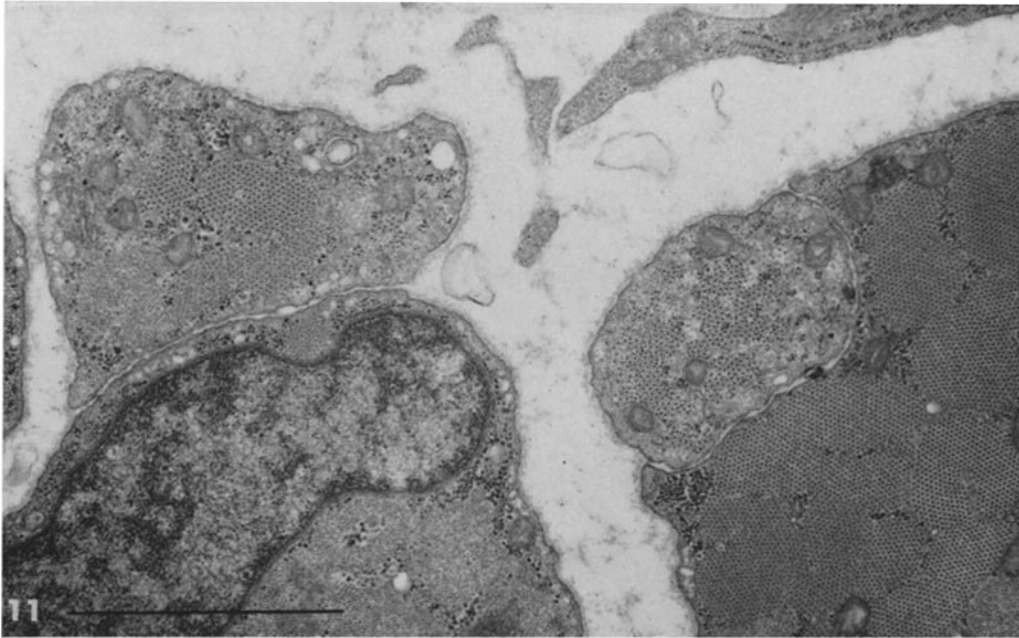


FIGURE 11 Birth. Two small myofibers border the plasma membranes of large myofibers. Each large and small myofiber group is peripherally ensheathed by basal laminae. $\times 35,800$. Marker = 1μ .

manner, the several orders of muscle cells which compose the mature muscle are aligned parallel to each other. Thus, intercostal muscle cells which, during differentiation, are clustered together in groups may develop in one of two directions. Some may fuse with one another as large multinucleated cells are built up, while others separate and become independent muscle cells. This pattern of intercostal muscle histogenesis is summarized in Fig. 15. To what extent it correlates with patterns of development of other muscles in the rat must await further study.

Many light microscopists have interpreted protrusions in the contour of large myotubes as buds formed by primary myotubes which, with differentiation, separate by longitudinal fission and become individual new generations of myotubes. Morpurgo (1898), Meves (1909), and Coueteaux (1941) have conflicted with this theory and proposed that new generations of muscle cells develop from mononuclear cells lying along the walls of primary myotubes. Clearly, the present study supports the latter hypothesis. Though we found no evidence of budding of the walls of large myotubes, the origin of this theory can be readily explained. Apposed plasma membranes of large

primary myotubes and new small myotubes followed a tortuous course, were separated by an intercellular space measuring 100–300 Å, were focally interconnected by specialized membrane junctions, and were enwrapped by a common basal lamina (Figs. 7 and 8). To a light microscopist, these neighboring cells might readily be misinterpreted as one myotube and their subsequent separation as fission of that cell.

In developing human muscle, the process of separation of myotubes from one another has been associated with the intervention of pseudopodia from mesenchymal cells into the interspace between neighboring myotubes. Haggquist (1956) and Ishikawa (1966), for example, suggest that these intervening cells cleave the myotubes apart. In the present study, cells which we described as undifferentiated were constantly found similarly disposed between neighboring myotubes at each of the developmental stages examined (Figs. 5 and 6). Though these undifferentiated cells may play some role in mechanically separating myotubes from one another, we interpret their distribution in a different manner. In 1962, Moscona reported that the ability to adhere is peculiar to cell membranes during early stages of differentiation and is

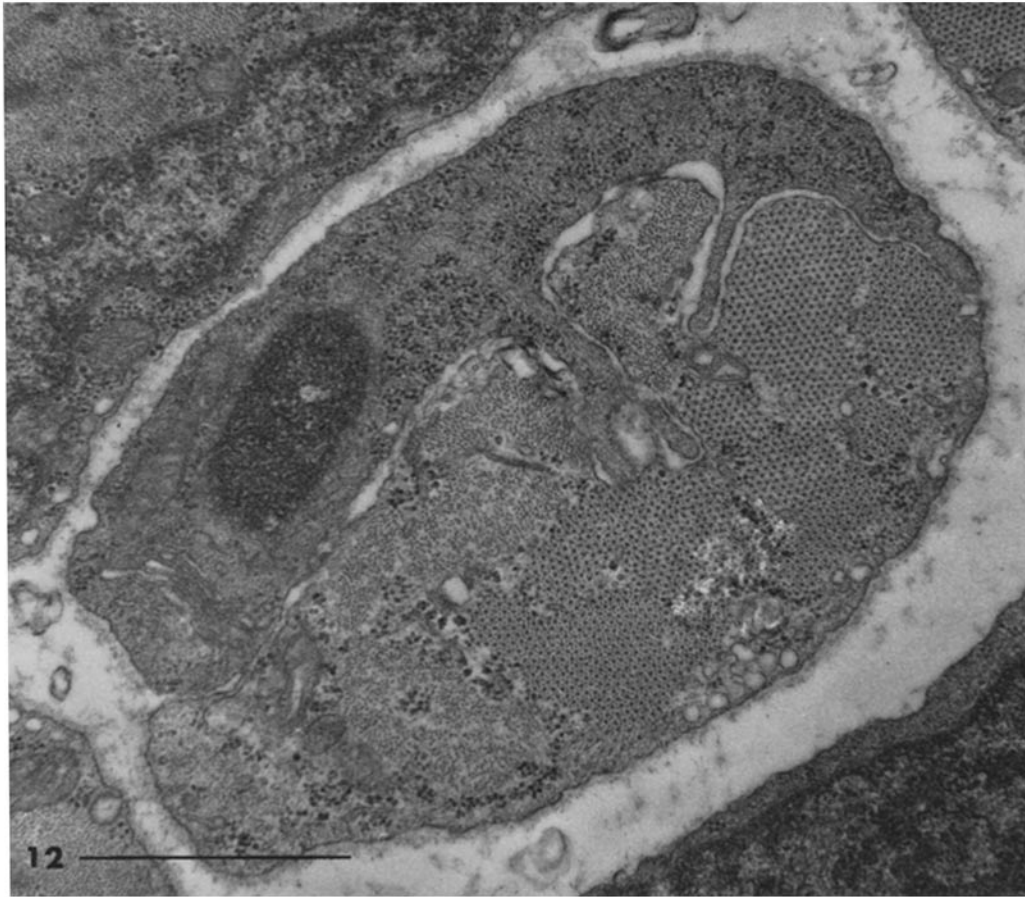


FIGURE 12 Birth. An undifferentiated cell lies beneath the basal lamina ensheathing a small myofiber. Processes from the undifferentiated cell enter invaginations of the wall of the myofiber. The invaginations occur in the region of the Z band and are interpreted as expanded T tubules. $\times 35,000$. Marker = 1μ .

lost as cells progress towards their specialized form. Thus, as myotubes differentiate, they may be expected to lose their membrane stickiness and cease to adhere to each other in groups. Membrane stickiness, for example, may rapidly decline as myotubes mature to myofibers, with the result that many cell groups disintegrate when this occurs. Accompanying the process of separation, pseudopodia from adjacent undifferentiated cells can successfully invade the interspaces between myotubes and appear to cleave the myotubes apart. If we are correct in this assumption, then why should processes of undifferentiated cells so frequently insinuate themselves between neighboring muscle cells? A partial answer may come from the studies of Weiss (1941) who observed that an interface is a favored site for pseudopodial growth.

In the developing intercostal muscle, the membranous juncture between neighboring myotubes or myofibers forms an interface, and there is little doubt that pseudopodia of undifferentiated cells commonly grow there.

The cells which Ishikawa (1966) observed intruding between neighboring myotubes in developing human muscle contained profiles of rough endoplasmic reticulum, and he described them as "fibroblast-like." Ishikawa concluded they were destined to become the satellite cells of mature muscle. In contrast, Mauro (1961) has suggested, amongst other possibilities, that satellite cells may be remnants of the embryonic development of multinucleated muscle cells and represent dormant myoblasts. This divergence of interpretation, no doubt, has arisen because mesenchymal cells of

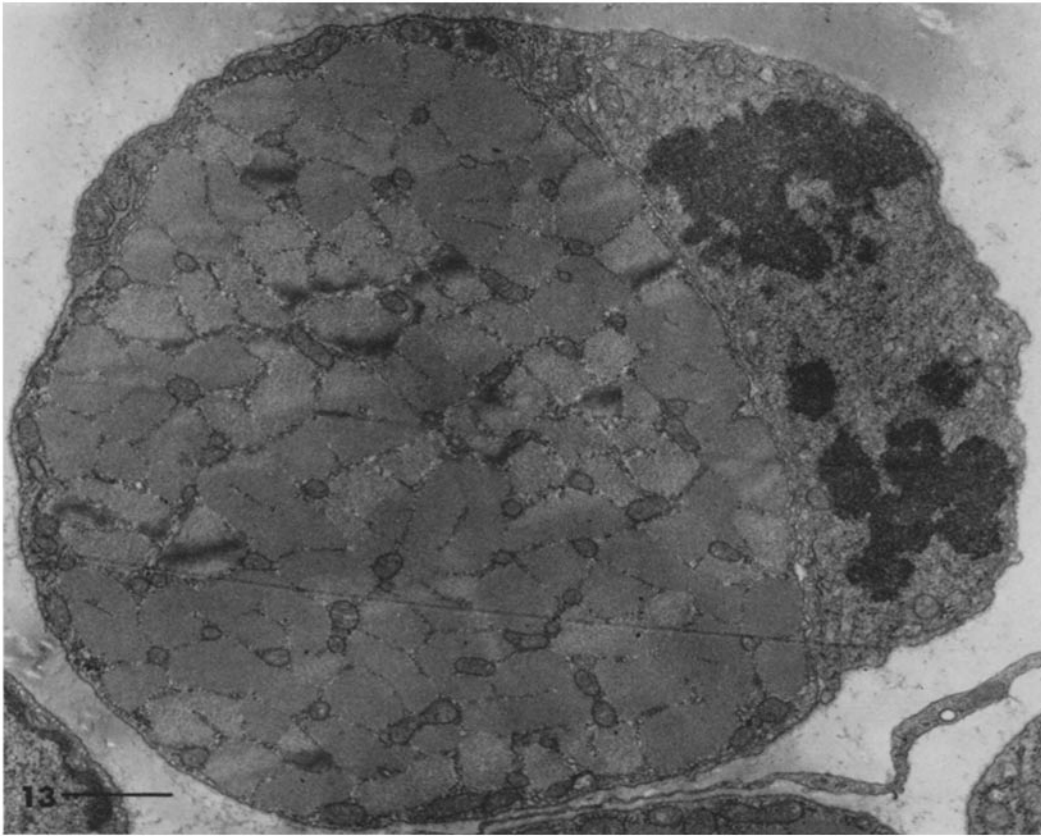


FIGURE 13 Birth. Mitotic division of an undifferentiated cell which borders the plasma membranes of a large myofiber. $\times 14,000$. Marker = 1μ .

muscle are a mixed population containing primordia of both connective tissue and muscle cells, and there are few morphological features by which these primitive cells can be distinguished (41). For this reason, we have termed basophilic cells with prominent nuclei, ribosome-studded cytoplasm and scant rough endoplasmic reticulum as "undifferentiated." Morphologically similar cells which occurred amongst groups of muscle cells in developing chick muscle were termed myoblasts by Fischman (1967), an interpretation which correlates with previous descriptions by Hay (1963) and Price et al. (1964). The present study supports their interpretation by indicating that those undifferentiated cells which lie beneath the basal lamina surrounding either myotubes or myofibers belong to a replicating population (Fig. 13) and occur in sites of formation of new generations of muscle cells. Possibly the interface between neighboring myotubes is a common site for insinuation

of these cells and early growth of myoblasts. Those mononucleated cells which do not differentiate and are situated beneath the basal lamina surrounding myofibers we interpret as the precursors of satellite cells.

Additional features of the cellular make-up of the muscle aggregates present at 18 and 20 days' gestation, birth, and 5 days postpartum are depicted in Histograms I-IV (Fig. 14). These histograms were constructed from planimetric measurements of the size of myotubes dominating each group plotted with the distribution and type of cells clustered around their walls. The histograms demonstrate that new generations of myofilament-containing cells occur only on the walls of myotubes that are over a certain size. By contrast, undifferentiated cells occur on the walls of myotubes of all sizes, but are the exclusive cell type associated with small myotubes. As has been discussed, there is a rapid decline in the incidence of

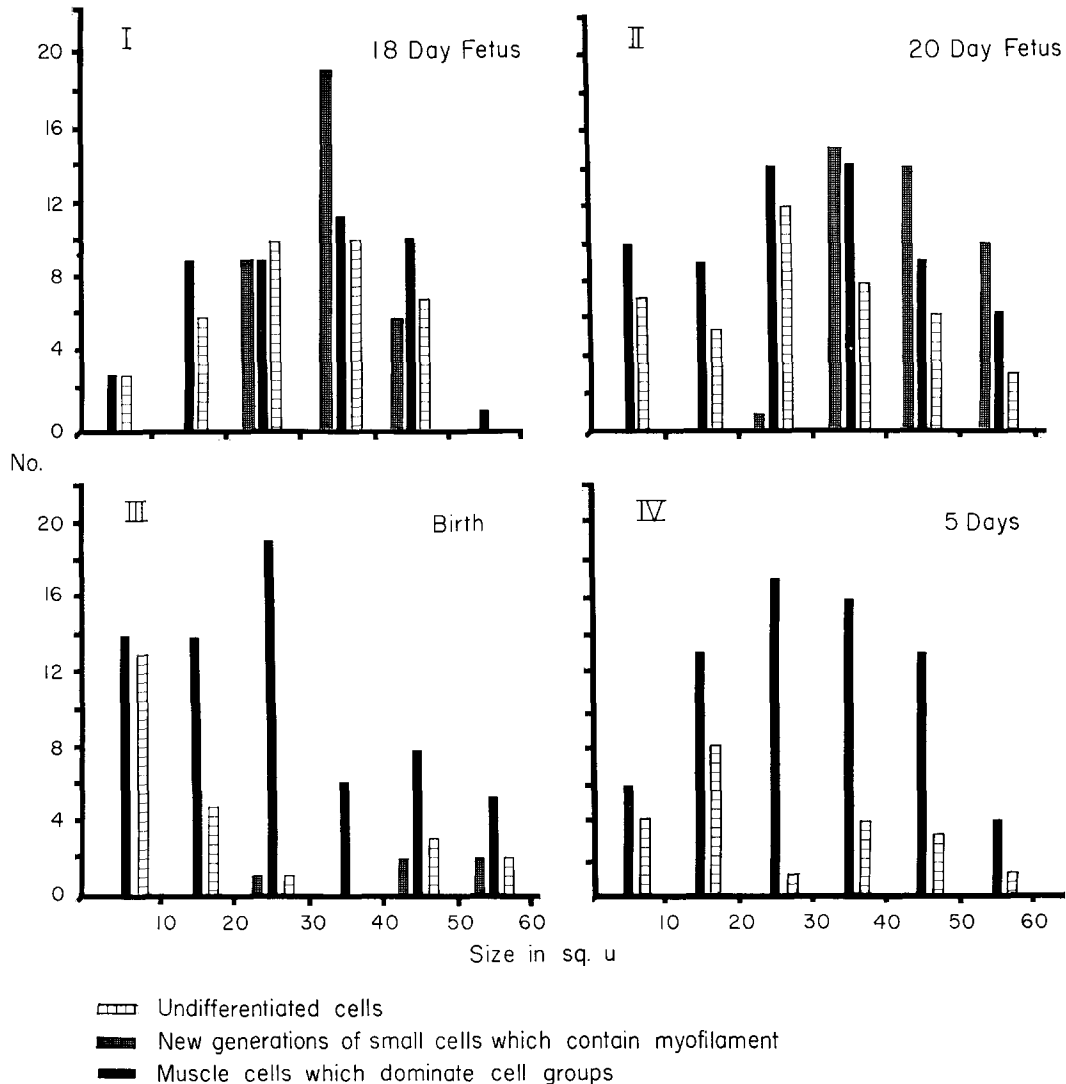


FIGURE 14 Histograms of size distribution of large muscle cells at four stages of intercostal muscle development. The incidence of new generations of small muscle cells and of undifferentiated cells related to their walls is also shown.

myofilament-containing cells surrounding muscle cells measuring over $20 \mu^2$ between 20 days' gestation and birth. There is a comparable decline of undifferentiated cells associated with large myofibers. However, the distribution of undifferentiated cells surrounding small muscle cells remains constant during this period. This can be interpreted as evidence of migration of the undifferentiated cells with the small muscle cells subsequent to separation. By interpreting the undifferentiated cells counted in the histograms as

myoblasts, it is possible to explain the constant association of these cells with small myotubes or myofibers and of myofilament-containing cells with large muscle cells. After separation from large myotubes, the small muscle cells will progressively grow into larger cells. As they do so, myoblasts applied to their walls may enter into myofilament synthesis and become definitive new generations of muscle cells. Therefore, small cells containing myofilaments will be found only on the walls of myotubes that are over a certain size.

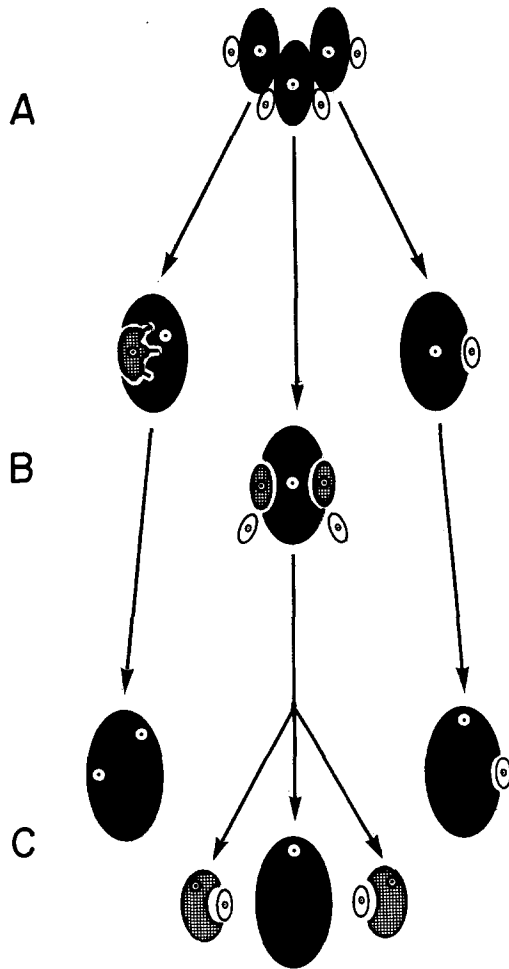


FIGURE 15 Summary of the histogenesis of rat intercostal muscle. *A*, 16-day fetus. Three early differentiating myotubes are aggregated in a group. Their membranes are closely apposed. Small morphologically undifferentiated cells (open cytoplasm) surround and lie in the interspace between adjacent myotubes. These undifferentiated cells are the precursors of new generations of muscle cells. *B*, 18-day fetus. The early differentiated myotubes have enlarged and separated from each other. New generations of small cells surround their walls, and some of them contain myofilaments (cross-hatched). On the left, the small cell occupies a depression in the wall of the large myotube and protrudes pseudopodia along expanded tubules farther into myotube substance. Two small myotubes are less intimately related to a large myotube in the center of the diagram. Undifferentiated cells lie in the interspace between these large and small muscle cells and contribute to their subsequent separation. An undifferentiated cell neighbors a large myotube to the right. *C*, Birth. All muscle cells have matured to myofibers with peripheral nuclei. To the left, the small cell has fused into the large myotube substance. In the center, the two small muscle cells have disengaged from the large myotube and lie independently. Undifferentiated cells lie adjacent to their walls and are interposed between the small muscle cells and the large central myofiber. An undifferentiated cell, or satellite cell, borders the myofiber to the right. As a result of this pattern of development, muscle cells differentiating early and late in myogenesis intermingle in a checker-board fashion.

In previous ultrastructure studies (24, 41, 52), specialized membrane interconnections have been reported in developing chick and human striated muscles which are similar to the membrane interconnections observed in the present account. The occurrence of desmosome-like "attachment plaques" in the primitive nervous system of chick (8, 33, 53), of tight junctions between primordial muscle cells of frog heart (23) and between cells of the primitive germ layers of chick embryos (50, 51) demonstrate that specialized junctions are common forms of intercellular relationships during morphogenesis. Trelstad et al. (1966, 1967) proposed that, in developing tissues, membrane specializations are sites of intercellular adhesion, a phenomenon which is little understood but is of fundamental importance to normal cell differentiation (1, 47). By analogy with respect to mature

cardiac or smooth muscle, the suggestion is that developing intercostal muscle cells which interconnect by membrane specializations, particularly tight junctions, contract and relax as one cell. The membrane interdigitations of the walls of large myotubes occupied by pseudopodia of adjacent small muscle cells support this conclusion, for it is unlikely that these could be formed and maintained if the cells moved independently. Recently, Sheridan (1966, 1968) and Potter et al. (1966) have reported that electrical couplings between cells occur extensively in both chick embryos and developing squid. These electrical couplings were thought to coincide with tight junctions. In developing intercostal muscle, electrical couplings via tight (29) and possibly close junctions would allow transmission of action potentials between cells within groups and permit their coordinated ac-

tivity. In contrast to those of cardiac and smooth muscle cells, however, membrane specializations between neighboring striated muscle cells are transient and are rarely seen between neighboring muscle cells after they have matured to myofibers. Presumably, maturation and separation of myofibers herald the onset of independent activity by each muscle cell.

On the basis of histochemical studies on fetal and neonatal mice, Wirsen and Larsson (1964) have previously proposed that the intermingling of separate generations of muscle cells correlates with the checkerboard pattern of distribution of histochemically distinct myofibers in mature muscle. These authors describe intense phosphorylase activity associated with primary generations of muscle cells, less intense reactivity with secondary generations, and no phosphorylase activity in tertiary muscle cells. The present study illustrates that rat intercostal muscle is built up from several

orders of cells which, owing to their growth pattern, intermingle with each other. In support of Wirsen and Larsson's histochemical data, the present study revealed that glycogen was widespread within large myotubes, but scant within small muscle cells differentiating late in muscle histogenesis. However, it is difficult to correlate their theory with the widely held belief that the histochemical characteristics of myofibers are determined by their nerve supply (14, 42), as the distribution of peripheral nerves to myofibers within a mixed muscle is at present unknown. This subject will be more fully discussed in an ensuing paper.

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